Stable Carbon Isotope Analysis of Amino Acid Enantiomers by Conventional Isotope Ratio Mass Spectrometry and Combined Gas Chromatography/Isotope Ratio Mass Spectrometry

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The application of a combined gas chromatography/isotope ratio mass spectrometry (GC/IRMS) method for stable carbon isotope analysis of amino acid enantiomers is presented. This method eliminates the numerous preparative steps integral to the isolation of amino acids and amino acid enantiomers from protein hydrolyzates that precede $\delta^{13}C$ analysis by conventional isotope ratio mass spectrometry. Unlike hydrocarbons, amino acids require derivatization prior to GC/IRMS analysis. Replicate $\delta^{13}C$ analyses of trifluoroacetyl (TFA) isopropyl ester derivatives of 22 amino acids by IRMS revealed that the derivatization process is reproducible, with an average error (1 standard deviation) of 0.10‰ ± 0.09‰. The average analytical error for analysis of amino acid derivatives by GC/IRMS was 0.26‰ ± 0.09‰. In general, absolute differences between IRMS and GC/IRMS analyses were less than 0.5‰. The derivatization process introduces a distinct, reproducible isotopic fractionation that is constant for each amino acid type. The observed fractionations preclude direct calculation of underivatized amino acid $\delta^{13}C$ values from their respective TFA isopropyl ester $\delta^{13}C$ compositions through mass balance relationships. Derivatization of amino acid standards of known stable carbon isotope compositions in conjunction with natural samples, however, permits computation of the original, underivatized amino acid $\delta^{13}C$ values through use of an empirical correction for the carbon introduced during the derivatization process.

INTRODUCTION

Conventional stable carbon isotope analyses of bulk organic fractions from terrestrial and extraterrestrial samples continue to provide information concerning the origin and significance of organic matter in the geologic record. A diverse spectrum of applications has evolved in recent years, including elucidation of Precambrian biological evolution (*1*), evaluation of dinosaur trophic levels (*2*), determination of paleodietary trends in hominids (*3*), and assessment of the origin of organic compounds in carbonaceous meteorites (*4*). The interpretation of stable isotope data for composite samples remains complex, however, because the preserved isotopic signal for a given bulk sample represents an average value for a multitude of compounds, some of which may be contaminants or alteration products of the original material. Fundamental isotopic trends may therefore be obscured.

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The stable isotope analysis of individual organic compounds, particularly amino acids from living systems and fossils, is a powerful method for probing modern (*5–8*) and ancient (*9*) biochemistries. Comparison of the stable isotope compositions of amino acid enantiomer pairs, furthermore, has been suggested as an independent method to assess amino acid indigeneity in fossils (*10, 11*) and carbonaceous meteorites (*12, 13*). Stable isotope values for individual amino acids and their respective enantiomers in modern and fossil systems are rarely reported, however, because of the difficulties inherent in isolating individual components from complex mixtures for stable isotope analysis by conventional combustion methods (e.g., ref 14).

The recent development of combined gas chromatography/isotope ratio mass spectrometry (GC/IRMS) systems (*15, 16*) has permitted the direct stable carbon isotope analysis of individual, volatile organic compounds (i.e., hydrocarbons) in chemically complex samples (*17, 18*). The application of this method to amino acid analysis, however, is complicated by the fact that amino acids are nonvolatile, multifunctional molecules that require derivatization prior to analysis. The derivatization process introduces additional carbon atoms and the potential for isotopic fractionation; consequently, alteration of the original stable carbon isotopic composition of the amino acids occurs. The relationship between the $\delta^{13}C$ of amino acid derivatives and the $\delta^{13}C$ of the underivatized amino acids must be established before GC/IRMS analysis of these compounds can assume any practical value.

In this paper, we report the effects of derivatization on amino acid $\delta^{13}C$ compositions as determined by both conventional isotope ratio mass spectrometry and GC/IRMS. Specifically, $\delta^{13}C$ values of trifluoroacetyl isopropyl esters of biologically significant amino acids and an amino acid that is present in carbonaceous meteorites were determined by conventional IRMS in order to establish the reproducibility of the derivatization method and to confirm $\delta^{13}C$ values obtained by GC/IRMS. Additionally, IRMS and GC/IRMS analyses of five racemic amino acids were performed to determine whether amino acid enantiomers with identical stable carbon isotope compositions retained their isotopic integrity during derivatization and analysis. The results of these studies and the implications for GC/IRMS analyses of amino acids in natural materials are presented below.

