L-Methionine Decarboxylase from *Dryopteris filix-mas*: Purification, Characterization, Substrate Specificity, Abortive Transamination of the Coenzyme, and Stereochemical Courses of Substrate Decarboxylation and Coenzyme Transamination†

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ABSTRACT: L-Methionine decarboxylase from the male fern *Dryopteris filix-mas* has been purified 256-fold from acetone powder extracts to very near homogeneity. The enzyme is membrane-associated and requires detergent for solubilization during the initial extraction. The enzyme is a homodimer of subunit M_r 57 000 and shows a pH optimum at ~5.0 with 20 mM (2*S*)-methionine as substrate. The specific activity, k_{cat}, for methionine is ~50 mol s^{-1} (mol of active site)$^{-1}$ at pH 4.5 and below. A wide range of straight- and branched-chain (2*S*)-alkylamino acids are substrates for the enzyme. The values for the rate of decarboxylation, V_{max}, and for the apparent Michaelis constant, K_m, however, vary with structure and with the chirality at C-3. The pH dependence of V and V/K has been examined for three substrates: (2*S*)-methionine, valine, and leucine. Pyridoxal 5′-phosphate (PLP) is required for activity, and in the absence of excess PLP, the activity of the enzyme in incubations reduces with respect to time. The addition of PLP fully restores the activity, indicating that an abortive decarboxylation–transamination accompanies the normal decarboxylation reaction. The occurrence of the abortive reaction was confirmed by showing that [^{35}S]methionine is converted to labeled 3-(methylthio)propionaldehyde while [4′-^{3}H]PLP is converted to labeled pyridoxamine 5′-phosphate (PMP). The decarboxylation of (2*S*)-methionine gave 3-(methylthio)-1-aminopropane. Preparation of the *N*-camphanamide derivative of the amine allowed the C-1 methylene protons to be distinguished by ^{1}H NMR spectroscopy. Synthetic samples of the camphanamide were prepared in which each of the C-1 methylene protons was replaced by deuterium. When (2*S*)-methionine and the C-2 deuteriated isotopomer were incubated with the enzyme in deuterium oxide and protium oxide, respectively, and the products were converted to their camphanamide derivatives and analyzed by ^{1}H NMR spectroscopy, it was evident that decarboxylation occurred with retention of configuration at C-2. When the decarboxylation of six other substrates was studied, examination of the *N*-camphanamide derivatives of the amines indicated that decarboxylation occurred stereospecifically and, by analogy, with retention of configuration at C-2. When tritiated pyridoxal phosphate was incubated with the enzyme, tritiated pyridoxamine phosphate was formed. Analysis of the chirality of the methylene group at C-4′ indicated that, during abortive transamination, protonation occurred from the 4′-*si* face of the coenzyme, the same stereochemical result as that obtained for several bona fide transaminase enzymes. These results are used to construct possible mechanistic schemes for both reactions, decarboxylation and transamination. The position and possible identities of active-site proton donors are discussed.

Determination of the mechanism of PLP[1]-dependent enzymes has been an area for intense research effort over the past two decades [see Floss and Vederas (1982) and Akhtar et al. (1984)]. Now, for the transaminases, a fairly detailed picture of the catalytic processes involved in the conversion of substrate to product can be envisaged (Kirsch et al., 1984). Decarboxylases, in contrast, are poorly understood. Much less effort has focused upon their mode of action, in spite of their ubiquity in nature and their importance in many biosynthetic pathways, including those leading to dopamine, γ-aminobutyric acid (GABA), and histamine [see Sukhareva (1986)]. Decarboxylases are usually highly substrate specific, and they catalyze essentially irreversible reactions via intermediates that are difficult to trap.

In the absence of the large bases of structural, stereochemical, and mechanistic information that are available for the transaminases, it was necessary to identify a decarboxylase that would be particularly amenable to mechanistic study. L-Alkylamino acid decarboxylases (EC 4.1.1.14, 4.1.1.57) from a variety of sources (Bast et al., 1971; Hartmann, 1972) had been shown to possess desirable properties including a wide substrate structure tolerance. Hartmann and co-workers in Germany (Hartmann et al., 1984) conducted a partial purification of L-methionine decarboxylase from the fern *Polypodium vulgare*. This fern species is not common in the U.K.; however, after screening a variety of ferns as acetone powders for L-methionine decarboxylase activity, the male fern

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[1] Abbreviations: PLP, pyridoxal 5′-phosphate; PMP, pyridoxamine 5′-phosphate; NMR, nuclear magnetic resonance; DEAE, (diethylamino)ethyl; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; UV, ultraviolet; TLC, thin-layer chromatography; AAT, aspartate aminotransferase; BSA, bovine serum albumin; FPLC, fast protein liquid chromatography; 2,4-DNP, 2,4-dinitrophenylhydrazine; Tris, tris(hydroxymethyl)aminomethane; ppm, parts per million (δ).

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Dryopteris filix-mas was identified as a suitable source of the enzyme.

Recently, we reported, in preliminary form, on the extent of substrate structure tolerance by the enzyme at 37.5 mM and also on the stereochemical course of the decarboxylation of L-methionine (Stevenson et al., 1986). Here we report on the purification of the protein to very near homogeneity, on the structural and chemical properties of the protein, including abortive transamination, and on the stereochemical courses of the decarboxylation of alternative substrates. We also report on the stereochemical course of the abortive transamination. In the following papers we describe the kinetic features of the decarboxylation and abortive reactions catalyzed by the fern enzyme, on the decarboxylation reaction catalyzed by a *Streptomyces* enzyme of similar specificity, and on details of the mechanism of these catalyzed reactions.

Materials and Methods

Fronds of *D. filix-mas* were collected from local areas during May to October and were stored, after removing midveins, at −70 °C prior to use. Amino acid substrates, PLP, PMP, buffers, salts, Triton X-100, deuterium oxide, alkaline phosphatase, glutamic acid decarboxylase, and acylase I were obtained from Sigma Chemical Co. (Poole, Dorset, U.K.). Cytosolic aspartate aminotransferase (glutamic-oxaloacetic transaminase) was obtained from Boehringer Mannheim (Lewes, Sussex, U.K.). Amberlite IR 45(OH) and Dowex 1X8(OH) ion-exchange resins were obtained from British Drug Houses (Poole, Dorset, U.K.), and [1-^{14}C]-L-amino acid substrates, [^{35}S]-L-methionine, sodium [^{3}H]borohydride, and tritiated water were obtained from Amersham International (Amersham, Bucks, U.K.). Water-miscible scintillant (ES-199) was obtained from Canberra Packard (Pangbourne, Berks, U.K.). Protein standards for electrophoresis and gel exclusion chromatography were obtained from British Drug Houses (Poole, Dorset, U.K.) and Bio-Rad (Watford, Herts, U.K.), respectively. All other chemicals were of analytical grade or were recrystallized or redistilled before use.

Protein concentrations were determined by the method of Spector (1978) by using solutions of BSA standards.

^{1}H NMR spectra were recorded by using either a Brucker AM360 or a Jeol JNM-GX270 instrument, and mass spectra were obtained by using a AEI MS30 spectrometer.

Enzyme Activity Assays. (*a*) *Electromanometric Assay.* Assay incubations contained substrate (70 mM), PLP (1 mM), and enzyme in a total volume of 2 mL in 0.2 M succinate buffer, pH 4.8, at 37 °C in a Warburg flask. Reactions were initiated by tipping the enzyme (contained in the Warburg side arm) into the substrate solution, after the apparatus had reached thermal equilbrium. The incubations were stirred under a nitrogen atmosphere. The rates of CO_2 evolution were monitored with a resetting diaphragm-cell electromanometer (Mercury M12) connected to a Kipp and Zonen chart recorder. Rates were calibrated against (2*S*)-methionine (100%) and standardized by comparison with the extent of $^{14}CO_2$ release from (2*S*)-[1-^{14}C]methionine in a similar experiment.

(*b*) *Radiochemical Assay.* Assay incubations contained L-methionine [20 mM containing (2*S*)-[1-^{14}C]methionine (20000 dpm)], PLP (1 mM), and enzyme in a total volume of 300 μL of 0.2 M succinate buffer, pH 4.8. The incubations were conducted in a scintillation vial (6.0-mL size) at 37 °C. Reactions were initiated by the addition of enzyme and were terminated at zero time and at suitable time intervals therafter by the addition of 8 M sulfuric acid (200 μL). After the reactions had stood for 30 min to ensure the complete evolution of CO_2, scintillant (3.0 mL) was added and the residual radioactivity was determined by using a Packard Tri-Carb 300 C scintillation counter. For purposes other than monitoring column eluate activity, 5–10 identical incubations were conducted. The rate of reaction was calculated by plotting the decrease in radioactivity against time for the incubations. Except where indicated, the radiochemical assay was used for the routine determination of activity. One unit of activity converts 1 μmol of L-methionine to products per minute under these assay conditions.

Table I: Summary of Purification of L-Methionine Decarboxylase from *D. filix-mas*[a]

stage	tot. protein (mg)	tot. act. (units)	sp act. (units mg^{-1})	purification factor	yield (%)
crude extract	472	37	0.078	1	100
butanol/Sephadex G-50	77	27	0.35	4.5	73
DEAE-Sephacel	1.75	12.5	7.1	91	34
TSK DEAE-5PW	0.3	4.5	15	192	12
TSK G3000 SWG	0.1	2.0	20	256	5.4

[a] The purification was started from 10 g of acetone powder, total activity 65 units with L-methionine as substrate. Specific activities were measured at a methionine concentration of 20 mM.

Enzyme Purification. (*1*) *Preparation of Acetone Powder.* Frozen fronds were homogenized in 5 volumes of acetone, precooled to −18 °C, in a Waring blender. The solid residue was collected by Büchner filtration and the extraction repeated. The resulting powder was lyophilized and the coarse particles were removed by using a 0.8-mm pore sieve. One hundred grams of fronds typically yielded 17–19 g of acetone powder. The dry acetone powder was stable for several years at −30 °C.

(*2*) *Preparation of Crude Extract.* Acetone powder (10 g) in 200 mL of sonication buffer [20 mM potassium phosphate, pH 8.0, containing 350 mM KCl, 1.0 mM diethyldithiocarbamate, 1% (v/v) Triton X-100, and 0.1 mM PLP] was sonicated at 0–5 °C for 15 min with a Heat Systems Ultrasonics W-200-F cell disruptor. The suspension was centrifuged at 30000*g* for 15 min, and the pellet was discarded to give crude extract.

(*3*) *Butanol Precipitation.* The crude extract (150 mL) was stirred at 0 °C and solid potassium chloride (11.2 g, 0.15 mol) was added portionwise over 5 min, followed by 1-butanol (90 mL, precooled to −18 °C) over 15 min. After being stirred for a further 5 min, the mixture was centrifuged at 30000*g* for 15 min and the organic phase discarded. The aqueous phase was immediately subjected to size-exclusion chromatography on Sephadex G-50 (4 × 50 cm) equilibrated with buffer A [50 mM potassium phosphate, pH 7.5, containing 10% (v/v) glycerol, 1.5 mM mercaptoethanol, and 0.1 mM PLP]. The column was eluted at 300 mL h^{-1}, and all protein-containing fractions were pooled (160 mL); see Table I.

(*4*) *DEAE-cellulose Chromatography.* The protein solution from the above procedure was applied to a column of DEAE-Sephacel (2.5 × 16 cm) equilibrated with buffer A and was eluted with a linear gradient of 0–0.6 M KCl in buffer A; total volume 400 mL, flow rate 25 mL h^{-1}. The active fractions were pooled (50 mL) (see Figure 1 in the supplementary material).

(*5*) *Ion-Exchange FPLC.* The DEAE-Sephacel fraction was desalted on Sephadex G-50 (2.5 × 25 cm) equilibrated with buffer B (50 mM potassium succinate, pH 6.0, containing 0.1 mM PLP). Fractions containing protein were pooled and subjected to FPLC anion-exchange chromatography on TSK

Table II: Relative Rates of the Alternative Substrates for L-Methionine Decarboxylase[a]

substrate	side-chain structure	relative rate 37.5 mM	relative rate 70 mM	Hartman et al. (1984)
L-methionine	$-CH_2CH_2SMe$	100	100	100
L-ethionine	$-CH_2CH_2SEt$	0	0	
D,L-methionine		25		
D-methionine		0	0	
S-ethyl-L-cysteine	$-CH_2SEt$		12	
O-ethyl-L-serine	$-CH_2OEt$	0	<5	
L-norvaline	$-CH_2CH_2Me$	36	55	39
L-leucine	$-CH_2CHMe_2$	28	40	32
L-isoleucine	$-CHMeEt$ (3*S*)	30	35	29
L-*allo*-isoleucine	$-CHMeEt$ (3*R*)		0	
L-norleucine	$-CH_2CH_2CH_2Me$	16	40	31
L-valine	$-CH(Me)_2$	17	16	6
L-alanine	$-CH_3$		0	0
L-phenylalanine	$-CH_2Ph$		0	0
γ-ethyl-L-glutamate	$-CH_2CH_2CO_2Et$	0		
β-methyl-L-aspartate	$-CH_2CO_2Me$	0		
β-ethyl-L-aspartate	$-CH_2CO_2Et$	0		

[a] The amino acids shown were incubated at concentrations of 37.5 and 70 mM with the enzyme in acetone powder form at pH 4.8, 37 °C. Rates of CO_2 production were measured electromanometrically.

DEAE-5PW (Pharmacia-LKB, 2.15 × 15 cm) equilibrated with buffer B and eluted with a linear gradient of 0–0.4 M NaCl in buffer B; total volume 120 mL, flow rate 2 mL min^{-1}. The fractions containing enzyme were pooled (12 mL) (see Figure 2 in the supplementary material).

(*6*) *Size-Exclusion FPLC.* The active fractions from the above procedure were pooled, desalted on Sephadex G-50 (1.5 × 30 cm) equilibrated with 5 mM potassium phosphate buffer, pH 6.5, and then freeze-dried. The lyophilized protein was redissolved in 500 μL of buffer C (100 mM potassium phosphate, pH 6.5, containing 0.1 mM PLP), and the solution was subjected to FPLC size-exclusion chromatography on Pharmacia-LKB TSK G3000 SWG (2.15 × 30 cm) equilibrated with buffer C, at a flow rate of 0.1 mL min^{-1}. The active fractions were pooled to give 2 mL of essentially homogeneous enzyme (specific activity routinely 15–20 units mg^{-1}). The protein was stable for several months when stored as a frozen solution at −30 °C. For long-term storage, the protein was kept as a lyophilized powder at −80 °C. The yield of the protein obtained after each step is given in Table I.

Electrophoresis. Electrophoretic analysis of the enzyme was performed on both SDS-containing and nondenaturing polyacrylamide gels, using the methods of Laemmli (1970). The polyacrylamide content was 12.5% for SDS gels and 10% for nondenaturing gels. Electrophoresis was carried out at pH 8.3, with Tris (25 mM) and glycine (200 mM) buffer. SDS (3 mM) was added to the buffer for SDS gels. The proteins were visualized with Coomassie Brilliant Blue stain. Both cylindrical and slab gels were used.

(2S)-O-Ethylserine. (2*S*)-*O*-Ethylserine was prepared by the method of Barlos et al. (1983), starting from (2*S*)-serine. The crystalline product was obtained in 37% overall yield, p 244–247 °C [lit. (Barlos et al., 1983) mp 243–245 °C] and showed the expected spectral parameters.

(4RS)-[4′-^{3}H]PMP and [4′-^{3}H]PLP. Tritiated PMP was prepared by the method of Voet et al. (1973). To a solution of pyridoxal 5′-phosphate (2 mg, 8.1 μmol) in aqueous ammonia (12 M, 1 mL) was added excess sodium borotritide (7 Ci $mmol^{-1}$) portionwise over 1 h. Water (2 mL) was added and the solution was lyophilized. The residue was dissolved in acetic acid (1 M, 2 mL) and the solution was relyophilized. The residue containing 22.2 mCi of (4*RS*)-[4′-^{3}H]PMP was dissolved in water (1.6 mL) and was stored frozen. The UV spectrum and TLC on cellulose, eluted with 1-butanol saturated with 1 M HCl, showed that the conversion to [4′-^{3}H]-PMP was essentially quantitative.

