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Revised version

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## **$^{13}\text{C}$ analysis of molecular organic substances, a novel breakthrough in analytical sciences**

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### **Abstract**

The recent development of a novel isotope method, Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (GC-C-IRMS), allows the direct determination of  $^{13}\text{C}/^{12}\text{C}$  ratios of any GC-amenable compound occurring at trace levels in very complex mixtures, and thus undoubtedly finds applications in every field aiming at the study of organic substances.

Keywords :  $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{18}\text{O}$ ,  $^{15}\text{N}$ , soil, sediment, alkane, acide gras, hopanoid.

### **Résumé**

La mise au point récente d'une nouvelle méthode isotopique, la Chromatographie en phase Gazeuse couplée à un four à Combustion puis à un Spectromètre de Masse de Rapport Isotopique (CG-C-SMRI), permet de mesurer les rapports  $^{13}\text{C}/^{12}\text{C}$  de substances présentes à l'état de traces dans les mélanges très complexes. Cette méthode va donc sans aucun doute créer de nouveaux domaines de recherche pour l'étude des substances naturelles.

Mots-clefs :  $^{13}\text{C}$ ,  $^2\text{H}$ ,  $^{18}\text{O}$ ,  $^{15}\text{N}$ , sol, sédiment, alcane, acide gras, hopanoïde.

### **A NOVEL HYPHENATED METHOD**

During the past decade, a novel hyphenated technique (1,2), coupling a gas chromatograph (GC) to a combustion furnace (C) then to an isotope ratio monitoring mass spectrometer (IRMS) has been designed (fig. 1). Typically, a complex mixture of substances is injected onto a high resolution fused-silica capillary column. After chromatographic separation under continuous helium flow, each compound is converted into  $\text{CO}_2$  and water by combustion with  $\text{CuO}$  at about  $850^\circ\text{C}$ . After water removal either by cryogenic trapping at  $-100^\circ\text{C}$  or by diffusion through a selective membrane, the  $\text{CO}_2$  is analysed by the mass spectrometer monitoring simultaneously ion currents at masses 44 ( $^{12}\text{C}^{16}\text{O}_2$ ), 45 ( $^{13}\text{C}^{16}\text{O}_2$ ,  $^{12}\text{C}^{16}\text{O}^{17}\text{O}$ ) and 46 ( $^{12}\text{C}^{16}\text{O}^{18}\text{O}$ ) with Faraday cups. Carbon isotope compositions are expressed in per mil relative to a geological carbonate, the Pee Dee Belemnite standard:  $\delta^{13}\text{C} = ([(^{13}\text{C}/^{12}\text{C}_{\text{sample}}) / (^{13}\text{C}/^{12}\text{C}_{\text{std}})] - 1) \times 10^3$ . It

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\*Lauréat du Prix de la Division Chimie Analytique de la Société Française de Chimie, en 1995.

should be also mentioned that modified systems are now able to measure  $^{15}\text{N}/^{14}\text{N}$ ,  $^{18}\text{O}/^{16}\text{O}$  and  $^2\text{H}/^1\text{H}$  ratios of individual substances.

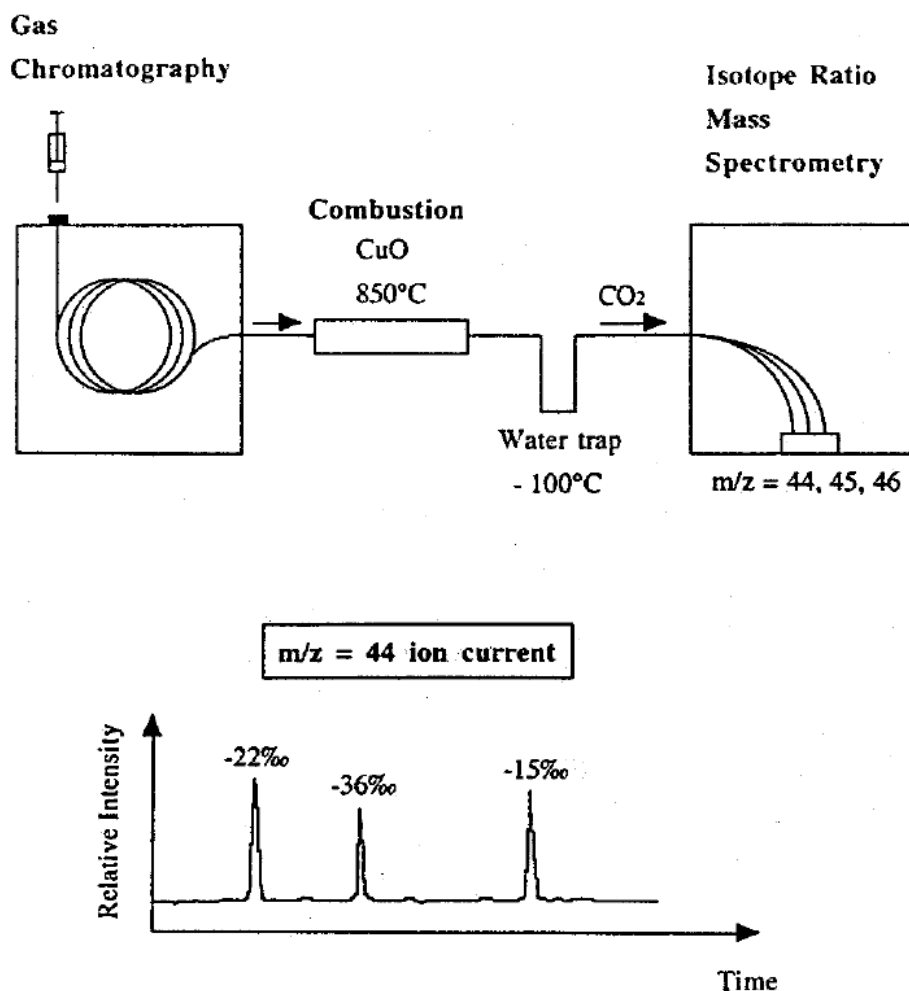


Fig. 1 Schematic view of a new hyphenated method, gas chromatography coupled to a combustion furnace and to an isotope ratio mass spectrometer (up) and typical ion current of carbon dioxide from the combustion of organic substances (down).

