Anal. Chem. **2006,** *78,* 2752−2757

Determination of Intramolecular $\delta^{13}C$ from Incomplete Pyrolysis Fragments. Evaluation of Pyrolysis-Induced Isotopic Fractionation in Fragments from the Lactic Acid Analogue Propylene Glycol

Christopher J. Wolyniak, Gavin L. Sacks, Sara K. Metzger, and J. Thomas Brenna*

Division of Nutritional Sciences, Savage Hall, Cornell University, Ithaca, New York 14853

Intramolecular carbon isotope ratios reflect the source of a compound and the reaction conditions prevailing during synthesis and degradation. We report here a method for determination of relative ($\Delta\delta^{13}C$) and absolute ($\delta^{13}C$) intramolecular isotope ratios using the volatile lactic acid analogue propylene glycol as a model compound, measured by on-line gas chromatography-pyrolysis coupled to GC-combustion-isotope ratio mass spectrometry. Pyrolytic fragmentation of about one-third of the analyte mass produces optimal fragments for isotopic analysis, from which relative isotope ratios ($\Delta\delta^{13}C$) are calculated according to guidelines presented previously. Calibration to obtain absolute isotope ratios is achieved by quantifying isotope fractionation during pyrolysis with an average fractionation factor, α, and evaluated by considering extremes in isotopic fractionation behavior. The method is demonstrated by calculating ranges of absolute intramolecular isotope ratios in four samples of propylene glycol. Relative and absolute isotope ratios were calculated with average precisions of SD($\Delta\delta^{13}C$) <0.84‰ and SD($\delta^{13}C$) <3.0‰, respectively. The various fractionation scenarios produce an average $\delta^{13}C$ range of 2‰ for each position in each sample. Relative isotope ratios revealed all four samples originated from unique sources, with samples A, B, and D only distinguishable at the position-specific level. Regardless of pyrolysis fractionation distribution, absolute isotope ratios showed a consistent pattern for all samples, with $\delta^{13}C^{(3)} > \delta^{13}C^{(2)} > \delta^{13}C^{(1)}$. The validity of the method was determined by examining the difference in relative isotope ratios calculated through two independent methods: $\Delta\delta^{13}C$ calculated directly using previous methods and $\Delta\delta^{13}C$ extracted from absolute isotope ratios. Deviation between the two $\Delta\delta^{13}C$ values for all positions averaged 0.1−0.2‰, with the smallest deviation obtained assuming equal fractionation across all fragment positions. This approach applies generally to all compounds analyzed by pyrolytic PSIA.

High-precision compound-specific isotope analysis (CSIA) is a routine method where the analyte molecule is purified on-line prior to isotope ratio measurement. CSIA has myriad applications, including sourcing[1] and determining the authenticity or purity of a sample.[2,3] A greater understanding of origin and biochemical history can be obtained through position-specific isotope analysis (PSIA), which enables observation of intramolecular isotopic variation.[4,5] Fractionation occurs during (bio)chemical processes at sites of bond breaking and formation, with molecules containing lighter isotopes reacting selectively over those with heavy isotopes.[6]

High-precision PSIA using isotope ratio mass spectrometry (IRMS) requires off-line or on-line fragmentation of the analyte molecule followed by isotope ratio measurement of the fragments. Most previous PSIA studies, with analytes such as amino acids,[7] glycerol,[8] and components of wine,[9] employed off-line fragmentation of samples prior to analysis. In these studies, a single position or moiety was chemically removed and the isotope ratio was measured independent from the rest of the compound structure; isotope ratios for all carbon positions were not determined. Site-specific natural isotopic fractionation-NMR has also been utilized for intramolecular isotope ratio measurements of hydrogen, carbon, nitrogen, and oxygen.[10] However, the low sensitivity of NMR to the heavier elements requires acquisition times significantly longer than hydrogen as well as sample size in the gram range,[11] preventing use in situations of limited sample size.

* To whom correspondence should be addressed. E-mail: jtb4@cornell.edu.

