Studies of Enzyme-mediated Reactions. Part 13.[1] Stereochemical Course of the Formation of Histamine by Decarboxylation of (2*S*)-Histidine with Enzymes from *Clostridium welchii* and *Lactobacillus* 30a

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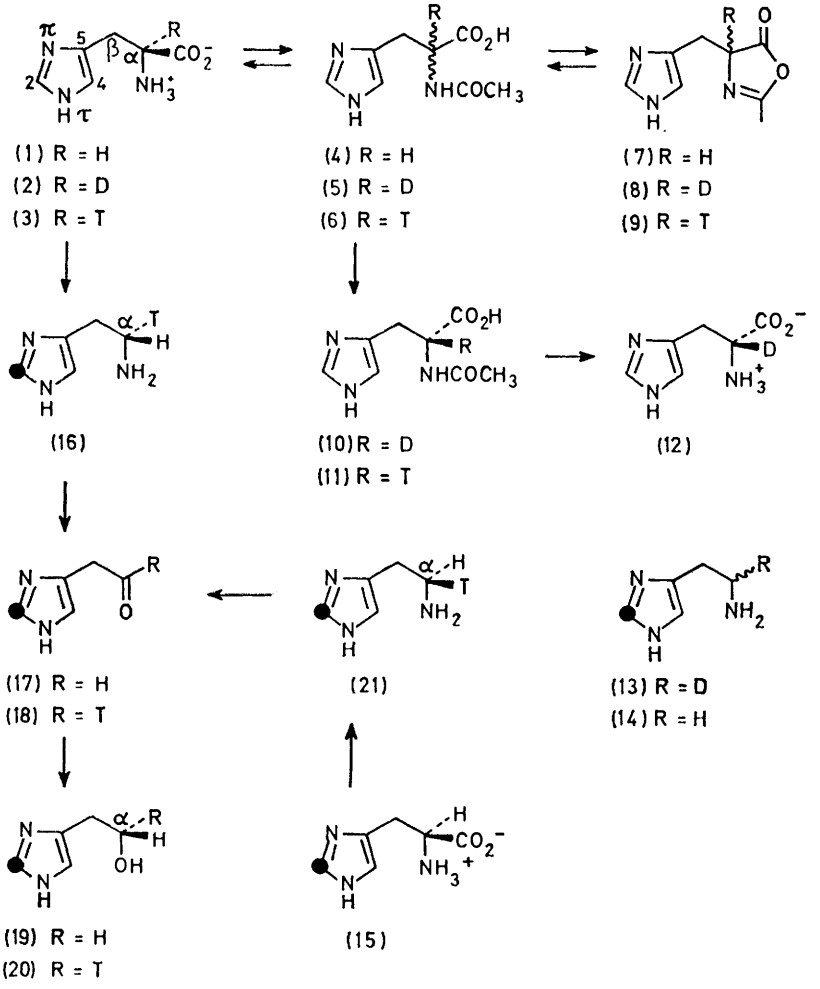
(αS)-[α-^3H]Histidine is prepared and the [α-$^3H_1$]histamine produced from it by the decarboxylating enzymes of *Clostridium welchii* and *Lactobacillus* 30a is proved by assay with diamine oxidase from pea seedlings to have the (αS)-configuration. The (*Si*)-stereospecificity of the latter enzyme is confirmed for the case of histamine by studying its action on (αR)-[α-$^3H_1$]histamine (21) which is synthesised rationally. The combined results prove that histidine decarboxylases from both sources bring about decarboxylation with overall *retention of configuration*. It is shown that histidine decarboxylase acts on histamine to bring about slow exchange with the medium of the α-*Re*-hydrogen, which occupies the same space (*Re*-space) as the carboxy-group of (αS)-histidine. Syntheses are described of (αS,βS)-[β-$^3H_1$]histidine (45) and (αS,βR)-[β-$^3H_1$]histidine (47).

HISTIDINE DECARBOXYLASE (E.C. 4.1.1.22) is unique among the bacterial amino-acid decarboxylases so far examined in that it does not require pyridoxal phosphate as coenzyme.[2] The decarboxylation is mediated by a pyruvoyl residue in the enzyme [3] which, as for the more common pyridoxal phosphate, undergoes Schiff's-base formation with histidine (1); a mechanism has been proposed for the decarboxylation process.[3] In contrast to the bacterial enzyme, histidine decarboxylase from mammalian sources appears to require pyridoxal phosphate.[4] This striking difference in mode of action of these enzymes adds considerable interest to comparative studies of the stereochemistry of the reaction brought about by enzymes of the two types. As a first step, we



SCHEME 1

have concentrated on the pyridoxal-free enzyme from *Lactobacillus* 30a [2] and on that from *Clostridium welchii*; the available evidence on the latter also points to be absence of pyridoxal phosphate.[5] Mammalian histidine decarboxylase will be the subject of future studies.

Chang and Snell had established [6] that decarboxylation of (αS)-histidine (1) in deuterium oxide by the enzyme from *Lactobacillus* 30a resulted in the introduction of *one* deuterium atom into the product histamine (13), which was optically active. This result pointed to a stereospecific process and it was tentatively concluded, on the basis of comparisons of signs of rotation, that the decarboxylation had occurred with retention of configuration. Professors E. E. Snell and R. S. Lane generously helped us to undertake a complete study of the stereochemical problem by providing crystalline enzyme from *Lactobacillus* 30a; a suitable preparation of histidine decarboxylase from *C. welchii* was commercially available.

Our approach, involving two complementary series of experiments, was outlined in the preceding paper.[1] Thus, the plan was to study the decarboxylation of (αS)-[α-^{3}H,2-^{14}C]histidine (3) in normal water and also the similar decarboxylation of (αS)-[2-^{14}C]histidine (15) in tritiated water. The first requirement was a specifically labelled sample of (αS)-[α-^{3}H]histidine (3) and as usual, the labelling procedures were monitored by carrying them out first in the deuterium series.

