



HAL
open science

Isotope and biosynthetic evidence for the origin of long-chain aliphatic lipids in soils

Eric Lichtfouse

► **To cite this version:**

Eric Lichtfouse. Isotope and biosynthetic evidence for the origin of long-chain aliphatic lipids in soils. Die Naturwissenschaften, 1998, 85 (2), pp.76-77. 10.1007/s001140050456 . hal-00193585

HAL Id: hal-00193585

<https://hal.science/hal-00193585>

Submitted on 4 Dec 2007

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Revised version

Naturwissenschaften 85, 76-77, 1998.

doi: 10.1007/s001140050456

Correspondence: Dr. Eric Lichtfouse, INRA-CMSE-PME, 17, rue Sully, 21000 Dijon, France

Eric.Lichtfouse@dijon.inra.fr

Isotope and Biosynthetic Evidence for the Origin of Long-Chain Aliphatic Lipids in Soils

Éric LICHTFOUSE

Abstract

Soil organic matter is a very complex mixture of compounds derived from numerous living organisms such as plants, fungi, algae, microfauna and microbes. During humification those biological compounds are transformed by processes such as biotic and abiotic degradation, polymerisation, reduction and aromatisation. Thus, the biological origin of soil organic molecules is often very difficult to assess. Knowledge of the biological origin of soil organic molecules is important, for instance, to understand the mechanisms of preservation of soil organic matter, which are still debated. Here I studied the origin of three classes of soil compounds, n-alkanes, n-alkanols (fatty alcohols), and n-alkanoic acids (fatty acids) that can be derived from numerous biological and anthropogenic sources. Those waxy compounds are of agronomical interest because they are believed to play a role in soil aggregates stability, as a glue, and in soil water retention due to their hydrophobic properties. Since the molecular structure of n-alkanes, n-alkanols and n-alkanoic acids does not yield enough information to assess their precise origin, I measured their ^{13}C isotope composition using a recently developed method, gas chromatography coupled to isotope ratio monitoring mass spectrometry (GC-IRMS) to gain more insight. The results show that long-chain n-alkanes (C_{27} , C_{29}), n-alkanols (C_{28} , C_{30}) and n-alkanoic acids (C_{28} , C_{30}) have similar ^{13}C isotope values averaging at -34.2‰. This finding evidences their common origin from leaf cuticular waxes of C_3 plants, in agreement with their biosynthetic pathway. Short-chain n-alkanoic acids (C_{16} - C_{18}) are ^{13}C -enriched with ^{13}C isotope values averaging at -26.6‰. This finding supports their microbial origin demonstrated in a previous report. Short-chain n-alkanes (C_{16} - C_{18}) are also ^{13}C -enriched with ^{13}C isotope values averaging at -28.3‰. This finding supports their fossil fuel origin demonstrated in a previous report. Overall the findings imply that plant leaf waxes are well preserved in soil, despite soil conditions that favors their degradation. Agricultural practices such as incorporating plant leaf residues in soil during tillage and amendment with plant-derived compost should therefore improve aggregate stability and water-holding capacity, on molecular grounds.

Key-words: n-alkane ; n-alkanol; n-alkanoic acid; fatty alcohol; fatty acid; leaf plant wax; fossil fuel pollution; ^{13}C ; GC-IRMS; biosynthesis; C_3 plant

Note from the author: this abstract was written in 2007 for indexing in the HAL archive.

Revised version

Naturwissenschaften 85, 76-77, 1998.

doi: 10.1007/s001140050456

Correspondence: Dr. Eric Lichtfouse, INRA-CMSE-PME, 17, rue Sully, 21000 Dijon, France

Eric.Lichtfouse@dijon.inra.fr

Isotope and Biosynthetic Evidence for the Origin of Long-Chain Aliphatic Lipids in Soils

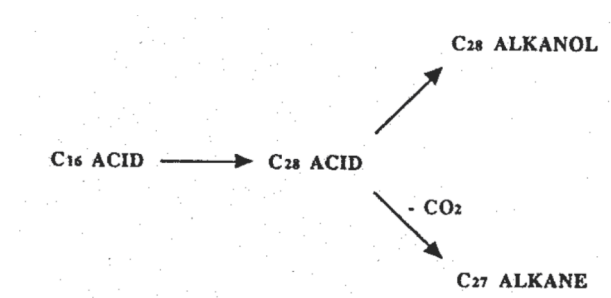
Éric LICHTFOUSE

Laboratoire Sols et Environnement, INRA/ENSAIA-INPL, 2, avenue de la Forêt de Haye,
BP 172, 54505 Vandoeuvre-Lès-Nancy, France
<lichtfouse@ensaia.u-nancy.fr>