### NEW FIELDS OF INVESTIGATIONS

Gas chromatography-combustion-isotope ratio mass spectrometry allows to measure  $^{13}\text{C}/^{12}\text{C}$  ratios of any GC-amenable substances, e.g. gases, lipids, aromatic hydrocarbons, pesticides, sugars, amino acids, etc. It thus enables scientists to use carbon 13 as a **stable tracer** at the molecular level in natural systems. Indeed, tremendous increases of performance have been gained in comparison to classical isotope ratio mass spectrometry analysis (IRMS). Firstly, the  $^{13}\text{C}/^{12}\text{C}$  ratio of each component from a complex mixture, e.g. more than 100 substances, can be measured in a single run, without earlier separation of each component in a pure form, which was difficult, time-consuming and in most cases impossible. This method opens fields aiming at the study of very complex mixtures such as petroleum, biological and environmental samples. Secondly, samples containing as low as 5 nmol of C can be analysed, which is about 100 times lower than the amount required by classical IRMS. GC-C-IRMS is therefore particularly well suited for trace analysis. Thirdly, the high resolution of the gas chromatographic step is usually one of the best way to purify each

substance before its conversion to CO<sub>2</sub>. Fourth, the good accuracy in isotope composition measurements of about 0.1‰ allows not only studies using artificially labelled substrates but also investigations at natural abundance. GC-IRMS has already found many applications in various academic and industry fields (table I). Several striking examples of biogeochemical studies using <sup>13</sup>C at the molecular level are outlined in the following sections.

Table I. Main fields of application of Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry (CG-C-IRMS).

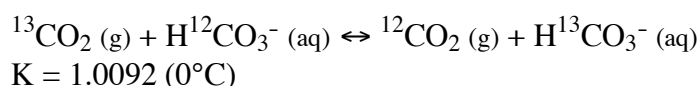
<b>FIELD</b>	<b>COMPOUND</b>	<b>APPLICATION</b>
Archaeology Environment Organic geochemistry	Alkanes, steroids, terpenoids, hopanoids, aromatic hydrocarbons, S-hydrocarbons, alkenones, carotenoids, fatty acids, amino acids, alcohols, carbohydrates, CO <sub>2</sub> , CH <sub>4</sub>	Palaeoenvironments Origin of molecular fossils Oil-source rock correlations Origin of life (meteorites) Tracer studies Carbon cycles Plant photosynthesis Paleodiets Soil and sediment pollution Climate changes
Food products Perfumes, aromas	Essential oils, flavors, vanillin, linalool	Adulteration
Sport	Steroids	Drug testing
Nutrition Medecine	Fatty acids, lipids, amino acids steroids, carotene, urea, ethanol, triglycerides, glucose, CO <sub>2</sub> , cholesterol	Metabolism studies Tests Biosynthesis
Forensic science	Heroin	Geographical origin

### **PUZZLING SEDIMENTARY MIXTURES**

Sedimentary organic matter is made up of remnants of various organisms such as plants, plankton and micro-organisms living millions years ago. During and after sedimentation, it undergoes multiple changes such as biodegradation, defunctionalization (loss of P, N, O), sulfurization and aromatisation, under the influence of increasing temperature, pressure and mineral catalysis. It occurs in soil, sediment, coal and petroleum as mixtures of organic molecules that are much more complex than what can be currently seen in biological fields. Lucky is the organic chemist who can follow in "real-time" the reaction of two pure compounds in a round-bottom flask by thin layer chromatography! The organic biogeochemist is dealing with hundreds of poorly defined natural substances reacting together million-years ago in a kind of paleobeaker. Moreover, our knowledge of microbial communities and food webs occurring in the water column and in the sediments is very poor. This implies that our present insight into ancient and modern biogeochemical processes is quite limited. For instance, the nature of the ancient living organisms that have contributed to the formation of petroleum is still unknown.

## CARBON ISOTOPES : POWERFUL TRACERS

Most modern biological substances have about 98.89% of  $^{12}\text{C}$ , 1.11% of  $^{13}\text{C}$ , and  $10^{-10}\%$  of  $^{14}\text{C}$ . Isotope compositions of natural substances are expressed in delta per mil ( $\delta^{13}\text{C}\%$ ) and measure small variations of  $^{13}\text{C}/^{12}\text{C}$  ratios. The use of stable carbon isotopes,  $^{12}\text{C}$  and  $^{13}\text{C}$ , to study present and ancient biogeochemical cycles is of great interest because the  $^{13}\text{C}/^{12}\text{C}$  ratios of natural substances are heterogeneous (fig. 2). Atmospheric  $\text{CO}_2$ , for example, has an actual  $\delta^{13}\text{C}$  value of about  $-8\%$ . It is depleted in  $^{13}\text{C}$  relative to marine carbonates,  $\sim 0\%$ , as the result of an equilibrium isotope effect that favours the concentration  $^{12}\text{CO}_2$  in the gas phase:



As a result, terrestrial  $\text{C}_3$  plants, which fix atmospheric  $\text{CO}_2$ , are usually  $^{13}\text{C}$ -depleted relative to marine algae, which grow partially on carbonate substrates. This difference may therefore be used to assess the contribution of marine versus terrestrial organic matter in sediments and petroleums.

