Phytochemistry, Vol 22, No 5, pp 1133–1135, 1983
Printed in Great Britain

0031–9422/83/051133–03$03 00/0

STEREOCHEMISTRY OF REACTIONS CATALYSED BY ARGININE DECARBOXYLASE AND ORNITHINE DECARBOXYLASE

DAVID J ROBINS

Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, U K

(*Received* 19 *August* 1982)

Key Word Index—*Escherichia coli*; *Pisum sativum*, Leguminosae, arginine decarboxylase, ornithine decarboxylase, diamine oxidase

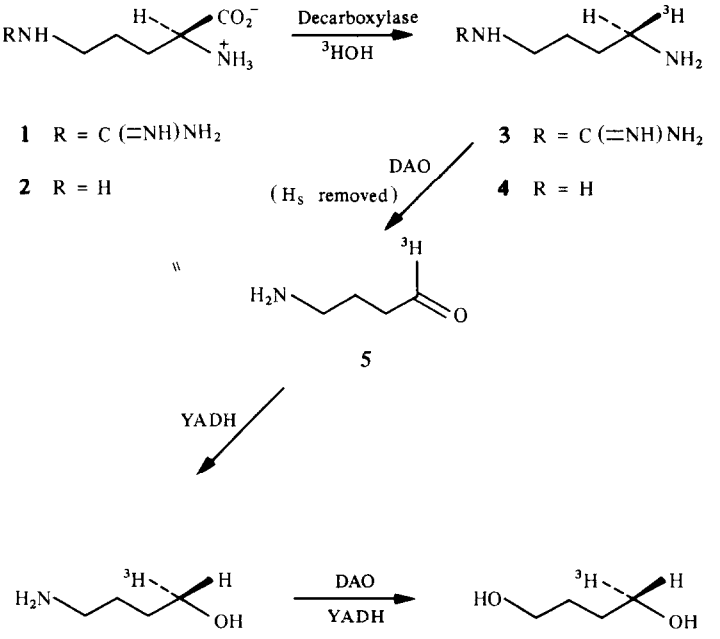
Abstract—The decarboxylations of L-arginine, catalysed by arginine decarboxylase (EC 4 1.1 19) and of L-ornithine, catalysed by ornithine decarboxylase (EC 4 1 1 17), both take place with retention of configuration

INTRODUCTION

Decarboxylation of basic α-amino-acids, such as lysine, arginine, and ornithine, followed by oxidation of the resulting diamines are key steps in the biosynthesis of many alkaloids [1]. We are interested in the stereochemistry of the conversion of L-arginine (**1**) and L-ornithine (**2**) into putrescine (1,4-diaminobutane) and the pyrrolizidine alkaloids [2]

The decarboxylation of L-lysine, catalysed by lysine decarboxylase with pyridoxal phosphate as co-factor, has been shown to proceed stereospecifically with retention of configuration [3–5], to yield cadaverine (1,5-diaminopentane) Chirally labelled samples of cadaverine were prepared by decarboxylation of L-lysine in ^2H_2O or ^3HOH using lysine decarboxylase. The new chiral centre was then assayed with the diamine oxidase (EC 1 4 3 6) from pea seedlings This enzyme is known to remove the *pro*-S hydrogen atom stereospecifically from the methylene group of the primary amine [6, 7] (see Scheme 1)

In their work, Gerdes and Leistner [4] observed 92% retention of ^2H on treatment of one chiral [1-^{2}H]cadaverine with diamine oxidase, indicating that the absolute configuration of this sample was (1*R*) (cf



DAO = Diamine oxidase

YADH = Yeast alcohol dehydrogenase

Scheme 1

Scheme 1) The retention of ^3H was 55% when [1-^3H]cadaverine of opposite absolute configuration was oxidized with diamine oxidase (50% loss of ^3H would be expected from the symmetrical diamine on oxidation of (1*S*)-[1-^3H]cadaverine, in the absence of any isotope effect)

