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STEREOCHEMISTRY OF REACTIONS CATALYSED BY L-LYSINE DECARBOXYLASE AND DIAMINE OXIDASE

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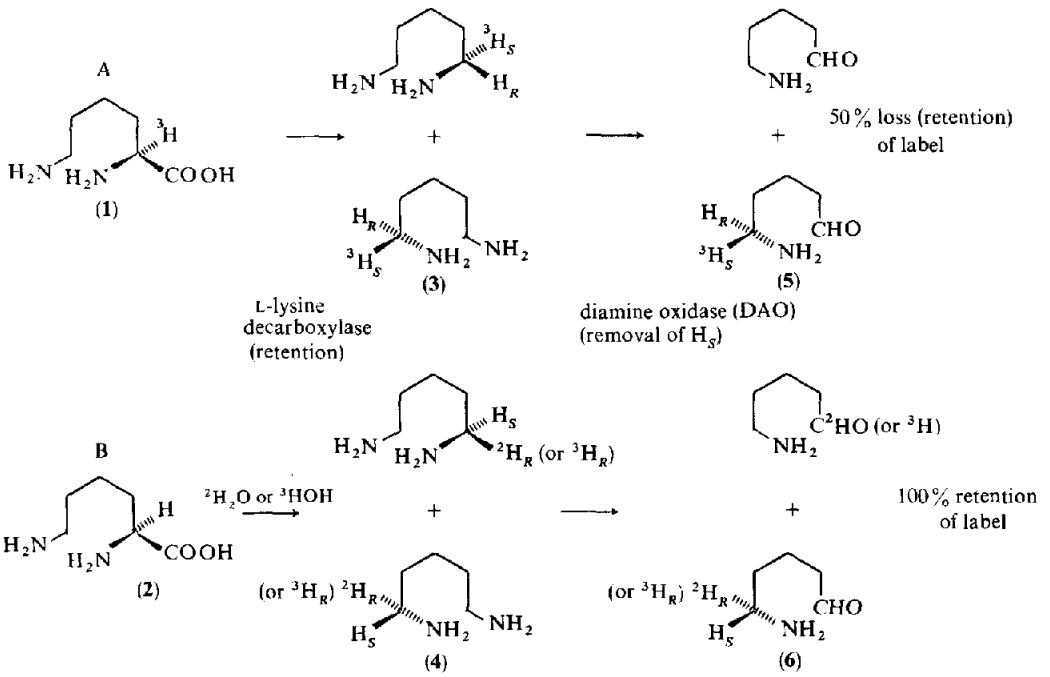
Key Word Index—*Bacillus cadaveris*; *Escherichia coli*; *Sedum acre*; *Pisum sativum*; L-lysine decarboxylase; diamine oxidase.

Abstract—The decarboxylation of L-lysine to yield cadaverine, catalysed by L-lysine decarboxylase (EC 4.1.1.18) takes place with retention of configuration. In the course of the dehydrogenation of cadaverine by diamine oxidase (EC 1.4.3.6) from pea seedlings, the pro-*S*-hydrogen is labilized.

INTRODUCTION

The decarboxylation of L-lysine and the deamination of the resulting cadaverine are likely to be key steps in the biosynthesis of piperidine alkaloids [1, 2]. While the stereochemical course of the decarboxylation of L-lysine by L-lysine decarboxylase (EC 4.1.1.18) from *Bacillus cadaveris* was found to proceed with retention of configuration [3], circumstantial evidence suggests that the decarboxylation of L-lysine in *Sedum* plants takes the opposite steric course [2]. Moreover, in a recent publication it had been demonstrated that the diamine oxidase from pea seedlings removes stereospecifically the pro-*S*-hydrogen atom from the methylene group of a primary amine [4]. In a previous publication, however, it had been shown that monoamine oxidase from rat liver removes the pro-*R*-hydrogen atom during deamination of tyramine [5].

The findings made it desirable to confirm both the results on the decarboxylation of L-lysine [3] and the deamination of a primary amine [4]. We have therefore prepared [3] enantiotopically labelled cadaverines by enzymatic decarboxlation of L-lysine and treated the cadaverines so obtained with diamine oxidase from pea seedlings. If the decarboxylation proceeds with retention of configuration and if the diamine oxidase from peas removes the pro-*S*-hydrogen of the amino group, activity from [2-^{3}H]-lysine should be removed from the substrate (Scheme 1, path A), whereas label which had been introduced into cadaverine during decarboxylation of L-lysine in the presence of ^2H_2O or HO^3H, should be retained (Scheme 1, path B).