[4′-^{3}H]PLP was prepared by a modification of the method of Metzler et al. (1954). A 400-μL portion of the above solution was treated with a solution of glyoxylic acid (40 mM) and potassium aluminum sulfate (10 mM) in acetate buffer (100 mM, 400 μL, pH 5.0), and the mixture was heated at 80 °C for 5 min. After the mixture cooled, the precipitated coenzymes were dissolved by carefully adjusting the pH to 11 with dilute ammonia solution. The UV spectrum of the products showed that 65% of the PMP had been converted to PLP. The mixture was diluted with water (4.5 mL) and was applied to a column of Dowex 1X8 acetate (1.5 × 7 cm) preequilibrated with ammonia solution (100 mM) adjusted to pH 10.6 with acetic acid. The PMP was eluted with ammonium acetate (100 mM) at pH 7, and the PLP was eluted with chloroacetic acid (100 mM) at pH 2. To ensure minimal contamination, the separated compounds were each repurified on fresh exchange resin as described above to give PLP (0.146 mg, 645 × 10^6 dpm) and unreacted PMP (0.126 mg, 615 × 10^6 dpm).

3-(Methylthio)propionaldehyde. Sodium methanethiolate (1 g, 14.28 mmol) and chloropropionaldehyde diethyl acetal (2.505 g, 15 mmol) were refluxed in dry methanol (20 mL) for 1 h. The solution was cooled, and hydrochloric acid (0.5 M, 2 mL) was added. The solvents were removed under reduced pressure, and the residual oil was distilled. The product was obtained in 51% yield after redistillation at atmospheric pressure (bp 165–170 °C) and showed the expected spectral parameters. Later batches of the aldehyde were obtained commercially from Fluka, Glossop, Derby, U.K. The aldehyde was characterized as the 2,4-dinitrophenylhydrazone, which crystallized from ethanol as pale orange needles, mp 121–122 °C [lit. (Catch et al., 1947) mp 122–123 °C].

Decarboxylation Reaction. The substrate specificity for the decarboxylation of 14 nonpolar amino acids was determined by incubating each of the substrates (37.5 and/or 70 mM) and PLP with the fern acetone powder at pH 4.8 and 37 °C. Activity was measured by the continuous electromanometric method; see Table II.

The kinetic parameters V_{max} and K_m for the membrane-associated enzyme were determined for the seven best substrates by incubating each amino acid at a range of concentrations with the fern acetone powder and PLP at pH 4.8 and 37 °C. Activity was measured by the continuous electromanometric method, and each determination was conducted in

Table III: Kinetic Parameters for Alternative Substrates[a]

substrate	V_{max} (% L-Met)	K_m (mM)	V/K
L-methionine	100	48	2.08
L-valine	100	1100	0.09
S-ethyl-L-cysteine	100	1000	0.10
L-leucine	59	124	0.48
L-isoleucine	45	153	0.29
L-norvaline	78	147	0.53
L-norleucine	51	155	0.33

[a] Determined in 0.2 M succinate buffer at pH 4.8 and 37 °C by using the electromanometric assays and fern acetone powder over the substrate concentration range 20–200 mM. Each determination was conducted six times. V_{max} is expressed as a percentage of the value for (2*S*)-methionine. Data were analyzed by direct linear plots; ±15% error on all values of V and K.

triplicate. The data were analyzed by the direct linear plot method (Eisenthal and Cornish-Bowden, 1974); see Table III.

The kinetic parameters V and K were also determined for L-methionine, L-valine, and L-leucine by using the partially purified soluble enzyme (15 units mg^{-1}) and the more accurate radioactivity assay. The incubations, which were conducted in quadruplicate in 0.2 M succinate buffer at 37 °C, contained the appropriate 1-^{14}C-labeled substrate at a range of concentrations, PLP, and enzyme. Each reaction was sequentially quenched by the addition of sulfuric acid, vide supra, and the initial rates were determined. For each substrate, the values of the kinetic parameters were determined over the pH range for which activity was measurable; see Table IV.

Abortive Decarboxylation–Transamination. Experiment 1: PLP Dependence. Ten identical 300-μL incubations contained enzyme (0.16 units, 6 units mg^{-1}, chromatographed on Sephadex G-25 to remove unbound coenzyme) and (2*S*)-[1-^{14}C]methionine (20 mM, 20 000 dpm) in succinate buffer (100 mM) at pH 4.8 and 37 °C. In the first experiment, 1A, the progress of the reaction was monitored by quenching individual reaction mixtures at regular intervals over a period of ~2 h with sulfuric acid (8 M, 200 μL). The residual substrate concentration was determined by scintillation counting.

In experiment 1B, coenzyme (1 mM in the incubation medium) was added at the start of the reaction, and in experiment 1C, coenzyme was added after 70 min; see Figure 3.

Experiment 2: Tritiated PMP Formation. Pure enzyme (1 unit, 14 units mg^{-1}, 0.33 nmol, freed of unbound coenzyme) was incubated with (2*S*)-methionine (25 mg, 167 μmol) and [4′-^{3}H]PLP (98 nmol, 1.07×10^8 dpm) in 100 mM succinate buffer (5 mL) at pH 5.0 and 37 °C for 20 h (experiment 2A). After this time, TLC analysis on cellulose eluted with 2-propanol/aqueous NH_3(sp gr 0.88)/H_2O (26:6:5) showed no unreacted methionine remained. The incubation solution was diluted to 10 mL with water, and unlabeled PMP (1.3 mg, 5.2 μmol) was added. The pH was adjusted to 11.0 with aqueous 500 mM sodium hydroxide, the solution was applied to a column of Dowex 1X8 acetate preequilibrated with 100 mM ammonia solution adjusted to pH 10.6 with acetic acid, and the PMP was eluted with 100 mM ammonium acetate at pH 7.0. The PMP-containing fractions, as determined by UV spectroscopy, were combined, and the total radioactivity was determined by scintillation counting.

The entire experiment was repeated at pH 6.0 but with 4 units of decarboxylase and 11.7 mg (78 μmol) of methionine (experiment 2B); see Table V.

Experiment 3: [^{35}S]-3-(Methylthio)propionaldehyde Formation. (2*S*)-[^{35}S]Methionine (7 mmol, 6×10^8 dpm) was incubated with enzyme (0.5 unit, 7 units mg^{-1}) at pH 5 and 37 °C in 100 mM succinate buffer containing 1 mM PLP, all in a total volume of 3 mL. After 24 h, 3-(methylthio)-propionaldehyde (15 mmol) and 3-(methylthio)-1-aminopropane hydrochloride (0.25 mmol) were added. Finally, 2,4-DNP reagent (1 mL) was added and the reaction was allowed to proceed for 1 h at room temperature. The phenylhydrazone was extracted with ethyl acetate and was recrystallized to constant specific activity. The pH was raised to 11 with 1 M NaOH, and the amine was extracted with dichloromethane, back-extracted into dilute HCl, and then recrystallized to constant specific activity; see Table VI.

Solvent Hydrogen Exchange. For (2*R*)-methionine, incubations contained (2*R*)-methionine (75 mg, 0.5 mmol), enzyme (3 units, acetone powder), and PLP (10 mM) in 0.1 M buffer containing tritium oxide (1.5×10^7 dpm mL^{-1}) at pH 5.5 (potassium succinate) or 8.0 (potassium phosphate) in a total volume of 4 mL. The reactions were stirred for 20 h at 37 °C, and then the particulate matter was removed by filtration and the filtrate was concentrated in vacuo. The residue was

Table IV: Kinetic Parameters for the Decarboxylation of (2*S*)-Methionine, (2*S*)-Leucine, and (2*S*)-Valine as a Function of pH[a]

		pH						
substrate	parameter	4.0	4.5	5.0	5.5	6.0	6.5	7.0
(2*S*)-methionine	V/K	1.1	3.72	3.03	1.88	2.25	5.1	4.95
	V^{app}	90	93	85	15	6.3	3.3	3.7
	K^{app}	80	25	28	8.0	2.80	0.65	0.75
(2*S*)-leucine	V/K	1.0	1.74	1.54	1.02	1.20	2.0	
	V^{app}	70	73	37	6.1	3.0	2.0	
	K^{app}	70	42	24	6.0	2.5	1.0	
(2*S*)-valine	V/K				0.17	0.24	0.38	
	V^{app}				10	6	3	
	K^{app}	*b*	*b*	*b*	60	25	8	

[a] V^{app} is the relative rate compared as a percentage of V_{max} for (2*S*)-methionine at pH 4.8; K^{app} is the measured K_m (mM). Rate determinations were conducted in 150 mM succinate and 150 mM phosphate buffer at the specified pH at 37 °C with 2*S* 1-^{14}C-labeled substrates. Data were analyzed by using direct linear plots. The maximum error is ±25% of the values of V and K. [b] Too large to measure.

Table V: Abortive Transamination of the Coenzyme

	pH 5.0	pH 6.0
amt of [4′-^{3}H]PLP incubated (nmol)	98 (1.07×10^8 dpm)	98 (1.07×10^8 dpm)
(2*S*)-methionine decarboxylated (μmol)	167	78
PMP carrier added before isolation (μmol)	5.2	5.2
sp act. of purified PMP (dpm μmol^{-1})	4.52×10^6	5.43×10^6
PMP produced by abortive reaction (nmol)	22	26
decarboxylation:transamination ratio	7590:1	3016:1

Table VI: Formation of 3-(Methylthio)propanaldehyde from (2S)-[^{35}S]Methionine

	amine hydrochloride	aldehyde or 2,4-DNP derivative
carrier added (μmol)	250	12.5
sp act. of purified material (dpm mmol^{-1})	1.29×10^9	2.31×10^7
tot. radioact (dpm)	3.22×10^8	2.89×10^5
ratio of amine to aldehyde	1115	1

dissolved in water (20 mL) and the solution was lyophilized; this process was repeated three times. The radioactivity of the resulting solid was determined by scintillation counting with reference to the result of no-enzyme control experiments.

For 3-(methylthio)-1-aminopropane, incubations contained 3-(methylthio)-1-aminopropane (75 mg, 0.7 mmol) enzyme (3 units, acetone powder), and PLP (10 mM) in the appropriate buffer containing tritium oxide (1.5×10^7 dpm mL^{-1}) at pH 5.5 or 8.0 in a total volume of 4 mL. The reactions were stirred for 20 h at 37 °C and then the particulate matter was removed by filtration. The pH of the filtrate was adjusted to 11, the solution was extracted with dichloromethane, and the amine hydrochloride was prepared as described above. The extent of tritium incorporation was determined by scintillation counting with reference to no-enzyme control experiments.

Chirality Assays: (*A*) *Stereochemical Course of Decarboxylation.* (*i*) *Synthesis of Chirally Deuteriated Standards.* 3-(Methylthio)-1-aminopropane hydrochloride was prepared from (2*S*)-methionine by using fern acetone powder. After the decarboxylation was complete, as judged by TLC, the particulate matter was removed by filtration on a pad of prewashed Celite, and the filtrate was adjusted to pH 11 by the addition of 2 M sodium hydroxide. The aqueous phase was extracted with dichloromethane (3 × 25 mL), and the organic phase was extracted with 500 mM hydrochloric acid (2 × 15 mL). The acid solution was reduced in volume in vacuo, and the residue was purified on Dowex 1X8(OH). The eluent was acidified with 6 M HCl to give the hydrochloride salt in 65% overall yield.

N-(−)-*Camphanoyl-3-(methylthio)-1-aminopropane.* The amine hydrochloride (140 mg, 0.99 mmol) was dissolved in 200 mM potassium hydroxide (5 mL), and the solution was extracted with chloroform (3 × 15 mL). The organic phase was dried over anhydrous magnesium sulfate, and the solvent was reduced to 2 mL in vacuo. The free amine solution was added to a solution of (1*S*,4*R*)-(−)-camphanic acid (160 mg, 0.81 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (142 mg, 0.74 mmol), and triethylamine (53 μL, 0.74 mmol) in acetonitrile (10 mL). The solution was stirred vigorously for 12 h and the solvents were removed in vacuo. The residue was dissolved in chloroform (10 mL), and the solution was washed with 50 mM HCl, 1 M sodium bicarbonate, and then water. The solution was dried over anhydrous sodium sulfate and concentrated in vacuo to give a dark-colored gum. The compound was purified by flash silica chromatography using chloroform/ethyl acetate (3:1) as eluent to give the thioether amide (120 mg, 43%) as a pale yellow oil. The 360-MHz ^{1}H NMR spectrum of the camphanamide showed an AB-type coupling pattern for the diastereotopic C-1 protons centered at 3.37 ppm, which we needed to assign by introducing deuterium stereospecifically into each position (see Appendix for full details).

(*4R*)-[*4-*^{2}*H*]-*4-Aminobutyric Acid.* (2*S*)-Glutamic acid (1.48 g, 10 mmol) was suspended in deuterium oxide, and the pH was adjusted to 5 with 35 M ammonia solution. PLP (3 mg) and *Escherichia coli* glutamate decarboxylase (150 units) were added, and the reaction was incubated at 37 °C. After 6 h the conversion was complete, as judged by cellulose TLC. The protein was denatured by boiling for 5 min and was removed by filtration through Celite. The solution was concentrated in vacuo, and the amine was converted to the hydrochloride salt by the addition of 6 M hydrochloric acid. The excess acid was removed in vacuo, and the residue was applied to a column of Amberlite IR45(OH). The column was eluted with water, and the fractions containing the product were combined and concentrated to give an oil (760 mg, 73%), which crystallized after standing for several days.

(*4S*)-[*4-*^{2}*H*]-*4-Aminobutyric Acid.* (2*S*)-Glutamic acid (2.94 g, 20 mmol) was dissolved in deuterium oxide (50 mL), and the pH was adjusted to 7.25 with 35 M ammonia solution. Aspartate aminotransferase (200 units) and PLP (5 mg, 0.02 mmol) were added, and the solution was incubated at 37 °C. The course of the reaction was monitored by ^{1}H NMR spectroscopy. When the C-2 hydrogen exchange was complete (~72 h), the solution was filtered through Celite and the filtrate was concentrated in vacuo. The residue was recrystallized from 6 M hydrochloric acid to give fine white crystals (2.90 g, 97%): mp 195 °C dec; $[\alpha]_D$ +22.5° (*c* 0.4 in 5 M HCl) {lit. (Greenstein & Winitz, 1961) $[\alpha]_D$ +46.8° for the undeuteriated compound}. C-2 contained >95 atom % deuterium as judged by NMR spectroscopy. The deuteriated glutamic acid was converted to (4*S*)-[4-^{2}H]-4-aminobutyric acid by using glutamate decarboxylase in protium oxide as described above for the 4*S* antipode.

N-(−)-*Camphanoyl-4-aminobutyric Acid.* To a solution of 4-aminobutyric acid (0.57 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol) in water (10 mL) was added a solution of freshly prepared (−)-camphanoyl chloride (1 g, 4.6 mmol) in toluene (10 mL). The reaction was shaken vigorously for 5 min and was stirred overnight. The aqueous phase was adjusted to pH 9, and the unreacted acid chloride was removed through extraction with chloroform (3 × 20 mL). The aqueous phase was acidified to pH 2 and then reextracted with chloroform (3 × 20 mL). The chloroformic solution was dried and concentrated to give the product as a clear oil in quantitative recovery. Crystallization occurred on standing; mp 93–95 °C.

N-(−)-*Camphanoyl-3-chloro-1-aminopropane.* A suspension containing *N*-(−)-camphanoyl-4-aminobutyric acid (450 mg, 1.6 mmol), lead tetraacetate (850 mg, 1.9 mmol), and lithium chloride (68 mg, 1.6 mmol) in dry benzene (20 mL) was purged with dry dinitrogen for 30 min and then heated under reflux for 90 min according to the method of Kochi (1965), during which time the suspension became colorless. The solvent was removed in vacuo, and the residue was partitioned between water (10 mL) and chloroform (30 mL). The organic phase was concentrated to give the crude chloride in quantitative recovery as an oil, which was 70–80% pure as judged by ^{1}H NMR spectroscopy. The remainder was essentially starting material. The product was characterized but not further purified and was reacted with excess sodium methanethiolate as described below.

