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Exploration of intramolecular ^{13}C isotope distribution in long chain *n*-alkanes (C_{11}–C_{31}) using isotopic ^{13}C NMR

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ABSTRACT

n-Alkanes are ubiquitous and useful biomarkers in the biogeochemistry field. Their carbon isotope composition in sedimentary organic matter is therefore of particular importance for inferring their origin. The commonly used technique for $\delta^{13}C$ determination, isotope ratio mass spectrometry (IRMS), gives access to the isotope composition of *n*-alkanes at the molecular level, but does not provide information on their intramolecular isotope distribution. Here, we evaluate the potential of isotopic ^{13}C nuclear magnetic resonance (NMR) spectrometry for the determination of the intramolecular isotope composition of long chain *n*-alkanes (C_{11}–C_{31}). The relative isotope composition of the three terminal carbon positions can be determined with a precision of 1.2‰ or better. The results from commercially available samples show that (i) the intramolecular ^{13}C isotope distribution is opposite between odd and even numbered *n*-alkanes in the C_{16}–C_{31} range and (ii) those in the C_{11}–C_{15} range show a ^{13}C depletion of ca. 12‰ in the methyl position and no difference between odd and even numbered compounds. The results are consistent with a biological origin of heavy *n*-alkanes whereas lighter ones are proposed to originate from abiogenic degradation such as thermal cracking. Overall, although only partial intramolecular ^{13}C patterns are obtained, the approach appears as promising tool in petroleum exploration and in the biogeochemistry field.

1. Introduction

n-Alkanes are ubiquitous compounds biosynthesized by several organisms such as terrestrial and aquatic plants, algae and insects. The stable carbon isotopic composition (noted as $\delta^{13}C$ and expressed in ‰ relative to the international standard Vienna Peedee Belemnite, V-PDB) of *n*-alkanes biosynthesized by such organisms is therefore a useful indicator for inferring the origin of *n*-alkanes in sedimentary organic matter (OM; Hayes, 1993; Chikaraishi and Naraoka, 2003; Chikaraishi et al., 2012).

The isotopic composition of *n*-alkanes is commonly determined using gas chromatography coupled with isotope ratio mass spectrometry via a combustion module (GC-C-IRMS; Hayes et al., 1990). The technique has the advantage of allowing determination of the $\delta^{13}C$ values of various compounds present in the same matrix with a precision as low as 0.2‰ and with a sample amount of the order of nmol. The main limitation is that it provides $\delta^{13}C$ values at the molecular level, i.e. the average isotopic composition of each carbon position of the analyte. Indeed, since chemical and biological reactions break or form specific C–C bonds, the isotope fractionation (variation in $\delta^{13}C$ values) expected in natural compounds must occur at specific carbon atom positions. As stated by Monson and Hayes (1982), "isotopic differences *between* whole molecules [...] must be the attenuated and superficial manifestations of isotopic differences *within* molecules". In other words, determining the $\delta^{13}C$ for each carbon position of a compound, namely the intramolecular isotope distribution, might lead to refined information about the chemical, physical and/or biological history of the compound.

Little is known about the intramolecular isotopic distribution in natural compounds. The pioneering work of De Niro and Epstein (1977) and later Monson and Hayes (1982), allowed a comprehensive view of the metabolic steps that could lead to a heterogeneous ^{13}C isotopic distribution in fatty acids (FAs). Given that *n*-alkanes are formed from the FAs of living organisms (Cheesbrough and Kolattukudy, 1984; Zhou et al., 2010), the ^{13}C intramolecular heterogeneity in FAs is likely to be preserved to a certain extent in *n*-alkanes and could thus be a valuable indicator of the sources and fate of organic compounds in sedimentary OM. Yet, to our knowledge, the intramolecular isotope distribution in *n*-alkanes has never been determined.

The most conventional way to obtain intramolecular isotope distribution at natural abundance levels consists in the degradation of the analyte into fragments, the $\delta^{13}C$ values of which are determined separately using IRMS. Nevertheless, the high stability of *n*-alkanes, that makes them valuable biomarkers in

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biogeochemistry, makes them difficult to degrade into specific fragments without any isotope fractionation. For instance, Corso and Brenna (1999) determined the isotopic composition of pyrolytic fragments from light *n*-alkanes (C_5–C_{10}) using an on-line pyrolysis system coupled with GC-C (combustion)-IRMS. Despite the high potential of the approach for fingerprinting purposes, the original ^{13}C pattern of *n*-alkanes cannot be reconstructed reliably due to potential isotope fractionation and occurrence of secondary reactions during pyrolysis.

