On the Stereochemistry of the Decarboxylation of (2*S*)-Histidine catalysed by Histidine Decarboxylase from *Clostridium welchii* (E.C. 4.1.1.22)

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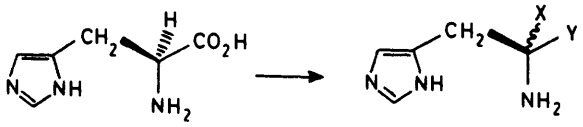
By decarboxylation of (2*S*)-histidine (1) in ^2H_2O in the presence of histidine decarboxylase from *Clostridium welchii*, monodeuteriated histamine (2a) was obtained. Ruthenium tetraoxide oxidation of (2a) furnished deuteriated β-alanine (3a), which was converted into the methyl ester of its phthaloyl derivative (6c). This compound showed a negative optical rotation, as did (6a) prepared from (3*R*)–(3c). From these results an (*R*)-configuration can be attributed to (2a) and consequently decarboxylation of (1) proceeds with retention of configuration.

WHEREAS most bacterial amino-acid decarboxylases are pyridoxal phosphate-dependent enzymes, histidine decarboxylase (histidine carboxy-lyase, E.C.4.1.1.22) does not require pyridoxal phosphate as coenzyme and contains a pyruvate residue covalently bound to the protein.[1] It had been previously shown that on decarboxylation of (2*S*)-histidine (1) in ^2H_2O in the presence of

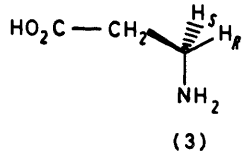


(1) (2) a; X = H, Y = ^{2}H
b; X = Y = H

histidine decarboxylase from Lactobacillus 30a, a single deuterium atom is stereospecifically introduced on the α-carbon to afford optically active monodeuteriohistamine (2a).[2] On the basis of the negative optical rotation of a sample of (2a), the authors suggested tentatively that an (*R*)-configuration could be assigned to the product, the decarboxylation having proceeded with retention of configuration.† Although very likely, this assignment was not established unequivocally, and we present in this paper our results concerning the absolute stereochemistry of (2a), using a purified preparation of histidine decarboxylase from *Clostridium welchii* (Sigma, Mo., U.S.A.).

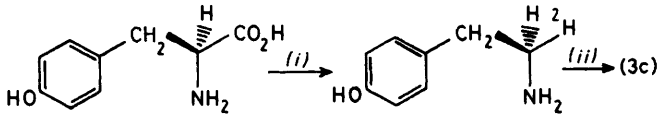
RESULTS AND DISCUSSION

Our plan was to transform (2a) into a deuteriated β-alanine, 3-aminopropanoic acid (3a), keeping intact the carbon atom which is chiral by isotopic substitution.



(3) a; H_R or H_S = ^{2}H
b; H_R = H_S = H
c; H_R = ^{2}H, H_S = H

Although the imidazole ring of (1) can undergo degradation by benzoylation and ozonolysis,[3] the same procedure on histamine (2b) did not afford (3b) in satisfactory yields for our purposes. Therefore we turned our attention to the ruthenium tetraoxide oxidation of aromatic amines, which proceeds in good yield when the aromatic group is a variously substituted benzene ring.[4] We have found that this method worked also on the imidazole moiety and from (2b) good yields of (3b) were obtained (60—70%). Furthermore, a reference compound of ascertained stereochemistry, *i.e.* (3*R*)-[3-^{2}H]-3-aminopropanoic acid (3c) could be, in principle, prepared by ruthenium tetraoxide oxidation of (1*R*)-[1-^{2}H]-tyramine (4). On the other hand, (2*S*)-tyrosine (5) could be stereospecifically decarboxylated in ^2H_2O with retention of configuration [5] and we outline the preparation of (3c) from (5) in Scheme 1. In fact, (5) was decarboxylated in ^2H_2O in the presence of commercially available tyrosine decarboxylase from *Streptococcus faecalis* (Sigma, U.S.A.) and the crude (4) was oxidized by $RuCl_3$–$NaIO_4$ to (3c) in 81% overall yield.



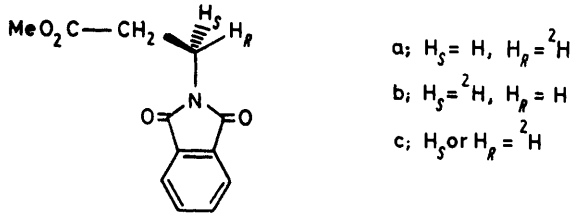
(5) (4)

SCHEME 1 (*i*) Tyrosine decarboxylase, ^2H_2O; (*ii*) $RuCl_3 \cdot 3H_2O$, $NaIO_4$