Treatment of (αS)-histidine [unlabelled (1)] with acetic anhydride in hot deuterioacetic acid (CH_3CO_2D) under a variety of conditions was shown by n.m.r. and mass spectrometry not only to label C_α of the side chain but also to introduce deuterium at C-2 of the imidazole ring. Not surprisingly, the hydrogens of the methyl group in the *N*-acetyl residue also exchanged for deuterium. The latter unwanted labelling would have been removed later but there was a risk of incomplete removal of that at C-2 of the heterocycle. Accordingly, we used a method developed by Dr. P. G. Strange [7] for the related case of tryptophan, which here involved ring-closure of *N*-acetylhistidine (4) by dicyclohexylcarbodi-imide in pyridine to the corresponding oxazolone (7). Addition of an excess of deuterium oxide then effected exchange at the chiral centre in a rapid step to produce (8) which without isolation then underwent slow hydrolytic opening of the oxazolone ring. This method gave deuterium incorporations of ≥90% and n.m,r. showed the label to be located exclusively at C_α of the product, *N*-acetyl-(αRS)-[α-^{2}H]histidine (5). This material was resolved with hog kidney acylase I [8] to yield (αS)-[α-^{2}H]histidine (2) and *N*-acetyl-(αR)-[α-^{2}H]histidine (10) which were separated and rigorously purified by ion-exchange chromatography. Acidic hydrolysis of the *N*-acetyl derivative (10) then afforded (αR)-[α-^{2}H]histidine (12). Both (αS)-, and (αR)-histidines contained 90 ± 3 atom % deuterium by n.m.r. and the specific rotation of each corresponded, within experimental error,* to configurational purity.

The preparation of (αS)-[α-^{3}H]histidine (3) followed exactly the foregoing methods save that tritiated water was used for the exchange step [forming (9)] and ring opening of the oxazolone (7). The resultant (αRS)-[α-^{3}H]-product (6) gave after resolution (αS)-[α-^{3}H]histidine (3) and *N*-acetyl-(αR)-[α-^{3}H]histidine (11).

After mixing the (αS)-[2-^{3}H]histidine (3) with a suitable quantity of (αS)-[2-^{14}C]histidine (15) to provide an internal standard, the now doubly-labelled sample (3 + 15) was incubated with histidine decarboxylase from *C. welchii* to yield histamine (16) labelled with tritium at C_α. Part of this product was converted into its crystalline phenylurea derivative for accurate determination of the ^{3}H : ^{14}C ratio (Experiments 1 and 2, Table) which showed that the tritium had been completely retained during the decarboxylation process. The rest of the labelled histamine (16) was then incubated with diamine oxidase from pea seedlings [9] (E.C. 1.4.3.6) to produce the aldehyde (17). This was trapped, as in earlier examples,[1,10] by reduction in a coupled enzymic reaction using liver alcohol dehydrogenase, a catalytic quantity of NADH, and an excess of ethanol or cyclohexanol [11] as the reducing donor. In this way histaminol (19) was produced.

Diamine oxidase from pea seedlings has been rigorously proved to remove the *Si*-hydrogen as it dehydrogenates benzylamine [10] or tyramine.[1] Similar proof is given below that it acts in the same stereochemical sense on histamine (14). Since the foregoing histamine on enzymic oxidation–reduction gave histaminol (19) which was devoid of tritium (Experiments 1 and 2, Table), it follows that the histamine produced by enzymic decarboxylation of (αS)-[α-^{3}H,2-^{14}C]histidine (3 + 15) has the (αS)-configuration (16). This corresponds to overall retention of configuration during the decarboxylation process.

Complementary results were obtained by decarboxylation of (αS)-[2-^{14}C]histidine (15) with the *C. welchii* enzyme in the appropriate quantity of tritiated water, found by trial runs to produce a satisfactory ^{3}H : ^{14}C ratio in the resultant histamine (21). Configurational assay as before using diamine oxidase gave a high retention of tritium in the final histaminol (20) but the values found (Experiments 3 and 4, Table) were significantly lower than the strictly complementary result of 99—100% retention. We comment on this point later.

When comparing the stereochemical preference of the crystalline enzyme from *Lactobacillus* 30a by an exactly similar series of experiments, it was again found that (αS)-[α-^{3}H,2-^{14}C]histidine (3 + 15) underwent decarboxylation to yield (αS)-[α-^{3}H$_1$]histamine (16) which lost essentially all its tritium on dehydrogenation by diamine oxidase (Experiment 5, Table). Further, decarboxylation of (αS)-[2-^{14}C]histidine (15) in tritiated water gave (αR)-[α-^{3}H$_1$]histamine (21) which largely retained its tritium over the enzymic dehydrogenation–reduction sequence to produce (αS)-[α-^{3}H$_1$]histaminol (20), though

* The qualification ' within experimental error ' is to be understood for all statements about results.

TABLE

Decarboxylation of labelled histidines and configurational assay of labelled histamines

Experiment no.	Substrate for enzyme (^3H : ^{14}C ratio)	Source enzyme and medium	Histamine [a] ^3H : ^{14}C ratio (% retention)	Histaminol [b] ^3H : ^{14}C ratio (% retention)
1	(αS)-[α-^3H,2-^{14}C]-Histidine (3) + (15) (12.3)	*C. welchii* in H_2O	12.2 (99)	0.11 (0.9)
2	As Expt. 1 (12.3)	*C. welchii* in H_2O	12.3 (100)	0.13 (1.0)
3	(αS)-[2-^{14}C]-Histidine (15)	*C. welchii* in HTO	5.3	4.5 (85)
4	As Expt. 3	*C. welchii* in HTO	16.5	14.6 (88)
5	(αS)-[α-^3H,2-^{14}C]-Histidine (3) + (15) (13.6)	*Lactobacillus* 30a in H_2O	14.3 (103)	0.5 (3.6)
6	(αS)-[2-^{14}C]-Histidine (15)	*Lactobacillus* 30a in HTO	2.60	2.31 (89)
7	[2-^{14}C]Histamine (14)	*C. welchii* in HTO	13.2	11.0 (83)
8	As Expt. 7	*Lactobacillus* 30a in HTO	1.71	1.41 (82)
9	(αR)-[α-$^3H_1$,2-^{14}C]-Histamine (21) from Scheme 2 synthesis		2.11	1.80 (85)

