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STRUCTURAL AND STEREOCHEMICAL STUDIES OF METHIONINE DECARBOXYLASE FROM DRYOPTERIS FELIX-MAS

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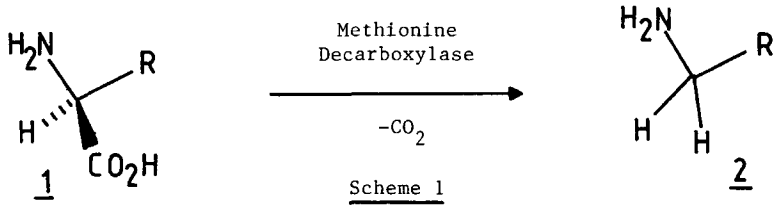
Summary Each diastereomer of N-(-)-camphanoyl-(3-methylthio)-1-aminopropane chirally deuteriated at C-1 has been synthesized and compared to deuteriated samples derived from L-methionine via enzymic decarboxylation. Here we report that decarboxylation occurs in a retentive mode.

The mechanisms by which pyridoxal 5'-phosphate (PLP) dependent enzymes operate have been subject to much investigation over recent years[1-3] and now for one group, the transaminases, a fairly detailed picture of the catalytic process can be envisaged.[4] On the other hand, for decarboxylases, an important group of enzymes which are responsible for the biosynthesis of several pharmacologically active amines, much less is known.[3]

Methionine decarboxylase[5,6] catalyses the conversion of L-methionine (1,R=$(CH_2)_2SMe$) into 3-methylthio-1-aminopropane (2,R=$(CH_2)_2SMe$).



1 2

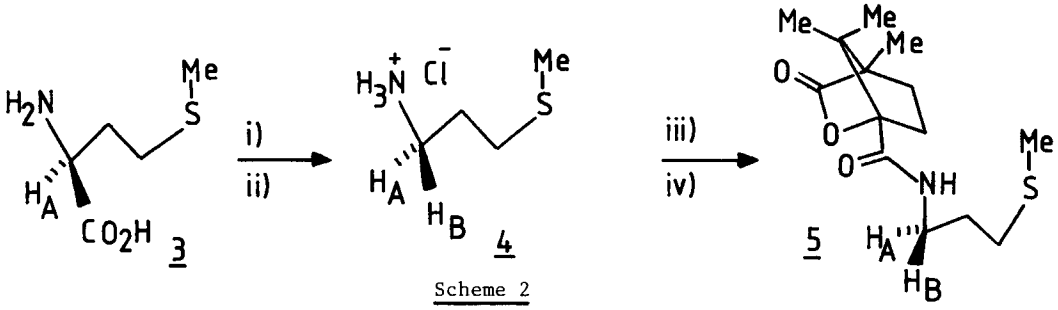
Scheme 1

Recently we have determined that the enzyme from the Male fern, Dryopteris felix-mas is particularly useful for mechanistic study as the enzyme shows a wide substrate tolerance, Table 1, and is readily available.[7]

To continue our studies we wished to determine the stereochemical course of the decarboxylation reaction and thus needed to develop a chirality assay for each of the protons at the prochiral C-1 centre of the product. Accordingly, L-methionine (3,H_A=H) was incubated with crude enzyme extracts at pH 4.5 to give 3-methylthio-1-aminopropane in good yield. The product was purified on Dowex 1x8 OH and was then converted to the hydrochloride salt (4, H_A=H_B=H) in 65% overall yield, δ_H (360 MHz, ^2H_2O) 2.92 (2H, t, J 7.5 Hz, 1-H_2); 2.42 (2H, t, J 7.5 Hz, 3-H_2); 1.92 (3H, s, S-Me); 1.78 (2H, qnt, J 7.5 Hz, 2-H_2); m/z (EI); 105(M^+). Conversion of the salt to the free base followed by reaction with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide, (1S,4R)-(-)-camphanic acid and triethylamine in acetonitrile gave the amide (5, H_A=H_B=H) in 43% yield as an oil after an aqueous work-up and chromatographic purification on flash silica, m/z (EI); 285 (M^+); 238 ([M-CH_3S]$^+$); $[\alpha]_D^{20}$-16.72 (c 0.6, $CHCl_3$).