Degraded organic matter in natural systems, such as soils [1, 2] and sediments [3, 4], is composed of a very complex mixture of individual organic substances inherited from living organisms, termed "biomarkers" [5]. Although studies of molecular structures have provided several insights on the fate of biological substances after the death of living organisms, their origin and transformation are still a matter of discussion [1-7]. Indeed, until recently only few methods based on stereochemical and distribution comparisons were available to give some insights on the genetic relationships among various classes of linear lipids. This is particularly critical whenever the biomarker molecular structure is poorly informative in terms of biological precursor and of type of processes which led to its presence in soils and sediments [5, 8, 9]. For instance, *n*-alkanes from soils and sediments may be derived either from direct input of biosynthesized *n*-alkanes, e.g. from higher plants [3, 10-12]; or by reduction of biological alkenes, e.g. from algae [3, 13, 14]; or by reduction of fatty alcohols, e.g. from bacteria and higher plants [10, 11, 15]; or by reduction or decarboxylation [3, 8, 16, 17] of the almost ubiquitous biological fatty acids [15]; or by degradation of highly aliphatic biopolymers [18, 19]. Alternatively, a substantial portion of soil *n*-alkanes can also represent an exogenous input of pollutants such as fossil fuels [20]. Therefore, there is an obvious need for new approaches in order to try to decipher these multiple origins and transformation processes.

A recently developed method, isotope ratio monitoring gas chromatography mass spectrometry (irm-GC-MS) [21], using capillary fused silica-gel columns of high chromatographic resolution, now allows the $^{13}\text{C}/^{12}\text{C}$ composition of GC-amenable substances to be measured at trace levels (~ 5 nmol of C). It is thus possible to undertake tracer studies using stable isotopes of carbon at the molecular level, at natural [14, 22] or artificial abundance [23], of organic substances occurring in complex mixtures, without the need to isolate the substance in a pure form. In this way, the isotope composition of fatty acids, fatty alcohols and linear alkanes from a crop soil at Grignon, France, were determined [23].

The average $\delta^{13}\text{C}$ value [24] of C_{27} - C_{30} soil aliphatic lipids, -34.2% , fall in the range values reported for modern plants fixing CO_2 by the C_3 photosynthetic pathway [14, 25], thus indicating a major contribution from C_3 plants. This plant derivation is in agreement with the absence of ^{13}C -labelling of long chain lipids that have been observed in an earlier study involving incubation of soils with ^{13}C -enriched glucose [23]. Moreover, the distributions of long chain soil aliphatic lipids show a strong carbon number predominance, either even for *n*-alkanoic acids and *n*-alkanols, or odd for *n*-alkanes, typical of biological material such as plant waxes [10]. This feature can be explained in terms of the biosynthetic pathways shown in scheme 1. Indeed, in all plants palmitic acid (C_{16}) is elongated by the addition of two carbon units until the appropriate chain length is reached, e.g. C_{28} [15, 26]. This C_{28} acid is then either reduced or decarboxylated to give the C_{28} alkanol and the C_{27} alkane, respectively. The observed predominances of long chain soil lipids are therefore consistent with derivation from plant waxes.



Scheme 1. Biosynthetic pathway of long chain *n*-alkanoic acids, *n*-alkanols and *n*-alkanes in plant waxes [15, 26].

Nonetheless, long chain aliphatic lipids may also be biosynthesized by other organisms than plants, such as algae, fungi and bacteria [11, 13, 15]. Also, in order to assess the genetic relationships of long chain aliphatic lipids in soils, the isotope composition of *n*-alkanols (e.g. C_{28}) and *n*-alkanes (e.g. C_{27}) were compared with that of their biosynthetic precursors *n*-alkanoic acids (e.g. C_{28}) (Fig. 1). It is shown that presumable acid precursors and both alcohol and alkane products have similar isotope values within the experimental error. Since the carbon isotopic composition of any naturally synthesized organic compound depends on many factors such as the carbon source utilized, isotope effects associated with assimilation, metabolism and biosynthesis, and cellular carbon budgets [27], observed similar isotope values of biomarkers from organisms located at different levels in the food web (e.g. plants and bacteria) and growing on different carbon sources are possible but very unlikely [21]. It is therefore concluded on biosynthetic and isotopic grounds that the long chain *n*-alkanes, *n*-alkanols, and *n*-alkanoic acids in our soil sample are derived from the same precursor organic material via the same photosynthetic pathway, that is plant fixation of CO_2 using the C_3 photosynthetic cycle.