Synthetic N-(−)-Camphanoyl-3-(methylthio)-1-aminopropane. The crude chloride (400 mg, 1.47 mmol) was dissolved in dry methanol (25 mL), and sodium methanethiolate (300 mg, 4.3 mmol) was added. The solution was refluxed for 1 h and the volume reduced in vacuo. The residue was dissolved in chloroform (20 mL), and the organic phase was washed with sodium bicarbonate solution and then dried and concentrated. The residue was purified by flash silica chro-

Table VII: Determination of the Stereochemistry of 4′-Tritiated PMP Derived from Abortive Transamination

	pH 5.0 (t = 14 h)		pH 6.0 (t = 14 h)		pH 6.0 (t = 3 h)	
	enzymic	nonenzymic[a]	enzymic	nonenzymic[a]	enzymic	nonenzymic[a]
dpm nmol^{-1} in pyridoxamine	50800 (100%)	50100 (100%)	50400 (100%)	50200 (100%)	50620 (100%)	50100 (100%)
dpm nmol^{-1} recovered in pyridoxal	23876 (47%)	26052 (52%)	39312 (78%)	26104 (52%)	48089 (95%)	26302 (52%)
dpm present in water	26924 (53%)	24048 (48%)	11088 (22%)	24096 (48%)	2531 (5%)	23798 (48%)

[a] Chemical method.

matography as described above to give the thioether amide as an oil (286 mg, 57%), identical in all respects with the compound prepared from 3-(methylthio)-1-aminopropane.

The entire synthesis was repeated for each of the chirally deuteriated 4-aminobutyric acids to give each diastereomer of the chirally deuteriated thioether amide; see partial spectra in Figure 4.

(*ii*) *Derivatization of Decarboxylation Products. (2S)-[2-^{2}H]Methionine.* (2*S*)-Methionine (3 g, 20 mmol) was dissolved in 1 M sodium deuterioxide (20 mL), and acetic anhydride (7 mL, 60 mmol) was added in three portions with vigorous shaking. The reaction wa sallowed to stand for 6 h, and 1 M sulfuric acid (20 mL) was added. The solvent was removed in vacuo, and the residue was extracted with warm ethyl acetate 5 times. The combined extracts were dried over sodium sulfate, and the solvent was removed to give crude (2*RS*)-[2-^{2}H]-*N*-acetylmethionine as a waxy solid (3.11 g, 81.5%). The crude DL-acetylmethionine (2 g, 10.4 mmol) was dissolved in water (100 mL), and the pH was adjusted to 7.4 with 1 M sodium hydroxide. Acylase I powder (4 mg) was added, and the formation of methionine was followed by TLC on cellulose. The pH of the reaction mixture was maintained at 7.4 by the addition of base. When the rate of methionine production fell sharply, the enzyme was denatured by boiling and the mixture was applied to a column of Amberlite IR45(OH). The column was eluted with water, and fractions containing methionine were pooled and concentrated in vacuo. The residue was recrystallized from methanol to give white flaky crystals of the deuteriated amino acid (400 mg, 26%): mp 200 °C, $[\alpha]_D$ +32.5° (*c* 0.4 in 5 M HCl) {lit. (Greenstein & Winitz, 1961) $[\alpha]_D$ +34.6° for the undeuteriated compound}.

Deuteriated 3-(Methylthio)-1-aminopropanes. These were prepared as described above for the unlabeled compound, with (2*S*)-methionine and (2*S*)-[2-^{2}H]methionine in deuterium oxide and protium oxide, respectively. These were each derivatized as their camphanoyl amides by the water-soluble carbodiimide method described above, and the ^{1}H NMR spectra of the derivatives were compared to those of the synthetic standards; see Figure 4. Other amino acid substrates [(2*S*)-leucine, isoleucine, norleucine, valine, norvaline, and *S*-ethylcysteine] were incubated with the enzyme in both protium and deuterium oxide. The camphanamide derivatives of these were prepared by the acid chloride method. The ^{1}H NMR spectra of these derivatives are shown in Figure 5.

(*B*) *Stereochemical Course of Transamination.* (4*RS*)-[4′-^{3}H]PMP and [4′-^{3}H]PLP were prepared and purified as described above.

(*i*) *Formation of Tritiated PMP.* Enzyme (2–4 units, freed of unbound coenzyme) was incubated with (2*S*)-methionine (25 mg, 0.17 mmol) and [4′-^{3}H]PLP (98 μmol, 10^8 dpm) in 100 mM succinate or phosphate buffer (5 mL) at (1) pH 5.0 for 14 h, (2) pH 6.0 for 14 h, and (3) pH 6.0 for 3 h, all at 37 °C. The reactions were followed by TLC analysis on cellulose eluted with 2-propanol/aqueous NH_3 (sp gr 0.88)/H_2O (26:6:5). When no unreacted methionine remained in experiments 1 and 2, but earlier for experiment 3, the incubation solutions were diluted to 10 mL with water, and unlabeled PMP (1.3 mg, 5.2 μmol) was added. The pH was adjusted to 11.0 with 500 mM sodium hydroxide, and the tritiated PMP was isolated exactly as described above.

(*ii*) *Conversion to Pyridoxamine.* Each sample of PMP was separately incubated with alkaline phosphatase (10 units) in 100 mM ammonia solution adjusted to pH 10 with acetic acid. After 20 h, the phosphate ester hydrolysis was complete as judged by TLC on silica. The samples of tritiated pyridoxamine were purified on Dowex 1X8 preequilibrated with 100 mM ammonia solution adjusted to pH 10.6. The samples were washed with equilibration buffer and were eluted from the column in the same buffer at pH 8.0. The fractions containing pyridoxamine were combined from each separate experiment. The radioactivity of each sample was determined by liquid scintillation counting.

(*iii*) *Incubation with ApoAAT.* In order to determine the stereochemical integrity at C-4′, a small portion of each sample of pyridoxamine (~50 000 dpm) was incubated with apoaspartate aminotransferase (1 mg) prepared according to the method of Yang and Metzler (1979) but under the reaction conditions of Dunathan et al. (1968), and the extent of tritium release into the water was determined. The tritium content at C-4′ of the pyridoxal produced was also determined; see Table VII. For each determination, a control containing synthetic (4*RS*)-[4′-^{3}H]PMP was also incubated with apoaspartate aminotransferase.

Results

Purification and Stability. L-Methionine decarboxylase has been purified 256-fold from fern acetone powder to very near homogeneity (Table I, Figure 1, and Figures 1 and 2 in the supplementary material), as judged by SDS–PAGE and by high-resolution size-exclusion chromatography. At very high loading, SDS–PAGE revealed additional faint bands, apparently less than 10% of the total protein. We were unable to purify the enzyme further without substantial activity loss. Affinity columns incorporating either 3′-*O*-linked PLP or (phosphopyridoxyl)methionine connected to a 1,6-diaminohexyl-Sepharose matrix were of little use, since the protein bound too tightly.

During the purification, denaturation was minimized through the use of phosphate buffer wherever possible and by the inclusion of coenzyme (PLP) in all buffer solutions. Mercaptoethanol did not stabilize the enzyme to activity loss but did prevent oxidative browning of the cruder protein solutions. The use of aqueous glycerol as the buffer medium prevented some loss of activity and was particularly useful in the DEAE-cellulose ion-exchange chromatography step, where the resolution was improved substantially. The improvement is probably due to enhanced solvation of the protein in the absence of detergent at high salt concentrations, leading to diminished hydrophobic interactions between protein mole-

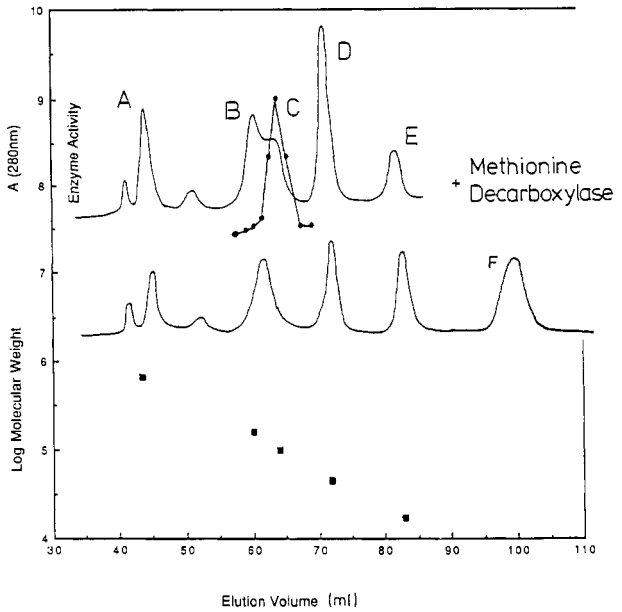


FIGURE 1: Determination of protein M_r. A sample of purified enzyme was mixed with standard proteins (shown in the lower trace) and was chromatographed on TSK G3000 SWG (2.15 × 30 cm) equilibrated with 0.1 M potassium phosphate, pH 6.5; flow rate 0.1 mL min^{-1}. Upper trace: A, bovine thyroglobulin (M_r 670 000); B, bovine δ-globulin (M_r 158 000); C, L-methionine decarboxylase; D, chicken ovalbumin (M_r 44 000); E, horse myoglobin (M_r 17 000); F, cyanocobalamin.

cules.

After the high-resolution gel-exclusion chromatography step, the specific activity of the enzyme was routinely 10–20 units (mg of protein)$^{-1}$. We have obtained a specific activity of 30 units mg^{-1} on one occasion, and thus purified protein apparently contains some inactive enzyme. This inactive enzyme is not reactivated by preincubation with the coenzyme or with the coenzyme and substrate prior to assay.

The enzyme eluted as a single band when subjected to FPLC on a precalibrated TSK G3000 SWG gel-exclusion chromatography column with a retention volume corresponding to M_r 100 000 ± 5000; see Figure 1.

Good reproducible electrophoretic analyses were obtained for SDS gels, but nondenaturing gels gave very poor and inconsistent results, possibly due to the hydrophobic nature of the protein. Typically the R_f of the active protein (purified to step 4) was ~0.5 at pH 8.3 on nondenaturing gels, relative to bromophenol blue, as determined by the coincidence of a staining band and activity in gel slices assayed with [^{14}C]-methionine. Highly purified enzyme showed essentially one band at R_f 0.5, but no activity could be detected in the gel. For routine analysis, SDS gels were used. The pure protein showed a single band except at very high loading when several other bands became apparent, representing up to ~5–10% of the total.

SDS–PAGE analysis against protein standards [equine cytochrome *c* (M_r 12 300), myoglobin (M_r 17 200), bovine erythrocyte carbonic anhydrase (M_r 30 000), hen egg ovalbumin (M_r 45 000), BSA (M_r 66 250), and hen egg ovotransferrin (M_r 76 000–78 000)] gave a value of M_r for the decarboxylase of 57 000 ± 3000.

Specific Activity. The highest specific activity obtained for the pure enzyme in any of the preparations was 30 units (mg of protein)$^{-1}$ as measured in the standard assay with 20 mM (2*S*)-methionine. Under saturating conditions, the activity is 50 μmol min^{-1} (mg of protein)$^{-1}$ and thus the maximum substrate turnover rate is 100 mol s^{-1} (mol of enzyme)$^{-1}$ at pH 4.8, assuming M_r is 100 000. Thus, below pH 4.5, where V_{max} is maximal, k_{cat} is 50 s^{-1}.

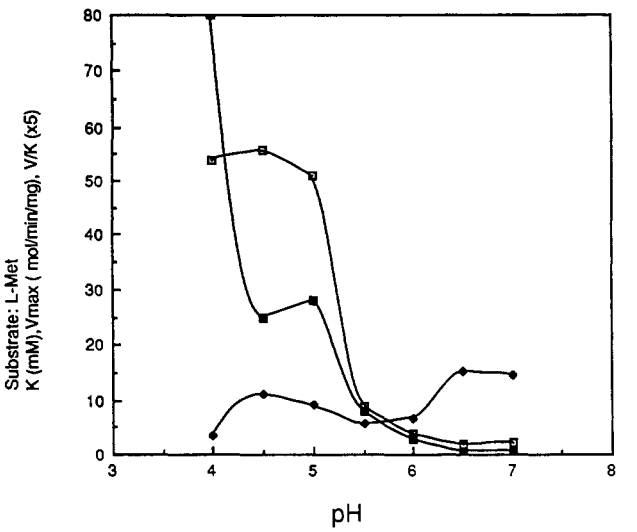


FIGURE 2: Variation of the kinetic parameters V (□), V/K (◆), and K (■).

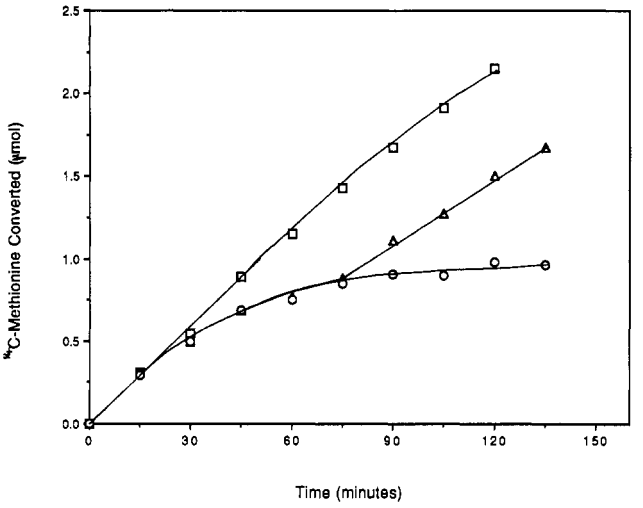


FIGURE 3: PLP requirement of L-methionine decarboxylase. The DEAE-Sephacel fraction was freed of unbound PLP by Sephadex G-50 chromatography and incubated with 20 mM L-methionine, under standard assay conditions but adding 1.0 mM PLP at t = 0 (□), t = 75 min (△), or not at all (○).

Substrate Specificity. Table II shows the relative rates of decarboxylation of 14 substrates at 37.5 and 70 mM, determined by using the continuous electromanometric assay and acetone powder. It is interesting to note that L-*allo*-isoleucine (absolute configuration 2*S*,3*R*), is a very poor substrate compared with L-isoleucine (absolute configuration 2*S*,3*S*). The kinetic parameters for the seven best substrates are shown in Table III. The kinetic parameters of three of these substrates were also determined with the DEAE-Sephacel-purified enzyme over the pH range at which activity was detectable, shown in Table IV. Figure 2 illustrates the pH dependence for (2S)-methionine.

Coenzyme Dependence. In order to assess the requirement of L-methionine decarboxylase for PLP, samples of the enzyme were incubated with (2*S*)-[1-^{14}C]methionine in three different experiments and the activity of the enzyme was monitored over a period of time, as shown in Figure 3. The incubation containing added coenzyme showed essentially no change in activity over the period, while the incubation containing no added coenzyme showed a gradual decrease in activity and was completely inactive after 120 min. Coenzyme added after

75 min restored the activity back to the starting level. Hydroxylamine completely inactivated the enzyme.

Abortive Transamination Products. The abortive decarboxylation–transamination products are PMP and 3-(methylthio)propionaldehyde; see experiments 2 and 3. The turnover:inactivation event ratios calculated from the two experiments are given in Tables V and VI.

Effect of Alternative Substrates. In order to assess the inhibitory effects of substrate analogues on the decarboxylation of (2*S*)-[1-^{14}C]methionine, the analogues (2*R*)-methionine, (2*S*)-isoleucine, (2*S*,3*R*)-L-*allo*-isoleucine, (2*S*)-norvaline, and (2*S*)-valine were incubated at a concentration of 10 mM with (2*S*)-[1-^{14}C]methionine at 10 mM. No reduction in rate was observed for any potential inhibitor, relative to control radiochemical assays. The experiments were repeated with (2*S*)-methionine (5 mM) and each of the inhibitors (20 mM). Under these conditions L-isoleucine, a good substrate, and (2*R*)-methionine inhibited to the extent of 25%. (2*S*)-Norvaline, a good substrate, showed 10% inhibition, while (2S)-valine, a poor substrate, and L-*allo*-isoleucine, a non-substrate, showed no effect. Note that unlabeled (2*S*)-methionine inhibits the observed reaction to the extent of 75% under these conditions.