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10.1021/ac0522198 CCC: $33.50
Published on Web 03/10/2006

We have previously reported a purely instrumental method for PSIA,[12] employing a continuous flow system of analyte separation by GC, fragmentation by pyrolysis, and GCC−IRMS for fragment separation and isotope ratio measurement. The method has been applied to the analysis of alkanes and fatty acids,[13] amino acids,[14,15] and low molecular weight organic acids.[16,17] In many cases, optimal signal is obtained at temperatures that produce incomplete pyrolysis (including amino acid studies) causing the analyte to fractionate at each carbon position to an indeterminate degree. Thus, absolute intramolecular isotope ratios ($\delta^{13}C$) cannot be determined by comparison to isotopically calibrated CO_2. However, each sample undergoes equivalent fractionation, which enables the reporting of a relative isotope ratio ($\Delta\delta^{13}C$) showing isotopic variability at a single position across multiple samples.[14,15] For example, a previous study of alanine illustrated variability at the carboxyl position among four samples.[15] A major disadvantage of $\Delta\delta^{13}C$ is that they do not reveal $\delta^{13}C$ at each position within a single sample to enable observation of intramolecular isotope distribution. Quantification of fractionation during pyrolysis would enable the calculation of absolute isotope ratios for each position and detection of isotopic distribution.

Isotope ratios of compounds are easily measured, and thus, the overall isotopic fractionation between a parent and a fragment is readily determined. Isotopic fractionation at each position depends on details of the potential energy surfaces and reaction mechanisms. For pyrolytic conditions, position-specific fractionation is not readily determined, and thus, it is unknown whether fractionation distribution is equal (E) across all sites or is due to fractionation at one site (S) with negligible fractionation at the others. These two extremes can be considered independently by assuming them in turn, calculating a fractionation factor, α, to be used for calculation of $\delta^{13}C$, and comparing the results to establish whether choice of fractionation factor has an important influence on determined $\delta^{13}C$'s. We evaluate the influence of these assumptions for propylene glycol, a volatile lactic acid analogue.

Lactic acid is a key metabolic intermediate produced in humans during anaerobic respiration, with an intramolecular isotope ratio that is likely to reflect fractionation in pyruvate. Figure 1 shows the conversion of pyruvate to acetyl-CoA by the pyruvate dehydrogenase complex in mammals. Isotope fractionation occurs for the carbons participating in the reaction, leaving the acetate carboxyl carbon as depleted in ^{13}C.[18] Mass balance predicts that the source pyruvate should be ^{13}C enriched at those two positions. During anaerobic respiration, pyruvate is converted to lactate and released into plasma. Lactic acid has been previously isolated from plasma and analyzed at the compound-specific level at natural ^{13}C abundance.[19,20] For GCC−IRMS analysis, the analyte was derivatized to a volatile methyl ester, altering the observed isotope ratio

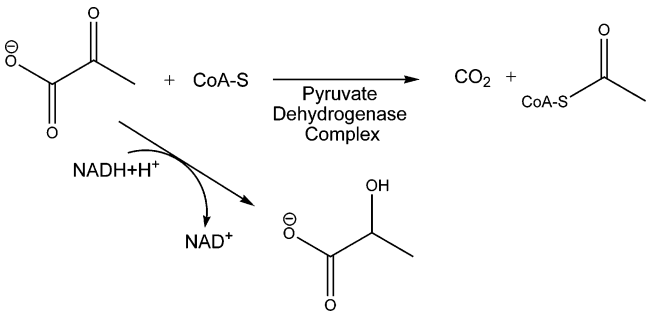

Figure 1. Biochemical oxidative decarboxylation of pyruvate to acetyl-CoA and conversion of pyruvate to lactate.

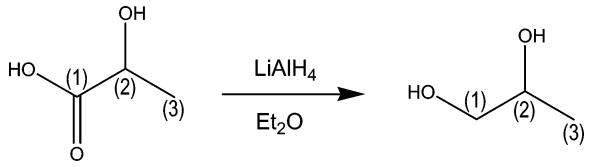

Figure 2. Chemical reduction of lactic acid to propylene glycol. Carbon positions (labeled as 1, 2, and 3) and isotope ratios are preserved during this reaction.

with an extraneous additional carbon atom. Pyrolysis of that compound is likely to yield fragments containing extraneous carbon that would interfere with fragments containing analyte carbon. Lactic acid can be quantitatively reduced to propylene glycol, which is amenable to GC analysis, and here we focus on intramolecular characterization of propylene glycol as a suitable derivative for lactic acid.