EXPERIMENTAL SECTION

Standards and Reagents. Standard solutions (0.05 M) of individual amino acid enantiomers and racemic amino acids were prepared by dissolving appropriate amounts of crystalline amino acids (Sigma, St. Louis, MO) in distilled 0.1 N HCl. The solutions were stored at 4 °C. Acidified (2.8 M HCl) 2-propanol was

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prepared in an ice bath by the addition of 250 μL of acetyl chloride (99+%, Aldrich, Milwaukee, WI) per milliliter of 2-propanol (HPLC grade, Fisher Scientific, Fairlawn, NJ). The acidified alcohol was used within 48 h of preparation. Trifluoroacetic anhydride (99+%, Pierce Chemical Co., Rockford, IL) was used for acylation. Reagents of the same lot numbers were used for all derivatizations.

Derivatization Procedure. For conventional IRMS analysis, 200-μL aliquots (10^{-5} mol) of the amino acid standard solutions were dispensed into individual 4-mL screw cap vials with Teflon cap liners. Three separate samples of each amino acid enantiomer were prepared. The samples were evaporated to dryness under a stream of N_2 at 40 °C. For GC/IRMS analysis, 100-μL aliquots of each standard solution were prepared in an identical manner.

The dried samples were esterified with 0.5 mL of the acidified 2-propanol for 1 h at 110 °C. After 1 h, the reaction was quenched by placing the vials in a freezer. Next, 0.25 mL of each sample was pipeted into a 20-cm × 7-mm-i.d. Pyrex tube. The solvent was removed by evaporation under a gentle stream of N_2 at 25 °C. Two successive 0.25-mL aliquots of CH_2Cl_2 were placed in each tube and evaporated to remove excess 2-propanol and water.

The remaining portions of the esterified samples were evaporated to dryness under N_2, redissolved in CH_2Cl_2, and dried again. The amino acid isopropyl esters were acylated with 0.5 mL of trifluoroacetic anhydride (TFAA) and 0.5 mL of CH_2Cl_2 for 10 min at 110 °C. Next, the vials were chilled in a freezer and then placed in an ice bath where the excess TFAA and CH_2Cl_2 were removed by evaporation under N_2. The derivatives were redissolved in 0.25 mL of CH_2Cl_2 and evaporated at 0 °C to remove residual traces of TFAA and trifluoroacetic acid. The derivatives were then dissolved in 0.5 mL of CH_2Cl_2, transferred to Pyrex tubes, and evaporated to dryness under N_2.

Conventional Isotope Ratio Mass Spectrometry. The amino acid isopropyl esters and the amino acid trifluoroacetyl isopropyl esters were transferred to individual Pyrex tubes (20 cm × 7 mm i.d.) as previously described. Five grams of copper oxide wire was added to each tube. The tubes and copper oxide wire were preheated (to 550 and 850 °C, respectively) and then cooled to room temperature just prior to loading the samples. The tubes were evacuated, sealed, and combusted at 550 °C for 2.5 h. The resultant CO_2 gas of each combusted sample was cryogenically purified and analyzed for its stable carbon isotope composition as previously described (*19*).

Stable carbon isotope data are presented by using the standard convention

$$\delta N_E‰ = [R_{sample}/R_{standard} - 1]10^3$$

where N is the heavier stable isotope of the element E and R is the abundance ratio of the heavy to light isotopes of the element. The standard for carbon is the Peedee Belemnite (PDB) that has been assigned a $\delta^{13}C$ value of 0.0‰. For routine measurement, samples are analyzed against a laboratory working standard tank of pure CO_2 gas that has been calibrated against NBS reference materials.