Recently, isotopic ^{13}C nuclear magnetic resonance (NMR) spectrometry has made possible the separation and quantification of each ^{13}C isotopomer in an analyte without prior degradation. The technique has been used to access the intramolecular isotopic composition of compounds such as EtOH (Caytan et al., 2007a; Gilbert et al., 2011), vanillin (Tenailleau et al., 2004), glucose (Gilbert et al., 2009) and paracetamol (Silvestre et al., 2009). Once the parameters are set to obtain quantitative conditions, the precision can reach 1‰ or less on the $\delta^{13}C$ scale (Caytan et al., 2007a). Since NMR is non-destructive, the intramolecular ^{13}C isotope distribution in *n*-alkanes can potentially be determined without any correction needed to account for isotope fractionation.

Here we have examined the feasibility of determining the intramolecular isotope distribution of long chain *n*-alkanes (C_{11}–C_{31}) using isotopic ^{13}C NMR. Preliminary results from commercially available samples are presented and discussed to illustrate the potential of the approach in biogeochemistry.

2. Material and methods

2.1. Chemicals

n-Alkanes (C_{11}–C_{31}) (purity > 99.5%) were from Tokyo Chemical Industry Co. (TCI, Tokyo, Japan). Deuterated chloroform ($CDCl_3$) and toluene (Tol-d_8) were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The relaxation agent, tris(2,4-pentadionato)chromium-III [$Cr(Acac)_3$] (97%) was from Kanto Chemical Co., Inc. (Tokyo, Japan).

2.2. Sample preparation

(i) For *n*-alkanes in the C_{11}–C_{27} range: to 100–600 mg of the compound were added 300–800 µl $CDCl_3$ and 100 µl of 0.1 M $Cr(Acac)_3$ in $CDCl_3$. The solution was homogenized before being filtered in a 5 mm outer diameter (o.d.) tube.

(ii) For *n*-alkanes in the C_{28}–C_{31} range: to 100–125 mg of the compound were added 500–700 µl Tol-d_8 and 100µL of 0.1 M $Cr(Acac)_3$ in Tol-d_8. The solution was gently heated and homogenized until complete dissolution of the compound before being filtered in a 5 mm o.d. tube.

2.3. NMR measurements

Quantitative ^{13}C NMR spectra were recorded using a Bruker 400 Avance III spectrometer fitted with a 5 mm i.d. $^{13}C/^{1}H$ probe carefully tuned at the recording frequency of 100.64 MHz. The temperature was set at 303 ± 0.1 K for the C_{11}–C_{27} *n*-alkanes and to 313 ± 0.1 K for the C_{28}–C_{31} *n*-alkanes. The sequence used was that described by Tenailleau and Akoka (2007). Briefly, a 90° pulse was applied on the ^{13}C channel followed by an acquisition period of 1 s. The number of repetitions of this sequence (number of scans, NS) was adapted for each compound in order to get a signal/noise ratio of 700. For each compound, the longitudinal relaxation times T_1 (^{13}C) were measured using an inversion-recovery sequence. The recovery delay, i.e. the delay between each 90° pulse, was set to $10 \cdot T_{1max}$ (^{13}C), where T_{1max} (^{13}C) is the largest T_1 (^{13}C) of the compound to be measured. ^{1}H decoupling was applied only during the acquisition period in order to avoid a Nuclear Overhauser effect inducing any change in the area of the carbon signals. The decoupling sequence used a cosine adiabatic pulse with appropriate phase cycles, as described by Tenailleau and Akoka (2007).

2.4. Spectra treatment

The free induction decay (FID) was multiplied by an exponential function with a line broadening factor of 1.6 Hz followed by Fourier transform. The curve fitting of the spectrum was carried out in accord with a Lorentzian mathematical model using Perch Software (Perch NMR Software: University of Kuopio, Finland). One measurement is the mean of the results obtained from 3 to 5 spectra.