The mode of inhibition by (2*R*)-methionine was investigated further by using the standard radiochemical assay with (2*S*)-[1-^{14}C]methionine as the substrate. The D-enantiomer acts as a competitive inhibitor with an apparent K_i of 20 mM at pH 5.5.

Hydrogen Exchange. No tritium from tritiated water was incorporated into either (*R*)-methionine or the product amine, 3-(methylthio)-1-aminopropane, when each of the compounds was incubated with the enzyme at pH 4.5 and 8.0.

Stereochemical Course of Decarboxylation. The synthesis of the chirally deuteriated *N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane standards is outlined in Scheme II. Full spectral and analytical data for each of the compounds and the intermediates are presented in the Appendix. Figure 4 shows a comparison of the 360-MHz ^{1}H NMR spectra of the synthetic and enzymically derived samples and indicates that decarboxylation occurs with retention of configuration at C-2 of the substrate, (2*S*)-methionine. Figure 5 shows a comparison of the spectra of the *N*-camphanoyl derivatives of the decarboxylation products of six alternative substrates, where each substrate was incubated in protium and deuterium oxide, respectively. The derivatized products from the incubations conducted in protium oxide gave signals centered at 3.10, 3.27, 3.29, 3.27, 3.15, and 3.50 ppm for valine, leucine, norleucine, norvaline, isoleucine, and (2*R*)-*S*-ethylcysteine respectively. The corresponding monodeuteriated compounds gave signals at 3.16, 3.28, 3.30, 3.30, 3.25, and 3.52 ppm, respectively. (Note that all of the deuteriated derivatives give signals at lower field than the unlabeled samples but that α-deuterium substitutions cause slight upfield shifts, which diminish the observable differences.) Each substrate is thus decarboxylated stereospecifically, and by analogy with methionine, decarboxylation occurs with retention of configuration at C-2 in each case.

Assessment of the sterochemical course of the reaction at high pH (7.0 and above) with methionine and *S*-ethylcysteine as substrates indicated that the reaction was completely stereospecific. The products were identical with those derived from the incubations conducted at pH 4.8.

Stereochemical Course of Transamination. 4′-Tritiated PLP and racemic 4′-tritiated PMP were prepared by the method of Voet et al. (1973) and were used to assess the

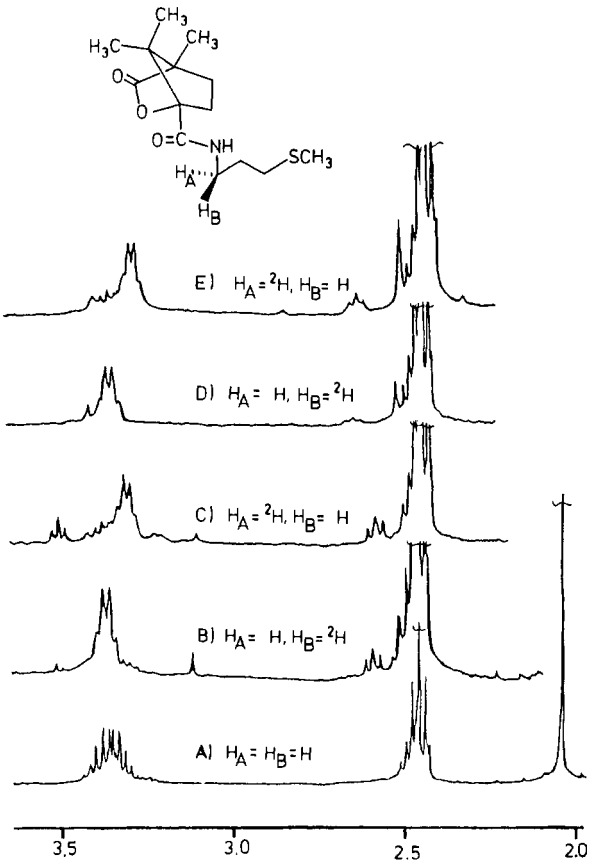


FIGURE 4: Stereochemical course of the methionine decarboxylase reaction with (2*S*)-methionine as substrate. Shown are ^{1}H NMR spectra of (A) synthetic *N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane, (B) synthetic (1*R*)-[1-^{2}H]-*N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane, (C) synthetic (1*S*)-[1-^{2}H]-*N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane, (D) *N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane derived from the incubation of unlabeled (2*S*)-methionine with the enzyme in deuterium oxide, and (E) *N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane derived from the incubation of (2*S*)-[2-^{2}H]methionine with the enzyme in protium oxide (see Materials and Methods and Appendix for details).

stereochemical course of the protonation of the quinoid intermediate during the abortive transamination catalyzed by the decarboxylase. Analysis of the chirality at C-4′ of the PMP produced from tritiated PLP after hydrolysis of the 5′-phosphate ester group showed that the PMP possessed the *R* absolute configuration at C-4′. Therefore, proton transfer to the quinoid intermediate ocurred from the 4′-*si* face of the quinoid intermediate. The low chiral integrity of the tritiated PMP produced in the 14-h incubations, especially at pH 5.0, probably results from non-enzyme-catalyzed hydrogen exchange at C-4′ with the solvent during the incubation with the decarboxylase. Racemization via proton removal is expected to be ~20 times faster than that by loss of tritium. Attempts to assess the chirality of tritiated PMP derived from abortive transamination incubations conducted with unlabeled decarboxylase holoenzyme in tritiated water were abandoned. The tritium incorporation was ~40-fold lower than expected.

Discussion

The purification of L-methionine decarboxylase from fern acetone powders is summarized in Table I. The enzyme is membrane-associated and on no occasion were we able to solubilize all of the activity of the acetone powder into detergent-containing buffer solutions. In agreement with Hartmann et al. (1984), we were unable to extract any activity from acetone powders by stirring with buffer in the absence

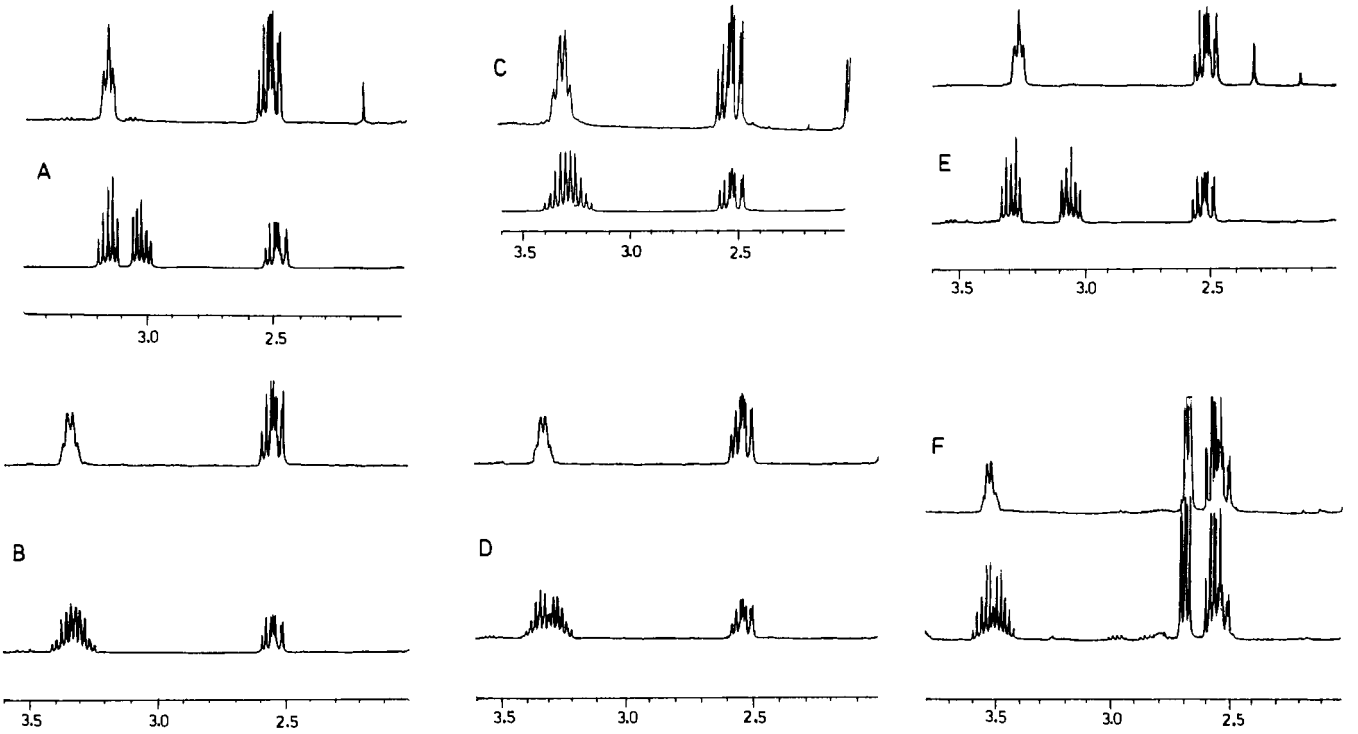


FIGURE 5: Stereochemical course of methionine decarboxylase reaction using (A) valine, (B) leucine, (C) norleucine, (D) norvaline, (E) isoleucine, and (F) *S*-ethylcysteine as substrate. The lower trace of each pair of spectra corresponds to the (−)-camphanoyl derivative of the amine produced from the incubation of the amino acid with the enzyme in protium oxide. The upper trace of each pair is for the derivative of the amine produced upon the incubation of the amino acid with the enzyme in deuterium oxide (see Materials and Methods and Appendix for details).

of detergent. In our hands sonication proved to be a much more efficient method for solubilizing activity than just stirring. The active protein appears to be a homodimer ($M_r \sim 100000$) and is thus similar to other PLP-dependent decarboxylases (Haddox & Russell, 1981; Ando-Yamamoto et al., 1987). The pH optimum of the enzyme is ~5.0 at 20 mM methionine. This value is somewhat lower than the value of pH 6.9 quoted for a similar enzyme from *Streptomyces* (Misono et al., 1980) but is near to the value reported for partially purified alkylamino acid decarboxylase from *P. vulgare* (Hartmann et al., 1984).

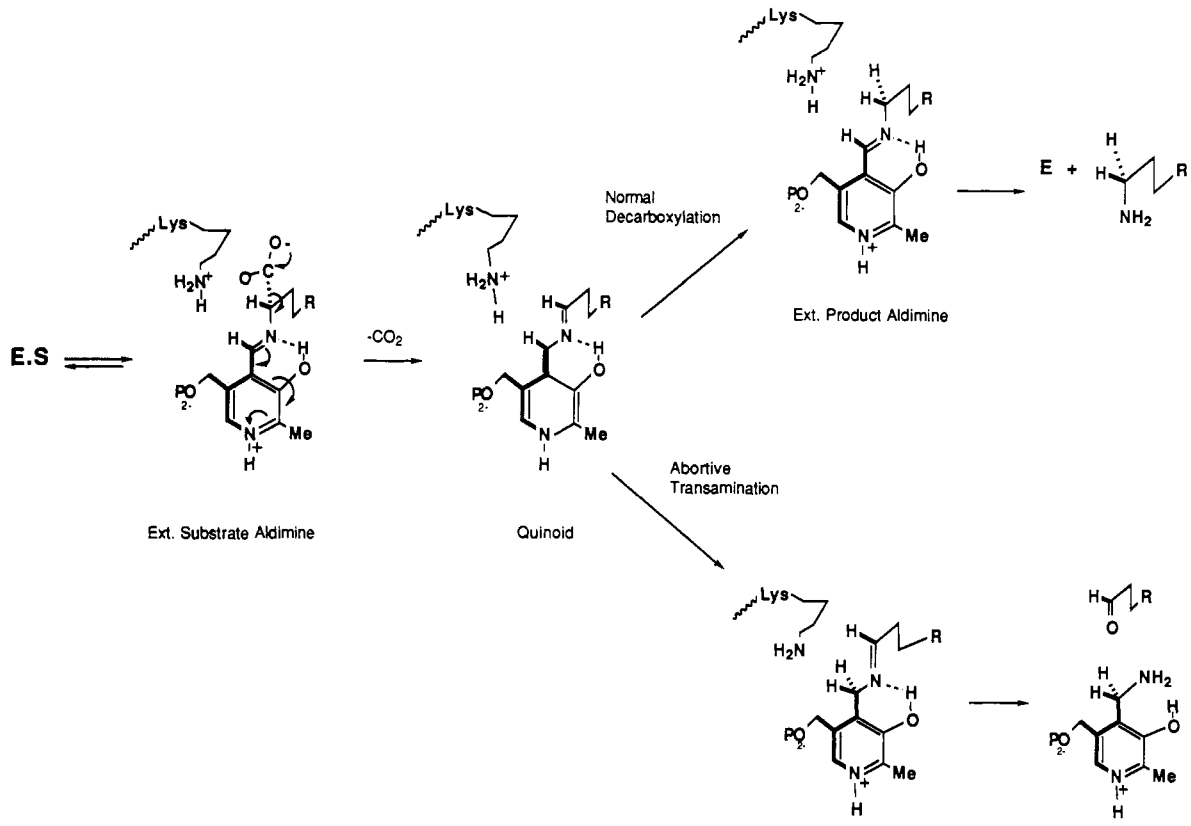
At pH 4.8 the K_m for the best substrate, L-methionine, is 24 mM. At low pH K_m increases, as does V_{max}, whereas at pH 7.0 K_m is ~1 mM; see Table IV. In the physiological pH range, V_{max} is also low but V/K is almost identical with its value at pH 4.8, and thus the V/K profile shows two maxima; see Figure 2. The pH dependence of the kinetic parameters is similar for (2*S*)-leucine except that K_m is slightly higher across the range. (2*S*)-Valine shows a very similar pH dependence for V_{max}, but K_m is extremely high and at low pH is difficult to measure. All of the pH profiles show a pronounced decrease for V_{max}, which titrates at pH 5.2. It is reasonable to expect that the decrease in activity corresponds to deprotonation of the pyridinium heteroatom of the coenzyme, since it is not able to function as an electron sink in its neutral pyridine form (O'Leary et al., 1981). However, there are other reasonable alternatives (see following paper in this issue). Other features are quite complex, although the behavior of V/K at low pH suggests that the carboxyl group of the substrate should be ionized.

Many other L-amino acids bearing hydrophobic side chains are substrates, as shown in Table II, but their K_m values are very large, as shown in Table III. Note that the values of K_m are generally higher for the membrane-associated form of the enzyme but that V_{max} values are qualitatively the same as those determined with the soluble form; see Table IV. (2*R*)-*S*-Ethylcysteine is a substrate for the enzyme, but interestingly, the oxygen-containing analogue (2*S*)-*O*-ethylserine is not. (2*S*)-Ethionine is not a substrate for the enzyme, although the enantiomer of the physiological substrate, (2*R*)-methionine, inhibits the decarboxylation of (2*S*)-methionine. (2*S*,3*R*)-L-*allo*-isoleucine is an extremely poor substrate for the enzyme, although the other L-diastereoisomer, (2*S*,3*S*)-isoleucine, is a good substrate. Thus, for the C-3 branched-chain alkylamino acids, there is a high regard for the absolute configuration of the substrate at C-3. (2*S*)-Valine, which contains a prochiral center at C-3 bearing two methyl groups, reacts at 15% of the rate of (2*S*)-methionine. Replacement of the *pro-S* methyl group by an ethyl group (L-isoleucine) increases the reaction rate 2-fold, whereas replacement of the *pro-R* methyl group of valine by ethyl (L-*allo*-isoleucine) reduces the rate to near zero. Preliminary results from our laboratory indicate that the two diastereomers of L-3-bromobutyrine, isosteric analogues of L-valine in which each of the methyl groups has been separately replaced by a bromine atom (Akhtar & Gani, 1987), irreversibly inhibit the enzyme differentially.