Gas Chromatography/Isotope Ratio Mass Spectrometry. Several of the TFA isopropyl esters of the individual enantiomers and racemic amino acids were analyzed directly for their stable carbon isotope compositions by using the VG Isochrom GC/IRMS system. The GC/IRMS system consists of a Hewlett-Packard 5890 gas chromatograph interfaced to a VG SIRA isotope ratio mass spectrometer via a combustion furnace/water trap. Details concerning the system hardware and software have been previously reported (*15*). In this study, the gas chromatograph was equipped with a 50-m × 0.25-mm-i.d. fused silica capillary column coated with an optically active stationary phase (Chirasil-Val; Alltech Assoc., Deerfield, IL) capable of resolving the TFA isopropyl esters of amino acid enantiomers (*20*). The CO_2 combustion products of the compounds eluting from the capillary column are introduced directly into the mass spectrometer ion source: this instrument configuration permits stable carbon isotope analysis at nanomole levels.

RESULTS AND DISCUSSION

$\delta^{13}C$ Analysis of Amino Acid Derivatives. The preparation of TFA isopropyl ester derivatives is, as previously discussed, a two-step process that alters the original stable carbon isotope compositions of the amino acids. In order to correct for the introduction of carbon during derivatization, it is necessary to establish the isotopic reproducibility of the derivatization method. The $\delta^{13}C$ values of 17 amino acids and their respective isopropyl esters and TFA isopropyl esters as determined by conventional IRMS are presented in Table I. In general, the esterification of three separate samples of each amino acid was accomplished with a reproducibility (1 standard deviation) of less than 0.3‰, with the exception of D-lysine (0.41‰). The average experimental error for the preparation and IRMS analysis of amino acid isopropyl esters was 0.12‰ ± 0.11‰. Similarly, the complete derivatization of the amino acids resulted in an experimental error of less than 0.2‰, with the exceptions of D-phenylalanine (0.25‰) and D-lysine (0.33‰). The average reproducibility for the preparation and IRMS analysis of the TFA isopropyl esters was 0.10‰ ± 0.10‰. The average experimental error for the preparation and IRMS analysis of the five racemic amino acid derivatives was 0.08‰ ± 0.03‰ (Table II). These reproducibilities are well within the the error reported for GC/IRMS $\delta^{13}C$ determinations of compounds that do not require derivatization prior to analysis (*16*).

The $\delta^{13}C$ compositions of TFA isopropyl ester derivatives of selected amino acids and amino acid enantiomers were also

Table I. Stable Carbon Isotopic Compositions of Amino Acids, Amino Acid Isopropyl Esters, and N-TFA Isopropyl Esters

	$\delta^{13}C$		
amino acid	underiv amino acid[a,b]	amino acid isopropyl ester[a-c]	amino acid N-TFA isopropyl ester[a-c]
α-Aiba[e]	−28.19	−27.87	−31.94
		0.05	0.09
D-Ala	−26.23	−25.99	−29.93
		0.17	0.08
L-Ala	−20.26	−23.12	−28.02
		0.06	0.07
D-Val	−31.47	−31.13[d]	−32.69
		0.10	0.04
L-Val	−25.97	−26.53	−29.65[d]
		0.23	0.23
Gly 1[f]	−32.67	−28.38	−32.42[d]
		0.01	0.29
Gly 2[f]	−25.85	−25.66	−30.43
		0.08	0.17
D-Leu	−25.25	−25.82	−28.76[d]
		0.09	0.04
L-Leu	−23.69	−24.83	−27.90
		0.22	0.02
D-Asp	−21.04	−23.81	−27.20
		0.08	0.01
L-Asp	−24.46	−25.18	−28.43
		0.02	0.01
D-Glu	−23.19	−24.25	−27.09
		0.03	0.11
L-Glu	−27.68	−26.45	−28.94
		0.05	0.03
D-Phe	−31.89	−30.84	−32.40
		0.08	0.25
L-Phe	−27.68	−27.63	−29.79[d]
		0.12	0.06
D-Lys	−22.62	−24.30	−29.44
		0.41	0.33
L-Lys	−11.48	−16.98	−24.47
		0.29	0.13

[a] Stable carbon isotopic compositions reported in per mil relative to PDB. [b] $\delta^{13}C$ determined by static combustion and conventional IRMS analysis. [c] Values represent means and standard deviations for three separate samples unless specified otherwise. [d] Average of two samples and their range. [e] α-Aminoisobutyric acid. [f] Gly 1 and Gly 2 represent glycines from two different commercial sources.