2.5. Expression of results

Since the signals corresponding to the central CH_2 atoms overlap in the 28–30 ppm region (Figs. 1 and 2), only the isotopomers bearing a ^{13}C atom in one of the three terminal positions (CH_3, CH_{2a} and CH_{2b}) could be quantified. For each of these three carbon atom positions, the relative ^{13}C abundance was determined using the molar fraction f_i (where *i* is the C atom position considered) as follows: $f_i = S_i/S_{tot}$, where S_i is the area of the peak corresponding to the ^{13}C isotopomer in the position *i* and S_{tot} the total area of the peaks corresponding to the three ^{13}C isotopomers ($S_{tot} = S_{CH3} + S_{CH2a} + S_{CH2b}$). Each S_i was corrected to compensate for the slight loss of intensity caused by satellites (interactions due to the presence of ^{13}C–^{13}C isotopologues) by multiplying by $(1 + n \times 0.011)$, where *n* is the number of carbons directly attached to the C atom position *i* (*n* = 1 for the CH_3 position and *n* = 2 for the CH_{2a} and CH_{2b} positions) and 1.1% (= 0.011) is the average natural ^{13}C abundance [see Tenailleau et al. (2004) and Silvestre et al. (2009) for a detailed explanation]. Eventually, if F_i denotes the statistical mole fraction (homogeneous ^{13}C distribution, i.e. $F_i = 1/3$ for each carbon position in the case of *n*-alkanes) at any C atom position *i*, then the site-specific relative deviation from a ^{13}C homogeneous distribution is d_i (‰)= $(f_i/F_i - 1) \times 1000$.

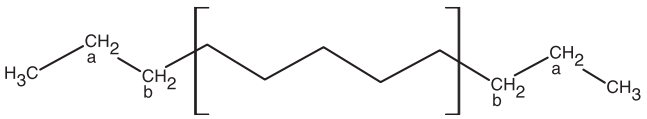

Fig. 1. *n*-Alkane structure and notation used. Only the three terminal carbon atom positions (CH_3, CH_{2a}, CH_{2b}) were considered.

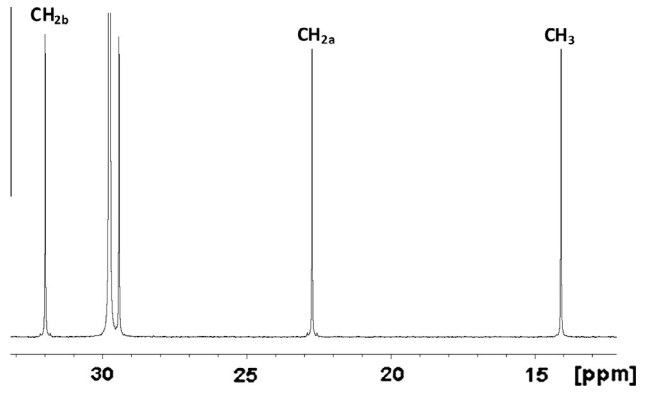

Fig. 2. ^{13}C NMR spectrum (^{1}H decoupled) of tetracosane (*n*-$C_{24}H_{50}$). Only the isotopomers with a ^{13}C on the three terminal atom positions (CH_3, CH_{2a}, CH_{2b}) could be quantified.

It is worth noting that the patterns thus obtained are an average of the isotopic patterns at each end of the carbon chain.

2.6. Bulk $\delta^{13}C$ determination of n-alkanes

The bulk $\delta^{13}C$ values of all *n*-alkanes were determined using cavity ring down spectroscopy (CRDS, G1121-I analyzer, Picaro Inc., Santa Clara , CA, USA) after their combustion to CO_2.

3. Results

The purpose was to evaluate the potential of isotopic ^{13}C NMR for determination of the intramolecular isotope distribution in *n*-alkanes (C_{11+}). Since *n*-alkanes up to C_{31} can be found in petroleum and plant wax, we focus here on the analysis of all the compounds in the C_{11}–C_{31} range. As can be seen from Fig. 2, only three isotopomers can be reliably quantified, the other signals overlapping in the 28–30 ppm region of the ^{13}C NMR spectrum. The precision required (ca. 1‰) prevents the use of an internal reference for the absolute determination of $\delta^{13}C$ values, so that only relative ^{13}C abundance for each of the three positions, d_i, can be determined. Only the d_i value for these three carbon atom positions was considered.

3.1. Experimental time

The precision of an isotopic ^{13}C NMR analysis is mainly governed by the SNR (Caytan et al., 2007a). Accordingly, the concentration of each analyte was made as high as possible by dissolving the maximum amount of the compound in the minimum of deuterated solvent. The NS was then adjusted to reach a SNR of at least 700 (required for 1‰ precision; see Caytan et al., 2007a). The duration of each scan is governed by the recovery delay, RD, which has to be adjusted from the maximum relaxation time T_1 (^{13}C) of the compound. In order to reduce RD, a relaxation agent was used for all the measurements. It allows the reduction of the longitudinal relaxation times T_1 (^{13}C) and thus the experimental time, without affecting the accuracy of the measurement (Caytan et al., 2007b).