We propose here a method to quantify pyrolysis fractionation in propylene glycol, permitting calculation of isotope ratios for individual carbon positions. We hypothesize that reasonable assumptions about the range of fractionation factors, α, used to calibrate relative isotope ratios, $\Delta\delta^{13}C$, will result in small differences in calculated $\delta^{13}C$ and compare this with calculations performed independently. Finally, we characterize the absolute $\delta^{13}C$ intramolecular in four samples of propylene glycol to test whether the method can reliably detect natural variability.

EXPERIMENTAL SECTION

Synthesis of Chemical Analogue. To enable GC analysis, lactic acid was reduced to propylene glycol (boiling point, 190 °C). Compounds were purchased as propylene glycol or lactic acid. For convenience, each source was given a one-letter code (with "C" omitted to prevent confusion with carbon); "A" Acros (Geel, Belgium), "B" Aldrich (St. Louis, MO), "D" Alfa Aesar (Ward Hill, MA), and "E" Mallinckrodt (Phillipsburg, NJ) were all purchased as propylene glycol and used without further reaction. ^{13}C-Labeled samples were purchased as lactic acid from Cambridge Isotope Laboratories (Cambridge, MA) and reduced to the derivative.

Figure 2 shows the reduction reaction based upon the methods of Nystrom[21] and Wood.[22] The reaction is performed in a three-neck, round-bottom flask, with one neck used for a condenser and one with a calcium chloride drying tube. Briefly, 0.5 g of lactic

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acid was dissolved in 10 mL of diethyl ether. The lactic acid solution was added dropwise over 5 min to a solution of 0.16 g of $LiAlH_4$ in 6 mL of diethyl either. The resulting solution was stirred for 30 min with the reaction quenched by adding 2 mL of distilled water. Six milliliters 15% (w/w) of NaOH followed by 6 mL of distilled water was added and the resultant mixture stirred for an additional 15 min. The ether layer containing the product was separated and vacuum filtered, rinsing three times with 4 mL of ether. The resulting white solid product was dissolved in 100 mL of methanol. A 40-μL aliquot of the solution was diluted with 60 μL of methanol to create a concentration appropriate for analysis (2 μg/μL). The reaction produces product at 97% yield.

Instrumentation. Analysis was done using a home-built GC-Py-GCC-IRMS system previously described.[12] A 1-μL aliquot of solution (2 μg of propylene glycol) was injected splitless using a Varian 8200 autosampler (Varian, Inc., Walnut Creek, CA). The analyte was separated from the solvent in GC1 (HP 5890, Hewlett-Packard, Palo Alto, CA) on a VB-1 capillary column (15 m × 0.32 mm × 3 μm, VICI, Gig Harbor, WA). The oven was initially held at 60 °C for 5 min and ramped at 20 °C/min to 180 °C. An electronically triggered rotary valve (Valco, Houston, TX) was used to direct column flow either to isotope analysis or to a flame ionization detector (FID). The FID was used to determine retention time of the analyte peak and, therefore, trigger times of the rotary valve to send the analyte to pyrolysis and isotope analysis.

One continuous length of 0.32-mm fused-silica capillary column stretched from the output of GC1, through the pyrolysis furnace, to the input of GC2. A resistively heated Fibercraft furnace (Thermcraft, Winston-Salem, NC) created a pyrolysis zone of ~20 cm. To secure the capillary in the pyrolysis furnace, the capillary was held in a 0.5-mm-i.d. ceramic tube. The pyrolysis furnace was held at 700 ± 1 °C and controlled by a CN9000A series temperature controller (Omega Engineering, Stamford, CT). Following pyrolysis, the effluent passed through a heated transfer line to the GC2 column.

Pyrolysis fragments were separated in GC2 (Varian 3400) on a CarbonPLOT column (30 m × 0.32 mm × 1.5 μm, J&W Scientific, Folsom, CA). The oven was initially held at 30 °C for 10 min, ramped at 10 °C/min to 200 °C, and held for 3 min. A manually controlled rotary valve directed separated peaks either to isotope ratio measurement or molecular analysis for fragment identification. Molecular analysis was accomplished using a Varian Saturn III QISMS ion trap operating in positive ion electron impact mode. Fragments were identified with the assistance of the Wiley mass spectral database (Palisades, Newfield, NY). For isotope ratio measurement, fragments were quantitatively combusted in a second resistively heated Fibercraft furnace (940 °C) consisting of a 30 cm × 0.5 mm i.d. ceramic tube containing oxidized Cu. Products continued through a Nafion water trap to an open split with a 10:1 split ratio into a Finnigan-MAT 252 IRMS (Bremen, Germany) for isotope ratio measurement.