Initial efforts to differentiate the C-3 enantiotopic hydrogens of (3b) by ^{1}H n.m.r. were unsuccessful, although the same kind of resolution has been achieved with glycine [6] and 4-aminobutanoic acid.[7] However, optical rotations of *N*-phthaloylamino-acids such as (*S*)-(+)-[2-^{2}H]glycine, (*S*)-(+)-[3-^{2}H]-3-aminopropanoic and (*S*)-(+)-[4-^{2}H]aminobutanoic acids have been reported.[8] We therefore prepared the *N*-phthaloyl methyl ester of (3c), in turn prepared following Scheme 1, by reaction with *N*-(ethoxycarbonyl)phthalimide [9] and esterification with diazomethane. This (*R*)-isomer (6a) showed a rotation of −0.82° at 589 nm and of −3.50° at 365 nm

† While this manuscript was in preparation, a paper by Professor Battersby appeared, in which decarboxylation of (1) by histidine decarboxylase from *Clostridium welchii* and *Lactobacillus* 30a was shown to occur with retention of configuration (A. R. Battersby, M. Nicoletti, J. Staunton, and R. Vleggaar, *J.C.S. Perkin I*, 1980, 43).

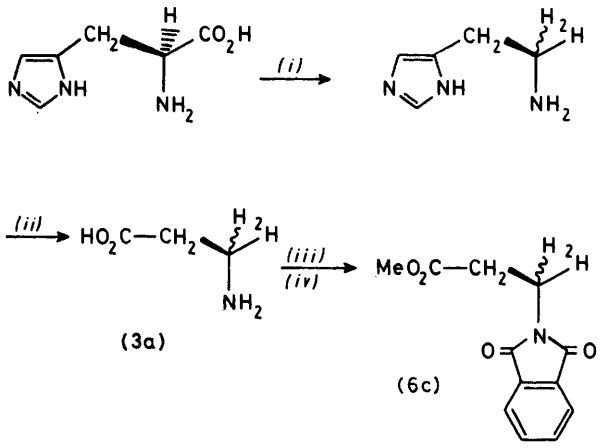
[lit.,[8] +0.83° for the *S*-isomer, (6b)]. By an analogous procedure, monodeuteriohistamine (2a) was prepared from (1) in ^2H_2O in the presence of histidine decarboxylase from *Clostridium welchii*. Compound (2a) was then



a; H_S = H, H_R = ^{2}H
b; H_S = ^{2}H, H_R = H
c; H_S or H_R = ^{2}H

(6)

oxidized by ruthenium tetraoxide to (3a), and (3a) was converted into (6c) (Scheme 2). The optical rotation of (6c) was recorded at 589 and 365 nm and the values were of −0.70 and −3.00°, respectively.



(3a)

(6c)

SCHEME 2 (*i*) Histidine decarboxylase, ^2H_2O; (*ii*) $RuCl_3 \cdot 3H_2O$, $NaIO_4$; (*iii*) *N*-(ethoxycarbonyl)phthalimide, Na_2CO_3; (*iv*) diazomethane

Moreover, mass spectrographic analysis of the sample of (6a) from (3c) showed a deuterium content of 90 ± 2%, and (6c) from (2a) a content of 80 ± 2%. In spite of the difference in the isotopic abundance of the deuteriated samples, from the negative rotations of both (6a) and (6c) it was concluded that (3a) was indeed (3*R*)-[3-^{2}H]-3-aminopropanoic acid, and that deuteriated histamine (2a) enzymically obtained from (1), was of (*R*)-configuration. It is concluded that decarboxylation of (1) catalysed by histidine decarboxylase proceeds with retention of configuration, according with the general mechanism proposed by Dunathan for the pyridoxal phosphate-dependent α-amino acid decarboxylases.[10] Moreover, our findings are in perfect agreement with the results obtained by Battersby *et al.* in an entirely different way (see footnote on title page).

EXPERIMENTAL

I.r. spectra were obtained in $CHCl_3$ solutions with a Perkin-Elmer 157 spectrometer. ^{1}H N.m.r. spectra (60 MHz) were determined in deuteriochloroform solutions (tetramethylsilane as internal standard) on a Hitachi–Perkin-Elmer R-24 spectrometer. The progress of the reactions was monitored by silica gel microplates; g.l.c. determinations were on a 2-m silanized glass column of 1% SE 30 on Gas-Chrom Q operating at 150—250 °C. Optical rotations were performed on a Perkin-Elmer 241 polarimeter in a 1-ml cell (10-cm path length). The enzymes were purchased from Sigma (Mo., U.S.A.) and deuteriated materials from Merck (Darmstadt, Germany).

(1R)-[1-^{2}H]-*Tyramine* (4) *from* (2S)-*Tyrosine* (5).—The incubation mixture was prepared as follows: compound (5) (50 mg, 0.276 mmol), pyridoxal phosphate (15 mg), fatty-acid-free albumin (40 mg), and ^2H_2O (50 ml). To this mixture at 37 °C, tyrosine decarboxylase (50 mg, 0.7 units/mg) was added and the pH kept at 6.5 by additions of ^{2}HCl diluted with ^2H_2O. The enzymic reaction, carried out under nitrogen, was followed by t.l.c. [butanol–water–acetic acid (8 : 2 : 2, v/v); spots developed with ninhydrin] and was complete within 1 h. The incubation was stopped by the addition of ethanol and boiling the mixture (5 min). After centrifugation (3 000 g, 5 min) the supernatant was evaporated *in vacuo* and the residue was oxidized.