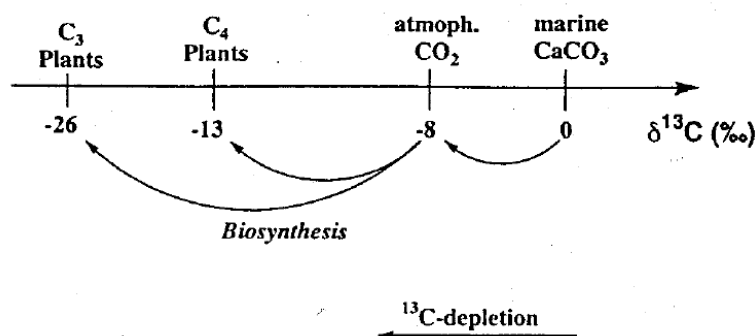


Fig. 2 Average  $^{13}\text{C}/^{12}\text{C}$  ratios of some modern compounds.

A peculiar example of the use of  $^{13}\text{C}$  as an **environmental tracer** is the dramatic decrease of the  $\delta^{13}\text{C}$  composition of atmospheric  $\text{CO}_2$  from  $-6.3\%$  to  $-8.0\%$  in the two last centuries, as deduced from air trapped in polar ice cores and from old maize collections (3). This change is thus associated with the industrial revolution and is mainly explained by the input of  $^{13}\text{C}$ -depleted fossil-fuel-derived  $\text{CO}_2$  ( $\sim -27\%$ ) by human activities.

Kinetic isotopic effects associated with incomplete chemical and biochemical reactions is another source of isotopic variation of organic carbon. A *normal* effect is characterised by a faster reaction of  $^{12}\text{C}$ - versus  $^{13}\text{C}$ -compounds, leading to the  $^{12}\text{C}$ -enrichment of the product and to the depletion in  $^{13}\text{C}$  of the reactant. Most biochemical reactions thus give products that are usually depleted in  $^{13}\text{C}$  relative to the reactant. Carbon of terrestrial plants, for example, is depleted in  $^{13}\text{C}$  relative to atmospheric  $\text{CO}_2$  (Figure 2). Remarkably, the depletion in  $^{13}\text{C}$  of about  $-20\%$  of living organisms relative to carbonates is a peculiar feature of biological processes. Since this difference has been observed between carbonates and associated organic matter in

sediments as old as  $3.8 \cdot 10^9$  years, it has been inferred that life was already present at that time.

It is also possible to identify the photosynthetic mode of plants by  $^{13}\text{C}$  analysis. Indeed,  $\text{C}_3$  plants, e.g. trees or wheat, fix  $\text{CO}_2$  using the Calvin cycle and have an average isotopic composition of  $-26\text{‰}$ . On the other hand,  $\text{C}_4$  plants, e.g. maize, fix  $\text{CO}_2$  using the Hatch-Slack cycle and have average isotopic composition of  $-13\text{‰}$ . An interesting consequence of this isotopic difference in forensic sciences is that one can simply distinguish corn-eaters, e.g. Americans, from wheat-eater, e.g. Europeans, by isotopic analysis. In the same way, the isotopic analysis of ancient human bones can provide clues to reconstruct ancient diet. In a more general way, the carbon isotopic composition of any naturally synthesised organic compound depends on: 1- the carbon source utilised; 2- isotope effects associated with assimilation of carbon by the producing organisms; 3- isotope effects associated with metabolism and biosynthesis; and 4- cellular carbon budgets (4). In the following sections, peculiar geochemical and environmental examples of isotopic studies at the molecular level are presented.

### A 60 MILLION-YEARS OLD CHEMICAL REACTION

*n*-Alkanes are an important class of natural substances occurring almost ubiquitously in living organisms, soils, sediments and petroleums. Although extensively studied, the ancient mode of formation of *n*-alkanes in deep sediments is still a matter of speculation. Indeed, fossil *n*-alkanes might derive from numerous biological precursors such as wax *n*-alkanes, *n*-alcohols, and *n*-fatty acids. They could also be formed by thermal breakdown of the remnants of highly resistant biopolymers. In order to test the possible production of fossil *n*-alkanes by decarboxylation of fossil *n*-fatty acids, we have analysed their individual  $\delta^{13}\text{C}$  values in a 60 million-years-old Eocene sediment from the Green River formation, Colorado (5) (fig. 3).

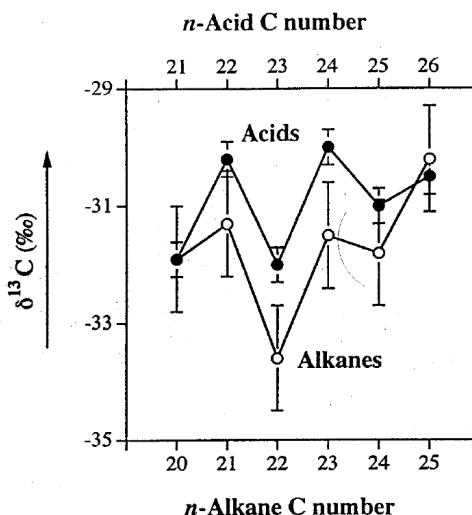


Fig. 3 Isotope values of individual *n*-alkanes and *n*-alkanoic acids from a 60 millions years old Eocene sediment.

The isotopic values of fossil *n*-fatty acids and *n*-alkanes are very close and are covariant with increasing carbon number. This striking feature indicates that these two classes of chemical fossils are biogenically linked. Furthermore, *n*-fatty acids with  $x + 1$  carbons are correlated with *n*-alkanes with  $x$  carbons. Such a correlation demonstrates

that *n*-alkanes in deep sediments can be produced by decarboxylation of *n*-fatty acids (fig. 4). Hence, this approach provides novel clues to understand the nature of ancient chemical reactions.