The system was controlled and data collected using SAXICAB,[23] a home-written Labview 6i-based program[24] with controls to trigger GC ovens, GC1 rotary valve, pyrolysis furnace, and standard gas pulses and with data reduction routines to calculate $\delta^{13}C_{V-PDB}$ values. It has been previously demonstrated that SAXICAB produces isotopic results identical to ISODAT, the proprietary software from Thermo Finnigan.[23] Data were collected using high-precision NI435x data acquisition boards (National Instruments, Austin, TX). Background levels were taken into account using "dynamic" background correction.[25] The mass 45 signal was adjusted to account for the presence of ^{17}O prior to final $\delta^{13}C_{V-PDB}$ calculation.[26]

Isotope Ratio Reporting. Absolute isotope ratios are reported using conventional $\delta^{13}C$ notation,

$$\delta^{13}C_{V-PDB} = \left[\frac{R_{spl} - R_{V-PDB}}{R_{V-PDB}}\right] \times 1000 \qquad (1)$$

where R_{spl} is the isotope ratio of the sample and R_{V-PDB} is the isotope ratio of Vienna-PeeDee Belemnite, the international standard for carbon with $R_{V-PDB} = 0.011\,179\,6$. In bulk and compound-specific analysis, the analyte is assumed to be quantitatively combusted, so that the resulting CO_2 $\delta^{13}C$ is identical to the analyte. Subsequent transfer of the analyte CO_2 to the ion source is assumed to introduce no major isotopic fractionation differing from that of the isotopically calibrated CO_2 gas, which typically takes a transfer path different from that of the analyte CO_2. By this reasoning, the resulting $\delta^{13}C_{V-PDB}$ is considered accurate. Incomplete pyrolysis introduces isotopic fractionation to the analyte to which the CO_2 calibrant gas is not subject. A relative isotope ratio, $\Delta\delta^{13}C$, can be accurately calculated comparing a single position across several samples, making the reasonable assumption that pyrolysis-induced fractionation is constant. When close to natural abundance levels, $\Delta\delta^{13}C$ can be calculated as the difference of the sample and standard $\delta^{13}C$ values as shown in eq 2,

$$\Delta\delta^{13}C^{(x)} = \delta^{13}C^{spl,(x)} - \delta^{13}C^{ref,(x)} \qquad (2)$$

where $\delta^{13}C^{ref,(x)}$ is the δ value for the reference source at carbon position x (source D in the current study) and $\delta^{13}C^{spl,(x)}$ is the δ value for the sample source at carbon position x. This notation has been discussed in detail previously.[14] Pyrolysis introduces artifactual isotopic fractionation, which will be taken into account using the method discussed later.

Fidelity of Pyrolysis Fragments. Calculation of $\Delta\delta^{13}C$ and $\delta^{13}C_{V-PDB}$ values requires knowledge of pyrolysis fragment origin in the parent molecule. We use the term *isotopic fidelity* to describe the extent to which the isotope ratio of a fragment reflects the isotope ratio of a specific position or moiety in the parent compound.[14,15] The fidelity of a compound is the percent of a fragment originating from a position or moiety in the parent. Utilizing previously established methods based on isotopic labeling, fidelity was calculated for each fragment formed as follows.

Separate standards were made with lactic acid labeled exclusively in the 1 or 3 position. The derivatized compound was added to an unlabeled solution with total ^{13}C label at a concentration between 0 and 200‰. Three labeled solutions were used, one

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Table 1. Fidelity of Fragments Formed during Pyrolysis

	$C^{(1)}$	$C^{(2)}$	$C^{(3)}$
methanol[a]	77.9 ± 0.7%	10.2 ± 0.7%	11.9 ± 0.4%
CH_4	35.9 ± 1.0%		64.1 ± 0.8%
ethylene	6.9 ± 0.5%	38.9 ± 0.5%	54.2 ± 0.2%
acetaldehyde	10.9 ± 0.2%		89.1 ± 1.3%
propylene	35.2 ± 3.6%	30.1 ± 3.7%	34.7 ± 0.7%
ethanol[a]	11.6 ± 1.9%	16.6 ± 2.6%	71.8 ± 1.7%
propanol[a]	27.3 ± 0.7%	35.4 ± 1.1%	37.4 ± 0.8%

