STABLE CARBON ISOTOPE RATIOS OF LIPID BIOMARKERS AND THEIR APPLICATIONS IN THE MARINE ENVIRONMENT

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Abstract. Studies on the distribution of lipid biomarkers in the environment help elucidate biogeochemical processes, but recent findings have significantly reduced the specificity of some biomarkers. The analytical development of Gas Chromatography-Combustion-IRMS (GC-C-IRMS) allows the determination of the $\delta^{13}C$ of specific biomarkers, thereby improving the veracity of source apportionment. In this report, we present a brief description of the analytical approach for sample preparation and carbon isotope measurements of individual biomarkers. Selected examples of the applications in the use of GC-C-IRMS for biomarker source elucidation in the marine environment and potential applications to paleoclimatological studies are reviewed.

1. INTRODUCTION

Biomarkers, or molecular markers, are compounds with structures that can be related to specific biological sources due to their own biosynthesis [1, 2]. An important fact in considering their fate is that the transformation of dissolved, suspended and deposited lipids in any environment (oxidizing or reducing) tends towards a selective preservation of low-polar compounds, including hydrocarbons, fatty acids, and sterols. Hence, organic biomarker compounds are often used as tracers of sedimentary organic matter sources [3]. Long-chain n-alkanes, n-alkanols and n-fatty acids are used as biomarkers for terrigenous input; unsaturated alkenones and dinosterol as tracers for marine production and branched-chain fatty acids of the *iso-* and *anteiso* series as bacterial markers [1, 2, 4]. Other biomarkers of high thermodynamic stability, such as terpanes and sterane isomers, are commonly found in petroleum and are widely used to identify anthropogenic contamination sourced from petroleum [5].

The use of lipid biomarkers has permitted useful perspectives in organic biogeochemical studies, but some care about the assumptions used in the different relationships is required. Over recent years, it has been apparent that some biomarkers are more widely distributed in the environment than previously thought, and thus, their specificity has been reduced. Sterols are a good example of this; some of them have been found only in a few classes while others are now known to be quite widely distributed [6]. Certain general markers are still considered unambiguous, but the usefulness of those derived from a variety of sources needs to be explored. The advent of compound specific isotopic analyses (CSIA) through the development of gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) [7, 8] allows the determination of the $\delta^{13}C$ composition of individual compounds. Consequently, $\delta^{13}C$ of specific biomarkers should be useful to improve the veracity of source identification, thereby allowing the origin of ubiquitous compounds with multiple potential sources to be determined.

Stable isotope ratios can also be used to distinguish between the photosynthetic pathways of terrestrial species (C_3 vs C_4 type) [9, 10]. Photosynthesis in the marine environment occurs via the C_3 pathway. However, $\delta^{13}C$ values of photosynthetic organisms in the ocean do not always resemble $\delta^{13}C$ values of terrestrial C3 plants. The reasons are that aquatic plants can also utilize bicarbonate, which is known to be ^{13}C enriched relative to dissolved CO_2, and the slower diffusion of CO_2 in water may reduce the extent of fractionation by the enzyme RuBP carboxylase [11]. Other factors that may influence $\delta^{13}C$ values of marine photosynthetic organisms include the salinity, temperature, phytoplankton growth rate and CO_2 availability [12, 13]. All these natural variations in $\delta^{13}C$ can be used for the identification of sedimentary carbon sources and for the differentiation between marine and terrestrial sources.

This report presents a brief description of the analytical approach for carbon isotopic analyses of individual biomarkers. It also provides selected examples of the applications of $\delta^{13}C$ biomarkers to the environment, including the elucidation of biological sources and palaeoclimate studies.

2. SAMPLE PREPARATION AND MASS SPECTROMETRIC MEASUREMENTS

An excellent gas chromatography performance, including baseline separation and complete peak integration, are extremely important for achieving accurate $\delta^{13}C$ measurements [14]. Thus, isolation of the target compounds from the co-eluting compounds that typically comprise a background matrix or unresolved complex mixture (UCM), is essential. Care should be taken, however, during the isolation of the 3 compounds from the complex sample matrix to ensure that no isotopic alteration of the products occurs. The quantitative collection of the compounds during the extraction and purification processes is required [15, 16].

Applications of size-exclusion techniques (e.g., urea adduction and molecular sieving) have been shown to improve the accuracy of the n-alkyl lipid profiles from complex organic mixtures. These techniques, which show no measurable isotope fractionation effect, work effectively in removing the UCM [17].

Some of the pitfalls encountered in the GC-IRMS technique have been discussed by Meier-Augenstein [18]. Depending on the polarity of the stationary phase used in the chromatographic column, isotopic fractionation for some families of compounds might occur. In general, chromatographic isotope effects are minimized when the polarity of the stationary phase matches the polarity of the sample [19]. However, the use of stationary phases of high polarity have a relatively low maximum operating temperature (250°C or less) and show considerable column bleed that might adulterate isotope ratio measurements.