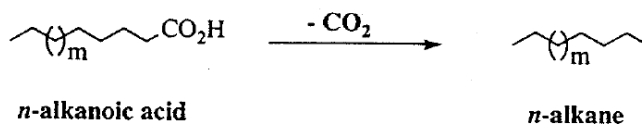


Fig. 4 Postulated mode of formation of fossil *n*-alkanes by decarboxylation of fossil fatty acids.

### RECONSTRUCTION OF ANCIENT FOOD WEBS

Our knowledge of modern and ancient food webs in waters and sediments is poor. For instance, the pathways of carbon flow from atmospheric CO<sub>2</sub> to photosynthetic and heterotrophic organisms are far from being well understood. Also, since the isotopic composition of biological molecules depends on carbon sources of living organisms ("we are what we eat"), isotopic investigations of specific molecular fossils should give new insights on the diet and on the food web level of ancient organisms.

Isotopic investigations (6,7) of specific molecular fossils in Eocene sediments from ancient lakes have shown an unprecedented wide range of isotopic values, from about -20‰ to -90‰. These data have been interpreted in terms of ancient food webs and paleoenvironment (fig. 5). Pristane, for example, is a typical transformation product of phytol from chlorophyll. The isotopic composition of pristane, around -27‰, is in good agreement with a derivation from ancient photosynthetic organisms, e.g. algae and cyanobacteria using atmospheric CO<sub>2</sub> and carbonates as main carbon source. On the other hand, hopanes, typical transformation products of bacterial hopanols, have extremely <sup>13</sup>C-depleted isotope compositions of about -85‰. Since the sole bacterial food on earth strongly <sup>13</sup>C-depleted is biogenic methane (-40‰ to -100‰), it has been inferred that hopanes are the fossils of methanotrophic bacteria.

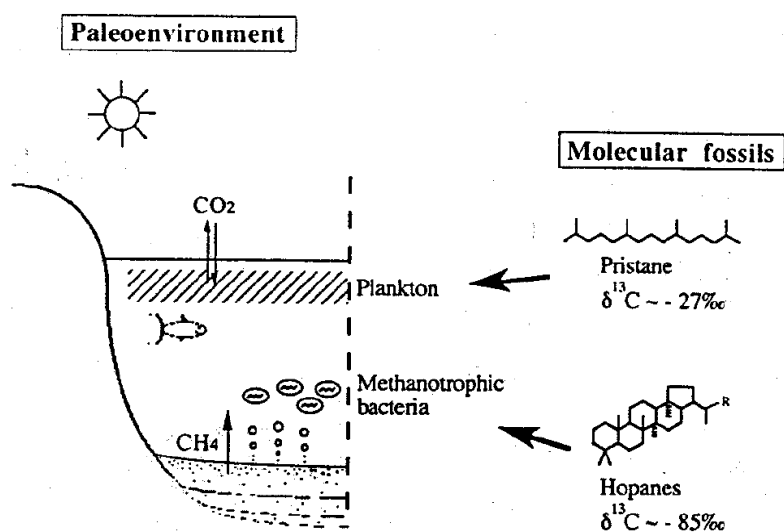


Fig. 5 Reconstruction of ancient food webs of a 60 million-years-old lake using carbon isotope composition of specific molecular fossils.

Another remarkable example of paleoenvironmental work has been reported in a recent isotopic study of molecular fossils of various geological age (8). It was observed that *n*-heptadecane from proterozoic sediments aged of 1,055 millions years is unusually enriched in <sup>13</sup>C relative to the bulk organic debris of the same sample, a feature which is not usually observed in younger sediments. These data have suggested that the oxic-anoxic interface was located high in the water column and that there was an extensive reworking of slow-sinking planktonic matter by heterotrophic organisms at the time of deposition. Paleoenvironmental conditions are therefore recorded by molecular fossils. Deciphering the parameters which control their <sup>13</sup>C content will undoubtedly give new insights on environmental changes that happened in the past. Moreover, such knowledge is of fundamental importance not only to understand the modern environment, but also to predict future evolution such as climatic changes.

### SOIL BIOGEOCHEMISTRY

Soil organic matter is a major pool of labile carbon on earth and plays a peculiar role in the global carbon cycling. Hence, its behaviour under changing factors is of agronomical, environmental and biogeochemical importance. For example soil organic matter acts as a filter of anthropogenic substances such as pesticides. However, little is known about the structure, the origin and the transformation of soil organic molecules. Using <sup>13</sup>C as a tracer at the molecular level, we have designed a new method to follow and to measure the fate of plant carbon into soil molecules (9).

Cuticular *n*-alkanes from C<sub>3</sub> plant waxes have average δ<sup>13</sup>C values of -36‰. Therefore, long-chain *n*-alkanes (C<sub>25</sub>-C<sub>35</sub>) from soils cultivated with C<sub>3</sub> plants show δ<sup>13</sup>C values around -36‰. If this soil is cultivated at a later stage with maize, a C<sub>4</sub> plant, the isotopic composition of soil *n*-alkanes will slowly increase with time because *n*-alkanes from maize waxes have δ<sup>13</sup>C values around -20‰ (fig. 6). The isotopic composition of soil *n*-hentriacontane (C<sub>31</sub>), for instance, increases from -35.7‰ to -28.1‰ after 23 years of maize cultivation. Hence, such a field experiment allows soil organic molecules to be labelled at natural abundance. The observed <sup>13</sup>C enrichment of long-chain *n*-alkanes from maize soils demonstrates that these compounds are derived from plant waxes. Furthermore, a simple calculation by isotope balance allows to determine the percentage M of maize *n*-alkane into each soil *n*-alkane:

$$M = 100 \cdot (\delta - \delta_0) / (\delta_m - \delta_0)$$

δ : δ<sup>13</sup>C value of the soil *n*-alkane at a given time of cultivation

δ<sub>0</sub> : δ<sup>13</sup>C value of the soil *n*-alkane before maize cultivation (-35.7‰)