Table II. Stable Carbon Isotopic Compositions of Amino Acids and Their Respective N-TFA Isopropyl Esters Determined by IRMS and GC/IRMS Analysis

	$\delta^{13}C$				
amino acid	underivatized amino acid[a,b]	amino acid N-TFA isopropyl ester[a-c]	amino acid	amino acid N-TFA isopropyl ester[a,d,e]	Δ^g
α-Aiba	−28.19	−31.94		−32.33	0.39
		0.09		0.08	
D-Ala	−26.23	−29.93		−30.10	0.17
		0.08		0.14	
L-Ala	−20.26	−28.02		−27.52	−0.50
		0.07		0.17	
D-Val	−31.47	−32.69		−32.62[f]	−0.07
		0.04		0.08	
L-Val	−25.97	−29.65		−29.75[f]	0.10
		0.23		0.03	
Gly 1	−32.67	−32.42		−32.09[f]	−0.33
		0.29		0.45	
D,L-Ala	−25.45	−29.82	D-Ala	−30.17	0.27
		0.05		0.32	
			L-Ala	−30.00	
				0.31	
D,L-Val	−26.99	−30.20	D-Val	−31.01	0.75
		0.11		0.28	
			L-Val	−30.88	
				0.40	
D,L-Leu	−24.93	−28.48	D-Leu	−29.13	0.39
		0.05		0.20	
			L-Leu	−28.61	
				0.26	
D,L-Asp	−22.32	−27.47	D-Asp	−28.47	0.90
		0.08		0.29	
			L-Asp	−28.27	
				0.25	
D,L-Glu	−23.59	−27.39	D-Glu	−28.22	0.73
		0.06		0.29	
			L-Glu	−28.01	
				0.33	

[a] Stable carbon isotopic compositions reported in per mil relative to PDB. [b] $\delta^{13}C$ determined by static combustion and conventional IRMS analysis. [c] Values represent means and standard deviations for three separate samples except for L-Val, Gly 1, and D,L-Ala, for which the mean and range for two samples is reported. [d] $\delta^{13}C$ determined by GC/IRMS analysis. [e] Values represent means and standard deviations for a minimum of three analyses of the same sample unless specified otherwise. [f] Values represent the mean and range for two analyses of the same sample. [g] IRMS $\delta^{13}C$ − GC/IRMS $\delta^{13}C$; note that for the racemic amino acids the average $\delta^{13}C$ value of the D- and L-enantiomers was used to compute the differences.

determined by GC/IRMS analysis. The results of these analyses are presented in conjunction with the $\delta^{13}C$ values for the derivatives determined by IRMS (Table II). The analytical error obtained for replicate GC/IRMS analyses of the same sample ranged from 0.08‰ to 0.40‰ and averaged 0.26‰ ± 0.09‰. The average error for GC/IRMS analysis of amino acid derivatives is consistent with analytical errors previously reported for other volatile organic compounds (*16*).

Following consultations with Sigma Chemical Co. (St. Louis, MO), it was concluded that the stable carbon isotope compositions of the individual enantiomers of their racemic amino acids should be identical, in the absence of stereospecific fractionation effects. The results of the GC/IRMS analyses confirm that, within the margin of analytical error, the $\delta^{13}C$ values of the enantiomers are in fact nearly indistinguishable (Table II).

The $\delta^{13}C$ values determined by GC/IRMS in general compare favorably with the stable carbon isotope compositions obtained by conventional IRMS analysis. The absolute differences between the $\delta^{13}C$ values obtained by the two analytical methods are less than 0.5‰ and in three instances are less than 0.2‰, for all amino acids except D,L-valine, D,L-aspartic acid, and D,L-glutamic acid. The presence of trace contaminants from the amino acid standards or the derivatizing reagents or side products resulting from derivatization could have influenced the IRMS analyses, whereas the chromatographic separation employed by GC/IRMS would have effectively removed these trace components.