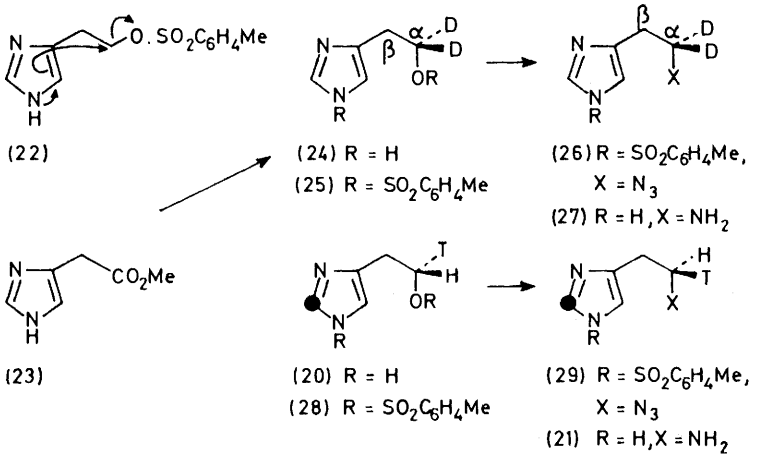
[a] Radio-assay as *N*-phenylurea derivative. [b] Radio-assay as phenylurethane derivative.

once more a significantly low value for tritium retention was observed (Experiment 6, Table).

The major findings are thus clear. Decarboxylation of (αS)-histidine (1) by the enzymes from *C. welchii* and from *Lactobacillus* 30a occurs with overall *retention of configuration* as has been found for (αS)-tyrosine [12,1] and (αS)-lysine.[13]

Brief comment is needed on the subsidiary finding that some ^3H-loss (relative to an internal ^{14}C-standard) unexpectedly occurs when (αR)-[α-$^3H_1$]histamine (21) is converted into (αS)-[α-$^3H_1$]histaminol (20) by the diamine oxidase–liver alcohol dehydrogenase system. Experiments 1, 2, and 5 (Table) show that the enzymic decarboxylation process producing the (αS)-amine (16) is highly stereospecific; indeed, the configurational control is almost certainly complete. We therefore feel that the complementary (αR)-amine (21) produced in Experiments 3, 4, and 6 is of similar configurational purity and would be expected to retain all its tritium in the diamine oxidase dehydrogenase step. However, the ^3H-retention values found in the final histaminol (20) have been consistently $14 \pm 3\%$ lower than the expected 100%. Similar results were reported in the preceding paper for the conversion of (αR)-[α-$^3H_1$]tyramine into (αS)-[α-$^3H_1$]tyrosol and there some additional experiments were described. A possible explanation is that we are observing an accumulation of small secondary isotope effects over the sequence amine ⟶ imine ⟶ aldehyde ⟶ alcohol which cause marginal discrimination against the tritium-carrying molecules. However, fuller study will be needed before this interesting secondary observation is fully understood.

Proof that diamine oxidase from pea seedlings removes the *Si*-hydrogen from C_α of histamine (14) was obtained by a synthesis of (αR)-[α-$^3H_1$]histamine (21) as follows. We accepted at the planning stage that attempted nucleophilic displacement of the *O*-tolenesulphonyl derivative of histaminol [*e.g.* (22)] would involve serious risk of *aryl*-participation as illustrated with resultant loss of control over site and stereospecificity of the labelling. To avoid participation the imidazole ring was deactivated by *N*-tosylation forming the *NO*-bis(toluene-*p*-sulphonyl)



SCHEME 2

derivative (25); these pilot experiments were carried out in the dideuterio-series. The starting material (24) was prepared by reducing imidazolyl acetic ester (23) with lithium aluminium deuteride. Azide anion then smoothly displaced the *O*-tolenesulphonyl residue to yield the azide (26). This was shown within the limits of detection by n.m.r. and mass spectrometry, to contain >98% $^2H_2$-species and still to be labelled solely at C_α. This is important since *aryl*-participation would have led to scrambling of the label across C_α and C_β. Catalytic hydrogenation of the azide (26) and acidic hydrolysis of the *N*-toluenesulphonyl group then afforded [α-$^2H_2$]-histamine (27).

These findings allowed the synthesis of the required (α*R*)-[α-$^3H_1$]histamine (21) to be undertaken with confidence. First, (α*S*)-[α-$^3H_1$,2-^{14}C]histaminol (20) was prepared enzymically from (α*S*)-[2-^{14}C]histidine (15) by the sequence (1) ⟶ (21) ⟶ (18) ⟶ (20) (Scheme 1).

At first it may appear odd to carry out a synthesis of the amine (21) using as starting material a substance which is apparently made from it. But it must be remembered that the present synthesis is necessary to prove rigorously that the histamine from the decarboxylation process in tritiated water in fact has the configuration (21). For our synthetic purposes, the *only stereochemical assumption* * in the preparation of (20) by the sequence (1) ⟶ (21) ⟶ (18) ⟶ (20) is that liver alcohol dehydrogenase adds ' hydride ' to the *Re*-face of [*formyl*-^{3}H]imidazolylacetaldehyde (18); there is ample precedent [14] to support this.

The rest of the synthesis involved following the steps (20) ⟶ (28) ⟶ (29) ⟶ (21), Scheme 2, exactly as had been developed in the deuterium series, to produce (α*R*)-[α-$^3H_1$,2-^{14}C]histamine (21), now of known configuration. Interestingly, treatment of this product (21) with the diamine oxidase–liver alcohol dehydrogenase enzyme system produced (α*S*)-[α-$^3H_1$,2-^{14}C]histaminol (20) with a high, but not complete retention of tritium (Experiment 9, Table). This result (*a*) established that diamine oxidase from pea seedlings removes the *Si*-hydrogen from C_α of histamine and this adds rigour to the experiments on the decarboxylation reaction; and (*b*) interlocks with the slightly low tritium content found above for the (α*R*)-[α-$^3H_1$,2-^{14}C]histamines from the decarboxylation reactions (Experiments 3, 4, and 6, Table).