[a] Used in calculation of relative and absolute δ values.

containing no labeled compound, one at ~50‰ and one at ~150‰. The observed isotope ratio of a fragment (R_{obs}) is a weighted sum of the isotope ratios of the carbons it contains (R_i),

$$R_{obs} = \sum_i R_i[X_i] \quad (3)$$

where X_i is the fraction of carbon from carbon i. Separating the labeled position yields,

$$R_{obs} = \sum_i R_i[X_i] + R_{lab}[X_{lab}] \quad (4)$$

where R_{lab} is the isotope ratio of the labeled compound and X_{lab} is the mole fraction of the labeled compound. Equation 4 can be plotted linearly as R_{obs} versus R_{lab}, where slope is the fidelity of the labeled carbon position for the fragment. Errors of slopes were determined using the linear regression tool in Microsoft Excel 2000.

RESULTS AND DISCUSSION

Pyrolysis Fragmentation. At 700 °C, 33% of the propylene glycol present was pyrolyzed. Table 1 shows fidelities of pyrolysis fragments formed from lactic acid. Each value listed is the quantity of a fragment originating from the specified carbon. For example, in the methanol fragment, 77.9 ± 0.7% of the carbon originated from position 1 ($C^{(1)}$). Lower temperatures do not produce the quantities of fragments necessary for high-precision measurements, while higher temperatures exhibit more carbon atom scrambling, and therefore, less ideal fidelity.[14]

Fidelities of the fragments formed reflect the parent compound structure, though evidence of scrambling is present. The majority of methanol is formed from carbons that contain hydroxyl groups in the parent compound, $C^{(1)}$ (77.9 ± 0.7%) and $C^{(2)}$ (10.2 ± 0.7%). Ethanol had a significant contribution from $C^{(3)}$ (71.8 ± 1.7%), which presumably contributed to the methyl carbon, while $C^{(1)}$ (11.6 ± 1.9%) and $C^{(2)}$ (16.6 ± 2.6%) primarily contribute to the hydroxyl-containing carbon. Propanol was formed from all three carbons with $C^{(2)}$ (35.4 ± 1.1%) and $C^{(3)}$ (37.4 ± 0.8%) slightly favored over $C^{(1)}$ (27.3 ± 0.7%). Methanol, ethanol, and propanol were used for the calculation of positional isotope ratios; average precisions of the fragment isotope ratios were SD(δ^{13}C) <0.14‰, SD(δ^{13}C) <0.34‰, and SD(δ^{13}C) <0.28‰, respectively. These fragments had the most desirable combination of orthogonal fidelities and isotope ratio precision to minimize propagated error in positional isotope ratio calculations.

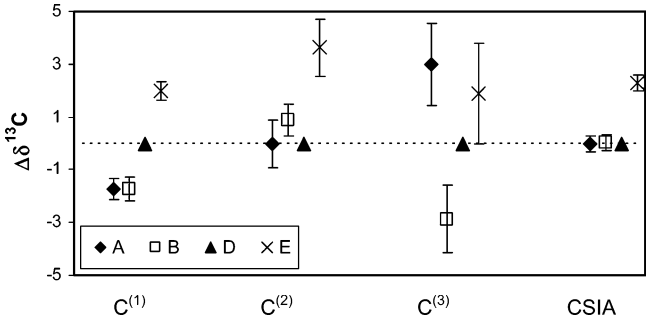

Figure 3. $\Delta\delta^{13}$C for the three positions of propylene glycol measured for four different sources. Error bars represent SD. Within a position (1, 2, or 3), error bars that do not overlap are significantly different (p <0.05). - - - represents $\Delta\delta^{13}C = 0$.