Consequently, the experimental time is governed by (i) the concentration of the analyte (i.e. the NS necessary to reach a S/N value of 700) and (ii) the RD. The relaxation times for *n*-alkanes in the C_{11}–C_{31} range were of the same order of magnitude (within 3 s). Accordingly, the RD did not affect the experimental time between two compounds to a great extent. Hence, the main factor affecting the experimental time was the concentration of the analyte. Since the solubility of *n*-alkanes decreases with increasing chain length, the higher the molecular weight of the analyte, the longer the experimental time. The latter thus increases gradually from *n*-C_{11} (ca. 1 h) to *n*-C_{27} (ca. 90 h). Tol-d_8 was used instead of $CDCl_3$ for C_{28+} compounds in order to allow working at higher temperature, and thus to increase the concentration of the analyte. In these conditions, compounds up to C_{31} can be analyzed within a reasonable time (i.e. < 90 h for one measurement). The results obtained using both Tol-d_8 and $CDCl_3$ were identical for C_{17} and C_{22} (data not shown), suggesting that both solvents could be used for our purpose. Nevertheless, because of its lower price, $CDCl_3$ was preferred when possible. Thus, the C_{11}–C_{27} compounds were analyzed using $CDCl_3$ and the C_{28}–C_{31} compounds using Tol-d_8.

3.2. Repeatability of measurements

The standard deviation of repeated experiments ($n > 3$) was determined for each compound. As shown in Fig. 3, the standard deviation from the mean d_i for each of the three C atom positions did not exceed 1.2‰ for all the compounds; this is in good accord with data for vanillin using the same S/N ratio (Caytan et al., 2007a). Furthermore, it is worth noting that the carbon chain length, and thus the experimental time, had no influence on the precision.

3.3. Application to commercially available samples

The results typically obtained for *n*-C_{21} and *n*-C_{22} are depicted in Fig. 4. Clearly, the intramolecular isotope distribution is not homogeneous, with differences between each C atom position of up to 10‰. To compare the data from one compound to another, the relative d_i values cannot be used. For instance, the d_i value of CH_{2b} for C_{21} and C_{22} (ca. 0‰) cannot be seen as equal, since the absolute $\delta^{13}C$ value is not known. Nevertheless, the variation within a compound (i.e. the isotopic pattern) can be used for a comparison. Therefore, the results are expressed subsequently as:

$$\Delta_1 = d_{CH3} - d_{CH2a}$$

$$\Delta_2 = d_{CH2a} - d_{CH2b}$$

For instance, the Δ_1 and Δ_2 values for C_{22} (Fig. 3) are 10.2 ‰ and −5.6 ‰, respectively.

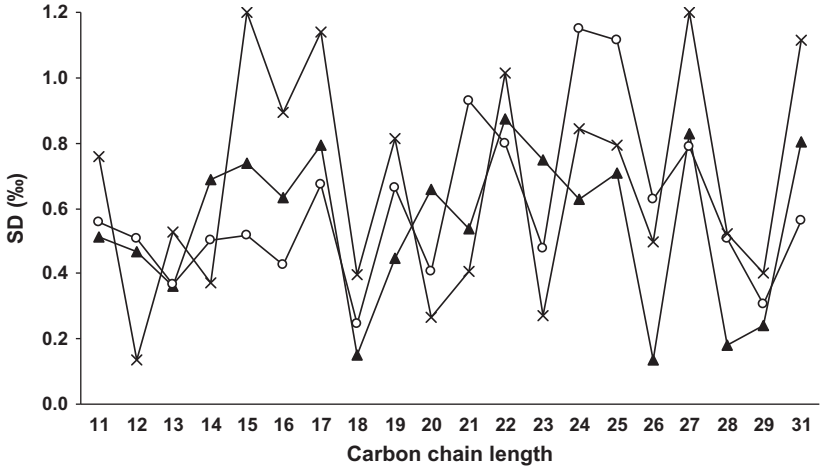

Fig. 3. Standard deviation from the mean d_i (expressed in ‰; $n \geqslant 3$) for each of the three terminal carbon atom positions obtained for *n*-alkanes as a function of the chain length. Black triangles, CH_3: white circles, CH_{2a}; black stars, CH_{2b}.