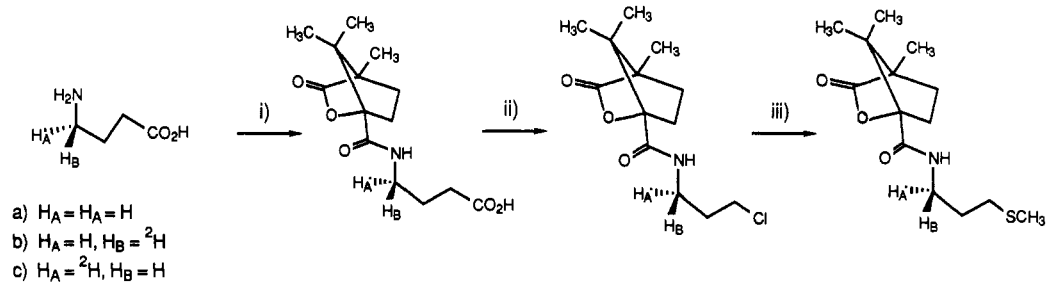
L-Methionine decarboxylase requires coenzyme PLP for activity, and in the absence of excess coenzyme, the activity of the enzyme decreases during sustained substrate turnover. The activity can be almost completely restored by the addition of coenzyme; see Figure 3. These observations are consistent with the occurrence of an abortive decarboxylation–transamination reaction in which, once in several turnover events, the product aldimine is protonated at C-4′ of the coenzyme to give, after hydrolysis, the PMP–apoenzyme complex (see Scheme I). For decarboxylases, such complexes are inactive until a new PLP molecule replaces the PMP to regenerate holoenzyme. Determination of the rates of turnover and inactivation allow the partition ratio between the normal and abortive reactions to be calculated.

The occurrence of an abortive transamination reaction was verified by the isolation and identification of both transamination products, PMP and 3-(methylthio)propionaldehyde; see Materials and Methods. The formation of tritiated PMP

Scheme I



Scheme II



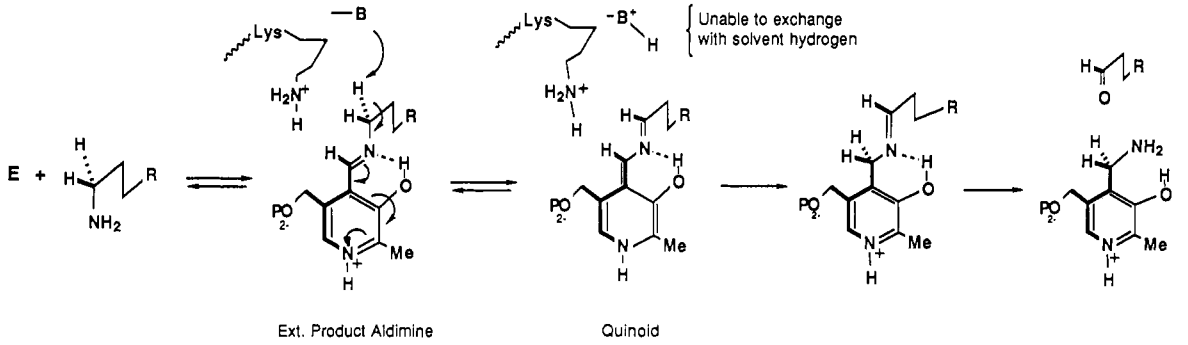
Reagents: i) Camphanoyl chloride, $PhCH_3$, NaOH(aq); ii) $Pb(OAc)_4$, LiCl, C_6H_6, reflux; iii) $NaSCH_3$, CH_3OH, reflux.

from [4′-^{3}H]PLP after the conversion of a known amount of substrate also allowed the partition ratio between the normal and abortive reactions to be calculated, and furthermore, the ratios of ^{35}S-containing aldehyde to ^{35}S-containing amine produced from [^{35}S]methionine allowed a further independent assessment; Tables V and VI. Calculation of the actual turnover:inactivation ratio for each of the three types of experiment (1, 2, and 3; vide supra) gave grossly different answers—approximately 100 000:1 from the rate of the decrease in enzyme activity calculated from experiment 1 conducted at pH 4.8 (see second paper of three in this issue) compared with 7600:1 from experiment 2 conducted at pH 5.0. Other than the small difference in pH, the reaction conditions were essentially identical. Experiment 2B, conducted at pH 6.0, gave a ratio of 3000:1, compared to a value of 1100:1 obtained from experiment 3 at the same pH.

Although the results of experiment 2 indicate that the turnover:inactivation ratio is sensitive to pH, there is no obvious explanation for the discrepancy in the values of the ratios for experiments conducted at similar or identical pH. Here the only difference in the reaction conditions are the concentrations of substrate used and the length of the incubation time. On the basis of previous work with DOPA decarboxylase (O'Leary & Baughn, 1977), it would seem to be unnecessary to consider such variables in setting up these essentially irreversible reactions, since it would be expected that the products, once formed, could not react. Two possible explanations, however, cannot be discounted.

First, it is reasonable to expect a priori that the amine product might react with the holoenzyme complex to give an external aldimine. If this were to occur, then occasionally it might be possible for the active-site base to remove a proton from C-1 to give the quinoid intermediate, identical in structure with the quinoid derived from the decarboxylation of the external substrate aldimine; see Scheme I. Since abortive events during the steady-state reaction must involve the quinoid intermediate, where the partition ratio between abortive and normal events is influenced by the relative protonation rates at C^α and C-4′, the formation of the quinoid intermediate by alternative pathways should increase the apparent frequency of the abortive events. This analysis predicts that experiments in which the amine was allowed to accumulate would show higher abortive transamination ratios than experiments that were maintained under steady-state conditions. Porcine brain

Scheme III



glutamate decarboxylase is known to be able to transaminate 4-aminobutyric acid to give succinate semialdehyde and PMP (Porter et al., 1985).

A second alternative is that a contaminating transaminase is present in the decarboxylase preparation and that the transaminase is able to convert PLP and 3-(methylthio)-1-aminopropane to PMP and the aldehyde. Currently there is no evidence to support the existence of such a transaminase, and indeed, the inclusion of a range of hydrophobic aldehydes in the medium of experiments of type 1 did not prevent inactivation.

(2*R*)-Methionine is a competitive inhibitor for (2*S*)-methionine, but the product amine, 3-(methylthio)-1-aminopropane, is a very poor inhibitor. The side chain of (2*R*)-methionine might be expected, therefore, to occupy the substrate's side-chain binding pocket. In this conformation the C^{α}-hydrogen atom of the inhibitor would be positioned correctly for C–H bond cleavage, according to the Dunathan postulate (Dunathan, 1966). Since a suitably poised enzyme-bound conjugate acid is able to protonate the imine formed during normal decarboxylation at C^{α} to give the product imine, it was reasoned that the enzyme might catalyze solvent hydrogen exchange with the C^{α}-hydrogen of (2*R*)-methionine. A similar rationale, based upon the principle of microscopic reversibility, has been used to account for the inhibition of *E. coli* glutamate decarboxylase of the D-enantiomer of the suicide inhibitor acetylenic GABA, (4*R*)-4-aminohex-5-ynoic acid (Jung et al., 1978). However, after prolonged incubation of the fern acetone powder in buffer solutions containing tritiated water, at either pH 5.5 or 8.0, no enzyme-catalyzed solvent hydrogen incorporation into (2*R*)-methionine was detected. We were also unable to detect any enzyme-catalyzed solvent hydrogen incorporation into the decarboxylation product 3-(methylthio)-1-aminopropane at pH 5.5 or 8.0, although the result is less informative because the value of K_i for the amine is very large. Nevertheless, this result rules out the possibility that purer preparations contain a transaminase activity.

The lack of solvent hydrogen exchange by the decarboxylase indicates that either the C^{α} protons are inaccessible (or not acidic enough) or that the conjugate acid of the enzyme-bound base is monoprotic and is therefore only able to return the proton that was originally removed from the C^{α} position, as shown in Scheme III. Since our own results indicate that the frequency of abortive events is pH dependent and that the quinoid intermediate is accessible through reverse steps, we expect that the C^{α} protons are accessible. The observations that mammalian brain L-glutamate decarboxylase converts 4-aminobutyric acid to succinate semialdehyde (Porter et al., 1985) and that the apoenzyme converts (phosphopyridoxyl)-ethanolamine phosphate to PLP (Choi & Churchich, 1986) presumably via C^{α} abstraction (Gani, 1986), together with the finding that the *E. coli* enzyme activates itself to irreversible inhibition on treatment with D-acetylenic GABA (Jung et al., 1978), are in accord with this notion. Furthermore, there is now a good deal of indirect evidence that has come from pyridoxyllysine active-site peptide sequence alignments (Morino & Nagashima, 1984; Fecker et al., 1986; Vaaler et al., 1986; Everleth et al., 1986; Kobayashi et al., 1987) and solvent deuterium incorporation studies (Yamada & O'Leary, 1977; Akhtar et al., 1990; Stevenson et al., 1990), which imply that histidine serves as an active-site conjugate acid in many reactions catalyzed by PLP-dependent decarboxylases.

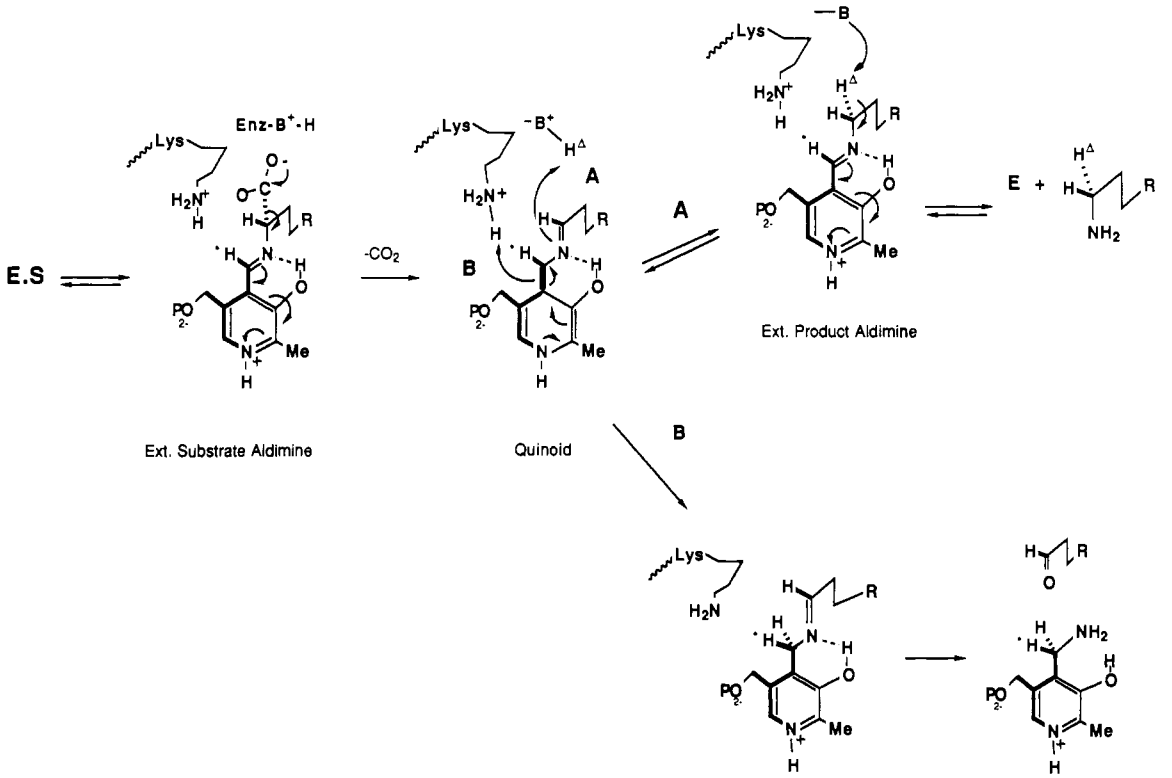
In order to gain some insight into the position of proton donors at the active site of the enzyme, and ultimately to allow a comparison with the transaminase enzymes, the stereochemical courses of substrate decarboxylation and coenzyme transamination were determined.

In spite of the fact that so many different enzymes use PLP as a cofactor, very little is known about the way in which the protein modulates the chemistry of the coenzyme. Indeed, only aspartate aminotransferase has been studied to the extent that it is possible to assign specific binding and catalytic roles to various amino acid residues at the active site of the enzyme. For AAT it is known that two arginine residues, Arg292 and Arg386, interact electrostatically with the α- and β-carboxylate groups of the various dioic acid substrates, aspartate, glutamate, oxaloacetate, and ketoglutarate, and that Lys258, which forms part of the internal aldimine with the coenzyme, resides on the 4′-*si* face of the coenzyme. The same ϵ-ammonium group of Lys258 shuttles protons between the α-carbon of the substrate and the C-4′ position of the coenzyme in the external aldimine in a suprafacial manner (Kirsch et al., 1984; Julin et al., 1989; Julin & Kirsch, 1989). Thus, for AAT, and indeed for transaminases in general (Floss & Vederas, 1982), proton transfer reactions seem to occur only on the 4′-*si* face of the coenzyme.

The majority of PLP-dependent decarboxylases, in fact all those that catalyze the decarboxylation of L-amino acids, replace the carboxyl group of the substrate with retention of configuration at C^{α} (Floss & Vederas, 1982; Gani, 1985; Stevenson et al., 1986; No et al., 1988). The results of stereochemical studies described here show that for seven different substrates, (2*S*)-methionine, valine, leucine, norleucine, norvaline, isoleucine, and (2*R*)-*S*-ethylcysteine, the carboxyl group is replaced with a proton derived from the solvent with retention of configuration at C-2 during decarboxylation. Therefore, the structure of the substrate does not influence the stereospecificity of the protonation of the quinoid at C^{α}, and the enzyme follows the same stereochemical pathway as other PLP-dependennt decarboxylases that operate at an α-amino acid center of the L-configuration.

In isolation this information tells us nothing about the relationship between the transaminase group and the de-

Scheme IV



carboxylase group, since the decarboxylation of an external aldimine could, in principle, occur with equal facility on either face of the coenzyme. Fortunately, once during several thousand turnovers of the physiological substrate, fern methionine decarboxylase transaminates the coenzyme to give PMP and 3-(methylthio)propionaldehyde, vide supra. The stereochemical course of this side reaction was assessed by allowing the enzyme to convert 4′-tritiated coenzyme to tritiated PMP in the presence of (2*S*)-methionine at both pH 5.0 and 6.0. The isolated PMP was converted to pyridoxamine through treatment with alkaline phosphatase, and the chirality at C-4′ was determined by incubating the sample with aspartate aminotransferase. AAT is known to selectively equilibrate the *pro*-4′-*S* hydrogen of pyridoxamine with the solvent (Tobler et al., 1987). The sample derived from the abortive transamination reaction conducted at pH 5.0 was essentially racemic at C-4′, while the samples derived from the incubations conducted at pH 6.0 were predominantly of the 4′*R* absolute configuration. PMP is known to exchange its 4′-benzylic hydrogens with solvent at moderately low pH (Dunathan et al., 1968), and we have found that the presence of PLP enhances this process, presumably through the formation of PLP–PMP aldimines. Thus, the incubation conditions seem to cause racemization of the PMP formed during the abortive reaction. In short incubations at pH 6.0, it was clear that (4′*R*)-[4′-^{3}H]PMP was formed via proton transfer to the 4′-*si* face of the coenzyme. This result is of special significance, since it indicates that protonation at C-4′ of the quinoid intermediate derived from the L-antipode of a physiological substrate occurs with the same stereospecificity as the analogous reaction catalyzed by bona fide transaminases, from the 4′-*si* face of the coenzyme, as shown in Scheme IV.

In accord with many PLP-dependent enzymes, including transaminases, decarboxylases are known to exist as internal lysine–coenzyme aldimine complexes. The finding that abortive transamination in decarboxylases occurs with the same stereospecificity as the identical lysine ϵ-ammonium group mediated protonation at C-4′ in bona fide transaminases strongly suggests that the equivalent active-site lysine in decarboxylases is responsible for the abortive protonation at C-4′. Further support for this notion is offered by the finding that, in abortive transamination incubations conducted in tritium oxide, tritium incorporation into the 4′-position of PMP was very much lower than expected, ~40-fold lower than the label content of the water. This discrepancy is too large to be accounted for by typical tritium isotope effects, even if the full intrinsic effect was realized, but can be rationalized if the proton donor is polyprotic, as it is for the ϵ-ammonium group of Lys, where each proton is accessible to the quinoid intermediate at C-4′.