Relative Isotope Calculation. Relative isotope ratios ($\Delta\delta^{13}$C) were calculated using previously developed methods,[15] arbitrarily using source D as the standard and setting relative isotope ratios at each position to zero. Equations 5−7 show the mass balance equations for methanol, ethanol, and propanol, respectively, used for the calculation.

$$\Delta\delta^{13}C(MeOH) = 0.78\Delta\delta^{13}C^{(1)} + 0.10\Delta\delta^{13}C^{(2)} + 0.12\Delta\delta^{13}C^{(3)} \quad (5)$$

$$\Delta\delta^{13}C(EtOH) = 0.12\Delta\delta^{13}C^{(1)} + 0.17\Delta\delta^{13}C^{(2)} + 0.72\Delta\delta^{13}C^{(3)} \quad (6)$$

$$\Delta\delta^{13}C(Prop) = 0.27\Delta\delta^{13}C^{(1)} + 0.35\Delta\delta^{13}C^{(2)} + 0.37\Delta\delta^{13}C^{(3)} \quad (7)$$

The system of three equations and three unknowns is solved for the relative isotope ratios for each position. Errors were calculated via standard propagation methods and took into account errors in fidelities and fragment isotope ratio measurements.[27] The isotope ratio of each position was calculated independently to eliminate compounded errors.

Figure 3 shows the relative isotope ratios for each position and relative compound-specific data for each of the samples analyzed. At the compound-specific level, all sources were within a δ^{13}C range of 2.3‰, with sources A ($\Delta\delta^{13}C_D = -0.04 \pm 0.31$‰), B ($\Delta\delta^{13}C_D = 0.03 \pm 0.32$‰), and D ($\Delta\delta^{13}C_D = 0.00$‰) statistically indistinguishable; E ($\Delta\delta^{13}C_D = 2.28 \pm 0.31$‰) was the only unique source. In contrast, at the position-specific level, the sources were unique, with source D distinguished from all other sources at $C^{(1)}$ and source B distinguished at $C^{(3)}$. The unique origin of source A only becomes apparent in examining differences at two positions; at $C^{(1)}$, A is isotopically unique from source D, while at $C^{(3)}$, A is isotopically unique from source B. Relative isotope ratios were calculated with an average precision of SD($\Delta\delta^{13}$C) <0.84‰.

Absolute δ^{13}C Calculation. Calibration for pyrolytic isotopic fractionation is necessary for calculation of δ^{13}C. The critical component of absolute isotope ratio calculation is the pyrolysis fractionation factor (α) quantifying the influence of incomplete fragmentation on isotope ratio due to partial pyrolysis. Ideally,

(27) Meyer, S. L. *Data Analysis for Scientists and Engineers*; Wiley: New York, 1975.

fractionation factors would be available for each site; however, in practice, it is only possible to determine isotope ratios at the compound-specific level. The thus determined average fractionation can be the result of equal (E) fractionation occurring at all sites, in which case a single α can be applied to calibrate to each position. The opposite extreme is to assume that fractionation occurs only at one site (S), with negligible fractionation at the other sites. If fractionation occurs at a single site within a fragment containing n carbons, fractionation for that fragment changes by a factor of $1/n$ as the isotope ratio of all other carbons remains constant. Each fragment may independently fractionate between the S and E extremes. For propylene glycol, we evaluate the magnitude of uncertainty by calculating α and $\delta^{13}C$ for both extreme scenarios.

The isotope ratio of pyrolyzed carbon used in the calculation was compared to that of the parent compound, with each factor weighted appropriately for the pyrolysis scenario used, to obtain α values, as follows. To accomplish this, first the relative abundances of carbon contributed by methanol, ethanol, and propanol in the pyrogram were calculated. Fractional abundances, χ_n, of the fragments were calculated through eq 8,

$$\chi_n = \frac{A_n}{A_{MeOH} + A_{EtOH} + A_{Prop}} \quad (8)$$

where A is peak area of fragment n in the m/z 44 pyrogram. The isotope ratio of carbon used in the absolute $\delta^{13}C$ calculations, $\delta^{13}C_{pyr}$, was calculated using the weighted sum in eq 9,

$$\delta^{13}C_{pyr} = \chi_{MeOH}\delta^{13}C_{MeOH} + \chi_{EtOH}\delta^{13}C_{EtOH} + \chi_{Prop}\delta^{13}C_{Prop} \quad (9)$$

with fragment $\delta^{13}C_n$ values directly measured. Total fractionation is the difference between the parent δ value, $\delta^{13}C_{CSIA}$, and $\delta^{13}C_{pyr}$. Including the $1/n$ term to account for site-specific fractionation (as shown in Appendix 1, Supporting Information), α is calculated as,

$$\delta^{13}C_{CSIA} - \delta^{13}C_{pyr} = \chi_{MeOH}\frac{\alpha}{n_{MeOH}} + \chi_{EtOH}\frac{\alpha}{n_{EtOH}} + \chi_{Prop}\frac{\alpha}{n_{Prop}} \quad (10)$$

where n is the number of carbons in the specified fragment for site-specific fractionation, or in the case of average fractionation, $n = 1$. Solving eq 10 for α yields eq 11.