Two topics related to the foregoing work were also studied. It seemed that a simple method for preparing histamines rendered chiral by isotopic substitution at C_α would be available if histidine decarboxylase would catalyse exchange at C_α of unlabelled histamine with hydrogen of the medium. Such exchange is known [15] to be mediated by glutamate decarboxylase and lysine decarboxylase working on the normal products of their action, *viz.* γ-aminobutyric acid and cadaverine respectively. Earlier attempts to demonstrate an analogous exchange on histamine using deuterium as the label had not led to detectable incorporation,[6] so the process is at best very slow. By incubating [2-^{14}C]histamine (14) in tritiated water with histidine decarboxylase from *C. welchii* or from *Lactobacillus* 30a, the exchange process could be demonstrated due to the greater sensitivity of tritium detection. The incorporation of tritium was very slow with both enzymes. Configurational assay of the now doubly-labelled histamine as above showed in each case that (α*R*)-[α-$^3H_1$]histamine (21) had been produced (Experiments 7 and 8, Table). This corresponds to labilisation of the hydrogen atom of histamine which occupies the same space (*Re*-space) as the carboxy-group of (α*S*)-histidine (1); note again the somewhat low tritium content of the histaminol samples from the enzymic assay (Experiments 7 and 8).

This demonstration that the *Re*-hydrogen of histamine is labilised by histidine decarboxylase is of mechanistic interest but not of preparative importance. It is easier to obtain (α*R*)-[α-$^3H_1$]histamine (21) by decarboxylation of histidine (1) in tritiated water and this method gives material of much higher specific activity than that from the exchange process. Also, the best method for preparing (α*S*)-[α-$^3H_1$]histamine (16) is undoubtedly by enzymic decarboxylation of (α*S*)-[α-^{3}H]histidine (3) and *not* by attempted ' wash out ' in H_2O of the *Re*-tritium from (α*RS*)-[α-$^3H_1$]histamine.

The second related study led to syntheses of (α*S*,β*S*)-[β-$^3H_1$]histidine (45) and of (α*S*,β*R*)-[β-$^3H_1$]histidine (47). The latter had been prepared previously [16] by an enzymic exchange process using histidine ammonia lyase.

Earlier work had shown that catalytic hydrogenation of acylaminoacrylic acids of general structure (30) is a cleanly *syn*-stereospecific process.[17] Based on this knowledge, the plan for synthesis of the labelled histidines (45) and (47) involved preparation of the labelled acrylic acids (39) and (40). As usual, the exploratory work was carried out with deuterium (Scheme 3).

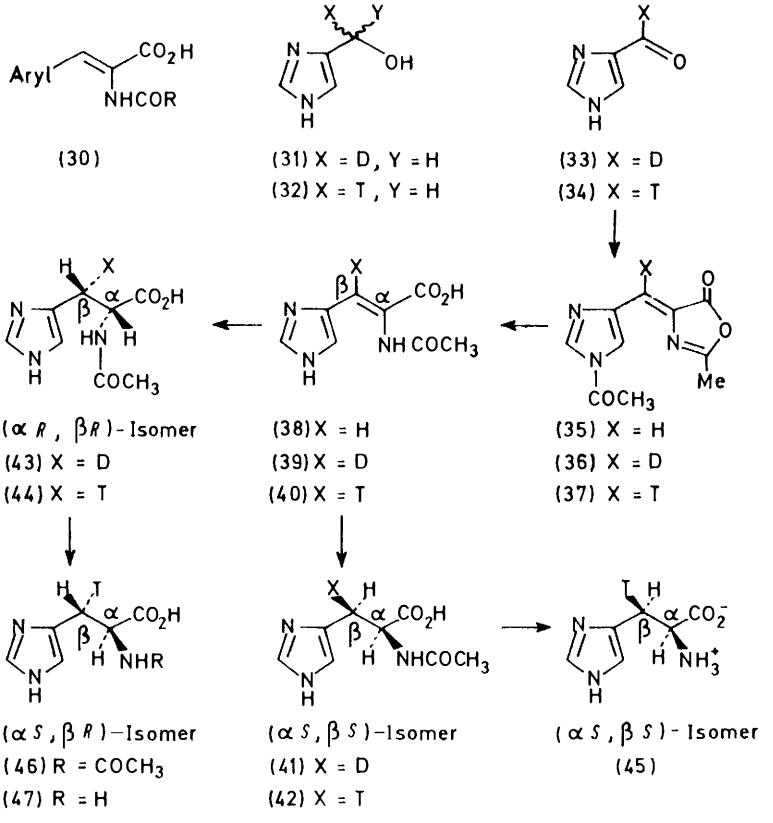
4(5)-Hydroxymethylimidazole prepared from fructose [18] was oxidised by activated manganese dioxide to 4(5)-formylimidazole. Reduction of this aldehyde with sodium borodeuteride gave the alcohol (31) which by oxidation with either activated manganese dioxide or barium manganate [19] formed the deuterioaldehyde (33) in good yield (⩽80% $^2H_1$-species). Condensation of this aldehyde with *N*-acetylglycine in acetic anhydride afforded the oxazolinone (36) which was converted into the required acrylic acid (39) by mild basic hydrolysis. Hydrogenation over palladium then gave a mixture of *N*-acetyl-(α*S*,β*S*)-[β-$^2H_1$]histidine (41) and the (α*R*,β*R*)-isomer (43).

The materials in this sequence were assayed for deuterium by n.m.r. and mass spectrometry and the loss of deuterium, if any, was negligible. These findings allowed synthesis of the ^{3}H-series to be undertaken as follows.

Reduction of unlabelled 4-formylimidazole with boro-

* Observation tells us that the aldehyde (18) contains tritium and that is all the knowledge needed; (18) *could* have arisen by the decarboxylation occurring with inversion and the diamine oxidase removing the *Re*-hydrogen. The sum of evidence in this paper shows this is not true; but for the synthesis, it would have been immaterial.

tritiide gave the (*RS*)-alcohol (**32**) which was oxidised as described above for (**31**). The kinetic isotope effect operated in our favour so that the resultant [*formyl*-^{3}H]aldehyde (**34**) retained 88% of the tritium present in the alcohol (**32**). This aldehyde was then worked up as above through the steps (**34**) ⟶ (**37**) ⟶ (**40**) ⟶ (**42**) + (**44**) and the final (α*RS*,β*RS*)-material was treated with hog kidney acylase-I. The hydrolysed product was (α*S*,β*S*)-[β-^{3}H$_1$]histidine (**45**), the first desired labelled product, which was readily separated from the still acylated (α*R*)-material (**44**) by ion-exchange.



SCHEME 3

Racemisation of the latter acylated product (**44**) with hot acetic anhydride gave a mixture of the (α*R*,β*R*)-isomer (**44**) with the (α*S*,β*R*)-isomer (**46**). Acylase-I then specifically hydrolysed the latter amide to afford the second required product, (α*S*,β*R*)-[β-^{3}H$_1$]histidine (**47**).