Given that the coenzyme binding residues in decarboxylases and transaminases should be similar, it is likely that the active-site Lys residue is disposed to the coenzyme in a similar manner in both groups of enzymes. Nevertheless, on the basis of our arguments so far, it is still not possible to determine on which face of the coenzyme decarboxylation occurs, since it is not evident that the Lys residue is responsible for the reprotonation of the quinoid intermediate at C^{α} to give the product aldimine. Indeed, there is growing evidence to suggest that a His residue fulfills this role (O'Leary, 1977). However, since it is now established that at least one proton donor resides on the 4′-*si* face of the coenzyme, further analysis is possible. A 4′-*re* face specific decarboxylation would lead to the formation of a quinoid intermediate that could be, potentially, protonated on the *si* face by the ϵ-ammonium group of the active-site Lys residue. This reaction would result in inversion of configuration at C^{α}. Recalling that the decarboxylation catalyzed by fern methionine decarboxylase occurs with retention of configuration and appears to be completely stereospecific for a range of different substrates, it is likely that decarboxylation does indeed occur on the 4′-*si* face of the coenzyme. Decarboxylation on the 4′-*re* face would almost certainly lead to some loss of stereospecificity for some substrates because the ϵ-ammonium group of the active-site Lys

residue *would* be able to provide a proton at C^{α} (on the *si* face) to any long-lived quinoid intermediate, that is, to any quinoid intermediate that is protonated at C^{α} from the 4′-*re* face slowly. This situation would almost certainly arise at high pH if a His residue was the proton donor for C^{α} of the quinoid intermediate. Interestingly, *meso*-α,ω-diaminopimelic acid decarboxylase, which is known to contain an active-site Lys residue, possibly residue Lys287 (Martin et al., 1988), does catalyze decarboxylation with inversion of configuration at C^{α} (Asada et al., 1981; Kelland et al., 1985). However, it is the D-center of the substrate that is processed, and by the above analysis, decarboxylation in this system should occur on the 4′-*re* face of the coenzyme.

The identity of catalytically important residues at the active site of decarboxylases and their positions with respect to the coenzyme are addressed in the following papers.

Acknowledgments

We thank the Science and Engineering Research Council for financial support and for a studentship to M.A. and the Royal Society for a Royal Society University Fellowship to D.G.

Appendix

N-(−)-*Camphanoyl-4-aminobutyric Acid.* A solution of 4-aminobutyric acid (0.57 g, 5.5 mmol) and potassium carbonate (0.76 g, 5.5 mmol) in water (10 mL) was added to a suspension of (1*S*,4*R*)-(−)-camphanoyl chloride (1 g, 4.6 mmol) in toluene (10 mL). The mixture was vigorously shaken for 5 min and left stirring overnight. The pH was adjusted to 9 and the layers were separated. The aqueous layer was acidified to pH 2, extracted with chloroform (3 × 20 mL), dried (Na_2SO_4), and concentrated in vacuo to give a clear oil, which crystallized over a period of 36 h to give a white solid in quantitative yield: mp 93–95 °C; $[\alpha]_D$ −24.4° (*c* 1 in $CHCl_3$); IR ($CHCl_3$) ν_{max} 3600–3000 (OH), 3440 (NH), 2980 (CH_2, CH_3), 1790 (C=O of acid), 1680 (C=O of amide), and 1535 cm^{-1} (C–O of lactone); ^1H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 1.07 (6 H, s, 2 7′-CH_3), 1.6–1.9 (5 H, m, 3-CH_2, 5′-CH_2, and 6′-C*H*), 2.39 (2 H, t, *J* = 7.6 Hz, 2-CH_2), 2.50 (1 H, m, 6′-C*H*), 3.36 [2 H, m (AB-type coupling), *J* = 7.3 Hz, 4-CH_2], and 6.63 (1 H, br s, 4-N*H*); EI-MS *m*/*z* (rel intensity, assignment) 283 (24, M^+), 236 [45, $(M - CH_3O_2)^+$], 109 (50, $C_8H_{13}^+$), 102 (82, $C_4H_8NO_2^+$), and 83 (100, $C_6H_{11}^+$). Anal. Calcd for $C_{14}H_{21}NO_5$: C, 59.35; H, 7.47; N, 4.95%. Found: C, 59.27; H, 7.55; N, 4.55.

N-(−)-*Camphanoyl-3-chloro-1-aminopropane.* Lithium chloride (68 mg, 1.6 mmol) was added to a solution of *N*-(−)-camphanoyl-4-aminobutyric acid (450 mg, 1.6 mmol) and lead tetraacetate (850 mg, 1.9 mmol) in dry benzene (20 mL). The mixture was purged with nitrogen for 30 min and then heated to 80 °C under reflux according to the method of Kochi (1965). Completion of the reaction was characterized by a change in color from yellowish brown to colorless (approximately 90 min). Benzene was removed under reduced pressure. The solid residue was redissolved in water (10 mL), extracted with chloroform (3 × 10 mL), dried (Na_2SO_4), and concentrated in vacuo to give crude chloride: IR ($CHCl_3$) ν_{max} 3450 (NH), 2980 (CH_2, CH_3), 1790 (C=O of lactone), 1680 (C=O of amide), and 1535 cm^{-1} (C–O of lactone); ^1H NMR (360 MHz, C^2HCl_3) δ 0.87 (3 H, s, 4′-CH_3), 1.08 (6 H, s, 2 7′-CH_3), 1.6–2.0 (5 H, m, 2-CH_2, 5′-CH_2, and 6′-C*H*), 2.50 (1 H, m, 6′-C*H*), 3.38 [2 H, m (AB-type coupling), 1-CH_2], 3.52 (2 H, dt, 3-CH_2), and 6.81 (1 H, br s, −N*H*); EI-MS *m*/*z* (rel intensity, assignment) 275 and 273 (5 and 15, M^+, chlorine isotopes), 228 and 226 [28 and 72, $(M - CH_3O_2)^+$], 109 (55, $C_8H_{13}^+$), and 55 (60, C_2HNO^+).

Synthetic N-(−)-Camphanoyl-3-(methylthio)-1-aminopropane. The crude 3-chloro compound from above (0.4 g, 1.47 mmol) was dissolved in methanol (25 mL). To this was added sodium methanethiolate (300 mg, 4.3 mmol), and the mixture was heated under reflux for 1 h. Excess methanol was removed, the residue was dissolved in chloroform (20 mL), washed with sodium bicarbonate, and dried (Na_2SO_4), and the solvent was removed in vacuo to give the amide. This was purified by using silica flash chromatography (3:1 $CHCl_3$/EtOAc) to give the thioether as an oil (286 mg, 57%): $[\alpha]_D$ −16.7° (*c* 0.6 in $CHCl_3$); IR ($CHCl_3$) ν_{max} 3445 (NH), 2980 (CH_2, CH_3), 1790 (C=O of lactone), 1680 (C=O of amide), and 1535 cm^{-1} (C–O of lactone); ^1H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 1.07 (6 H, s, 2 7′-CH_3), 1.6–1.9 (5 H, m, 2-CH_2, 5′-CH_2, and 6′-C*H*), 2.06 (3 H, s, 3-SCH_3), 2.43 (3 H, m, 3-CH_2 and 6′-C*H*), 3.37 [2 H, m (AB-type coupling), *J* = 7.4 Hz, 1-CH_2], and 6.63 (1 H, br s, −N*H*); EI-MS *m*/*z* (rel intensity, assignment) 285 (30, M^+), 238 [100, $(M - SCH_3)^+$], 109 (63, $C_8H_{13}^+$), and 83 (84, $C_6H_{11}^+$). Anal. Calcd for $C_{14}H_{23}NO_3S$: C, 58.92; H, 8.12; N, 4.91%. Found: C, 59.12; H, 7.95; N, 4.67.

(2*S*)-[2-^{2}*H*]*Glutamic Acid Hydrochloride.* (2*S*)-Glutamic acid (2.94 g, 20 mmol) was dissolved in deuterium oxide (50 mL) and the pH adjusted to 7.25 with concentrated ammonia solution. To this solution was added glutamic-oxaloacetic transaminase (aspartate aminotransferase, 200 units) and pyridoxal 5′-phosphate (5 mg). The mixture was incubated at 37 °C and the reaction followed by NMR. On completion of the reaction (ca. 72 h), the enzyme was denatured. The solution was filtered and then concentrated under reduced pressure. The solid residue was dissolved in water (25 mL) and concentrated in vacuo to 5 mL, diluted to 10 mL with water, and crystallized by using hydrochloric acid (6 M) to yield fine white crystals (2.90 g, 97%): mp 195 °C dec [lit. (Greenstein & Winitz, 1961) mp 205 °C dec]; $[\alpha]_D$ +22.5° (*c* 0.4 in 5 M HCl) {lit. (Greenstein & Winitz, 1961) $[\alpha]_D$ +46.8° (*c* 2 in 5 M HCl) for the undeuterated free amino acid}; ^1H NMR (360 MHz, ^2H_2O) δ 1.55 [2 H, m (AB-type coupling), 3-CH_2], and 1.92 (2 H, t, *J* = 6.5 Hz, 4-CH_2); FAB-MS (+ve) *m*/*z* (rel intensity, assignment) 149 [55, $(M - Cl)^+$], 103 (10, $C_4H_9NO_2^+$), 100 (15, $C_4H_6NO_2^+$), and 75 (25, $C_2H_5NO_2^+$). Anal. Calcd for $C_5H_{11}ClNO_4$: C, 32.53; H, 6.00; N, 7.60. Found: C, 32.40; H, 5.52; N, 7.60.

(4*R*)-[4-^{2}*H*]-*4-Aminobutyric Acid.* (2*S*)-Glutamic acid (1.48 g, 10 mmol) was suspended in deuterium oxide (25 mL) and the pH adjusted to 5 with concentrated ammonia solution. Glutamate decarboxylase (from *E. coli*) (150 units) and pyridoxal 5′-phosphate (4 mg) were added, and the reaction was incubated at 37 °C. The course of the reaction was followed by TLC. After 6 h the protein was denatured and filtered, and the filtrate was concentrated in vacuo. The hydrochloride salt of the amine was made by using hydrochloric acid. The resultant residue was dissolved in water (100 mL), applied to a column of Amberlite IR-45(OH) weakly basic anion-exchange resin, and eluted with water (300 mL at a rate of 1 drop/3 s). The ninhydrin-positive fractions of the eluant were combined and concentrated in vacuo to give an oil: ^1H NMR (360 MHz, ^2H_2O) δ 1.71 (2 H, dt, *J* = 7.4 Hz, 2-CH_2), 2.13 (2 H, t, *J* = 7.1 Hz, 3-CH_2), and 2.83 (1 H, t, *J* = 7.4 Hz, 4-C*H*).

(4*S*)-[4-^{2}*H*]-*4-Aminobutyric Acid.* This was prepared as described above but with (2*S*)-[2-^{2}H]glutamic acid and water as the starting materials: ^1H NMR (360 MHz, ^2H_2O) δ 1.70

(2 H, dt, J = 7.5 Hz, 2-CH_2), 2.10 (2 H, t, J = 7.1 Hz, 3-CH_2), and 2.80 (1 H, t, J = 7.5 Hz, 4-CH).

Synthetic (1R)-[1-^{2}H]-N-(−)-Camphanoyl-3-(methylthio)-1-aminopropane. This was prepared from (4*R*)-[4-^{2}H]-4-aminobutyric acid, and the procedure outlined for *N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane was followed: IR ($CHCl_3$) ν_{max} 3450–3440 (NH of amide), 2980 (CH_2, CH_3), 1790 (C=O of lactone), 1680 (C=O of amide), and 1535 cm^{-1} (C–O of lactone); ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 1.07 (6 H, s, 2 7′-CH_3), 1.6–1.9 (5 H, m, 2-CH_2, 5′-CH_2, and 6′-CH), 2.06 (3 H, s, 3-SCH_3), 2.39 (3 H, m, J = 7.5 Hz, 3-CH_2 and 6′-CH), 3.41 (1 H, m, 1-CH), and 6.63 (1 H, br s, 1-NH).

Synthetic (1S)-[1-^{2}H]-N-(−)-Camphanoyl-3-(methylthio)-1-aminopropane. This was prepared from (4*S*)-[4-^{2}H]-4-aminobutyric acid, and the procedure outlined for *N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane was followed: IR ($CHCl_3$) ν_{max} 3445 (NH of amide), 2980 (CH, CH_3), 1790 (C=O of lactone), 1680 (C=O of amide), and 1535 cm^{-1} (C–O of lactone); ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 1.07 (6 H, s, 2 7′-CH_3), 1.6–1.9 (5 H, m, 2-CH_3, 5′-CH_2, and 6′-CH), 2.06 (3 H, s, 3-SCH_3), 2.39 (3 H, m, J = 7.5 Hz, 3-CH_2 and 6′-CH), 3.35 (1 H, m, 1-CH), and 6.63 (1 H, br s, 1-NH).

(2S)-[2-^{2}H]Methionine. To (2*S*)-methionine (3 g, 20 mmol) dissolved in 1 M sodium deuterioxide (20 mL) was added acetic anhydride (7 mL, 60 mmol) in three portions with vigorous shaking. After allowing the mixture to stand at 37 °C for 6 h, sulfuric acid (1 M, 20 mL) was added, the solution was concentrated in vacuo, and the residue was extracted several times with ethyl acetate. The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo to give a waxy solid: ^{1}H NMR (60 MHz, ^2H_2O) δ 1.96 (3 H, s, 2′-COCH_3), 2.02 (3 H, s, 4-SCH_3), 2.09 (2 H, m, 4-CH_2), and 2.49 (2 H, m, 3-CH_2).

The crude DL-acetylmethionine (2 g, 10.4 mmol) was dissolved in water (100 mL), the pH was adjusted to 7.4, and acylase I powder (4 mg) was added. The progress of the reaction was followed by TLC (compared with methionine). On completion of the reaction the enzyme was denatured, and the mixture was applied to a basic ion-exchange column (Amberlite IR45(OH)] (the column was prewashed with water) and then further eluted with water (~100 mL). The eluant fractions containing methionine were concentrated under reduced pressure to give a solid, which was recrystallized from methanol to yield white flaky crystals (0.4 g, 26%): mp 200 °C; $[\alpha]_D$ +32.5° (*c* 0.4 in 5 M HCl) {lit. (Greenstein & Winitz, 1961) $[\alpha]_D$ +34.6° (*c* 1–2 in 5 M HCl) for the undeuteriated compound}; ^{1}H NMR (360 MHz, ^2H_2O) δ 1.65 (3 H, s, 4-SCH_3), 1.79 [2 H, m (AB-type coupling), 3-CH_2], and 2.24 (2 H, t, J = 7.5 Hz, 4-CH_2); *m/z* FAB-MS (+ve) *m/z* (rel intensity, assignment) 151 [100, $(M + H)^+$] and 75 (55, $C_2H_5NO_2^+$). Anal. Calcd for $C_5H_{12}NSO_2$: C, 40.00; H, 8.05; N, 9.30. Found: C, 39.70; H, 8.00; N, 9.50.

Enzymic N-(−)-camphanoyl-3-(methylthio)-1-aminopropane was prepared from the product of the fern enzyme incubation. L-Methionine (100 mg, 0.67 mmol) was suspended in aqueous sodium acetate (0.2 M, 15 mL, pH 4.8). Pyridoxal 5′-phosphate (5 mg) and methionine decarboxylase (1 g of fern acetone powder) were added. The reaction flask was gently rotated at room temperature for 24 h. The solution was filtered through a Celite pad. The pH of the filtrate was adjusted to 11 with sodium hydroxide, and the solution was extracted with dichloromethane (3 × 25 mL). The organic layer was extracted with 0.5 M hydrochloric acid (2 × 15 mL), and the water was removed in vacuo to give 3-(methylthio)-1-aminopropane hydrochloride (50 mg, 53%): ^{1}H NMR (360 MHz, C^2HCl_3) δ 1.82 (2 H, m, J = 7 and 14 Hz, 2-CH_2), 1.91 (3 H, s, 3-SCH_3), 2.45 (2 H, t, J = 7.1 Hz, 3-CH_2), and 2.90 (2 H, t, J = 7.3 Hz, 1-CH).