Isotopic Fractionation during Derivatization. Theoretically, the amino acid isopropyl esters and N-TFA isopropyl esters should exhibit $\delta^{13}C$ compositions that reflect the relative contributions of carbon from each component and their respective $\delta^{13}C$ values. The generalized stoichiometric mass balance relationship for amino acid isopropyl esters, for example, may be written as

$$\delta^{13}C_{ESTER} = X\delta^{13}C_{AA} + (1 - X)\delta^{13}C_{ISO} \quad (1)$$

where $\delta^{13}C_{ESTER}$, $\delta^{13}C_{AA}$, and $\delta^{13}C_{ISO}$ represent the stable carbon isotope compositions of the isopropyl ester, the underivatized amino acid, and the 2-propanol, respectively, and X and $1 - X$ are the mole fractions of carbon from each of the sources. Similarly, the $\delta^{13}C$ composition of the TFA isopropyl derivatives, which contain additional carbon from trifluoroacetic anhydride, can be expressed as

$$\delta^{13}C_{DER} = X\delta^{13}C_{AA} + Y\delta^{13}C_{ISO} + (1 - X - Y)\delta^{13}C_{TFAA} \quad (2)$$

where $\delta^{13}C_{DER}$ and $\delta^{13}C_{TFAA}$ represent the stable carbon isotope compositions of the TFA isopropyl derivative and TFAA, respectively, and X, Y, and $1 - X - Y$ represent the mole fractions of carbon from each component. Comparison of the stable carbon isotope compositions of amino acid isopropyl esters and TFA isopropyl esters determined by IRMS analysis and the respective values predicted by mass balance consid-

Table III. Stable Carbon Isotopic Compositions of Amino Acid Isopropyl Esters and N-TFA Isopropyl Esters versus $\delta^{13}C$ Mass Balance Predictions for Each

	$\delta^{13}C$			$\delta^{13}C$		
amino acid	amino acid isopropyl ester[a,b]	predicted isopropyl ester[c]	Δ[d]	amino acid N-TFA isopropyl ester[a,b]	predicted N-TFA isopropyl ester[c]	Δ[d]
α-Aiba	−27.87	−27.34	−0.53	−31.94	−28.63	−3.31
D-Ala	−25.99	−26.22	0.23	−29.93	−27.96	−1.97
L-Ala	−23.12	−23.24	0.12	−28.02	−25.72	−2.30
D-Val	−31.13	−29.50	−1.63	−32.69	−30.23	−2.46
L-Val	−26.53	−26.06	−0.47	−29.65	−27.48	−2.17
Gly 1	−28.38	−28.79	0.41	−32.42	−30.05	−2.37
Gly 2	−25.66	−26.07	0.41	−30.43	−28.09	−2.34
D-Leu	−25.82	−25.57	−0.25	−28.76	−26.94	−1.82
L-Leu	−24.83	−24.53	−0.30	−27.90	−26.09	−1.81
D-Asp	−23.81	−24.14	0.33	−27.20	−25.64	−1.56
L-Asp	−25.18	−25.51	0.33	−28.43	−26.78	−1.65
D-Glu	−24.25	−24.84	0.59	−27.09	−26.10	−0.99
L-Glu	−26.45	−26.88	0.43	−28.94	−27.83	−1.11
D-Phe	−30.84	−30.47	−0.37	−32.40	−30.85	−1.55
L-Phe	−27.63	−27.31	−0.32	−29.79	−28.14	−1.65
D-Lys	−24.30	−23.82	−0.48	−29.44	−26.67	−2.77
L-Lys	−16.98	−16.39	−0.59	−24.47	−21.52	−2.95

[a] Stable carbon isotopic compositions reported in per mil relative to PDB. [b] $\delta^{13}C$ determined by static combustion and conventional IRMS analysis. [c] 2-Propanol $\delta^{13}C$ = −26.21‰; TFAA $\delta^{13}C$ = −33.10‰. [d] Analytical $\delta^{13}C$ − predicted $\delta^{13}C$.

erations, however, reveals that an apparent isotopic fractionation occurs during each derivatization step (Table III).