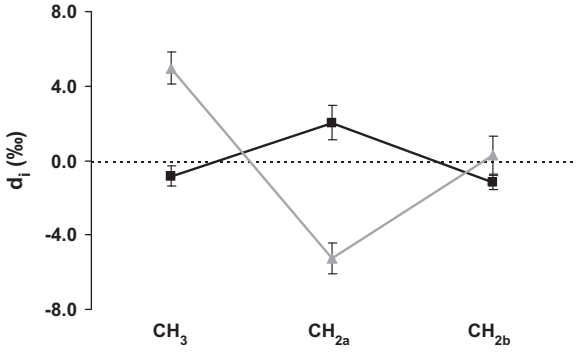

Fig. 4. Partial isotopic pattern, expressed as d_i (‰), of the three terminal carbon positions of *n*-C_{21} (black line) and *n*-C_{22} (gray line) from TCI. The dotted line represents a homogeneous ^{13}C distribution ($d_i = 0$).

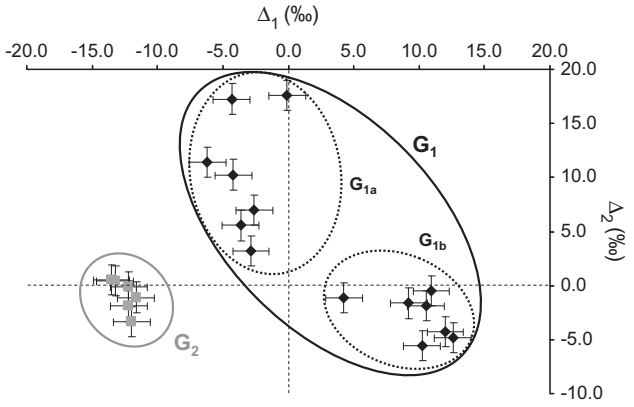

Fig. 6. Representation of Δ_2 values as a function of Δ_1 values for *n*-alkanes analyzed. Three groups could be distinguished according to their Δ_1 and Δ_2 values: G_{1a} (OCN *n*-alkanes in the C_{16}–C_{31} range, excluding *n*-C_{17}), G_{1b} (ECN *n*-alkanes in the C_{16}–C_{31} range) and G_2 (OCN *n*-alkanes + ECN-*n*-alkanes in the C_{11}–C_{15} range, including *n*-C_{17}).

The results for all *n*-alkanes from TCI in the range C_{11}–C_{29} and C_{31} are reported in Fig. 5. Two features arise from these results:

(i) For all the compounds in the C_{16}–C_{31} range (except *n*-C_{17}), the intramolecular pattern was alternate between odd carbon numbered and even carbon numbered *n*-alkanes (denoted subsequently as OCN *n*-alkanes and ECN *n*-alkanes, respectively): for OCN *n*-alkanes, the CH_3 position was ^{13}C depleted vs. the CH_{2a} position (Δ_1 negative) whereas the CH_{2a} position was ^{13}C enriched vs. CH_{2b} (Δ_2 positive). The opposite tendency was observed for ECN *n*-alkanes.

(ii) All the lighter *n*-alkanes (*n*-C_{11} to *n*-C_{15} including *n*-C_{17}) had a similar pattern which was not dependent on the number of carbons in the compound: CH_3 was ^{13}C-depleted vs. CH_{2a} (Δ_1 negative) whereas CH_{2a} and CH_{2b} had similar isotope composition (Δ_2 ca. 0‰).

4. Discussion

The *n*-alkanes were from commercial sources. Unfortunately, obtaining clear information about their origin (petroleum distillates, biological extracts) from the supplier turned out to be difficult. Hence, rather than deciphering their origin, the following discussion aims at showing the potential information obtained using relative isotopomer concentration and how the approach might be used in future studies.

Two groups could be distinguished, denoted as G_1 ("heavy" group: C_{16}–C_{31} range, excluding C_{17}) and G_2 ("light" group: C_{11}–C_{15} range, including C_{17}). The G_1 group could be subdivided into two sub-groups: G_{1a} (OCN *n*-alkanes) and G_{1b} (ECN *n*-alkanes). The arbitrary distinction between the groups was straightforward when the Δ_2 values were plotted as a function of the Δ_1 values for each compound, as shown in Fig. 6. The mean $\delta^{13}C$, Δ_1 and Δ_2 values and the standard deviation associated with each group are listed in Table 1 (see Supplementary material for a detailed list).