Battersby and co-workers recently carried out similar enzymic experiments [5], in which they converted their enantiomeric samples of cadaverine into 5-aminopentan-1-ol Both samples of this material were found to have retained most (> 95%) of the ^3H They attributed this result for the (1*S*)-[1-^3H]cadaverine sample to the operation of a substantial isotope effect which favours oxidation of the diamine at the unlabelled end This problem was overcome by oxidizing both amino groups of each enantiomeric [1-^3H]cadaverine sample in the presence of yeast alcohol dehydrogenase (EC 1 1 1 1) and ethanol, yielding pentane-1,5-diol Nearly all the ^3H was retained in the diol obtained from one chiral cadaverine sample, whereas the enantiomeric material lost virtually all its ^3H This clearly established the absolute configuration of the two [1-^3H]cadaverine samples as (1*R*) and (1*S*), respectively (cf Scheme 1)

Initially, we attempted to use the method of Gerdes and Leistner [4] to establish the stereochemistry of the enzymic decarboxylations of arginine and ornithine, but we encountered the same problem of high ^3H retention on enzymic oxidation of the enantiomeric samples of putrescine We, therefore, adapted the procedure of Battersby and co-workers [5] for the current study

RESULTS AND DISCUSSION

Chirally labelled [1-^3H]putrescine (**4**) was prepared by the action of ornithine decarboxylase on L-ornithine (**2**) in the presence of ^3HOH (Scheme 1) In order to make the enantiomeric material, L-ornithine was racemized by heating in acid solution containing ^3HOH [8] The DL-[2-^3H]ornithine so produced was not resolved before incubation with ornithine decarboxylase, because this enzyme is known to be specific for the L-isomer [9] Each sample of [^3H]putrescine was mixed with [1,4-$^{14}C_2$]putrescine to give a $^3H/^{14}C$ ratio of *ca* 10 Aliquots of each sample were diluted with inactive putrescine, and the *bis*-benzoyl derivatives were prepared and recrystallized to constant $^3H/^{14}C$ ratio (Table 1, experiments 1 and 2)

Enantiomeric samples of [1-^3H]putrescine were also obtained from arginine Decarboxylation of L-arginine (**1**) with arginine decarboxylase in ^3HOH yielded [1-^3H]agmatine (**3**) The enantiomeric material was prepared by decarboxylation of DL-[2-^3H]arginine [8] with arginine decarboxylase Again, the DL-arginine was not resolved because this enzyme is specific for the L-isomer [9] The samples of chirally labelled [1-^3H]agmatine were hydrolysed to putrescine [10] and each sample of [1-^3H]putrescine was then treated, as outlined above, to furnish doubly labelled material (Table 1, experiments 3 and 4)

Each sample of [1,4-$^{14}C_2$,1-^3H]putrescine was incubated with diamine oxidase isolated from pea seedlings [11] The product of this enzymic reaction is 4-aminobutyraldehyde (**5**) which cyclizes spontaneously to 1-pyrroline However, in the presence of yeast alcohol dehydrogenase and ethanol, the aldehyde (**5**) is reduced to 4-aminobutan-1-ol This amine is also a substrate for diamine oxidase and after incubation for an extended period the major product is butane-1, 4-diol (Scheme 1) Incubation of each of the four samples of doubly labelled putrescine previously obtained (Table 1) yielded butane-1, 4-diol, isolated and characterized as the *bis*-*p*-nitrobenzoyl derivative The $^3H/^{14}C$ ratio for each product was measured (Table 1) The almost complete retention of ^3H in experiments 1 and 3 demonstrates that these samples of putrescine are (1*R*) (**4**), whereas the virtually complete loss of ^3H in experiments 2 and 4 shows that these putrescine samples are (1*S*) Thus, the enzymic decarboxylations of both arginine and ornithine proceed stereospecifically with retention of configuration

Results published in preliminary form after these studies were complete led to similar conclusions about the stereochemistry of the reactions involving arginine and ornithine decarboxylases, although different methods were employed [12, 13]