In general, amino acid esterification produces relatively small isotopic discrepancies that, with the exception of D-valine, range from +0.59‰ to −0.59‰. For amino acid isopropyl esters in which 2-propanol contributes more than 50% of the carbon (e.g., glycine, aspartic acid, glutamic acid), the predicted $\delta^{13}C$ values are light relative to the values measured by IRMS and GC/IRMS. In contrast, for the isopropyl esters in which 2-propanol contributes less than 50% of the carbon (e.g., valine, phenylalanine), the mass balance predictions are consistently heavier than the analytical values. The discrepancy between the predicted and analytical $\delta^{13}C$ values for alanine isopropyl esters, for which alanine and 2-propanol contribute identical moles of carbon, is approximately zero. The stable carbon isotope compositions of the TFA isopropyl esters determined by IRMS, however, are consistently depleted relative to the $\delta^{13}C$ values predicted from mass balance considerations. The isotopic discrepancies between the analytical and predicted values for the amino acid derivatives range from −0.99‰ to −3.31‰ (Table III).

The observed isotopic fractionations are consistent for each amino acid type and are independent of differences in original $\delta^{13}C$ compositions (Table III). For example, although glycine 1 and glycine 2 (1 and 2 designate the two commercial suppliers) differ by nearly 7‰, the observed fractionation for preparation of the glycyl isopropyl esters was 0.41‰ for both samples. The preparation of the N-TFA glycyl isopropyl esters resulted in a fractionation of −2.37‰ for glycine 1 and −2.34‰ for glycine 2. Similarly, the original $\delta^{13}C$ values for D-lysine and L-lysine prior to derivatization differ by approximately 11‰, yet the differences between the analytical and predicted values for the isopropyl esters were −0.48‰ and −0.59‰ and for the TFA isopropyl ester derivatives were −2.77‰ and −2.95‰, respectively. For the amino acids investigated, the isotopic fractionations observed for each amino acid type differ by no more than 0.16‰ for esterification (D-glutamic acid, L-glutamic acid) and 0.33‰ for preparation of the TFA isopropyl esters (D-valine, L-valine) and in most instances are less.

Origin of the Isotopic Fractionation. The origin of the isotopic discrepancies between the analytical and predicted $\delta^{13}C$ compositions of the amino acid derivatives is not apparent at present. The conditions employed for esterification and acylation have been demonstrated to produce quantitative derivatization of the amino acids investigated (*20* and references therein). Assuming that the reactions were quantitative, it is possible that a kinetic fractionation occurs during the acylation step, whereupon isotopically lighter reagent molecules react preferentially and deplete the derivative $\delta^{13}C$ value beyond that predicted by mass balance (eq 2). The fractionations for esterification, however, range from +0.59‰ to −0.59‰, with the exception of D-valine, and apparently cannot be ascribed simply to kinetics.

Variations of the magnitudes of the fractionations may result from differences in amino acid reactivity. Future detailed investigations of the effects of reagent $\delta^{13}C$ compositions, reaction time and temperature, reagent:amino acid molar ratios, and alternative derivatization methods on the magnitude of the isotopic fractionations should provide indirect evidence of their origin(s).

Calculation of Amino Acid $\delta^{13}C$ Values from Derivative $\delta^{13}C$ Values. The carbon isotope fractionation that occurs during derivatization precludes direct calculation of the $\delta^{13}C$ values of amino acids in natural samples by stoichiometric mass balance relationships (e.g., eq 2). The constancy of the derivatization fractionation effect observed for each amino acid, however, implies that original amino acid stable carbon isotope compositions ultimately can be derived from TFA isopropyl ester $\delta^{13}C$ values. To this end, the series of mass balance equations relating the $\delta^{13}C$ values of the amino acid derivatives to the stable isotope compositions and stoichiometric contributions of carbon from the amino acid, 2-propanol, and TFAA were modified:

$$\delta^{13}C_{DER} = X\delta^{13}C_{AA} + (1 - X)\delta^{13}C_{ISO,TFAA} \quad (3)$$

In essence, the mole fractions of carbon contributed to the amino acid derivative by 2-propanol and TFAA were summed and the variable $\delta^{13}C_{ISO,TFAA}$ was defined as the "effective" stable carbon isotope composition of the 2-propanol and trifluoroacetic acid introduced during derivatization. $\delta^{13}C_{ISO,TFAA}$ incorporates the observed isotopic fractionation for each amino acid type and is not related to the actual carbon isotope compositions of the two reagents. Consequently, $\delta^{13}C_{ISO,TFAA}$ must at the present time be determined empirically for each amino acid from standards of known stable