The amine hydrochloride from above (140 mg, 0.99 mmol) was dissolved in water (5 mL), and potassium hydroxide (1 M) was added until the solution became basic. The mixture was then extracted with chloroform (3 × 15 mL), dried, and concentrated in vacuo to 2 mL (solution A).

To (1*S*,4*R*)-(−)-camphanic acid (160 mg, 0.81 mmol) suspended in acetonitrile (10 mL) were added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide (142 mg, 0.74 mmol), triethylamine (53 μL, 0.74 mmol), and solution A. The mixture was then left vigorously stirring overnight (generally for 10 h). After completion of the reaction, the mixture was concentrated in vacuo, redissolved in chloroform (10 mL), washed successively with 0.05 M hydrochloric acid (10 mL), 1 M sodium bicarbonate (10 mL), and water (10 mL), dried (Na_2SO_4), and finally concentrated in vacuo to give a dark oil. The crude compound was purified by using silica flash chromatography (3:1 $CHCl_3$/EtOAc) to yield a light yellow oil (120 mg, 43%): ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 1.07 (6 H, s, 2 7′-CH_3), 1.6–1.9 (5 H, m, 2-CH_2, 5′-CH_2, and 6′-CH), 2.06 (3 H, s, 3-SCH_3), 2.43 (3 H, m, 3-CH_2 and 6′-CH), 3.37 [2 H, m (AB-type coupling), J = 7 Hz, 1-CH_2], and 6.63 (1 H, br s, 1-NH). All other spectral data were identical with those for the synthetic material.

Enzymic (1R)-[1-^{2}H]-N-(−)-camphanoyl-3-(methylthio)-1-aminopropane was prepared from the product of the fern enzyme incubation. This was prepared in a manner identical with that described above for *N*-(−)-camphanoyl-3-(methylthio)-1-aminopropane, starting with the hydrochloride derived from the incubation of (2*S*)-methionine with the fern enzyme in deuterium oxide: ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 1.07 (6 H, s, 2 7′-CH_3), 1.6–1.9 (5 H, m, 2-CH_2, 5′-CH_2, and 6′-CH), 2.06 (3 H, s, 3-SCH_3), 2.39 (3 H, m, J = 7.5 Hz, 3-CH_2 and 6′-CH), 3.41 (1 H, m, 1-CH), and 6.63 (1 H, br s, 1-NH). All other spectral data were identical with those for the synthetic material.

Enzymic (1S)-[1-^{2}H]-N-(−)-camphanoyl-3-(methylthio)-1-aminopropane was prepared from the product of the fern enzyme incubation. This was prepared in a manner identical with that described above for the unlabeled compound, starting with the hydrochloride derived from the incubation of (2*S*)-[2-^{2}H]methionine with the fern enzyme in protium oxide: ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 1.07 (6 H, s, 2 7′-CH_3), 1.6–1.9 (5 H, m, 2-CH_2, 5′-CH_2, and 6′-CH), 2.06 (3 H, s, 3-SCH_3), 2.39 (3 H, m, J = 7.5 Hz, 3-CH_2 and 6′-CH), 3.37 (1 H, m, 1-CH), and 6.63 (1 H, br s, 1-NH). All other spectral data were identical with those for the synthetic material.

N-(−)-Camphanoyl-2-methyl-1-aminopropane. Valine (100 mg, 0.85 mmol) was suspended in aqueous sodium acetate (0.2 M, 15 mL, pH 4.8). Pyridoxal 5′-phosphate (5 mg) and methionine decarboxylase (1 g of fern acetone powder) were added. The reaction flask was gently rotated at room temperature for 24 h. The solution was filtered through a Celite pad. The pH of the filtrate was adjusted to 11 with sodium hydroxide, and the solution was extracted with dichloromethane (3 × 25 mL). The organic layer was extracted with 0.5 M hydrochloric acid (2 × 15 mL) and then the water was removed in vacuo to give 2-methylpropylamine hydrochloride

(40 mg, 43%): ^{1}H NMR (270 MHz, ^2H_2O) δ 0.81 [6 H, d, J = 6.6 Hz, 2-CH(CH_3)$_2$], 1.78 [1 H, m, J = 6.6 and 7.1 Hz, 2-CH(CH_3)$_2$], and 2.67 (2 H, d, J = 7.1 Hz, 1-CH_2).

The amine hydrochloride (33 mg, 0.3 mmol) from above was dissolved in water (5 mL), and potassium carbonate (83 mg, 0.6 mmol) was added. This basic solution was then added to a suspension of camphanoyl chloride (65.2 mg, 0.3 mmol) in toluene (5 mL). The mixture was vigorously shaken for 5 min and left stirring overnight. The pH was adjusted to 9, and the layers were separated. The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give a white solid, which was purified by silica flash chromatography ($CHCl_3$) to give the amide (49.7 mg, 62%): mp 62–63 °C; $[\alpha]_D$ −28.9° (c 0.7 in $CHCl_3$); IR ($CHCl_3$) ν_{max} 3440 (NH), 2980 (CH, CH_2), 1790 (C=O of lactone), 1680 (C=O of amide), and 1540 cm^{-1} (C–O of lactone); ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.89 [9 H, m, 4′-CH_3 and 2-CH(CH_3)$_2$], 1.08 (6 H, d, J = 1.2 Hz, 2 7′-CH_3), 1.6–2.0 (4 H, m, 2-CH, 5′-CH_2, and 6′-CH), 2.50 (1 H, m, 6′-CH), 3.04 (1 H, m, 1-CH), 3.16 (1 H, m, 1-CH), and 6.49 (1 H, br s, −NH). EI-MS m/z (rel intensity, assignment) 253 (93.6, M^+), 238 [33.7, (M − CH_3)$^+$], 206 [89.1, (M − CH_3O_2)$^+$], 182 (66.0, $C_{10}H_{16}NO_2^+$), 153 (68.1, $C_9H_{13}O_2^+$), 109 (53.8, $C_8H_3^+$), and 83 (100, $C_6H_{11}^+$). Exact Mass Calcd for $C_{14}H_{23}NO_3$: 253.1678. Found: 253.1672. Anal. Calcd for $C_{14}H_{23}NO_3$: C, 66.37; H, 9.15; N, 5.53. Found: C, 66.27; H, 9.25; N, 5.52.

N-(−)-*Camphanoyl-3-methyl-1-aminobutane*. This was prepared in a manner analogous to that described above for the aminopropane, starting with leucine as the amino acid. The intermediate 3-methyl-1-aminobutane hydrochloride was obtained in 44% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.7 [6 H, dd, J = 1.5 and 6.6 Hz, 3-CH(CH_3)$_2$], 1.37 (2 H, m, J = 7.32 and 1.5 Hz, 2-CH_2), 1.48 [1 H, m, J = 6.4 and 6.6 Hz, 3-CH(CH_3)$_2$], 2.83 (2 H, t, J = 8.06 and 7.3 Hz, 1-CH_2).

The camphanamide was obtained in 65% yield (from the amine hydrochloride): mp 66–67 °C; $[\alpha]_D$ −31.2° (c 0.5 in $CHCl_3$); IR ($CHCl_3$) ν_{max} 3440 (NH), 2980 (CH, CH_2, CH_3), 1790 (C=O of lactone), 1675 (C=O of amide), and 1540 cm^{-1} (C–O of lactone); ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.86 (3 H, s, 4′-CH_3), 0.88 [6 H, dd, J = 0.5 and 6.6 Hz, 3-CH-(CH_3)$_2$], 1.07 (6 H, d, J = 1.2 Hz, 2 7′-CH_3), 1.3–2.0 (6 H, m, 3-CH, 2-CH_2, 5′-CH_2, and 6′-CH), 2.50 (1 H, m, 3′-CH), 3.27 [2 H, m (AB-type coupling), 1-CH_2], and 6.42 (1 H, br s, 1-NH); EI-MS m/z (rel intensity, assignment) 267 (74.2, M^+), 220 [96.8, (M − CH_3O_2)$^+$], 211 [71.3, (M − C_4H_8)$^+$], 109 (59.4, $C_8H_{13}^+$), and 83 (100, $C_6H_{11}^+$). Exact Mass Calcd for $C_{15}H_{25}NO_3$: 267.1834. Found: 267.1830. Anal. Calcd for $C_{15}H_{25}NO_3$: C, 67.38; H, 9.43; N, 5.24. Found: C, 67.13; H, 9.50; N, 5.37.

N-(−)-*Camphanoyl-1-aminopentane*. This was prepared in a manner analogous to that described for *N*-(−)-camphanoyl-2-methyl-1-aminopropane, with (2*S*)-norleucine as the substrate. The intermediate 1-aminopentane hydrochloride was obtained in 27% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.71 (3 H, t, J = 7.32 and 6.84 Hz, 5-CH_3), 1.17 (4 H, m, 3-CH_2 and 4-CH_2), 1.48 (2 H, m, J = 7.1 and 5.86 Hz, 2-CH_2), and 2.80 (2 H, t, J = 7.6 and 7.3 Hz, 1-CH_2).

The camphanamide was obtained in 30% yield (from the amine hydrochloride): IR ($CHCl_3$) ν_{max} 3445 (NH), 2985 (CH, CH_2, CH_3), 1790 (C=O of lactone), 1675 (C=O of amide), and 1545 cm^{-1} (C–O of lactone); ^{1}H NMR (270 MHz, C^2HCl_3) δ 0.83 (6 H, m, 4′-CH_3 and 5-CH_3), 1.05 (6 H, d, J = 1.2 Hz, 2 7′-CH_3), 1.2–1.9 (9 H, m, 5′-CH_2, 6′-CH, 2-CH_2, 3-CH_2, and 4-CH_2), 2.50 (1 H, m, 6′-CH), 3.29 [2 H, m (AB-type splitting), 1-CH_2], and 6.45 (1 H, br s, 1-NH).

N-(−)-*Camphanoyl-1-aminobutane*. This was prepared in an analogous manner to that described for *N*-(−)-camphanoyl-2-methyl-1-aminopropane, with norvaline as the starting amino acid. The intermediate butylamine hydrochloride was obtained in 42% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.7 (3 H, t, J = 7.3 Hz, 4-CH_3), 1.16 (2 H, sextet, J = 7.81 and 7.32 Hz, 3-CH_2), 1.42 (2 H, quintet, J = 7.8 and 7.6 Hz, 2-CH_2), and 2.77 (2 H, t, J = 7.6 and 7.3 Hz, 1-CH_2).

The camphanamide was obtained in 58% yield (from the amine hydrochloride) after purification: mp 58–59 °C; IR ($CHCl_3$) ν_{max} 3440 (NH), 2980 (CH, CH_2, CH_3), 1790 (C=O of lactone), 1675 (C=O of amide), and 1540 cm^{-1} (C–O of lactone); ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.88 (3 H, s, 4′-CH_3), 0.91 (3 H, t, J = 7.3 Hz, 4-CH_3), 1.08 (6 H, d, J = 0.7 Hz, 2 7′-CH_3), 1.32 (2 H, m, 3-CH_2), 1.48 (2 H, m, 2-CH_2), 1.6–2.0 (3 H, m, 5′-CH_2 and 6′-CH), 2.50 (1 H, m, 6′-CH), 3.27 [2 H, m (AB-type coupling), 1-CH_2], and 6.44 (1 H, br s, 1-NH); EI-MS m/z (rel intensity, assignment) 253 (66.4, M^+), 207 [52.8, (M − CH_2O_2)$^+$], 206 [100, (M − CH_3O_2)$^+$], and 83 (72.7, $C_6H_{11}^+$). Exact Mass Calcd for $C_{14}H_{23}NO_3$: 253.1678. Found: 253.1668. Anal. Calcd for $C_{14}H_{23}NO_3$: C, 66.40; H, 9.15; N, 5.55. Found: C, 66.60; H, 9.35; N, 5.70.

(2*S*)-*N*-(−)-*Camphanoyl-2-methyl-1-aminobutane*. This was prepared in a manner analogous to that described for *N*-(−)-camphanoyl-2-methyl-1-aminopropane, with (2*S*,3*S*)-isoleucine as the amino acid. The intermediate (2*S*)-2-methyl-1-aminobutane hydrochloride was obtained in 41% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.68 (3 H, t, J = 7.3 Hz, 4-CH_3), 0.72 (3 H, d, J = 6.8 Hz, 2-CHCH_3), 1.04 (1 H, m, J = 7.3 and 6.8 Hz, 3-CH), 1.19 (1 H, m, J = 7.3 and 6.8 Hz, 3-CH), 1.52 (1 H, octet, J = 13.2, 6.6, and 6.8 Hz, 2-CH), 2.60 (1 H, m, J = 12.7 Hz, 1-CH), and 2.74 (1 H, m, J = 12.7 Hz, 1-CH).

The camphanamide was obtained in 57% yield (from the amine hydrochloride): IR ($CHCl_3$) ν_{max} 3440 (NH), 2980 (CH, CH_2, CH_3), 1790 (C=O of lactone), 1675 (C=O of amide), and 1540 cm^{-1} (C–O of lactone); ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.87 (9 H, m, 4′-CH_3, 4-CH_3, and 2-CHCH_3), 1.09 (6 H, d, J = 1.2 Hz, 2 7′-CH_3), 1.0–2.0 (6 H, m, 5′-CH_2, 6′-CH, 2-CH, and 3-CH_2), 2.50 (1 H, m, 6′-CH), 3.04 (1 H, m, 1-CH), 3.27 (1 H, m, 1-CH), and 6.46 (1 H, br s, 1-NH); EI-MS m/z (rel intensity, assignment) 267 (72.1, M^+), 252 [11.6, (M − CH_3)$^+$], 238 [64.8, (M − CH_2CH_3)$^+$], 220 [57, (M − CH_3O_2)$^+$], 182 (53, $C_{10}H_{16}NO_2^+$), 153 (67.1, $C_9H_{13}O_2^+$), 109 (53.8, $C_8H_{13}^+$), and 83 (100, $C_6H_{11}^+$). Exact Mass Calcd for $C_{15}H_{25}NO_3$: 267.1834. Found: 267.1828. Anal. Calcd for $C_{15}H_{25}NO_3$: C, 67.38; H, 9.43; N, 5.24. Found: C, 66.80; H, 9.03; N, 5.17.

N-(−)-*Camphanoyl-2-(ethylthio)-1-aminoethane*. This was prepared in a manner analogous to that described for *N*-(−)-camphanoyl-2-methyl-1-aminopropane, with (2*S*)-*S*-ethylcysteine as the substrate. The intermediate 2-(ethylthio)-1-aminoethane hydrochloride was obtained in 46% yield; ^{1}H NMR (360 MHz, ^2H_2O) δ 0.74 (3 H, t, J = 7.5 Hz, 2-SCH$_2$CH_3), 2.44 (2 H, q, J = 7.2 Hz, 2-SCH_2CH$_3$), 2.71 (2 H, m, J = 6.8 Hz, 2-CH_2), and 3.07 (2 H, m, J = 6.8 Hz, 1-CH_2).

The camphanamide was obtained in 42% yield (from the amine hydrochloride): $[\alpha]_D$ −14.9° (c 0.7 in $CHCl_3$); IR ($CHCl_3$) ν_{max} 3440 (NH), 2985 (CH_2, CH_3), 1790 (C=O of lactone), 1695 (C=O of amide), and 1545 cm^{-1} (C–O of lactone); ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.93 (3 H, s, 4′-CH_3), 1.11 (6 H, d, J = 1.7 Hz, 2 7′-CH_3), 1.27 (3 H, t, J

= 7.35 Hz, 2-SCH_2CH_3), 1.6–2.0 (3 H, m, 5′-CH_2 and 6′-C*H*), 2.5 (3 H, m, 6′-C*H* and 2-SCH_2), 2.68 (2 H, t, J = 6.6 Hz, 2-CH_2), 3.50 [2 H, m (AB-type coupling), 1-CH_2)], and 6.80 (1 H, br s, 1-N*H*); EI-MS m/z (rel intensity, assignment) 285 (12.2, M^+), 198 (7, $C_{10}H_{16}NO_3^+$), 109 (5, $C_8H_{13}^+$), and 88 (100, $C_4H_8S^+$). Exact Mass Calcd for $C_{14}H_{23}NO_3S$: 285.1399. Found: 285.1388. Anal. Calcd for $C_{14}H_{23}NO_3S$: C, 58.92; H, 8.12; N, 4.91; S, 11.24. Found: C, 58.71; H, 7.90; N, 4.90; S, 10.95.