4.1. Preliminary considerations

As stated in Section 3, the patterns obtained for *n*-alkanes are the average of that at each end of the carbon chain. If the pattern at each end of the chain is perfectly opposite (e.g. one end has an isotopic pattern *x*–*y*–*x* and the other *y*–*x*–*y*, where *x* and *y* are ^{13}C abundances), the method employed here will lead to a homogeneous pattern ($\Delta_1 = \Delta_2 = 0$). Any deviation from this pattern thus requires (i) a symmetrical arrangement in which both ends of the alkane have the same pattern *x*–*y*–*x* or (ii) marked inequality between patterns of each end so that one dominates the averaged signal.

4.2. Intramolecular ^{13}C isotope distribution in n-alkanes from G_1 group

The ^{13}C isotopic pattern of odd numbered, long chain *n*-alkanes (G_{1a} group) can be attributed to an alternation in $\delta^{13}C$ values between odd and even carbon atom positions in FAs, as observed

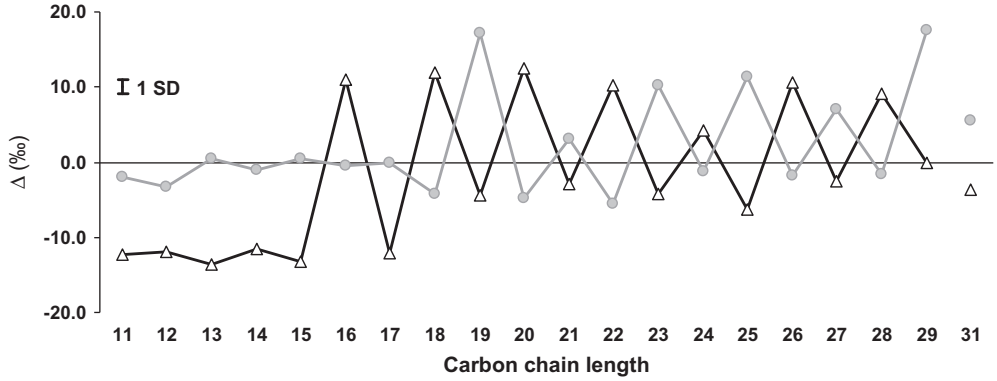

Fig. 5. Representation of Δ_1 (‰) (black line) and Δ_2 (‰) (gray line) from the isotopic ^{13}C NMR measurement of *n*-alkanes as a function of carbon chain length.