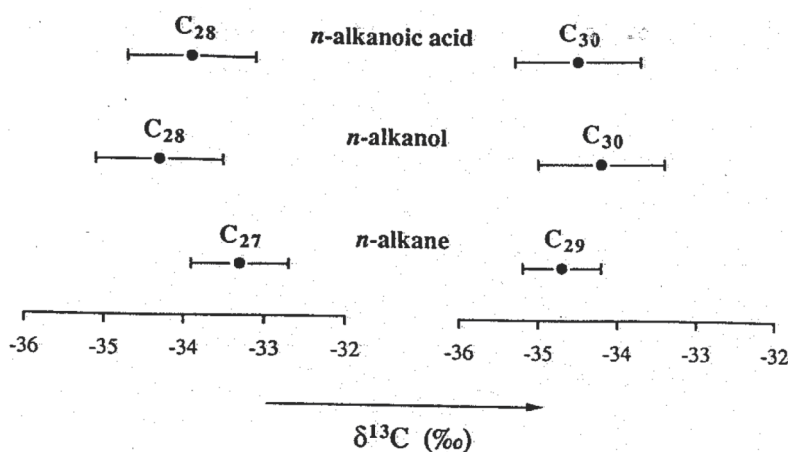


Figure 1. Carbon isotope composition [24] of long chain soil lipids. Data and detailed experimental procedures are reported elsewhere [23, 28, 29]. Typically, soil samples (120 g) from a field cultivated with C_3 plants (Grignon, France) were dried, finely ground, then extracted with CHCl_3 -MeOH (3/1 v/v). The extract was fractionated into neutral and acid fraction by column chromatography on KOH impregnated silica gel. The neutral fraction was then fractionated into polar, alcohol, ketone, and hydrocarbon-ester fractions by silica-gel thin layer chromatography using CH_2Cl_2 as developer and 1,2;3,4-dibenzanthracene, friedelin, and cholesterol as reference compounds. The hydrocarbon-ester fraction was fractionated into alkane-alkene, aromatic, and ester fractions by silica-gel thin layer chromatography using *n*-hexane as developer and 1-phenyldodecane, 2-methylphenanthrene, and 1,2;3,4-dibenzanthracene as reference compounds. The alcohol fraction was acetylated with a large excess of acetic anhydride in pyridine (1/1, v/v) 1 h at 50°C. After the addition of water and CH_2Cl_2 (3 x), the organic phases were mixed then washed with water (3 x), dried overnight over CaCl_2 , concentrated under reduced pressure and fractionated by silica-gel thin layer chromatography using CH_2Cl_2 as developer and cholesterol acetate as reference compound, to give a mono-acetate fraction. Acid fractions were treated overnight with an excess of diazomethane in diethyl ether. After concentration under a ventilated hood, the residue was fractionated by silica-gel thin layer chromatography using ethyl acetate-*n*-hexane (5/95 v/v) as developer and octacosanoic acid methyl ester as reference compound. Isotopic analyses were carried out by isotope ratio monitoring gas chromatography-mass spectrometry (irmGC/MS) as described elsewhere [28, 29]. Isotope composition are expressed in per mil. relative to the PDB standard [24]. $\delta^{13}\text{C}$ values of alcohols and carboxylic acids, and their respective errors, have been corrected for the contribution of the added methyl group ($\delta = -79.7 \pm 3.9\text{‰}$) and acetyl group ($\delta = -50.1 \pm 1.8\text{‰}$), respectively, using pure isotopic standards : palmitic acid, arachidic acid, *n*-hexacosanol, cholesterol, 5 α -cholestan-3 β -ol, and ergosterol.

On the other hand, C_{16} - C_{18} soil *n*-alkanoic acids are strongly enriched in carbon 13 relative to long-chain homologues, giving $\delta^{13}\text{C}$ values ranging from -28.4 to -24.8‰ [23]. This feature suggest that short-chain acids do not represent a plant input. Indeed, it has been demonstrated by labelling of the soil sample with ^{13}C -enriched glucose that C_{16} - C_{18} soil *n*-alkanoic acids are biosynthesized by the microbial biomass such as fungi and bacteria [23]. On the other hand, the absence of labelling of the C_{16} - C_{18} *n*-alkanes in the same experiment allows to exclude microbes as their main biological precursors of these compounds [23]. Further, their $\delta^{13}\text{C}$ values, ranging from -27.1 to -29.4‰ allows to exclude plant as their main biological precursors [30]. In fact, these substances most likely represent an atmospheric input of fossil fuel pollutants, as shown by a 23-year labelling experiment [29]. A such approach using ^{13}C as a tracer at the molecular level represents a powerful tool to decipher the origin and transformation pathways of biomarkers and pollutants occurring in natural media [30].