δ<sub>m</sub> : δ<sup>13</sup>C value of maize *n*-alkane (-20.6‰)



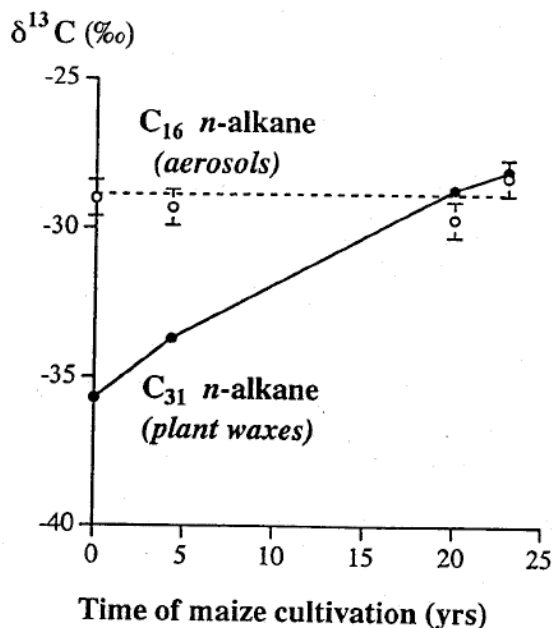


Fig. 6 Isotope composition of *n*-alkanes from soils cultivated with maize. Note the enrichment in  $^{13}\text{C}$  of the  $\text{C}_{31}$  *n*-alkane with time. Note also the absence of isotopic variation of the  $\text{C}_{16}$  *n*-alkane.

In a more general way, if a component A is made of a mixture of two isotopically distinct components B and C, the proportions of B and C can be determined if the isotope composition of B and C are known. It was found that *n*-hentriacontane ( $\text{C}_{31}$ ) contains 50.3% of maize waxes after 23 years of maize cultivation. Hence, 49.7% of this compound derive from wheat waxes and thus represent the proportion of carbon older than 23 years. This low turnover is probably due to the resistance of long chain *n*-alkanes to biodegradation, in comparison with more functionalized substances such as amino acids and carbohydrates. Therefore, such an approach using isotope analysis represents a novel way to study the transformation of soil organic matter at the molecular level.

### $^{13}\text{C}$ BETRAY FOSSIL FUEL POLLUTION

As shown in the previous section, some light on the origin and fate of soil organic compounds has been brought using *variations* of isotope compositions during a field labelling experiment. But the *absence of variation* of isotope compositions in this experiment could have considerable significance, as well. We have found that short-chain *n*-alkanes from the soils cultivated with maize do not show any variation with time of cultivation (see  $\text{C}_{16}$  on fig. 6). These *n*-alkanes cannot derive from the plant and soil biomass, because if it was so, they should have been labelled by  $^{13}\text{C}$ -enriched carbon from maize. By contrast, the isotopic variation of short-chain soil *n*-alkanes gives evidence for an allotochthonous origin. Their isotope composition of about -29‰ is also typical of values observed for fossil fuels such as petroleums. Furthermore, to sustain the relatively high concentration of short-chain *n*-alkanes in soils, a regular input of fossil fuel and combustion products carried by airborne particles is the most likely source.

The discovery of a petroleum spill in cultivated soils is another striking example of the use of  $^{13}\text{C}$  as an environmental and molecular test (10). During our investigations of soil lipids, we have been surprised by the unusual distribution of *n*-alkanes from a soil sample (fig. 7). Most soil samples show *n*-alkane distributions with a predominance of odd-carbon *n*-alkanes in the  $\text{C}_{25}$ - $\text{C}_{35}$  range. Such a fingerprint is typical of an input of plant cuticular waxes into the soil. However, one soil sample, named "polluted soil", shows a *n*-alkane distribution with few odd/even carbon predominance, suggesting a major input from fossil fuels and a minor input from plant waxes. This hypothesis was confirmed by isotope analysis.

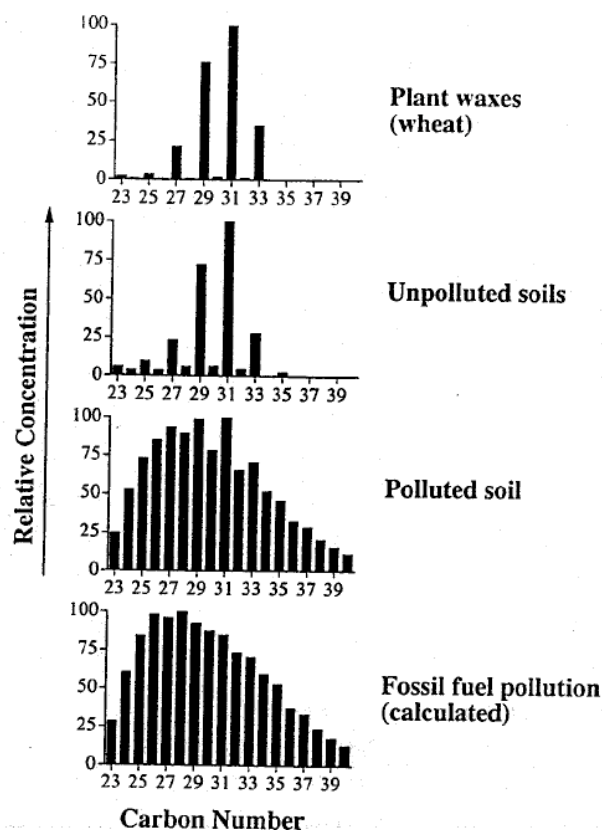


Fig. 7 *n*-Alkane distributions of plant, soils and fossil fuel pollution. (Data from Lichtfouse and Eglinton, *Org. Geochem.*, in press.)