These (β*S*)-, and (β*R*)-labelled samples of (α*S*)-histidine, (**45**) and (**47**), are readily prepared in quantity by the foregoing sequence. They are thus available to allow complementary experiments on any enzymic reaction which in some way removes one hydrogen atom from C_β of (α*S*)-histidine (**1**).

EXPERIMENTAL

General directions are given in ref. 10*b*.

N-*Acetyl*-(αRS)-[α-^{2}H]*histidine* (**5**).—*N*-Acetyl-(α*RS*)-histidine monohydrate (**4**) was prepared [20] by standard acetylation of (α*S*)-histidine, *m/e* 197 (M^+), δ(CF_3CO_2D), 2.26 (3 H, s, NCOMe), 3.48 and 3.55 (2 H, AB part of ABX, J_{AB} 15.7, J_{AX} 5.5, J_{BX} 7.1 Hz), 5.08 (1 H, X part of ABX, J_{AX} 5.5, J_{BX} 7.1 Hz), and 7.45 and 8.61 (each 1 H, s, imidazole H), $[\alpha]_D^{20}$ 0.0° (*c*, 1.0 in H_2O).

A solution of anhydrous *N*-acetyl-(α*RS*)-histidine (2.96 g) in anhydrous pyridine (100 ml) was treated with dicyclohexylcarbodi-imide (3.28 g) and the solution was stirred at 80 °C for 3 h. Deuterium oxide (6 ml) was then added and after the mixture had been stirred for 1 h, the solvents were evaporated off. The residue was dissolved in water and after filtration the solution was evaporated; crystallisation of the product twice from aqueous acetone gave N-*acetyl*-(αRS)-[α-^{2}H]*histidine monohydrate* (2.32 g), δ(CF_3CO_2D) 2.26 (3 H, s, NCOMe), 3.39 and 3.62 (2 H, dd, *J* 15.5 Hz), and 7.45 and 8.61 (each 1 H, s, imidazole H), D content 90 ± 3% (n.m.r.).

(αS)-[α-^{2}H]*Histidine* (**2**) and (αR)-[α-^{2}H]*Histidine* (**12**).—A solution of the foregoing racemate (1.29 g) in water (80 ml) was adjusted to pH 7.2 with lithium hydroxide. Hog kidney acylase-I (Sigma) (600 units mg^{-1}, 40 mg) was then added and after the solution had been kept at 37 °C for 12 h, more acylase-I (5 mg) was added and incubation was continued for a further 8 h. The solution was adjusted to pH 5 with acetic acid, boiled briefly with absorbent charcoal (*ca.* 20 mg), filtered, and adjusted to pH 7.

After concentration to *ca.* 10 ml, this solution was applied to a column (2 × 50 cm) of Amberlite IRC-50 (H-form); the *N*-acetyl-(α*R*)-[α-^{2}H]histidine (**10**) was eluted with water and recovered by evaporation. 3N-Acetic acid eluted (α*S*)-[α-^{2}H]histidine (**2**) and the appropriate fractions were combined and concentrated to *ca.* 10 ml. This solution was run onto Amberlite IR-120 (H-form, 2 × 20 cm)

and after thorough washing of the column with water, the amino-acid was eluted with aqueous 2N-ammonia and the eluate was evaporated. Crystallisation of the residue from aqueous ethanol gave (αS)-[α-^{2}H]*histidine* (2) (285 mg), deuterium content 90 ± 3% (n.m.r.); $[\alpha]_D^{21}$ −38.1° (*c*, 0.94 in H_2O); an authentic sample of (α*S*)-histidine showed $[\alpha]_D^{21}$ −38.2° (*c*, 1.10 in H_2O).

All the foregoing *N*-acetyl-(α*R*)-[α-^{2}H]histidine (10) was heated under reflux for 3 h with an excess of 2N-hydrochloric acid and the solution was evaporated to dryness. The evaporation was repeated thrice with portions (5 ml) of water. A solution of the residue in water (10 ml) was adjusted to pH 7.2 with lithium hydroxide and run onto Amberlite IR-120 as above to yield, by the same work-up, (αR)-[α-^{2}H]*histidine* (12) (310 mg), deuterium content 90 ± 3% (n.m.r.); $[\alpha]_D^{21}$ +38.2° (*c*, 1.06 in H_2O).

(αS)-[α-^{3}H]*Histidine* (3).—*N*-Acetylhistidine (4) (480 mg) was stirred at 85 °C in pyridine (20 ml) with dicyclohexylcarbodi-imide (525 mg) for 3.5 h and then treated with tritiated water (48 mg, 5 Ci ml^{-1}). The mixture was stirred for an additional 3 h, evaporated, and the residue re-evaporated 6 times with portions (10 ml) of water. The final residue was crystallised from aqueous acetone to afford the *N*-acetyl derivative (6) (365 mg) of which part (344 mg) in water (10 ml) was incubated with hog kidney acylase-I (20 mg, 600 units mg^{-1}) and worked up as for the ^{2}H-series to yield (αS)-[α-^{3}H]*histidine* (87 mg), 4.8 mCi mmol^{-1}.

(αS)-[α-^{3}H,2-^{14}C]*Histidine*.—Part of the foregoing product was diluted with unlabelled histidine and recrystallised to give material of lower specific activity. An aliquot (38 mg, total activity *ca.* 0.6 mCi) was dissolved in water together with (α*S*)-[2-^{14}C]histidine (total activity 50 μCi). The residue after evaporation was recrystallised thrice from aqueous ethanol to yield the doubly-labelled material (17 mg), ^{3}H : ^{14}C ratio 12.3.

Derivatisation of Histamine [*as* (14)] *and Histaminol* [*as* (19)].—A solution of histamine (444 mg) and phenyl isocyanate (490 mg) in anhydrous pyridine (5 ml) was heated at 80 °C for 30 min and after evaporation the residue was dissolved in 6N-hydrochloric acid (10 ml). After filtration, the solution was evaporated and the residue in water (5 ml) was adjusted to pH 8 with 6N-potassium hydroxide. Recrystallisation of the precipitate from aqueous ethanol gave the *N*-phenylureido-derivative of histamine (730 mg), m.p. 182—184° (lit.,[21] 170°) (Found: C, 62.5; H, 6.2; N, 24.1%; M^+, 230. Calc. for $C_{12}H_{14}N_4O$: C, 62.6; H, 6.1; N, 24.3%; M^+, 230), δ(CD_3SOCD_3) 2.72 and 3.37 (each 2 H, t and m, *J* 7.5 Hz, CH_2CH_2NH), 6.16 (1 H, m, NHCO), 6.8—7.52 (5 H, m, Ph), 6.83 and 7.57 (each 1 H, s, imidazole), and 8.51 (1 H, S, PhNH).