Scheme 1. Stereospecificity of the reactions catalysed by L-lysine decarboxylase and diamine oxidase.

Table 1. Treatment of chirally labelled samples of cadaverine with diamine oxidase to yield pelletierine (Scheme 2)

Experiment No.	Substrate	$^3H/^{14}C$ ratios (expt 1) or atom % excess (expt 2)		Loss of isotopic H (%)
		Cadaverine	Pelletierine (13)	
1	(1*S*)-[1-^{3}H]cadaverine [1,5-^{14}C]cadaverine	10	5.5	45
2	(1*R*)-[1-^{2}H]cadaverine	85.7%	78.5%	8

The chirally labelled samples of cadaverine were obtained by decarboxylation of [2-^{3}H]-lysine (expt 1), and of L-lysine in ^2H_2O (expt 2), respectively using L-lysine decarboxylase from *B. cadaveris*.

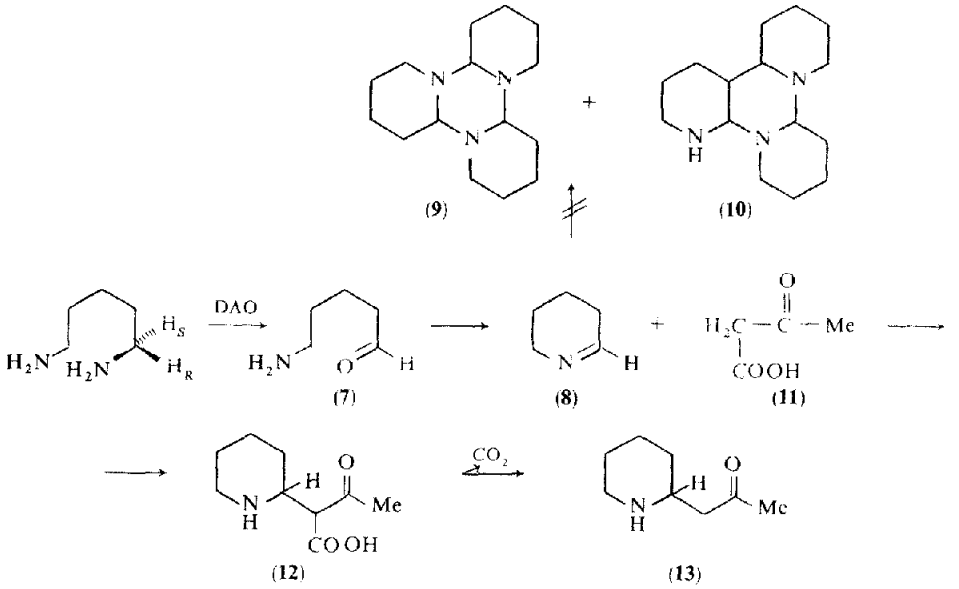
RESULTS AND DISCUSSION

Chirally labelled cadaverines were prepared by treatment of [2-^{3}H]-lysine (**1**) in H_2O, or L-lysine (**2**) in HO^3H or ^2H_2O with L-lysine decarboxylase [2, 3]. The L-lysine decarboxylase was derived from either *Escherichia coli* or *B. cadaveris*. The radioactive cadaverine (**3**, **4**) samples were mixed with [1,5-^{14}C]-cadaverine and the $^3H/^{14}C$ ratio determined by liquid scintillation counting. The deuterium content of the ^{2}H-labelled cadaverine (**4**) was determined by MS. The sample was found to contain 85.7 atom % excess deuterium (Table 1). The labelled cadaverine samples so obtained were incubated with a preparation [6] of diamine oxidase from pea seedlings. The resulting reaction product 5-aminopentanal (**5**–**7**) undergoes ring closure to give Δ^1-piperideine (**8**). This compound is known [7] to trimerize spontaneously yielding at least two isomers (**9**, **10**). In order to prevent formation of these isomers, acetoacetic acid (**11**) was included in the reaction mixture [8]. The hypothetical reaction product, a β-keto acid (**12**), has never been isolated, but decarboxylates spontaneously. When the reaction was terminated by acidification and heating, pelletierine (**13**) was isolated (Scheme 2), the identity of which was established by comparison (mp, MS, IR of the hydrochloride) with an authentic sample.