EXPERIMENTAL

All radiochemicals were purchased from the Radiochemical Centre, Amersham Activities of ^{14}C and ^3H were measured by liquid scintillation counting using toluene MeOH solns Sufficient counts were taken to give a s e of less than 1% for each determination The enzymes were obtained from Sigma, except

Table 1 Incubations of samples of chirally labelled putrescine with diamine oxidase

Expt No	Origin of putrescine	$^3H/^{14}C$ ratios: Putrescine*	$^3H/^{14}C$ ratios: Butane-1,4-diol† (^3H retention %)
1	L-Ornithine in ^3HOH	9 2	8 6 (94)
2	DL-[2-^3H]Ornithine	9 9	0 4 (4)
3	L-Arginine in ^3HOH	8 7	8 4 (96)
4	DL-[2-^3H]Arginine	10 5	0 3 (3)

The samples of chirally labelled putrescine were obtained by enzymic decarboxylation of ornithine (experiments 1 and 2), or by enzymic decarboxylation of arginine followed by hydrolysis of the resulting agmatine (experiments 3 and 4)

*Counted as its *bis*-benzoyl derivative

†Counted as its *bis*-*p*-nitrobenzoyl derivative

for diamine oxidase which was extracted from pea seedlings and purified up to step 4 as described in ref [11]

Expt 1 (1R)-[1-^{3}H]*Putrescine* (**4**) *from* L-*ornithine* (**2**) L-Ornithine hydrochloride (16 9 mg, 0 1 mmol) in acetate buffer (0.2 M, pH 5 0, 1 ml) containing ^{3}HOH (100 mCi) was decarboxylated with ornithine decarboxylase (5 units) at 37° After 18 hr the mixture was acidified with 5 M HCl (1 ml), and evaporated to dryness *in vacuo* The residue was purified by TLC on cellulose-coated plates developed in *iso*-PrOH–conc NH_3 (5.3) The band corresponding to putrescine, R_f 0 55, was eluted with MeOH and converted into the dihydrochloride of (1*R*)-[1-^{3}H]putrescine (**4**), 6.3 mg (39 %), 76 7 μCi/mmol. [1,4-$^{14}C_2$]Putrescine dihydrochloride (42.7 mg, 0 3 μCi) was added to an aq. soln (1 ml) of this sample and an aliquot (0 1 ml) was further diluted with inactive putrescine dihydrochloride and converted into the dibenzoate of (1*R*)-[1,4-$^{14}C_2$,1-^{3}H]putrescine, and crystallized (EtOH) to constant ^{3}H/^{14}C ratio, mp 178 5–179° (lit [14] mp 179–180°)

Expt 2 (1S-[1-^{3}H]*Putrescine from* L-*ornithine* (**2**). L-Ornithine hydrochloride (67 4 mg, 0.4 mmol) was racemized in acid soln containing ^{3}HOH (100 mCi) as described in ref [8] The product was purified by TLC, as in expt 1, R_f 0 25, and converted into DL-ornithine [2-^{3}H]hydrochloride, 39 mg (58 %), 186 4 μCi/ mmol This material was decarboxylated as in expt 1, but omitting the ^{3}HOH from the incubation, to give (1*S*)-[1-^{3}H]putrescine dihydrochloride, 6.1 mg (36 % based on the L-isomer), 162 9 μCi/mmol Doubly labelled material was prepared and the ^{3}H/^{14}C ratio measured as in expt 1

Expt 3 (1R)-[1-^{3}H]*Putrescine* (**4**) *from* L-*arginine* (**1**) L-Arginine (52.2 mg, 0 3 mmol) in acetate buffer (0.2 M, pH 5.2, 2 ml) containing ^{3}HOH (100 mCi) was decarboxylated with arginine decarboxylase (25 units) at 37° After 16 hr, the mixture was worked-up as in expt 1 (1*R*)-[1-^{3}H]Agmatine was obtained, R_f 0.4, 14 7 mg (35 %), 97.9 μCi/mmol This material was hydrolysed [12] to give (1*R*)-[1-^{3}H]putrescine dihydrochloride, 7 2 mg (43 %), 96.3 μCi/mmol. Doubly labelled material was prepared and the ^{3}H/^{14}C ratio measured as in expt 1