Histaminol (448 mg) was treated in exactly the same way to yield its *phenylurethane derivative* (803 mg), m.p. 132—134° (from aqueous ethanol) (Found: C, 62.2; H, 5.8; N, 17.9; M^+, 231. $C_{12}H_{13}N_3O_2$ requires C, 62.3; H, 5.7; N, 18.15%; M^+, 231), δ(CD_3COCD_3) 2.92 and 4.34 (each 2 H, t, *J* 7.5 Hz, CH_2CH_2O), 6.8—7.6 (5 H, m, Ph), 6.9 and 7.56 (each 1 H, s, imidazole), and 9.58 (1 H, s, NH).

Enzymic Assay of Histamine using Diamine Oxidase.—*Method* (*a*) The procedure was developed using unlabelled histamine hydrochloride (73 mg). This was incubated at 37 °C for 48 h in 0.05M-phosphate buffer (35 ml, pH 7.0) containing ethanol (2 ml), NAD^+ (22 mg), liver alcohol dehydrogenase (10 mg), catalase (1 500 units, 0.5 mg), and diamine oxidase [10] from pea seedlings (2 ml of solution containing 2.15 mg protein ml^{-1} and of activity 4.95 × 10^{-2} μmol benzylamine oxidised min^{-1}, mg protein^{-1}). More NAD^+ (11 mg) and liver alcohol dehydrogenase (5 mg) were added after 24 h from the start.

The final solution was boiled briefly with charcoal (20 mg), filtered, and adjusted to pH 8 with 2N-potassium hydroxide. Extraction with n-butyl alcohol (2 × 10 ml) of the residue from evaporation of the solution gave histaminol (34 mg, M^+, 112) which as the picrate, crystallised from water, m.p. 143—144° (lit.,[22] 144°) (Found: C, 38.8; H, 3.4; N, 20.5. Calc. for $C_{11}H_{11}N_5O_8$: C, 38.7; H, 3.25; N, 20.5%).

Method (*b*). The incubation was repeated on the same scale as for method (*a*) but ethanol-free liver alcohol dehydrogenase (17 mg) was used with cyclohexanol (0.2 ml) as the reducing agent. The quantity of additional dehydrogenase after 24 h as above was 8.5 mg. Work-up as before gave histaminol (30 mg) which was characterised as above.

Decarboxylation of (αS)-[α-^{3}H,2-^{14}C]*Histidine by Histidine Decarboxylase from* C. welchii.—The doubly-labelled histidine prepared above (1.55 mg) in 0.2M-sodium acetate buffer (2 ml, pH 5) was incubated at 37 °C for 1 h with bovine serum albumin (1 mg) and histidine decarboxylase (10 mg) from *C. welchii* (Sigma; activity 0.05 units mg^{-1}). After the mixture had been adjusted to pH 2 with 2N-hydrochloric acid, unlabelled histamine dihydrochloride (53.4 mg) was added, and the solution filtered and evaporated.

Half the residue was dissolved in ethanol (10 ml) and the solution passed through Amberlite IRA-400 (OH phase, 8 × 50 cm). Histamine base from the percolate was converted into its phenylureido-derivative as earlier save that after adjustment to pH 8, the solution was evaporated. Extraction of the residue with chloroform–ethanol (1 : 4) (4 × 10 ml) gave the crude derivative which was purified by preparative t.l.c. on silica with chloroform–ethanol (3 : 1). After dilution of the radioactive urea with unlabelled material, it was recrystallised thrice from aqueous ethanol to give material of constant ^{3}H : ^{14}C ratio (11 mg), m.p. 182—184°.

Enzymic Assay of Configuration of [α-$^3H_1$]*Histamine*.—The remaining half of the histamine dihydrochloride from the foregoing experiment was treated on about half the previous scale with the diamine oxidase–liver alcohol dehydrogenase system. Work-up was as previously save that after adjustment to pH 8, the solution was evaporated and the residue extracted with chloroform–ethanol (4 : 1; 4 × 10 ml) to afford crude histaminol which was purified by preparative t.l.c. on alumina in chloroform–ethanol (3 : 1).

After the labelled histaminol had been diluted with three times its weight of radio-inactive histaminol, it was converted into the corresponding phenylurethane (see above) and at the stage of the work-up when adjustment to pH 8 was carried out, the solution was evaporated. The product extracted from the residue by chloroform–ethanol (4 : 1; 4 × 10 ml) was purified by preparative t.l.c. on silica with chloroform–ethanol (3 : 1) and then crystallised from aqueous ethanol to constant specific activity and ^{3}H : ^{14}C ratio (13 mg), m.p. 133—134°.

Decarboxylation of (αS)-[2-^{14}C]*Histidine* (15) *in Tritiated Water*.—A solution of the ^{14}C-labelled histidine (1.71 mg, 145 μCi mmol^{-1}) in 0.2M-sodium acetate buffer (1.0 ml, pH 5.0) containing tritiated water (37.6 mg, 5 Ci ml^{-1}) was incubated for 3 h at 37 °C with *C. welchii* histidine decarboxylase (8 mg; Sigma; 0.05 units mg^{-1}). After the solution had been adjusted to pH 2 with 2N-hydrochloric acid, the solvent was removed by vacuum transfer for re-use. The

residue was dissolved in water (2 ml), diluted with ten times its weight of unlabelled histamine dihydrochloride (typically 55—60 mg) and the methods described above for derivatisation and configurational assay were then used.

Experiments with Histidine Decarboxylase from Lactobacillus 30a.—These runs were carried out as for the ones described above; typical quantities used were as follows: (αS)-[α-$^{3}H_1$,2-^{14}C]histidine (1.5 mg), 0.2M-sodium acetate buffer (2 ml, pH 4.8), with a solution of the decarboxylase (0.1 ml of solution containing 7 mg ml^{-1} of crystalline enzyme); incubated at 37 °C for 14 h.