Table IV. Calculated $\delta^{13}C$ Values for Racemic Amino Acids

amino acid	$\delta^{13}C$ underivatized amino acid[a,b]	$\delta^{13}C$ calcd amino acid[c]
D,L-Ala	−25.45	−25.49
D,L-Val	−26.99	−26.78
D,L-Leu	−24.93	−24.75
D,L-Asp	−22.32	−21.70
D,L-Glu	−23.59	−23.81

[a] Stable carbon isotopic compositions reported in per mil relative to PDB. [b] $\delta^{13}C$ determined by static combustion and conventional IRMS analysis. [c] Calculated from respective N-TFA isopropyl ester $\delta^{13}C$ values determined by IRMS analysis and eq 3.

carbon isotope compositions. By use of the IRMS $\delta^{13}C$ values for the TFA isopropyl esters and underivatized amino acids from Table I, the empirical corrections for the carbon introduced during the derivatization process were derived for each amino acid enantiomer. The average $\delta^{13}C_{ISO,TFAA}$ for each amino acid type was then calculated from the separate enantiomer values.

To assess the accuracy of this method, the $\delta^{13}C$ compositions of the underivatized racemic amino acids D,L-alanine, D,L-valine, D,L-leucine, D,L-aspartic acid, and D,L-glutamic acid were calculated from their respective IRMS TFA isopropyl ester $\delta^{13}C$ values. The $\delta^{13}C_{AA}$ values were computed by substituting the $\delta^{13}C_{DER}$ compositions from Table II and the appropriate $\delta^{13}C_{ISO,TFAA}$ correction factors into eq 3. The analytically determined $\delta^{13}C$ values and the calculated $\delta^{13}C$ values for the five amino acids are presented in Table IV. The calculated $\delta^{13}C$ values of the racemic amino acids are, with the exception of D,L-aspartic acid, within 0.25‰ of the original, underivatized $\delta^{13}C$ compositions determined by IRMS. These data suggest that, at present, the most suitable method to determine amino acid $\delta^{13}C$ compositions in natural samples is to establish empirical correction factors for each amino acid by derivatization of amino acid standards of known stable isotope composition.

SUMMARY AND CONCLUSIONS

The effects of derivatization on the stable carbon isotope compositions of amino acids were investigated by conventional IRMS and GC/IRMS. The following conclusions can be drawn:

(1) Preparation and IRMS analysis of isopropyl esters and TFA isopropyl esters of 22 amino acids revealed that the derivatization process is reproducible. The average error for preparation and analysis of three separate samples of each amino acid was 0.12‰ ± 0.11‰ for isopropyl esters and 0.10‰ ± 0.09‰ for TFA isopropyl esters of individual enantiomers and racemic amino acids.

(2) The average analytical error for replicate analyses of a given amino acid derivative by GC/IRMS was 0.26‰ ± 0.09‰. In general, $\delta^{13}C$ compositions of amino acid derivatives determined by IRMS compared favorably with GC/IRMS $\delta^{13}C$ values. With three exceptions, the absolute differences between the two methods was less than 0.5‰. The exceptions may have resulted from incomplete derivatization and consequent elimination of impurities from the bulk samples by the chromatographic separation employed in the GC/IRMS system.

(3) The derivatization procedures introduce distinct but reproducible fractionations that presently preclude direct computation of the original, underivatized amino acid $\delta^{13}C$ compositions by stoichiometric mass balance. However, derivatization of amino acid standards of known stable carbon isotope composition in conjunction with natural samples permits computation of the original, underivatized amino acid $\delta^{13}C$ values through use of an empirical correction for the carbon introduced during the derivatization process.

ACKNOWLEDGMENT

We thank E. C. P. Gillyon and N. Crossley for permitting us to use the GC-IRMS system at VG Isotech.

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RECEIVED for review August 2, 1990. Accepted November 16, 1990. This work is supported by the National Science Foundation, Division of Earth Sciences (Grant No. EAR-8904215).