$$\alpha = \frac{\delta^{13}C_{CSIA} - \delta^{13}C_{pyr}}{\frac{\chi_{MeOH}}{n_{MeOH}} + \frac{\chi_{EtOH}}{n_{EtOH}} + \frac{\chi_{Prop}}{n_{Prop}}} \quad (11)$$

For methanol, S and E are equivalent since there is only one carbon, and $n_{MeOH} = 1$ always. We then use a two-letter code to describe specific pyrolysis scenarios for the three fragments. The four possible combinations are SS, SE, ES, and EE, which correspond to values of n_{EtOH} and n_{Prop} of (2,3), (2,1), (1,3), and (1,1), respectively. Calculated α's range from 3.3 to 8.2‰, and their specific values can be found in Table 2.

Table 2. Calculated α Values Used in Eq 12 To Correct $\delta^{13}C$ of Pyrolysis Fragments

	fractionation scenario			
source	EE	ES	SE	SS
A	3.6	5.3	4.1	6.6
B	4.5	6.6	5.2	8.2
D	3.4	5.1	3.9	6.3
E	3.4	5.0	3.9	6.2

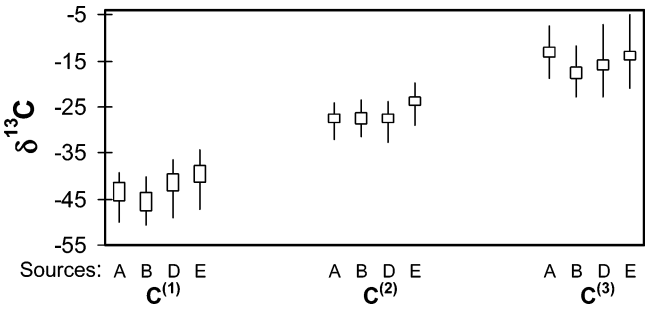

Figure 4. Range of absolute isotope ratios enabling comparison between positions within the same molecule. The central box spans the range of $\delta^{13}C$ values, while the error lines extend to the extremes of standard deviation.

Following calculation of α, fragment δ values are corrected for pyrolysis fractionation using eq 12,

$$\delta^{13}C_{corr} = \delta^{13}C_{uncorr} - \alpha/n \quad (12)$$

where $\delta^{13}C_{uncorr}$ is the uncorrected fragment isotope ratio, $\delta^{13}C_{corr}$ is the corrected fragment isotope ratio, and n is either the number of carbons in the fragment (for site-specific fractionation, S) or 1 (for equal fractionation, E).

Utilizing corrected fragment isotope ratios, absolute position-specific isotope ratios were calculated using a method similar to that of the relative isotope ratio calculation. Equations 13−15 show weighted sum equations with coefficients based on fidelity for methanol ($\delta^{13}C_{MeOH}$), ethanol ($\delta^{13}C_{EtOH}$), and propanol ($\delta^{13}C_{Prop}$), respectively,

$$\delta^{13}C_{MeOH} = 0.78\delta^{13}C^{(1)} + 0.10\delta^{13}C^{(2)} + 0.12\delta^{13}C^{(3)} \quad (13)$$

$$\delta^{13}C_{EtOH} = 0.12\delta^{13}C^{(1)} + 0.17\delta^{13}C^{(2)} + 0.72\delta^{13}C^{(3)} \quad (14)$$

$$\delta^{13}C_{Prop} = 0.27\delta^{13}C^{(1)} + 0.35\delta^{13}C^{(2)} + 0.37\delta^{13}C^{(3)} \quad (15)$$

where $\delta^{13}C^{(1)}$, $\delta^{13}C^{(2)}$, and $\delta^{13}C^{(3)}$ are δ values of $C^{(1)}$, $C^{(2)}$, and $C^{(3)}$, respectively. Calculated $\delta^{13}C$ values for all pyrolysis scenarios are shown in Figure 4, to be discussed below.