Table 1 Relative Rates of Decarboxylation Measured Electromanometrically at pH 4.5, 30°C

Substrate (at 37.5 mM), see Scheme 1	Relative Rate
L-Methionine; 1,R=CH_2CH_2SMe	100
L-Ethionine; 1,R=CH_2CH_2SEt	0
DL-Methionine; -	25
D-Methionine; -	0
O-Ethyl L-Serine;[8] 1,R=CH_2OEt	0
L-Norvaline; 1,R=CH_2CH_2Me	35
L-Leucine; 1,R=CH_2CHMe_2	30
L-Isoleucine; 1,R=CHMeEt	30
L-Norleucine; 1,R=$CH_2CH_2CH_2Me$	15
L-Valine; 1,R=$CH(Me)_2$	15
γ-Ethyl L-glutamate; 1,R=$CH_2CH_2CO_2Et$	0
β-Methyl L-aspartate;[9] 1,R=CH_2CO_2Me	0
β-Ethyl L-aspartate;[9] 1,R=CH_2CO_2Et	0

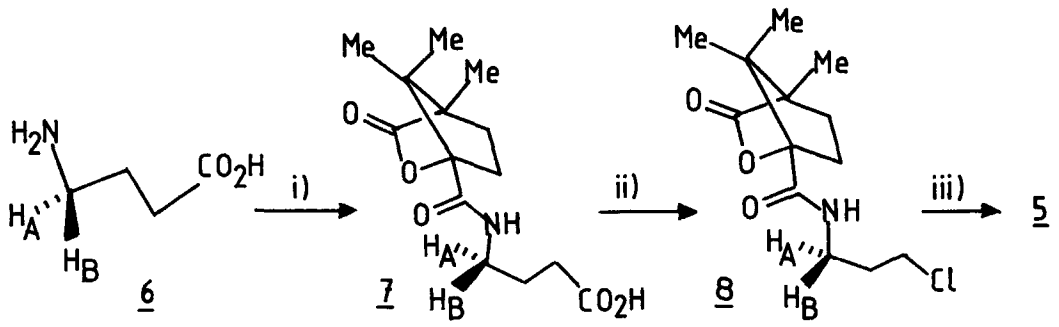


Scheme 2

i) Methionine decarboxylase, PLP, pH 4.5; 25°C; ii) 6M HCl; iii) Na_2CO_3(aq); iv) (1S,4R) camphanic acid, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide, triethylamine, acetonitrile, 37°C.

The 360 MHz ^{1}H-nmr spectrum of the amide showed an AB-type coupling pattern for the diastereotopic C-1 protons centred at 3.37 ppm, (partial spectrum A) which we needed to assign by introducing deuterium stereospecifically into each position. As 4-aminobutyric acid (6) could be prepared with deuterium in either of the two C-4 positions,[10] we chose to use this compound as a starting material for the preparation of our reference standards.

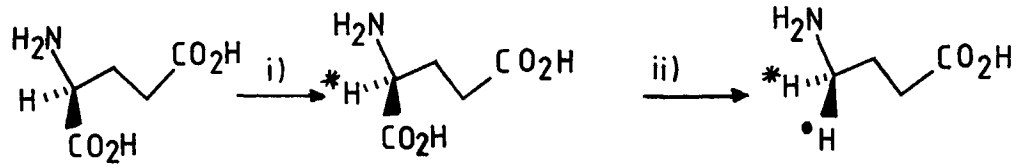
To synthesize the amide (5, H_A=H_B=H), 4-aminobutyric acid was converted quantitively to the N-camphanoyl derivative[11] (7, H_A=H_B=H), as outlined in Scheme 3, m.p. 92-5°C; m/z (EI) 283 (M^+); 237 ($[M-HCO_2H]^+$, $[\alpha]_D^{20}$-24.4° (c 1, $CHCl_3$). Chlorodecarboxylation[12] of the acid gave the 3-chloro- compound (8, H_A=H_B=H), m/z (EI); 275 and 273 (M^+, Cl isotopes), which was reacted directly with sodium methanethiolate in methanol to give the desired thioether (5, H_A=H_B=H) in 57% overall yield after chromatographic purification. This sample was identical in all respects to the enzymically derived compound.



Scheme 3

i) Camphanoyl chloride, toluene, NaOH(aq); ii) $Pb(OAc)_4$, LiCl, benzene, reflux; iii) NaSMe, MeOH, reflux.

In order to synthesize the chirally deuteriated reference samples (5, H_A= H, H_B=^{2}H), and (5, H_A=^{2}H, H_B=H), (4R)- and (4S)- [4-$^2H_1$]-4-aminobutyric acid (6, H_A=H, H_B=^{2}H) and (6, H_A=^{2}H, H_B=H) were prepared from (2S)- and (2S)-[2-$^2H_1$]glutamic acid respectively using commercial preparations of E.coli glutamate decarboxylase and the appropriately labelled water. This enzyme is known to catalyse decarboxylation with retention of configuration at C^{α}.[10] (2S)-[2-$^2H_1$]Glutamic acid was prepared through deuterium oxide exchange with the C^{α}-proton of (2S)-glutamic acid using commercial preparation of glutamic-oxaloacetic transaminase. The product was recrystallised from 6M HCl $[\alpha]_D^{20}$ +22.5° (c 0.4, 5M HCl); lit.[13] $[\alpha]_D^{25}$ +46.8° (c 2, 5M HCl) for undeuteriated free base.