When [2-^{3}H]-lysine (**1**) was treated with L-lysine decarboxylase from *B. cadaveris*, the formation of (1-*S*)-[1-^{3}H]-cadaverine (**3**) was expected [3]. Since the cadaverine molecule is symmetrical, removal of only one amino group and only 50% of the tritium would occur if diamine oxidase reacts with cadaverine and removes the pro-*S*-hydrogen (Scheme 1). This was observed (Table 1) when the $^3H/^{14}C$ ratio of pelletierine (**13**) was determined.

In the complementary experiment in which (1-*R*)-[1-^{2}H]-cadaverine (**4**) was treated with diamine oxidase, the resulting pelletierine was found to contain essentially the same enrichment in deuterium (78.5% excess) as the original cadaverine (85.7% excess, i.e. 91.6% retention; Table 1).

In previous work the stereochemistry of the enzymic decarboxylation of an amino acid has been determined in 3 cases [3, 9, 10]. Samples of tyrosine, glutamic acid or lysine were decarboxylated with the requisite decarboxylase in the presence of an isotopic label. The chirality of the resulting chirally labelled amines, viz. tyramine, γ-aminobutyric acid or cadaverine, was then determined using different techniques. A kinetic method was employed for the analysis of tyramine [9]. The chirality of the γ-aminobutyric acid was carried out by measurement of the optical rotation of a derivative, methyl 4-phthalimido-[4-^{2}H]-butyrate [10]. The chiral-



Scheme 2. Method employed for the analysis of chirally labelled cadaverine. DAO = diamine oxidase.

Table 2. Application of chirally labelled cadaverines in admixture with [1,5-^{14}C]-cadaverine to cuttings of *Sedum acre*

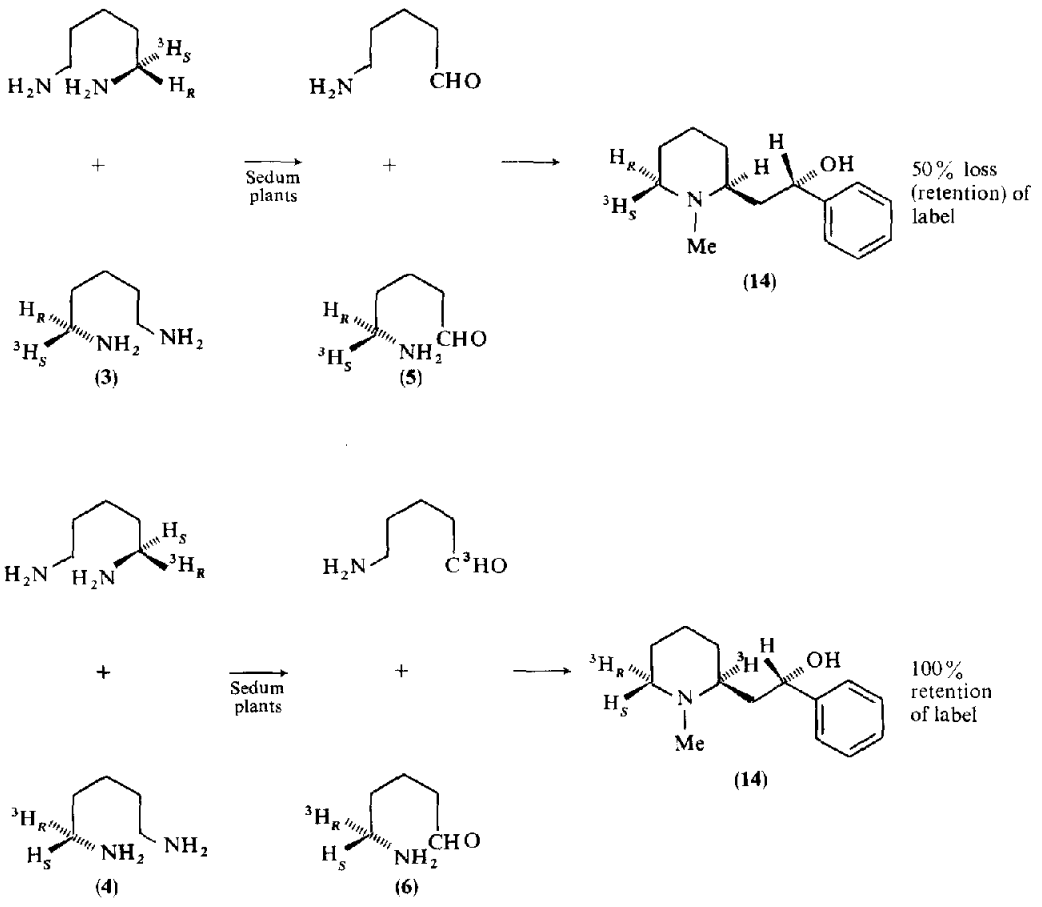
Expt No.	Substrate	Cadaverine administered	$^{3}H/^{14}C$ ratios of Cadaverine reisolated	Sedamine	Loss of ^{3}H relative to ^{14}C (%)
3	(1*S*)-[1-^{3}H]-cadaverine	10.0	9.5	5.3	47
4	(1*R*)-[1-^{3}H]-cadaverine	10.0	12.1	10.6	no loss
5	(1*S*)-[1-^{3}H]-cadaverine	10.0	10.3	5.2	48
6	(1*R*)-[1-^{3}H]-cadaverine	10.0	9.7	12.1	no loss