(αS)-[2-^{14}C]Histidine (1.5 mg) was decarboxylated as above but with the addition of tritiated water (total 300 mCi, 5 Ci ml^{-1}).

The purification and derivatisation of the histamine and histaminol samples were as described earlier.

Enzymic Exchange of the Re-*Hydrogen Atom at* C_α *of Histamine.*—The exchange reactions using histidine decarboxylase from both sources were carried out simply by replacing the histidine (*ca.* 1.5 mg) in the foregoing experiments by histamine (1.5 mg). The rest of the various procedures for isolation, purification, and configurational assay were unchanged.

[αα-$^{2}H_2$]*Histaminol* (24).—Dry hydrogen chloride gas was passed for 4 h through a boiling solution of 4(5)-imidazole acetic acid hydrochloride (1 g) in anhydrous methanol (20 ml). A solution of the residue from evaporation, in methanol (20 ml), was adjusted to pH 7 by addition of solid sodium hydrogencarbonate. Evaporation and extraction of the residue with methanol–chloroform (1 : 19; 4 × 75 ml) gave the methyl ester (718 mg), *m/e* 140 (M^+), δ (CD_3SOCD_3) 3.58 and 3.62 (3 H, and 2 H, s, CH_2, and CO_2Me), and 6.90 and 7.52 (each 1 H, s, imidazole).

This ester (560 mg) in anhydrous dioxan (20 ml) was added dropwise during 30 min to a suspension of lithium aluminium deuteride (168 mg) in dioxan (40 ml) which was being heated under reflux. After a further 1 h of heating, the cooled suspension was treated with sufficient 2N-sodium hydroxide to destroy the deuteride and the solids were removed and washed with dioxan. Evaporation of the combined organic solutions left an oil which was chromatographed on silica in chloroform–ethanol (4 : 1) to give [αα-$^{2}H_2$]*histaminol* which was purified as its picrate (930 mg), m.p. and mixed m.p. with protio-sample, 143—144°, *m/e* 114 (M^+), δ(CD_3SOCD_3) for picrate 2.80 (2 H, s, CH_2CD_2OH), 8.62 (2 H, s, ArH), and 7.28 and 8.86 (each 1 H, s, imidazole).

Synthesis of [αα-$^{2}H_2$]*Histamine* (27).—The route was developed using unlabelled materials (see end of Experimental section) as follows. Histaminol (112 mg) in dry pyridine (8 ml) was cooled to −23 °C (CO_2–CCl_4 bath) and treated with *p*-tolylsulphonyl chloride (1.9 g). The mixture was stirred at −23 °C for 2 h, then mixed with water (10 ml) and extracted with chloroform (2 × 20 ml). The combined organic solution was washed with 2N-hydrochloric acid and then evaporated. Purification of the product by preparative t.l.c. on silica in chloroform–ethanol (3 : 1) gave NO-*bis*-p-*tolylsulphonylhistaminol* (336 mg), m.p. 78.5—80° (from aqueous ethanol) (Found: C, 53.4; H, 4.8; N, 6.3; *m/e* 420.079 0. $C_{19}H_{20}N_2O_5S_2 \cdot 0.5H_2O$ requires C, 53.15; H, 4.9; N, 6.5%; *M* for anhydrous material, 420.079 2). δ 2.44 (6 H, s, ArMe), 2.85 and 4.24 (each 2 H, t, *J* J Hz, CH_2CH_2O), 7.02 (1 H, s, imidazole), and 7.20—7.82 (9 H, overlapping Ar and imidazole).

The foregoing [αα-$^{2}H_2$]histaminol (24) was converted by the same procedure into NO-*bis*-p-*tolylsulphonyl*-[αα-$^{2}H_2$]-*histaminol* (25), m.p. 78—80°, δ 2.44 (6 H, s, ArMe), 2.84 (2 H, s, CH_2CD_2O), 7.01 (1 H, s, imidazole), 7.19—7.82 (9 H, overlapping, Ar and imidazole), *m/e* 422 (M^+), 98% $^{2}H_2$-species.

A solution of the above unlabelled bistoluene-*p*-sulphonyl derivative (336 mg) in dry dimethylformamide (20 ml) was stirred with lithium azide (98 mg) for 18 h at 21 °C. Water was then added and ether extraction afforded the unlabelled N-p-*tolylsulphonyl-azide* [as (26)] which was purified by preparative t.l.c. on silica in ether–dichloromethane (1 : 1) to give a gum (180 mg) (Found: *m/e* 291.079 2. $C_{12}H_{13}N_5O_2S$ requires *M*, 291.079 0), δ 2.40 (3 H, s, ArMe), 2.74 and 3.50 (each 2 H, t, *J* 6 Hz, $CH_2CH_2N_3$), 7.08 (1 H, d, *J ca.* 1 Hz, imidazole), 7.30 and 7.76 (each 2 H, dd, ArH), and 7.90 (1 H, d, *J ca.* 1 Hz, imidazole), $\nu_{max.}$ 2 101 cm^{-1}.

The corresponding [αα-$^{2}H_2$]azide showed δ 2.40 (3 H, s, ArMe), 2.74 (2 H, s, $CH_2CD_2N_3$), 7.08 and 7.90 (each 1 H, br s, imidazole), and 7.30 and 7.76 (each 2 H, d, *J* 8 Hz, ArH), *m/e* 293 (M^+), 98% $^{2}H_2$-species.

A solution of the unlabelled azide (180 mg) in ethanol (15 ml) and concentrated hydrochloric acid (0.06 ml) was shaken with 10% palladium–charcoal (100 mg) and hydrogen. After 8 h, the catalyst was removed and the solution evaporated. The residue in ethanol (5 ml) was treated with a stream of dry hydrogen chloride for 3 min, and the solution was kept at 20 °C for 12 h and then evaporated to give histamine dihydrochloride. Crystallisation from ethanol–ether gave the pure salt (90 mg), m.p. 240—245° (lit.,[23] 244—246°), which was identified by direct comparison with authentic material, δ(CD_3OD) 3.26 (4 H, m, CH_2CH_2) and 7.52 and 8.88 (each 1 H, d, *J ca.* 1 Hz, imidazole), *m/e* 111 (M^+). The amine was also converted as above into its phenylureido-derivative, m.p. 182—184°, which was identical (i.r., n.m.r.) with an authentic sample.