$\delta^{13}\text{C}$  composition of *n*-alkanes from unpolluted soils of about - 36‰ are typical of an input from  $\text{C}_3$  plant waxes (fig. 8). By contrast, the  $\delta^{13}\text{C}$  values of *n*-alkanes from the polluted soil of about - 30‰ are typical of fossil fuels such as petroleum. Moreover, the slight depletion in  $^{13}\text{C}$  of  $\text{C}_{29}$  and  $\text{C}_{31}$  *n*-alkanes confirms that these compounds are made of a *mixture* of plant waxes *n*-alkanes and fossil fuel *n*-alkanes (see arrows on fig. 8). It is even possible to reconstruct the original fingerprint of the pollution using isotope composition and relative abundances. The fossil fuel contribution *x* of each *n*-alkane from the polluted soil can be calculated by:

$$x = (\delta_P - \delta_U) / (\delta_F - \delta_U)$$

- x : *n*-alkane fraction derived from the fossil fuel
- $\delta_P$  :  $\delta^{13}\text{C}$  value of the *n*-alkane from the polluted soil
- $\delta_U$  :  $\delta^{13}\text{C}$  value of the *n*-alkane from the unpolluted soil (plant-derived)
- $\delta_F$  :  $\delta^{13}\text{C}$  value of the *n*-alkane from the fossil fuel source (-29.5‰)

For instance, the x value for the C<sub>29</sub> *n*-alkane is 0.81 ( $\delta_P$  -30.7‰,  $\delta_U$  -35.7‰), which means that this compound is composed of 81‰ of fossil fuel *n*-alkane and 29‰ of plant-derived *n*-alkane. Hence, the relative concentration A<sub>F</sub> of each *n*-alkane from the fossil fuel pollution is simply obtained by  $A_F = x \cdot A_P$ , where A<sub>P</sub> is the relative concentration of each *n*-alkane in the polluted soil. The A<sub>F</sub> value for the C<sub>29</sub> *n*-alkane is thus 80.4 (A<sub>P</sub> 99.2). This calculation has been done for all *n*-alkanes from the polluted soil, allowing virtually to subtract the plant contribution. After normalisation of relative abundance to 100%, the reconstructed fingerprint of the pollution shows a distribution without predominance of odd or even *n*-alkanes, which is very characteristic of a fossil fuel (fig. 8). To conclude, such an approach using carbon isotopes and concentrations of biological markers is a novel way to track down the origin of pollutions in contaminated samples.

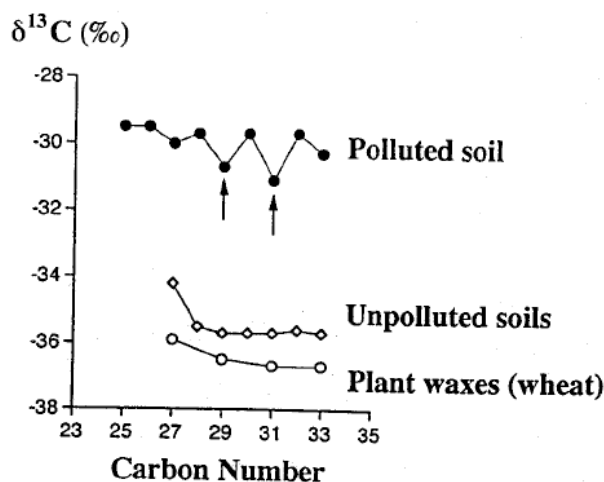


Fig. 8  $\delta^{13}\text{C}$  values of *n*-alkanes from plant and soils. The  $^{13}\text{C}$ -depletion of odd-carbon number *n*-alkanes from the polluted soil (arrows) gives evidence for a mixture of plant *n*-alkanes and fossil fuel *n*-alkanes.

### SEDIMENTARY STUDIES

The recent analytical progress and, more precisely, the improvements in deciphering molecular information have allowed the organic geochemistry to perform detailed qualitative analysis. This science studies molecular contents by interpreting structural (isomeric, stereochemical details) information. Numerous molecular tools have been constructed. They can be divided into two classes: some of them are individual compounds, others are molecular indices constructed as concentration ratios. These tools are used to reconstruct the fate of the organic matter in the environment. They can allow also to characterize the origin of the organic matter.

The measurement by GC-C-IRMS of isotopic composition of individual compounds in conjunction to such a molecular approach add a new dimension to these studies and provides the geochemist with a powerful tool to study the fate and the origin of the organic matter.

Various applications of this concept are found in the analysis of *n*-alkanes. For example, Ishiwatari and his co-workers (11), by using GC-C-IRMS to determine carbon isotope composition of individual long chain *n*-alkanes from Tokyo Bay sediments, were able to reconstruct a two end-member mixing model of indigenous higher plant wax *n*-alkanes with *n*-alkanes from oil pollution. The addition of  $^{13}\text{C}$  individual data to molecular criteria allows in some cases to correct currently assumed ideas. In this way, it has been shown via isotopic and molecular studies that the occurrence of odd-numbered *n*-alkanes in the range  $\text{C}_{25}\text{-C}_{35}$  in sediment extracts may reveal the input of microalgae at the time of deposition (12). Indeed, previous studies have systematically attributed the occurrence of those alkanes to an input of terrestrial plants.  $^{13}\text{C}$  analyses at the molecular level could therefore be a useful tool to try to resolve the multiple origins of molecular fossils.