Check of Method Validity. Method validity was checked through a comparison of relative isotope ratios ($\Delta\delta^{13}C$). One set was calculated directly from fragment $\delta^{13}C$ and fidelities in eqs 5−7 ($\Delta\delta^{13}C$-dir), and the other set was calculated from absolute isotope ratios of different pyrolysis scenarios (eqs 8−10) and

Table 3. Average Deviation of $\Delta\delta^{13}C$ Calculated by Assuming Various Scenarios Compared to Those Determined by Direct Calibration of Fragment Isotope Ratios Weighted by Fidelities

	$\Delta\delta^{13}C^{(1)}$ dev	$\Delta\delta^{13}C^{(2)}$ dev	$\Delta\delta^{13}C^{(3)}$ dev	av abs dev
EE[a]	−0.37	−0.20	0.23	0.27
ES	−0.55	−0.29	0.32	0.39
SE	−0.43	−0.23	0.26	0.31
SS	−0.69	−0.36	0.39	0.48

[a] Smallest average absolute deviation.

converted to relative isotope ratios using eq 2 ($\Delta\delta^{13}C$-check). This is an independent check because $\Delta\delta^{13}C$-check incorporates an independent mean α, and $\Delta\delta^{13}C$ values thus derived are independent of $\Delta\delta^{13}C$-dir. Table 3 presents all results for the differences ($\Delta\delta^{13}C$-dir) − ($\Delta\delta^{13}C$-check). The average absolute deviation is ~0.4‰, positions 1 and 2 are biased to lower values, and position 3 is biased to higher values. This value is modest compared to the magnitude of differences for the means and is about twice the precision expected for direct $\delta^{13}C$ measurement of fragments. The EE combination, where fractionation is distributed evenly across all carbon sites, yields the smallest deviation, which we take as evidence that variation among α are closer to E than to S.

Intramolecular Isotope Distribution. Figure 4 shows the intramolecular isotope ratios grouped by source incorporating α factors for all pyrolysis scenarios. In the figure, central rectangles span the range of $\delta^{13}C$ calculated by assuming all four fractionation scenarios, and the error lines extend to the extremes of means plus and minus SDs. Each source exhibits a similar pattern of isotope distribution, with $\delta^{13}C^{(3)} > \delta^{13}C^{(2)} > \delta^{13}C^{(1)}$, though the degree of variability is different. Although no biological statements can be made concerning these propylene glycol samples, we note that all samples show a clear pattern of increasing $\delta^{13}C$ with carbon number. Average precision was calculated with an average SD-($\delta^{13}C$) <3.0‰.

CONCLUSIONS

A method is presented to calculate absolute intramolecular isotope ratios, demonstrated using the lactic acid analogue propylene glycol as a model compound. Four samples of propylene glycol were analyzed, for which relative ($\Delta\delta^{13}C$) and absolute ($\delta^{13}C$) isotope ratios were calculated. Relative isotope ratios showed all samples originated from different sources, with sources A, B, and D distinguishable only at the position-specific level. Absolute isotope ratios revealed the same $\delta^{13}C$ pattern, with enrichment at the $C^{(3)}$ position, with $C^{(1)}$ most depleted in ^{13}C.

Absolute $\delta^{13}C$ calculations required quantification of fractionation due to pyrolysis. The degree of fractionation is dependent on whether fractionation is exclusive to one carbon site in a fragment or distributed among all carbons. Four different scenarios were explored, with different combinations of site-specific and average fractionation for each fragment. We find that use of an average fractionation factor yields minimal error and absolute $\delta^{13}C$ within ±1.5‰ of that calculated under assumptions of extreme fractionation. Although this figure is higher than commonly reported for high-precision IRMS, we note that the difference in $\Delta\delta^{13}C$ is determined with much less error and is usually the quantity of most interest. In addition, intramolecular variability is high compared to compound-specific or bulk isotope $\delta^{13}C$, providing greater tolerance for error. This analysis indicates that on-line PSIA by GC-Py-GCC-IRMS can provide useful intramolecular $^{13}C/^{12}C$ for a single analyte without resorting to intramolecularly calibrated isotopic standards.

ACKNOWLEDGMENT

This work was funded by NIH grant GM49209. C.J.W. acknowledges support from NIH training grant DK07158.

SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review December 15, 2005. Accepted February 7, 2006.

AC0522198