The combustion interface must also be closely monitored. Its performance might vary during the chromatographic run, giving good accuracy for low molecular weight compounds but compromising the accuracy and precision for the high molecular weight compounds due to an incomplete combustion (non-quantitative conversion of the analyte to CO_2). It can also be temporarily overloaded, particularly with the use of derivatization agents, which results in non-quantitative combustion. When derivatives are to be used for obtaining a good chromatographic performance (e.g. carboxylic acids, sterols, aminoacids), the possibility of isotope fractionation processes should be also considered [20]. The additional imprecision of delta values of compounds for derivative groups must be considered before conclusions are drawn.

In order to overcome these problems, the use of internal standards that have the same functional group as the sample compound is an advisable way to check for possible isotope effects. Standardization of the internal standards should be determined using conventional techniques (off-line combustion).

3. BIOMARKER SOURCES AND BIOGEOCHEMICAL PROCESSES

Carbon isotopic compositions of individual biomarkers have been interpreted in terms of biogeochemical processes in ancient depositional environments [7, 21–27]. The isotopic composition of individual lipid biomarkers can indicate the isotopic composition of the parent organism and infer the carbon source utilized by the producer, and thus its position within the ancient ecosystem.

Refined estimate of marine and terrigenous contributions to sedimentary organic carbon have also been provided by using the $\delta^{13}C$ of a marine organic biomarker, such as C_{37} alkadienone [28]. A great number of studies using the GC/C/IRMS technique has demonstrated its potential to support the simple molecular approach clarifying the source of some enigmatic biomarkers. Only some examples from selected lipid class will be presented below and discussed.

A separate analysis of isoprenoid-hydrocarbons, pristane and phytane, for which a common origin from chlorophyll had been generally assumed, seemed to indicate that these two components have different origins in sedimentary rocks from the Eocene Messel Shale [21]. The $\delta^{13}C$ for pristane fitted

with that expected for algal lipids (–25‰) and that of phytane (–31.8‰) with those from methanogenic bacteria.

The $\delta^{13}C$ of the regular C_{18} to C_{25} isoprenoids tentatively assigned an origin from halophilic archae agreed with the enrichment by up 7‰ compared to phytoplanktonic biomarkers of the same sediment [26]. The precursor of the C_{40}-isoprenoid hydrocarbon, lycopane, has yet to be identified but its carbon isotopic composition from water column and sediment samples (between –23.6‰ and –32.9‰) confirm its source from photoautotroph organisms [29].

By comparing the $\delta^{13}C$ of individual n-alkanes and n-alkanols from leaves of lakeside trees with those from the lake's sediments, it was possible to discriminate between the fresh-water algae and terrestrial plants [30]. Carbon isotopic ratios of n-alkanols from a saline sediment enabled to distinguish between the terrestrial long-chain n-alkanols (n-C_{24}, n-C_{26}: from –30 to –32‰) and the marine short-chain n-alkanols (n-C_{16} to n-C_{22}: 18 to –23‰) [31].

Resolution of biological sources of individual fatty acids in modern sediments were also provided by GC/C/IRMS. The isotopic compositions of long-chain fatty acids ($C_{20:0}$-$C_{26:0}$: from –31.0 to –30.7‰) reflect their sources from higher-land plants, whereas the $C_{16:0}$ and $C_{18:0}$ acids (–28.7‰ and –27.7‰) were from marine plankton, and $C_{14:0}$ and $C_{15:0}$ acids (–38.7‰ and –37.2‰) originated from bacteria [32].

Other classes of biomarkers, such as the hopanes, are also not always derived from a common precursor [33]. Stable carbon isotopic compositions for diploptene in sediment cores from the Japan Sea has inferred a cyanobacteria origin (–25‰) for the first section of the sediment and a methanotrophic source (–53.1‰) for the older section [34].

Alternatively, the $\delta^{13}C$ obtained for biomarkers commonly associated with terrestrial sources (e.g., long-chain n-alkanes, n-alkanols and $C_{29}\Delta^5$ sterol) have elucidated sources other than higher plants. The isotope profiles of the long-chain n-alkanes in Arctic surface sediments (no shift with chain length and a systematic difference between the odd-and even carbon numbered compounds) favored a non-continental source [35]. Other possible algal origin of long chain odd n-alkanes in immature sediments was revealed by distributions and carbon isotopic composition [36]. Similarly, the light $\delta^{13}C$ of long-chain n-alkanols in modern sediments from the China Sea area indicated sources (marine and bacterial) different of terrestrial [37]. Another example was recently presented for the commonly terrestrially derived sterol (24-ethylcholest-5-en-3β-ol) where its enriched $\delta^{13}C$ inferred a marine and not a terrestrial origin in saline sediments from Spain [31].