(*1R*)-[*1*-^{2}*H*]-*N*-(−)-*Camphanoyl-2-methyl-1-aminopropane.* Valine (100 mg, 0.85 mmol) was suspended in aqueous sodium acetate (0.2 M, 15 mL, pH 4.8). The solution was freeze-dried overnight and then resuspended in deuterium oxide (15 mL). Pyridoxal 5′-phosphate (5 mg) and methionine decarboxylase (1 g, fern acetone powder) was added. The reaction flask was gently rotated at room temperature for 24 h. The solution was filtered through a Celite pad. The pH of the filtrate was adjusted to 11 with sodium hydroxide, and the solution was extracted with dichloromethane (3 × 25 mL). The organic layer was extracted with 0.5 M hydrochloric acid (2 × 15 mL), and the water was removed in vacuo to give (1R)-[1-^{2}H]-1-aminopropane hydrochloride (34 mg, 37%): ^{1}H NMR (270 MHz, ^2H_2O) δ 0.75 [6 H, d, J = 6.6 Hz, 2-CH(CH_3)$_2$], 1.17 [1 H, octet, J = 6.8 Hz, 2-C*H*(CH_3)$_2$], 2.60 (1 H, d, J = 6.84 Hz, 1-C*H*).

The camphanamide was prepared in a manner analogous to that described for the unlabeled compound, in 45% yield (after purification): mp 66–67 °C; ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.91 [9 H, m, 4′-CH_3 and 2-CH(CH_3)$_2$], 1.10 (6 H, s, 2 7′-CH_3), 1.6–2.0 (4 H, m, 2-C*H*, 5′-CH_2, and 6′-C*H*), 2.50 (1 H, m, 6′-C*H*), 3.16 (1 H, m, 1-C*H*), and 6.49 (1 H, br s, 1-N*H*); see Figures 5A. Exact Mass Calcd for $C_{14}H_{24}NO_3$: 254.1756. Found: 254.1746.

(*1R*)-[*1*-^{2}*H*]-*N*-(−)-*Camphanoyl-3-methyl-1-aminobutane.* This was prepared as described above, with (2*S*)-leucine as the substrate. The intermediate (1*R*)-[1-^{2}H]-3-methyl-1-aminobutane hydrochloride was obtained in 56% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.74 [6 H, d, J = 6.6 Hz, 3-CH(CH_3)$_2$], 1.36 (2 H, t, J = 7.6 and 6.8 Hz, 2-CH_2), 1.48 (1 H, d of octet, J = 6.35 and 7.6 Hz, 3-C*H*), 2.82 (1 H, t, J = 7.6 and 7.8 Hz, 1-C*H*).

The camphanamide was obtained in 62% yield (from the amine hydrochloride) after purification: mp 67–68 °C; ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.85 (3 H, s, 4′-CH_3), 0.87 [6 H, dd, J = 0.7 and 6.6 Hz, 3-CH(CH_3)$_2$], 1.06 (6 H, d, J = 1.4 Hz, 2 7′-CH_3), 1.3–2.0 (6 H, m, 3-C*H*, 2-CH_2, 5′-CH_2, and 6′-C*H*), 2.48 (1 H, m, 6′-C*H*), 3.28 (1 H, m, 1-C*H*), and 6.41 (1 H, br s, 1-N*H*); see Figure 5B. Exact Mass Calcd for $C_{15}H_{26}NO_3$: 268.1913. Found: 268.1902.

(*1R*)-[*1*-^{2}*H*]-*N*-(−)-*Camphanoyl-1-aminopentane.* This was prepared as described above, with (2*S*)-norleucine as the substrate. The intermediate (1*R*)-[1-^{2}H]-1-aminopentane was obtained in 28% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.70 (3 H, t, J = 7.08 Hz, 5-CH_3), 1.15 (4 H, m, 3-CH_2 and 4-CH_2), 1.46 (2 H, m, J = 7.1 Hz, 2-CH_2), and 2.78 (1 H, t, J = 7.6 Hz, 1-C*H*).

The camphanamide was obtained in 35% yield (from the amine hydrochloride): ^{1}H NMR (270 MHz, C^2HCl_3) δ 0.90 (6 H, m, 4′-CH_3 and 5-CH_3), 1.10 (6 H, s, 2 7′-CH_3), 1.2–2.0 (9 H, m, 5′-CH_2, 6′-C*H*, 2-CH_2, 3-CH_2, and 4-CH_2), 2.50 (1 H, m, 6′-C*H*), 3.30 (1 H, m, 1-C*H*), and 6.47 (1 H, br s, 1-N*H*); see Figure 5C.

(*1R*)-[*1*-^{2}*H*]-(*N*)-(−)-*Camphanoyl-1-aminobutane.* This was prepared in a manner analogous to that described above, with (2*S*)-norvaline as the substrate. The intermediate (1*R*)-[1-^{2}H]-1-aminobutane hydrochloride was obtained in 35% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.73 (3 H, t, J = 7.3 Hz, 4-CH_3), 1.20 (2 H, sextet, J = 7.3 and 8.0 Hz, 3-CH_2), 1.44 (2 H, q, J = 7.3, 7.6 and 7.8 Hz, 2-CH_2), 2.80 (1 H, t, J = 7.1 and 7.3 Hz, 1-C*H*).

The camphanamide was obtained in 54% yield (from the amine hydrochloride) after purification: mp 62–63 °C; ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.87 (3 H, s, 4′-CH_3), 0.90 (3 H, t, J = 7.3 Hz, 4-CH_3), 1.09 (6 H, d, J = 0.7 Hz, 2 7′-CH_3), 1.32 (2 H, m, 3-CH_2), 1.46 (2 H, m, 2-CH_2), 1.6–2.0 (3 H, m, 5′-CH_2 and 6′-C*H*), 2.50 (1 H, m, 6′-C*H*), 3.30 (1 H, m, 1-C*H*), and 6.44 (1 H, br s, 1-N*H*); see Figure 5D. Exact Mass Calcd for $C_{14}H_{24}NO_3$: 254.1756. Found: 254.1750.

(*1R,2S*)-[*1*-^{2}*H*]-*N*-(−)-*Camphanoyl-2-methyl-1-aminobutane.* This was prepared as described above, with (2*S*,3*S*)-isoleucine as the substrate. The intermediate (1*R*,2*S*)-[1-^{2}H]-2-methyl-1-aminobutane hydrochloride was obtained in 38% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 0.66 (3 H, t, J = 7.3 and 7.6 Hz, 4-CH_3), 0.72 (3 H, d, J = 7.6 Hz, 2-CHCH_3), 1.01 (1 H, m, 3-C*H*), 1.16 (1 H, m, 3-C*H*), 1.50 (1 H, septet, J = 6.6 and 13.2 Hz, 2-C*H*), 2.71 (1 H, d, J = 5.6 Hz, 1-C*H*).

The camphanamide was obtained in 52% yield (from the amine hydrochloride): mp 56–57 °C; ^{1}H NMR (360 MHz, C^2HCl_3) δ 0.87 (9 H, m, 4′-CH_3, 4-CH_3, and 2-CHCH_3), 1.08 (6 H, d, J = 1.0 Hz, 2 7′-CH_3), 1.1–2.0 (6 H, m, 5′-CH_2, 6′-CH, 2-C*H*, and 3-CH_2), 2.51 (1 H, m, 6′-C*H*), 3.25 (1 H, m, 1-C*H*), and 6.46 (1 H, br s, 1-N*H*); see Figure 5E. Exact Mass Calcd for $C_{15}H_{26}NO_3$: 268.1913. Found: 268.1903.

(*1R*)-[*1*-^{2}*H*]-*N*-(−)-*Camphanoyl-2-(ethylthio)-1-aminoethane.* This was prepared as described above, with (2*S*)-*S*-ethylcysteine as the substrate. The intermediate (1*R*)-[1-^{2}H)-2-(ethylthio)-1-aminoethane hydrochloride was obtained in 40% yield: ^{1}H NMR (270 MHz, ^2H_2O) δ 1.05 (3 H, t, J = 7.4 Hz, 2-SCH_2CH_3), 2.41 (2 H, q, J = 7.4 Hz, 2-SCH_2), 2.67 (2 H, d, J = 6.6 Hz, 2-CH_2), 3.01 (1 H, t, J = 5.86 Hz, 1-C*H*).

The camphanamide was obtained in 33% yield (from the amine hydrochloride): $[\alpha]_D$ −17.4° (c 0.6 in $CHCl_3$); ^{1}H NMR (270 MHz, C^2HCl_3) δ 0.92 (3 H, s, 4′-CH_3), 1.11 (6 H, d, J = 1.64 Hz, 2 7′-CH_3), 1.27 (3 H, t, J = 7.4 Hz, 2-SCH_2CH_3), 1.6–2.0 (3 H, m, 5′-CH_2 and 6′-C*H*), 2.54 (3 H, m, 6′-C*H* and 2-SCH_2), 2.67 (2 H, d, J = 6.6 Hz, 2-CH_2), 3.52 (1 H, m, J = 6.4 Hz, 1-C*H*), and 6.78 (1 H, br s, 1-N*H*); see Figure 5F. Exact Mass Calcd for $C_{14}H_{24}NO_3S$: 286.1477. Found: 286.1456.

Supplementary Material Available

Figures showing the elution profile of L-methionine decarboxylase from DEAE-Sephacel and TSK DEAE-5PW columns (2 pages). Ordering information is given on any current masthead page.

References

Akhtar, M., & Gani, D. (1987) *Tetrahedron 43*, 5341–5349.

Akhtar, M., Emery, V. C., & Robinson, J. A. (1984) in *The Physical and Organic Basis for Biochemistry* (Page, M. I., Ed.) p 303, Elsevier, Amsterdam, The Netherlands.

Akhtar, M., Stevenson, D. E., & Gani, D. (1990) *Biochemistry* (second paper of three in this issue).

Ando-Yamamoto, M., Hayashi, H., Sugiyama, T., Iukui, H., Watanabe, T., & Wada, H. (1987) *J. Biochem. (Tokyo) 101*, 405–414.

Asada, Y., Tanizawa, K., Sawada, S., Suzuki, T., Misono, H., & Soda, K. (1981) *Biochemistry 20*, 6681–6886.

Barlos, K., Papaioannou, D., Cordopatis, P., & Theodoropoulos, D. (1983) *Tetrahedron 39*, 475–478.

Bast, E., Hartmann, T., & Steiner, M. (1971) *Arch. Mikrobiol. 79*, 12–24.
Catch, J. R., Cook, A. H., Graham, A. R., & Heilbron, I. (1947) *J. Chem. Soc.*, 1609–1612.
Choi, S. Y., & Churchich, J. E. (1986) *Eur. J. Biochem. 160*, 515–520.
Dunathan, H. C. (1966) *Proc. Natl. Acad. Sci. U.S.A. 55*, 712–716.
Dunathan, H. C., Davis, L., Kury, P. G., & Kaplan, M. (1968) *Biochemistry 7*, 4532–4537.
Eisenthal, R., & Cornish-Bowden, A. (1974) *Biochem. J. 139*, 715–720.
Everleth, D. D., Gietz, R. D., Spencer, C. A., Nargang, F. E., Hodgetts, R. B., & Marsh, J. L. (1986) *EMBO J. 5*, 2663–2672.
Fecker, L. F., Beier, H., & Berlin, J. (1986) *Mol. Gen. Genet. 203*, 177–184.
Floss, H. G., & Vederas, J. C. (1982) in *Stereochemistry* (Tamm, C., Ed.) p 161, Elsevier, Amsterdam, The Netherlands.
Gani, D. (1985) *Annu. Rep. Prog. Chem., Sect. B 82*, 287–310.
Gani, D. (1986) *Annu. Rep. Prog. Chem., Sect. B 83*, 303–330.
Greenstein, J. P., & Winitz, M. (1961) *Chemistry of the Amino Acids*, Vol. 3, John Wiley & Sons, Inc., New York.
Haddox, M. K., & Russell, D. H. (1981) *Biochemistry 20*, 6721–6729.
Hartmann, T., (1972) *Phytochemistry 11*, 1327–1336.
Hartmann, T., Bax, K., & Scholz, R. (1984) *Z. Naturforsch. 39C*, 24–30.
Jung, M. J., Metcalf, B. W., Lippert, B., & Casara, P. (1978) *Biochemistry 17*, 2628–2632.
Julin, D. A., & Kirsch, J. R. (1989) *Biochemistry 28*, 3825–3833.
Julin, D. A., Heinrich, W., Toney, M. D., & Kirsch, J. F. (1989) *Biochemistry 28*, 3815–3821.
Kelland, J. G., Palcic, M. M., Pickard, M. A., & Vederas, J. C. (1985) *Biochemistry 24*, 3263–3267.
Kirsch, J. F., Eichele, G., Ford, G. C., Vincent, M. G., Jansonius, J. N., Gehring, H., & Christen, P. (1984) *J. Mol. Biol. 174*, 497–525.
Kobayashi, Y., Kaufman, D. L., & Tobin, A. J. (1987) *J. Neurosci. 7*, 2768–2772.
Kochi, J. K. (1965) *J. Am. Chem. Soc. 87*, 2500–2502.
Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.
Martin, C., Cami, B., Yeh, P., Stragier, P., Parsot, C., & Patte, J.-C. (1988) *Mol. Biol. Evol. 5*, 549–559.
Metzler, D. E., Olivard, J., & Snell, E. E. (1954) *J. Am. Chem. Soc. 76*, 644–648.
Misono, H., Kawabata, Y., Toyosato, M., Yamamoto, T., & Soda, K. (1980) *Bull. Inst. Chem. Res. Kyoto Univ. 58*, 323–333.
Morino, Y., & Nagashima, F. (1984) *Methods Enzymol. 106*, 116–137.
No, Z., Sanders, C. R., Dowhan, W., & Tsai, M.-D. (1988) *Bioorg. Chem. 16*, 184–188.
O'Leary, M. H. (1977) in *Bioorganic Chemistry: Enzyme Action* (van Tamelen, E. E., Ed.) Vol. 1, p 259, Academic Press, New York.
O'Leary, M. H., & Baughn, R. L. (1977) *J. Biol. Chem. 252*, 7168–7173.
O'Leary, M. H., Yamada, H., & Yapp, C. J. (1981) *Biochemistry 20*, 1476–1481.
Porter, T. G., Spink, D. C., Martin, S. B., & Martin, D. L. (1985) *Biochem. J. 231*, 705–712.
Spector, T. (1978) *Anal. Biochem. 86*, 142–146.
Stevenson, D. E., Akhtar, M., & Gani, D. (1986) *Tetrahedron Lett. 27*, 5661–5664.
Stevenson, D. E., Akhtar, M., & Gani, D. (1990) *Biochemsitry* (third paper of three in this issue).
Sukhareva, B. S. (1986) in *Vitamin B_6 Pyridoxal Phosphate: Chemical, Biochemical and Medical Aspects* (Dolphin, D., Poulson, R., & Avramovic, O., Eds.) Part B, p 325, Wiley-Interscience, New York.
Tobler, H. P., Gehring, H., & Christen, P. (1987) *J. Biol. Chem. 262*, 8985–8989.
Vaaler, G. L., Brasch, M. A., & Snell, E. E. (1986) *J. Biol. Chem. 261*, 11010–11014.
Voet, J. G., Hindenlang, D. M., Blanck, T. J. J., Ulevitch, R. J., Kallen, R. G., & Dunathan, H. C. (1973) *J. Biol. Chem. 248*, 841–842.
Yamada, H., & O'Leary, M. H. (1977) *J. Am. Chem. Soc. 99*, 1660–1661.
Yang, B. J., & Metzler, D. E. (1979) *Methods Enzymol. 62*, 528–557.