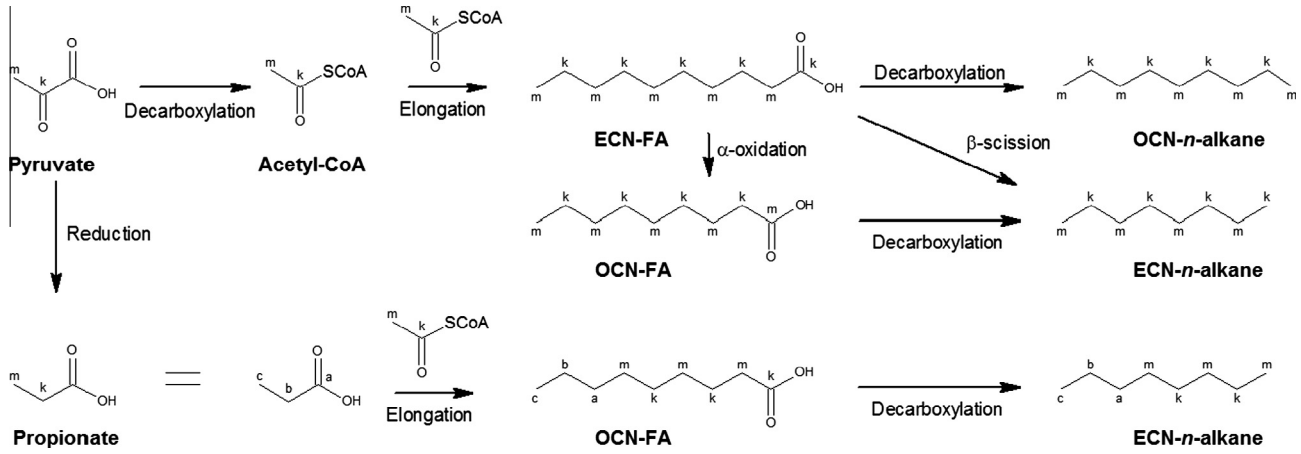

Fig. 7. Simplified scheme for formation of odd numbered and even numbered *n*-alkanes; m and k depict the ^{13}C isotope composition of methyl and carboxyl positions, respectively, of acetyl-CoA, the precursor of FAs; *c*, *b* and *a* represent the ^{13}C isotope composition of carbon atom positions in propionate (from the methyl position inwards). The term "decarboxylation" is used to express the loss of the terminal carboxyl carbon atom, either by direct decarboxylation or by reduction followed by decarbonylation. ECN, even carbon numbered; OCN, odd carbon numbered; FA, fatty acid.

Table 1
Average $\delta^{13}C$, Δ_1 and Δ_2 values for each of the 3 groups of *n*-alkanes (numbers in brackets indicate standard deviation from the mean).

	$\delta^{13}C$ (‰)	Δ_1 (‰)	Δ_2 (‰)
G_{1a} [a] (*n*=7)	-30.2 (1.1)	-3.9 (1.8)	11.0 (5.2)
G_{1b} [b] (n=7)	-31.1 (2.0)	10.4 (2.4)	-3.1 (1.9)
G_2 [c] (n=6)	-28.3 (1.7)	-12.5 (0.7)	-0.5 (1.9)

[a] OCN *n*-alkanes in the C_{16}-C_{31} range, excluding C_{17}.
[b] ECN *n*-alkanes in the C_{16}-C_{31} range.
[c] OCN *n*-alkanes + ECN *n*-alkanes in the C_{11}-C_{15} range, including C_{17}.

by Monson and Hayes (1980, 1982). Assuming an alternate pattern in even carbon numbered fatty acids (ECN FAs) inherited from the ^{13}C isotopic composition of carbonyl (*k*) and methyl (*m*) positions of acetyl-CoA, both ends of the *n*-alkanes would have the same isotope pattern, *m*–*k*–*m*, which corresponds to case *i* above (Fig. 7). This alternation was indeed observed for *n*-alkanes in the G_{1a} group, with the CH_3 and CH_{2b} positions ^{13}C depleted vs. the CH_{2a} position. It is worth noting that the expected pattern in the precursor FAs would show a ^{13}C depletion in odd positions (derived from the methyl position of acetyl-CoA) and a ^{13}C enrichment in the even ones (from the carboxyl position of acetyl-CoA). This finding is in good agreement with the data obtained by Monson and Hayes (1982) from FAs biosynthesized by *Saccharomyces cerevisiae*.

Remarkably, the Δ_1 and Δ_2 values for even long chain *n*-alkanes (G_{1b} group) were 10.4‰ and −3.1‰, respectively (see Table 1), which is significantly different from zero. This would not be compatible with α-oxidation or β-scission where the isotope pattern should be null ($\Delta_1 = \Delta_2 = 0$), with one end having an *m*–*k*–*m* pattern and the other one *k*–*m*–*k* (Fig. 7). It could be consistent with a biological origin of these compounds deriving from OCN FAs, the precursor of which is propionate (Zhou et al., 2010). In such a case, both ends of *n*-alkanes would have different patterns (i.e. *c*–*b*–*a* and *m*–*k*–*m*; see Fig. 7), leading to a non-null result (case ii above). Obviously the veracity of this conjecture cannot be assessed without knowing the clear origin of the compounds.

4.3. Intramolecular ^{13}C isotope distribution in n-alkanes from G_2 group

Comparing the intramolecular ^{13}C patterns for compounds from the G_2 group with those from the G_1 group, two features arise: (i) the ^{13}C pattern was different from both G_{1a} and G_{1b} groups and (ii) there was little or no alternation of Δ_1 and Δ_2 values between odd and even numbered compounds. Indeed, the ^{13}C pattern in G_2 *n*-alkanes was highly reproducible, with a standard deviation from the mean below 2‰ for both Δ_1 and Δ_2 values (Table 1). Moreover, the $\delta^{13}C$ values of *n*-alkanes from the G_2 group were different from those of *n*-alkanes from the G_1 group (Table 1; $p < 0.05$), suggesting they arose from a different process and/or source.