All these studies have claimed that in the future the molecular approach in carbon cycling should be pursued using combined biomarker and stable isotope techniques. However, it has also been shown that lipids synthesized by the same biosynthetic pathway might not have the same isotopic composition.
A striking example was shown by Summons et al. [38] where the isotopic compositions of squalene and hopan-29-ol produced by methanotrophic bacteria differed by more than 10‰. Others instances have recently been presented in different photosynthetic organisms [31, 39]. Small variations within and between biosynthetically related compound classes were observed in cyanobacteria. In algae, e.g. diatoms and dinoflagellates, remarkable differences between the average $\delta^{13}C$ composition of fatty acids and sterols were observed (7.5‰ and 2‰, respectively). Similarly, differences between biomarkers with linear carbon skeletons and those with steroid carbon skeletons may differ by up to 8‰.

Isotopic variations among homologues of the same lipid class were also observed. In diatoms, variations were up to 5‰ within each class of fatty acids and sterols and in the dinoflagellate species, these variations were lower than 3‰. In studies of modern leaf tissues, biosynthetically related n-

alkyl lipids (n-alkanes, n-alkanols and n-fatty acids) in epicuticular waxes have been shown to have similar carbon isotopic compositions, but variations of up to 6‰ were noted for homologous n-alkanes extracted from a single leaf [10].

Significant variations and temporal fluctuations in $\delta^{13}C$ of biogenic hydrocarbons were also documented in spring bloom samples of the NW Atlantic waters. These findings highlighted the influence of growth rate and timing of synthesis on the $\delta^{13}C$ of biomarkers over the course of phytoplankton blooms [40].

All these differences, in particular the intra-specific shifts in $\delta^{13}C$ lipid composition render the assignment and deconvolution of biomarker sources more complicated. It is important that more studies of the compound-specific isotopic composition of modern environments take place to ensure the correct interpretation of biomarkers signatures. Alternatively, the significant progress that occurred in GC/IRMS recently enabling the determination of H/D ratios in individual molecules, will probably provide a better understanding of all these processes. This might help facilitating the interpretation of the carbon isotopic biomarker record.

4. PALAEOCLIMATE APPLICATIONS

Applications of the isotope techniques to palaeoenvironmental studies have been explored in recent years due to the observed trends between dissolved CO_2 in water and fractionation of carbon isotopes during photosynthetic fixation of CO_2 [41–43]. More recently, it has been shown that this relationship is only reliable when typically autotrophic C_3 phytoplankton dominate the particulate organic carbon [44].

In this sense, investigations of the potential use of some marine biomarkers for the recovery of the CO_2 related signal, which would provide much better estimate of the palaeopCO_2 reconstruction for all sea-surface waters, have been done. Analyses of phytoplankton grown in chemostat cultures have shown the importance of many environmental variables (e.g., [CO_2 (aq)], cell growth rate, cell size, cell geometry) on the isotopic composition of phytoplankton [13, 45–47]. However, the long-chain C_{37}–C_{39} n-alkenones, derived exclusively from primary producers (Prymnesiophyte algae), are particularly suitable as recorders of CO_2 levels due to their constant isotopic fractionation [48]. Their isotopic analyses have allowed determination of the glacial-to-interglacial variation in the isotopic composition of the total biomass of the source organism and the recovery of a CO_2 related signal covering ~100 kyr [49]. Alternatively, its low concentration in the particulate matter from low productivity regions has fostered the study of other more abundant biomarkers, such as cholesterol, which has a general planktonic origin with a large proportion typically derived from zooplankton inputs. A significant correlation between $\delta^{13}C$ cholesterol of the suspended particulate fraction from the water column in the Indian Ocean and dissolved CO_2 concentrations was found [31]. Additional investigations are required because calibrations for the isotopic fractionation-[CO_2(aq)] relationship may vary significantly from one environment to another [50].

The development of carbon isotope signatures of biomarkers as a palaeoceanographic tool has also been considered. Interestingly, Schoell et al. [51] have demonstrated that $\delta^{13}C$ of organic constituents such as C_{35} hopanes and C_{27} steranes provided palaeoclimatic information from Monterey-type sediments that complemented palaeoclimatic studies from open ocean sediments. Alternatively, the $\delta^{13}C$ values of terrestrially derived n-alkanes in the marine environment have been used to asses basin-wide vegetation changes in adjacent river catchments on geological timescales [52]. More recently, the $\delta^{13}C$ of specific aromatic plant markers (e.g. cadalene) have contributed to interpreting the climatic conditions in ancient sediments [53]. Future work by biogeochemists will probably demonstrate that other biomarkers also have a similar potential to provide information about the paleoclimate and CO_2 exchanges between the ocean and atmosphere.

5. CONCLUSIONS

CSIA is a powerful tool allowing a more accurate assessment of the sources of organic carbon. The use of this technique in combination with biomarker studies can yield additional important environmental information on specific sources and palaeoclimate studies. Care must always be taken, however, in the interpretation of the carbon isotopic composition of individual biomarkers since isotopic variations within and between compound classes metabolized by the same organism can be substantial.

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