Expt 4. (1S)-[1-^{3}H]*Putrescine from* L-*arginine* (**1**). L-Arginine (104.4 mg, 0 6 mmol) was racemized in acid soln containing ^{3}HOH (100 mCi) [8]. The product was purified by TLC as in expt 1, R_f 0 3, and converted into DL-[2-^{3}H]arginine hydrochloride, 84.8 mg (67 %), 117.3 μCi/mmol Decarboxylation of this material as in expt 3, omitting the ^{3}HOH, gave (1*S*)-[1-^{3}H]agmatine, 9.9 mg (35 % based on the L-isomer), 109 8 μCi/mmol This material was hydrolysed [12] to yield (1*S*)-[1-^{3}H]putrescine dihydrochloride, 5 4 mg (46 %), 109 1 μCi/mmol Doubly labelled material was purified and the ^{3}H/^{14}C ratio measured as in expt 1

Stereochemical assay of (1R)-[1,4-$^{14}C_2$,1-^{3}H]*putrescine* (1*R*)-[1,4-$^{14}C_2$,1-^{3}H]Putrescine dihydrochloride (100 mg, 0.062 mmol, expt 1) was incubated with diamine oxidase and yeast alcohol dehydrogenase as described in ref. [5], except that the incubation was extended to 48 hr The *bis-p*-nitrobenzoyl derivatives of the reaction products were prepared and separated [5] The major product was (1*R*)-[1,4-$^{14}C_2$,1-^{3}H]butane-1,4-diyl bis-(*p*-nitrobenzoate), 6.9 mg (28 %), mp 173–174° (lit. [15] mp 175°, mmp 174–175° with an authentic sample prepared from butane-1,4-diol and *p*-nitrobenzoyl chloride) The remaining samples of [1,4-$^{14}C_2$, 1-^{3}H]putrescine were assayed in a similar way and the derivative of butane-1,4-diol was recrystallized to constant ^{3}H/^{14}C ratio (Table 1)

REFERENCES

1 Herbert, R B. (1979) *Alkaloid Biosynthesis in Comprehensive Organic Chemistry* (Barton, D H R. and Ollis, W D., eds.) Vol 5, p 1072 Pergamon Press, Oxford
2 Robins, D J (1982) *Fortschr Chem Org. Naturst.* **41**, 115
3 Leistner, E. and Spenser, I D (1975) *J Chem Soc Chem Commun* 378.
4. Gerdes, H J. and Leistner, E. (1979) *Phytochemistry* **18**, 771
5 Battersby, A. R , Murphy, R and Staunton, J. (1982) *J. Chem. Soc Perkin Trans* 1, 449
6. Battersby, A R , Staunton, J and Summers, M C (1976) *J Chem Soc Perkin Trans* 1, 1052
7 Battersby, A. R , Staunton, J. and Tippett, J (1982) *J Chem Soc Perkin Trans* 1, 455
8. Garnett, J. L., Halpern, B and Kenyon, R S (1972) *J. Chem Soc Chem. Commun* 135
9 Robins, D. J. and Sweeney, J R (1983) *Phytochemistry* **22**, 457
10 Ono, K (1946) *J Chem Soc Jpn.* **67**, 132.
11 Hill, J. M (1971) in *Methods in Enzymology* (Tabor, H and Tabor, C W., eds) Vol XVII, Part B, p 730 Academic Press, New York.
12 Orr, G. R and Gould, S. J (1982) *Tetrahedron Letters* **23**, 3139
13. Wigle, I. D , Mestichelli, L J J and Spenser, I D (1982) *J Chem Soc Chem Commun* 662.
14 Veith, H J , Guggisberg, A. and Hesse, M (1971) *Helv. Chim Acta* **54**, 653
15 Carothers, W H. and Van Natta, F J (1930) *J Am. Chem Soc* **52**, 314