REFERENCES AND NOTES

1. Schnitzer, M., Khan, S. U. (eds.): *Soil Organic Matter*. Amsterdam: Elsevier 1978
2. Hayes, M. H. B., MacCarthy, P., Malcolm, R. L., Swift, R. S. (eds.): *Humic Substances II. In Search of Structure*. Chichester: Wiley 1989
3. Tissot, B. P., Welte, D. H.: *Petroleum Formation and Occurrence*. Berlin: Springer 1984
4. Engel, M. H., Macko, S. A. (eds.): *Organic Geochemistry, Principles and Applications*. New York: Plenum 1993
5. Peters, K. E., Moldowan, J. M.: *The Biomarker Guide*. Englewood Cliffs: Prentice Hall 1993
6. Schnitzer, M.: *Soil Sci.* *151*, 41 (1991)
7. De Leeuw, J. W., Hatcher, P. G.: *Naturwissenschaft.* *79*, 330 (1992)
8. Lichtfouse, É., Collister, J. W.: *Tetrahedron Lett.* *33*, 8093 (1992)
9. Collister, J. W., Lichtfouse, É., Hieshima, G., Hayes, J. M.: *Org. Geochem.* *21*, 645 (1994)
10. Eglinton, G. and Hamilton, R. L.: *Science* *156*, 1322 (1967)
11. Diné, H., Schnitzer, M., Mehuys, G. R., in: *Soil Biochemistry*, p. 397 (J.-M. Bollag, G. Stotzky, eds.). New York: Marcel Dekker 1990
12. Lichtfouse, É., Elbisser, B., Balesdent, J., Mariotti, A., Bardoux, G.: *Org. Geochem.* *22*, 349 (1994)
13. Gelpi, E., Oró, J., Schneider, H. J., Bennett, E. O.: *Science* *161*, 700 (1968); Gelpi, E., Schneider, H., Mann, J., Oró, J.: *Phytochem.* *9*, 603 (1970)
14. Lichtfouse, É., Derenne, S., Mariotti, A., Largeau, C.: *Org. Geochem.* *22*, 1023 (1994)
15. Kolattukudy, P. E. (ed.): *Chemistry and Biochemistry of Natural Waxes*. Amsterdam: Elsevier 1976
16. Cooper, J. E., Bray, E. E.: *Geochim. Cosmochim. Acta* *27*, 1113 (1963)
17. Behar, F., Albrecht, P.: *Org. Geochem.* *6*, 597 (1984)
18. Tegelaar, E. W., Matthezing, R. M., Jansen, J. B. H., Horsfield, B., De Leeuw, J. W.: *Nature* *342*, 529 (1989)
19. De Leeuw, J. W., Largeau, C. in: *Organic Geochemistry, Principles and Applications*, chap. 2 (M. H. Engel, S. A. Macko, eds). New York: Plenum 1993

20. Lichtfouse, E., Bardoux, G., Mariotti, A., Balesdent, J., Ballentine, D. C., Macko, S. A.: *Geochim. Cosmochim. Acta* *61*, 1891-1898 (1997).
 21. Hayes, J. M., Freeman, K. H., Popp, B. N., Hoham, C. H.: *Org. Geochem.* *16*, 1115 (1990)
 22. Lichtfouse, É.: *Tetrahedron Lett.* *36*, 529 (1995)
 23. Lichtfouse, É., Berthier, G., Houot, S., Barriuso, E., Bergheaud, V., Vallaëys, T.: *Org. Geochem.* *23*, 849 (1995); Lichtfouse, É., Églinton, T.: *Org. Geochem.* *23*, 969 (1995); Lichtfouse, É., Budzinski, H.: *Analisis* *23*, 364 (1995)
 24. Carbon isotopic compositions are expressed in per mil. relative to the Pee Dee Belemnite standard: $\delta^{13}\text{C} = [({}^{13}\text{C}/{}^{12}\text{C}_{\text{sample}} - {}^{13}\text{C}/{}^{12}\text{C}_{\text{std}})/({}^{13}\text{C}/{}^{12}\text{C}_{\text{std}})] \times 10^3$.
 25. Rieley, G., Collier, R. J., Jones, D. M., Eglinton, G., Eakin, P. A., Fallick, A. E.: *Nature* *352*, 425 (1990); Rieley, G., Collister, J. W., Stern, B., Eglinton, G.: *Rapid Commun. Mass Spectrom.* *7*, 488 (1993); Collister, J. W., Rieley, G., Stern, B., Eglinton, G., Fry, B.: *Org. Geochem.* *21*, 619 (1994)
 26. Kolattukudy, P. E., Walton, T. J., in: *Progress in the Chemistry of Fats and other Lipids*, p. 147 (R. T. Holman, ed.). Oxford: Pergamon 1972
 27. Hayes, J. M.: *Marine Geol.* *113*, 111 (1993)
 28. Lichtfouse, E., Albrecht, P., Behar, F., Hayes, J. M.: *Geochim. Cosmochim. Acta* *58*, 209 (1994)
 29. Lichtfouse, E.: *Naturwissenschaft.* *84*, 23-25 (1997)
 30. Lichtfouse, E., Budzinski, H., Garrigues, Ph., Eglinton, T. I.: *Org. Geochem.* *26*, 353-359 (1997)
-