The chirally labelled samples were obtained by decarboxylation of [2-^{3}H]-lysine (expts 3 and 5) and of L-lysine in $HO^{3}H$ (expts 4 and 6), respectively, using L-lysine decarboxylase from *B. cadaveris* (expts 3 and 4) or *E. coli* (expts 5 and 6).

ity of the chirally labelled cadaverine was determined by degradation to glycine and analysis of the products obtained when this amino acid was incubated with L-alanine amino transferase (EC 2.6.1.2) and D-amino acid oxidase (EC 1.4.3.3) [3].

Since the steric course of the dehydrogenation of a primary amine by diamine oxidase from peas has been determined recently [4], another method thus became available by which the steric course of decarboxylation of an amino acid could be determined. The results are described in the present paper. In each case decarboxylation of the amino acids proceeded with retention of configuration. This is in agreement with current concepts on the stereobiochemistry of pyridoxal phosphate catalysed reactions [11]. It is believed that bond breaking and bond making occur on the same face of the amino acid–pyridoxal phosphate adduct. The data reported here are consistent with this view.

Since the decarboxylation of L-lysine has now been shown to proceed with retention by two different methods (*vide supra* and [3]), one of which employed the diamine oxidase from peas, the results reported here also confirm the finding that diamine oxidase from peas removes H_S during deamination. This result is at variance with the stereochemistry of the reaction catalysed by monoamine oxidase from rat liver [5].



Scheme 3. Incorporation of chirally labelled cadaverine (**3**, **4**) into sedamine (**14**) in *Sedum acre*.

Further confirmation of the observation that diamine oxidase labilizes the pro-*S*-hydrogen, and that decarboxylation proceeds with retention has been obtained using intact *Sedum* plants. Diamine oxidase is likely to be involved in the biosynthesis of sedamine [2]. During the conversion to sedamine of chirally labelled cadaverines applied to cuttings of *Sedum* plants, the sedamine derived from samples of (1-*R*)-[1-^{3}H)-cadaverine (expts 4 and 6) retained the $^3H/^{14}C$ ratio of the substrate, whereas that derived from samples of (1-*S*)-[1-^{3}H]-cadaverine (expts 3 and 5) lost *ca* 50% of the tritium relative to ^{14}C. This shows that again the pro-*S*-hydrogen was removed whereas the pro-*R*-hydrogen was retained (Table 2, Scheme 3).

The samples of chirally labelled cadaverine were obtained by decarboxylation of L-lysine with L-lysine decarboxylase from two sources, *E. coli* and *B. cadaveris*. Essentially the same results were obtained (Table 2). Thus decarboxylation of L-lysine proceeds with retention in *E. coli* as well as *B. cadaveris*.

The chirality of the conversion of (1-*R*)-[1-^{3}H]-cadaverine and (1-*S*)-[1-^{3}H]-cadaverine to sedamine also sheds light on a problem related to the stereochemistry of the decarboxylation of lysine in *Sedum* plants. When (2-*S*)-[2-^{3}H]-lysine is converted to sedamine in *Sedum acre* the label is retained [12]. But when (2-*S*)-[2-^{3}H]-lysine is first decarboxylated by bacterial L-lysine decarboxylase and the resulting chirally labelled cadaverine (viz. (1-*S*)-[1-^{3}H]-cadaverine) then applied to *Sedum* plants, the label is removed ([2] and Scheme 3). This suggests that the decarboxylation in *Sedum* plants takes the opposite steric course when compared to the bacterial decarboxylase. Since we know that the bacterial enzyme decarboxylates with retention of configuration, one may conclude that the enzyme from *Sedum* decarboxylates with inversion. This, however, would be at variance with the current concept [11] on the stereochemistry of pyridoxal phosphate catalysed reactions.