Ruthenium Tetraoxide Oxidation of (4).—To a solution containing the above crude (4) in dilute HCl (pH 2) were added sodium metaperiodate (0.7 g) and ruthenium trichloride trihydrate (1.5 mg). After 1 h at 10 °C, the reaction was complete as indicated by t.l.c. examination of the mixture (eluants as above). The reaction mixture was filtered and run through a cation-exchange resin column (AG 50W-X2, 100—200 mesh). After washing with water, the β-alanine formed was eluted with 1N NH_4OH. An aliquot of the ammonia fraction was evaporated to dryness and examined by g.l.c., β-alanine being analysed as the *N*-trifluoroacetyl n-butyl derivative.[11] The yield of (3c) was 20 mg.

Methyl (3R)-3-*Phthalimido*-[3-^{2}H]*propanoate* (6a).—To a solution of (3c) (20 mg, 0.22 mmol) in water (2 ml), sodium carbonate (24 mg) and *N*-(ethoxycarbonyl)phthalimide (60 mg, 0.27 mmol) were added, and the mixture was stirred at room temperature (10 min). After filtration, the solution was brought to pH *ca.* 4 and the precipitate of (3*R*)-3-phthalimido[3-^{2}H]propanoic acid washed with cold water and dried *in vacuo*. The residue was dissolved in methanol (1 ml) and esterified with ethereal diazomethane. The product, nearly pure by t.l.c. [benzene–ethyl acetate, (8 : 2, v/v)], was purified by preparative t.l.c. to yield pure (6a) (24 mg); $[\alpha]_{589}^{20}$ −0.82°, $[\alpha]_{365}^{20}$ −3.50° (*c* 2.4, $CHCl_3$); $\nu_{max.}$, 1 780, 1 740, and 1 720 cm^{-1}; δ 2.75 (d, 2 H, CH_2CO_2Me), 3.70 (s, 3 H, OMe), 4.10 (t, 1 H, CH^2H), and 7.90 (m, 4 H, aromatic hydrogens). Quantitative evaluation of the deuterium content of (6a) was performed by mass-spectrometric analysis, focusing the ions at *m/e* 233 [M^+ of unlabelled (6a)], 234, and 235. From the intensities of the three fragments the monodeuteriated species was determined to be 90 ± 2% of the total.

Decarboxylation of (2S)-*Histidine* (1) *by Histidine Decarboxylase.*—A solution of (2S)-histidine·HCl (50 mg, 0.32 mmol) in ^2H_2O was evaporated to dryness, and twice dissolved in ^2H_2O and again evaporated to remove exchangeable hydrogen. The residue was dissolved in 0.2N ammonium acetate buffer, pH 4.8, in ^2H_2O and to it were added: histidine decarboxylase (from *Clostridium welchii*, 50 mg, 0.04 units/mg), fatty-acid-free albumin (25 mg), and a 50% saturated ammonium sulphate solution in ^2H_2O (0.5 ml). Ammonium acetate and sulphate were previously

dissolved in ^2H_2O and evaporated to dryness three times. Aliquots of 0.1N ^2HCl in ^2H_2O were added in order to maintain the solution at pH 4.8. The reaction was carried out at 37 °C and followed by t.l.c. [acetone–27% ammonia (9 : 1, v/v); spots developed with ninhydrin]. A further 25 mg of the enzyme was added after 10 h and after 24 h the reaction was complete, and stopped by addition of 3M ^2HCl in ^2H_2O and boiling (3 min). After centrifugation (3 000 g, 5 min) the supernatant was evaporated to dryness.

Methyl 3-*Phthalimido*[3-^{2}H]*propanoate* (6c).—The above residue [corresponding to 35 mg of (2a), 0.032 mmol] was dissolved in water (10 ml) and the pH brought to 2.0 by addition of 1M HCl; sodium metaperiodate (0.97 g) and ruthenium trichloride trihydrate (1.5 mg) were added, and the reaction mixture was stirred at room temperature (20 h). The reaction course was followed by t.l.c. [butanol–water–acetic acid (8 : 2 : 2, v/v)]. The mixture was then filtered and the aqueous solution was purified by column chromatography (AG 50W-X2) as described previously. The fraction eluted with 1N ammonia contained (3a) (26 mg), whose purity was checked by g.l.c. as the *N*-trifluoroacetyl n-butyl derivative.[11] Compound (6c) was prepared from (3a) as described for (6a). Specific rotations were as reported in the text (*c* 2.4 in $CHCl_3$) and mass-spectrometric analysis showed a deuterium content of 80 ± 2%.

We thank the Italian Research Council for financial support, Professor A. Fiecchi for helpful discussions, and Professor G. Galli for the mass spectra.

[0/330 *Received*, 29*th February*, 1980

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