Generally, light *n*-alkanes arise from the thermal cracking of heavier ones. Thermal cracking of OM (heavy *n*-alkanes) can occur in situ, at temperatures above 100 °C and within geological timescales, or in industry, in order to obtain valuable commercial components (e.g. gasoline). In both cases, that might affect the intramolecular isotopic signature of the newly formed *n*-alkanes (light *n*-alkanes).

During thermal cracking, each precursor *n*-alkane will produce a comparable amount of OCN *n*-alkane and ECN *n*-alkane. Hence, the intramolecular isotopic pattern of newly formed *n*-alkanes can be considered to a first approximation as homogeneous, the heterogeneity inherited from both OCN *n*-alkanes and ECN *n*-alkanes being cancelled out. This is indeed the case for the CH_{2a} and CH_{2b} positions, which had similar ^{13}C isotope composition (Δ_2 ca. 0‰, Table 1). Although this is obviously not the case for the methyl position, which was ^{13}C depleted by ca. 12.5‰ compared with the two other carbon atom positions ($\Delta_1 = -12.5$‰, Table S1). Since the methyl position arises from the broken C–C bond of their precursor (a heavier *n*-alkane), we suggest that the depletion originates from an isotope effect associated with its formation. Tang et al. (2000) calculated an isotope effect ($^{13}k/^{12}k$) of 0.981 for the central C–C bond breaking of *n*-hexane at 500 K. Accordingly, the ^{13}C isotope composition in the methyl position of the product formed (propane in that case) should be ^{13}C-depleted compared with the parent hydrocarbon (*n*-hexane) to an extent depending on the rate of its formation. Assuming a single reaction where a single C–C bond is broken, the isotopic fractionation observed with our method would be diluted by a factor 2 due to the symmetry of *n*-alkanes: one methyl position is involved in the reaction whereas the other remains unaffected. Eventually, the two methyl positions cannot be distinguished from each other, and the final isotopic composition of the methyl position would be the average of the unaltered and altered positions. Therefore, the real isotope fractionation induced on the methyl position of newly formed *n*-alkanes

must be around 25‰, which is of the same order as that calculated by Tang et al. (2000).

Thus, it seems likely that the intramolecular isotope distribution in *n*-alkanes from the G_2 group is a consequence of the degradation of heavier *n*-alkanes. Yet, no indication can be obtained as to whether or not the thermal cracking giving rise to the light *n*-alkanes analyzed here occurred in situ or during industrial processing.

Interestingly, the ^{13}C depletion in the methyl position of *n*-alkanes might influence the isotopic signature of the natural gas formed therefrom. Mathematical models attempting to understand the isotopic composition of natural gas generally assume a homogeneous isotope distribution in the source components (e.g. Chung et al., 1988). Although intramolecular isotope heterogeneity has been thought to be partly responsible for the isotopic signature of natural gas, and especially the ^{13}C depletion of CH_4 from thermogenic natural gas (Waples and Tornheim, 1978; Galimov, 2006), to our knowledge, this feature has never been demonstrated. Here, we show that the methyl position of *n*-alkanes can be ^{13}C depleted by up to 13‰ compared with the other carbon atom positions. As a consequence, the $\delta^{13}C$ value of methane formed therefrom via thermal cracking would be ^{13}C depleted compared with that arising from *n*-alkanes with a homogeneous intramolecular distribution.

5. Conclusions

Isotopic ^{13}C NMR allows determination of the relative ^{13}C isotopomer ratios of the three terminal carbon atom positions of *n*-alkanes (C_{11}–C_{31}) with 1.2‰ precision. Using commercially available standards, we show that this approach can potentially give insights into the biochemical processes responsible for the formation and degradation of *n*-alkanes. Obviously, the conjectures made here will have to be tested using samples of known origin (e.g. plants, petroleum). Nevertheless, it appears that the approach allows a new type of information to be obtained, which may be of importance in biogeochemistry and in petroleum exploration.

Given the amount of sample necessary to run NMR measurements (> 100 mg), the technique could be used for the determination of the relative isotopomer concentration in matrices where the *n*-alkane concentration is relatively high (e.g. crude oil). It is worth noting, however, that isotopic ^{13}C NMR could be used to correct the $\delta^{13}C$ values of the fragments obtained by the on-line pyrolysis approach developed by Corso and Brenna (1999). The intramolecular isotope distribution in the parent *n*-alkanes could then be reconstructed from the pyrolytic fragments, which would considerably decrease the required amount of sample. The approach could then be used with a wider range of applications (e.g. biodegradation studies, lipid metabolism, biogeochemistry of sediments).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2013.07.004.

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