Since the 60s, major improvements in gas chromatography and mass spectrometry have enabled qualitative and quantitative analyses of very complex mixtures of aromatic compounds. This has led to the construction of several molecular criteria for distinguishing natural and anthropogenic Polycyclic Aromatic Hydrocarbon (PAH) inputs in marine sediments. These criteria were based on PAH molecular fingerprints or concentration ratios. Nevertheless, physical, chemical and microbial transformations can alter the characteristics of many sources during transport and deposition. Alternative methods are very desirable. In that prospect, GC-C-IRMS can be a complementary technique of source apportionment. Using this technique, O'Malley et al.(13) have been able to unravel distinct sources of PAH in sediments. Distinct isotopic patterns were found for 3-, 4- and 5-ring PAH in firesoots, carsoots and crankcase oils. They were used as source apportionment tools. The dominant signatures identified in surface sediments were attributed to a woodburning source but PAH from crankcase oil and other petroleum products were shown to contribute to the overall isotope signatures of secondary sources and sediments. Moreover, the isotopic approach offers the additional advantage that quantitative source apportionment may be possible by mass balance calculations.

$^{13}\text{C}$  data can also be of a great use in the study of macromolecular materials. The analysis of geomacromolecules by gas chromatography requires a previous chemical degradation of the macromolecular network to obtain GC-amenable low-molecular-weight subunits. Hydrogenolysis of carbon-sulfur bonds combined with compounds specific carbon isotope measurements has proven to be a valuable approach to provide information on sulfurization mechanisms and on the original depositional environment (14,15). More recently the combined use of chemical degradation techniques and GC-C-IRMS has allowed to differentiate between at least two organic sources contributing to the same macromolecular fraction (16). Moreover, this new approach has supplied information on the mode of formation of macromolecules which could not be deduced from the chemical degradation alone.

## FURTHER DEVELOPMENTS

### ***GC-C-IRMS***

(Gas Chromatography-Combustion-Isotope Ratio Mass Spectrometry)

Several novel methods that allow isotope analysis of molecular substances occurring at trace levels in complex media are actually rapidly developing(17). GC-C-IRMS has been modified to measure  $^{15}\text{N}/^{14}\text{N}$  ratios of organic compounds, e.g. amino acids, nicotine and caffeine, as well as inorganic compounds such as  $\text{N}_2\text{O}$  and nitrates.

Rapid  $^{18}\text{O}/^{16}\text{O}$  analysis of small water samples (0.5  $\mu\text{l}$ , ~250 sec.) has also been set up in a continuous-flow system by conversion of  $\text{H}_2\text{O}$  into  $\text{CO}$ , then monitoring  $m/z$  28 and 30 with an isotope ratio mass spectrometer. Measurements of water  $^2\text{H}/^1\text{H}$  ratios are also possible by conversion of  $\text{H}_2\text{O}$  into  $\text{H}_2$  in a continuous-flow system. Another development of interest is the isotope analysis of trace organic compounds in water using solid phase micro extraction (SPME), which will undoubtedly find many applications in geochemistry and environment (water samples) as well as in food chemistry (wine) and medicine (plasma).

### ***HPLC-IRMS***

(High Performance Liquid Chromatography-Isotope Ratio Mass Spectrometry)

Due to the relatively high temperatures required to separate compounds by gas chromatography (~ 50-350°C), GC-C-IRMS will be somewhat restricted to relatively small, volatile, and stable molecules of molecular mass lower than 800 uma. The recent development of HPLC coupled with IRMS (18,19,20) allows now to undertake isotope investigations of thermosensible and high molecular weight compounds such as proteins that are of great biological interest. Tracing studies at the molecular level of biological substances in human tissues using stable isotopes instead of radioactive atoms will therefore be a new research field.

### ***SNIF-NMR***

(Site-specific Natural Isotope Fractionation measured Nuclear Magnetic Resonance)

Isotope ratio mass spectrometry (IRMS) requires first the conversion of a compound into  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . The measured isotope ratios represent therefore an average of all carbon of this compound. A new method (21, 22), SNIF-NMR, now allows to measure isotope ratios at each atom position in the molecule. This analysis at the atomic level of  $^{13}\text{C}/^{12}\text{C}$  and  $^2\text{H}/^1\text{H}$  has already found several applications of interest in biosynthetic studies and food chemistry, e.g. for flavour adulteration.

### ***GC-AMS***

(Gas Chromatography-Accelerator Mass Spectrometry)

This is the "Jules Verne" section of this article. Indeed, the coupling of gas chromatography with an Accelerator Mass Spectrometer (AMS), thus allowing the  $^{14}\text{C}$  analysis of trace organic compounds occurring in complex media, has not yet been set up. However, with the current development of novel hyphenated techniques, one can predict that a such method have good chances to appear in the next 20 years.  $^{14}\text{C}$  analysis of trace molecules should be of particular interest in archaeology, in recent geochemistry and in food chemistry.

### ***GC-AED***

(gas chromatography- atomic emission detector)

A hyphenated system coupling the gas chromatography to an Atomic Emission Detector (GC - AED) has appeared few years ago. This detector was introduced on the market by Hewlett Packard in 1989 (23). This system enables the detection of atomic elements by using different wavelengths.

In the AED the molecules eluting from the chromatographic column are atomized in a helium-sustained plasma. The energy for the plasma generation is supplied by microwaves at 2.45 GHz, giving an energy input to the plasma of about 50 W. Some of the atoms of the eluting compounds are excited by the plasma. When the

excited electrons fall back to the ground level the excitation energy is emitted as light. By using a diode array to register the light, emissions can be recorded over a wide wavelength range from nitrogen at 174.3 nm to oxygen at 777.3 nm.

Dual isotope measurements can be obtained for elements which have sufficiently spaced emission lines (24, 25).  $^{13}\text{C}$  is recorded at 342 nm and  $^{12}\text{C}$  is recorded at 343 nm. Such a detector shows a limit of detectable isotopic dilution of about 2% which represents about 1000 times as high as the one of GC-C-IRMS (24). Its big advantages are its sensitivity (about at least 10 times as high as the one of the GC-CIRMS) and its linearity. This technique can be of a great use in the case of compounds which present large variations in  $\delta^{13}\text{C}$  and are present at trace levels.