The experimental data would be in agreement with this concept as well as the experimental data discussed here if it is assumed that D-lysine rather that L-lysine is decarboxylated with retention and the resulting cadaverine then converted to the piperidine alkaloid. Yet it had been shown conclusively that L-lysine rather than D-lysine is the precursor of piperidine alkaloids [1]. Another possibility, however, remains to be considered. One may assume that the decarboxylation of L-lysine in *Sedum* plants is not catalysed by an enzyme which uses pyridoxal phosphate as a cofactor. This, however, would invalidate a hypothesis in which pyridoxal phosphate plays a key rôle in the biosynthesis of piperidine alkaloids [2]. Since none of the 3 obvious assumptions on the biosynthesis of the piperidine nucleus seems to be correct, a problem arises which may only be solved by *in vitro* experiments using cell free extract isolated from *Sedum* plants.

EXPERIMENTAL

Sedum acre plants were grown in a growth chamber and feeding was carried out as described in ref. [1].

Enzymes. L-lysine decarboxylase from *B. cadaveris* (Type II) and *E. coli* (Type IV) were purchased from Sigma. Diamine oxidase was extracted from pea seedlings and purified up to step 4 as described in ref. [6].

Chirally labelled cadaverines. Radioactive samples were prepared as described in refs. [2, 3]. Deuterated cadaverine was prepared by decarboxylation of L-lysine (monoHCl) (100 mg) in ^2H_2O (5 ml, 99.75 atom % excess D) containing K_2HPO_4 (31 mg), KH_2PO_4 (194 mg) and L-lysine decarboxylase (9 mg) from *B. cadaveris*. After acidification (HCl) and heating, the cadaverine was isolated by PC (*iso*PrOH–NH_3–H_2O, 8:1:1, R_f cadaverine = 0.5) and crystallized from MeOH–Et_2O. Yield 36 mg (as diHCl).

MS. The deuterated samples of the HCl of cadaverine and pelletierine were analysed by MS at 70 eV (inlet temp. 200°). For determination of the isotope content of cadaverine, the peaks at *m/e* 85/86 representing the Δ^1-piperideine fragment [13] and containing 75% of the label of (1-*R*)-[1-^{2}H]-cadaverine was measured repeatedly (×10) and the isotope content calculated according to ref. [14]. For determination of the isotope content of pelletierine, the M^+ (*m/e* 141/142) was measured ×10 and the isotope content calculated as before.

Deamination with diamine oxidase. To the doubly labelled cadaverine (expt 1, Table 1) carrier (10 mg, diHCl) was added and the mixture dissolved in H_2O (1 ml). A soln (0.2 ml) containing Na acetoacetate (170 mg), K-Pi buffer (0.01 M, pH 7.6, 5 ml) and diamine oxidase (2 ml) derived from pea seedlings (100 g) [6] were added. The mixture was kept at 37° for 65 hr and the reaction terminated by acidification (HCl) and heating. The protein was removed by centrifugation, KOH added to the supernatant and the soln extracted with Et_2O. The Et_2O was dried, evapd, and the pelletierine isolated by TLC (Si gel; CH_2Cl_2–MeOH–NH_3, 50:20:3, R_f 0.8, yield 28%). Inactive pelletierine HCl (10 mg) as well as phenylisothiocyanate (10 mg) [15] was added to the eluate. The ppt. was recrystallized (EtOH), the crystals (mp 135°) collected and the $^3H/^{14}C$ ratio determined.

The deuterated cadaverine diHCl (expt 2, Table 1) (36 mg) was incubated similarly with H_2O (10 ml), buffer (10 ml), diamine oxidase soln (5 ml) and Na acetoacetate (255 mg). Pelletierine monoHCl was isolated, crystallized (MeOH, Et_2O) and the D content determined by MS [16].

Isolation of sedamine (**14**) *and reisolation of cadaverine.* This was carried out as described previously [2].

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