Scheme 4

i) Glutamic-oxalacetic transaminase, $^{*}H_2O$, 25°C; ii) Glutamate decarboxylase, $^{\bullet}H_2O$, 25°C.

The chirally deuteriated samples of 4-aminobutyric acid were then converted into the thioether camphanamides (5, H_A=H, H_B=^{2}H) and (5, H_A=^{2}H, H_B=H) according to Scheme 3. The 360 MHz ^{1}H-nmr spectrum of the (1R)-diastereomer showed a signal at 3.41 ppm (partial spectrum B) while the (1S)-diastereomer showed a signal at 3.35 ppm (partial spectrum C).

When (2S)-methionine was incubated with the fern enzyme in deuterium oxide and the product was derivatised as outlined in Scheme 1, the ^{1}H-nmr spectrum of the derivative was identical to that of the camphanamide (5, H_A=H, H_B=^{2}H) (partial spectrum D). On the other hand the derivative of the product of the decarboxylation of (2S)-[2-$^2H_1$]methionine[14] in water showed a spectrum identical to that of the camphanamide (5, H_A=^{2}H, H_B=H) (partial spectrum E).

These results clearly indicate that L-methionine decarboxylase from the fern Dryopteris felix-mas catalyses reaction with retention of configuration at C^{α}. cf [3]

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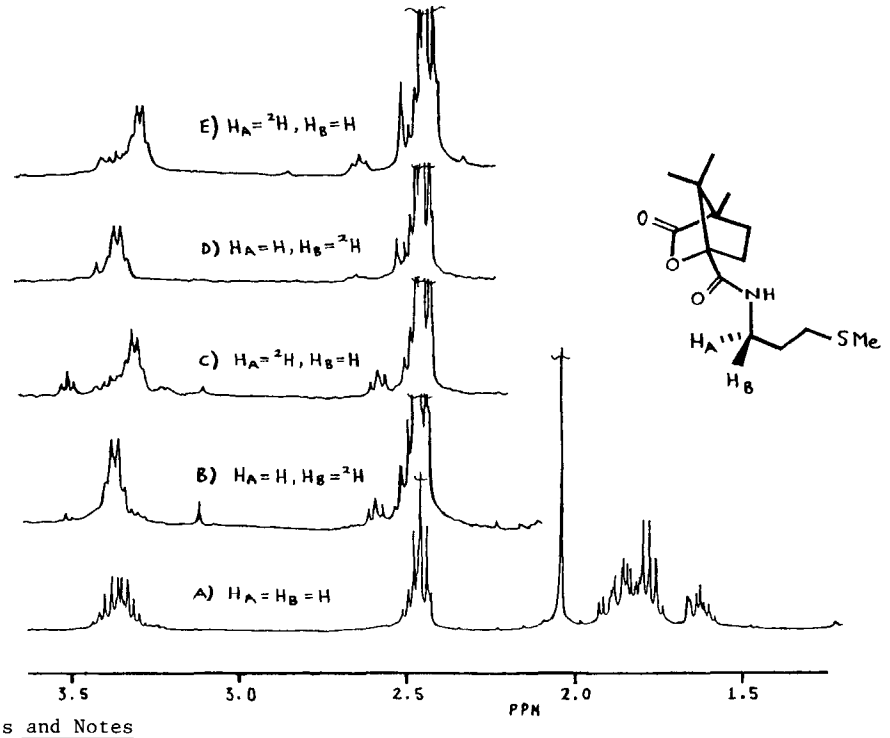

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14. (2S)-[2-$^2H_1$]Methionine was prepared via N-acetylation and base-catalysed racemisation[15] of L-methionine in the presence of deuterium oxide followed by selective deacylation of the L-antipode using acylase.[16] Purification on Amberlite IR45(OH) gave the desired product in 26% overall yield after recrystallisation from aqueous methanol, $[\alpha]_D^{20}$ +32.5° (C 0.4, 5M HCl), lit.[13] (for undeuteriated compound), $[\alpha]_D^{25}$ + 34.6° (C 1-2, 5M HCl). The sample contained better than 90 atom % deuterium at C-2 as judged by integration of the ^1H-nmr spectrum.
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