## REFERENCES

- 1- Matthews D. E., Hayes J. M. *Anal. Chem.*, 1978, 50, 1465-1473.
- 2- Barrie A., Bricout J., Koziat J. *Biomed. Mass Spectrom.*, 1984, 11, 583-588.
- 3- Marino B. D., McElroy M. B. *Nature*, 1991, 349, 127-131.
- 4- Hayes J. M. *Mar. Geol.*, 1993 113, 111-125.
- 5- Lichtfouse É., Collister J. W. *Tetrahedron Lett.*, 1992, 33, 8093-8094.
- 6- Collister J. W., Summons R. E., Lichtfouse E., Hayes J. M. *Org Geochem.*, 1992, 19, 265-276.
- 7- Freeman K. H., Hayes J. M., Trendel J.-M., Albrecht P. *Nature*, 1990, 343, 254-256.
- 8- Logan G. A., Hayes J. M., Hieshima G. B., Summons R. E. *Nature*, 1995, 376, 53-56.
- 9- Lichtfouse É. *Tetrahedron Lett.*, 1995, 36, 529-530.
- 10- Lichtfouse É., Eglinton T. *Org. Geochem.*, 1995, in press.
- 11- Ishiwatari R., Usaki M., Yamada K. *Org. Geochem.*, 1994, 21, 801-808.
- 12- Lichtfouse É., Derenne S., Mariotti A., Largeau C. *Org. Geochem.*, 1994, 22, 1023-1027.
- 13- O'Malley V. P., Abrajano Jr T. A., Hellou J. *Org. Geochem.*, 1994, 21, 809-822.
- 14- Kohnen M. E. L., Schouten S., Sinninghe Damsté J. S., de Leeuw J. W., Merritt D. A., Hayes J. M. *Science*, 1992 256, 358-362.
- 15- Schoell M., Hwang R. J., Carson R. M. K., Welton J. E. *Org. Geochem.*, 1994, 21, 673-683.
- 16- Hauke V., Hefter J., Wehrung P., Albrecht P., Richnow H. H., Michaelis W. A new approach to investigate sulphur-rich macromolecules: stepwise chemical degradations combined with IRMGC-MS, présentée au 17<sup>e</sup> congrès de Géochimie Organique, San Sebastian, 4-8 septembre 1995.
- 17- Brand W. A., Tegtmeyer A. R., Hilkert A. *Org. Geochem.*, 1994, 21, 585-594.
- 18- Caimi R. J., Brenna J. T. *Anal. Chem.*, 1993, 65, 3497-3500.
- 19- Caimi R. J., Houghton L. A., Brenna J. T. *Anal. Chem.*, 1994, 66, 2989-2991.
- 20- Caimi R. J. and Brenna J. T. *J. Mass Spectrom.*, 1995, 30, 466-472.
- 21- Caer V., Trierweiler M., Martin G. J., Martin M. L. *Anal. Chem.*, 1991, 63, 2306-2313.
- 22- Martin G. J., Martin M. L., Mabon F., Bricout J. J. *Amer. Chem. Soc.*, 1982, 104, 2658-2659.
- 23- Sullivan J. J., Quimby B. D. *Anal. Chem.*, 1990, 62, 1034-1043.

- 24- Deruaz B., Deruaz D., Bannier A., Desage M., Brazier J. L. *Analisis*, 1994, 22, 241-248.
- 25- Andersson J. T., Schmid B. *Fresenius J. Anal. Chem.*, 1993, 346, 403-409.

**Further readings:**

Craig H. "The geochemistry of the stable carbon isotopes".  
*Geochim. Cosmochim. Acta*, 1953, 3, 53-92.

Craig H. "Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide".  
*Geochim. Cosmochim. Acta* 1957, 12, 133-149.

Degens E. T. "Biogeochemistry of stable carbon isotopes".  
dans "Organic Geochemistry, Methods and Results", (Eglinton G., Murphy M. T. L.) Editeurs, Springer, Berlin, 1969.

Engel M. H., Macko S. A., Nagy B. "The organic geochemistry of carbonaceous meteorites. Amino acids and stable isotopes"  
dans "Organic Geochemistry, Principles and Applications", (Engel M. H., Macko S. A) Editeurs, Plenum Press, New York, 1993.

Fogel M. L., Cifuentes L. A. "Isotope fractionation during primary productions".  
dans "Organic Geochemistry, Principles and Applications", (Engel M. H., Macko S. A) Editeurs, Plenum Press, New York, 1993.

Galimov E. M. " $^{13}\text{C}/^{12}\text{C}$  in kerogen".  
dans "Kerogen, Insoluble Organic Matter from Sedimentary Rocks", (Durand B.) Editeur, Technip, Paris, 1980.

O'Leary M. H. "Carbon isotope fractionation in plants".  
*Phytochem.* 1981, 20, 553-567.

Schidlowski M. " $^{13}\text{C}/^{12}\text{C}$  ratios as indicators of biogenicity".  
dans "Biological Markers in the Sedimentary Record", (Johns R. B.) Editeur, Elsevier, Amsterdam, 1986.

Schidlowski M. "The initiation of biological processes on earth. Summary of empirical evidence".  
dans "Organic Geochemistry, Principles and Applications", (Engel M. H., Macko S. A) Editeurs, Plenum Press, New York, 1993.

Schoell M. "Recent advances in petroleum isotope geochemistry".  
*Org. Geochem.* 1984, 6, 645-663.

Summons R. E. "Biogeochemical cycles: a review of fundamental aspects of organic matter formation, preservation, and composition".  
dans "Organic Geochemistry, Principles and Applications", (Engel M. H., Macko S. A) Editeurs, Plenum Press, New York, 1993.

Handbook of Environmental Isotope Geochemistry  
(Fritz P., Fontes J. Ch.) Editeurs, Elsevier, Amsterdam, 1980.