The [αα-$^{2}H_2$]histamine dihydrochloride was prepared in the same way and showed δ(CD_3OD) 3.20 (2 H, s, CH_2CD_2) and 7.52 and 8.87 (each 1 H, d, *J ca.* 1 Hz, imidazole), *m/e* 113 (M^+), 98% $^{2}H_2$-species.

(αR)-[α-$^{3}H_1$]*Histamine* (21).—(αS)-[2-^{14}C]Histidine was decarboxylated in tritiated water as above using the enzyme from *C. welchii*. The resultant amine (^{3}H : ^{14}C ratio 2.46) was then treated with the diamine oxidase–liver alcohol dehydrogenase couple as earlier to produce (αS)-[α-$^{3}H_1$,2-^{14}C]histaminol [(20), ^{3}H : ^{14}C ratio 2.07, 84% retention]. After dilution with unlabelled histaminol (50 mg), it was then converted into (αR)-[α-$^{3}H_1$-2-^{14}C]*histamine* (^{3}H : ^{14}C ratio 2.11) by the steps described for the $^{2}H_2$-series in the previous section.

4(5)-*Formylimidazole.*—A solution of 4(5)-hydroxymethylimidazole [18] (1.96 g) in dioxan (80 ml) was heated under reflux for 1 h with active manganese dioxide [24] (19.6 g) and then filtered. The solids were washed with hot dioxan (3 × 50 ml) and the residue after evaporation of the organic solution was crystallised from methanol to yield 4(5)-formylimidazole (1.73 g), m.p. 174—175° (lit.,[25] 173—174°), δ(CD_3SOCD_3) 7.95 and 7.98 (each 1 H, s, imidazole), 9.81 (1 H, s, CHO), and 13.9 (1 H, NH).

[formyl-^{2}H]-4(5)-*Formylimidazole* (33) *and its* [formyl-^{3}H]-*Analogue* (34).—Sodium borodeuteride (462 mg) was added to a solution of 4(5)-formylimidazole (1.92 g) in ethanol (100 ml). After 30 min the solution was acidified with 6N-hydrochloric acid, the solvent was evaporated off, and the residue in water (20 ml) was adjusted to pH 8 with potassium carbonate. The residue from evaporation of the

solution was extracted with hot acetonitrile (4 × 50 ml), the extracts were evaporated, and the [*methylene*-$^2H_1$]-alcohol (31) was crystallised from acetonitrile (1.78 g), m.p. 90—92° (lit.,[26] 93—94°), δ(CD_3SOCD_3) 6.87 and 7.54 (each 1 H, s, imidazole), and 4.40 (1.05 H, CHD), 95% $^2H_1$-species; a synthesis of the analogous $^2H_2$-alcohol is described later.

A solution of this $^2H_1$-alcohol (1.65 g) in dioxan (100 ml) was oxidised with active manganese dioxide [24] as described above to yield [*formyl*-^{2}H]-4(5)-formylimidazole (33) (1.44 g), m.p. 174—175° (lit.,[25] 173—174°), δ(CD_3SOCD_3) 9.81 (0.18 H, CHO of unlabelled aldehyde), 83 ± 3% $^2H_1$-species. Oxidation of the alcohol was achieved in comparable yield using barium manganate [19] in dioxan. The crude 4(5)-formylimidazole could also be purified by sublimation (bath temperature 120 °C at 0.1 mmHg).

(*RS*)-[*methylene*-$^3H_1$]-4(5)-Hydroxymethylimidazole (32) was prepared from unlabelled 4(5)-formylimidazole (480 mg) which in ethanol (25 ml) was stirred with sodium borohydride (50 mg). Potassium borotritiide (*ca.* 20 mCi) was added and after the solution had been stirred for 18 h, sodium borohydride (60 mg) was added. The reaction mixture was worked up as described above to give the [*methylene*-$^3H_1$]alcohol (32) which was crystallised from acetonitrile (430 mg), m.p. 92—94°, total activity 9.98 mCi, 2.28 × 10^3 μCi $mmol^{-1}$.

This alcohol (380 mg) was oxidised as above with manganese dioxide (3.8 g) to give [*formyl*-^{3}H]-4(5)-formylimidazole (34), m.p. 174—175° (218 mg), 1.76 × 10^3 μCi $mmol^{-1}$.

[methylene-$^2H_2$]-4(5)-*Hydroxymethylimidazole*.—(a) *Benzylation of methyl imidazole*-4(5)-*carboxylate*. A solution of the ester [27] (9.14 g) in anhydrous dimethylformamide (200 ml) was added to a stirred suspension of sodium hydride (3.6 g, 50% dispersion) in dimethylformamide (50 ml) at −30 °C under nitrogen. The mixture was stirred for 30 min and then allowed to warm to 0 °C. Benzyl chloride (9.18 g) was then added dropwise and the solution was stirred at room temperature for 90 min. The solvents were evaporated off, the residue was partitioned between chloroform and water, and the product from the organic solvent was purified by column chromatography on t.l.c. silica gel (Merck type H) under pressure (100 kP cm^{-2}) using chloroform–methanol (96 : 4 v/v).

The major product, *methyl* 1-*benzylimidazole*-4-*carboxylate* (10.0 g), was obtained as a colourless oil, δ 3.80 (3 H, s, CO_2Me), 5.12 (2 H, s, PhCH_2), 7.25 (5 H, m, phenyl-H), and 7.56 and 7.58 (each 1 H, s, imidazole) (Found: M^+, 215.088 0. $C_{12}H_{12}N_2O_2$ requires *M*, 216,089 9).

The minor product, methyl 1-benzylimidazole-5-carboxylate (3.48 g), had m.p. 64—65° (lit.,[28] 63—64°), δ 3.81 (3 H, s, CO_2Me), 5.51 (2 H, s, PhCH_2) 7.25 (5 H, m, phenyl-H), and 7.62 and 7.78 (each 1 H, s, imidazole), M^+, 216.

(b) 1-*Benzyl*[methylene-$^2H_2$]-4-*hydroxymethylimidazole and* 1-*benzyl*[methylene-$^2H_2$]-5-*hydroxymethylimidazole*. A solution of methyl 1-benzylimidazole-4-carboxylate (3.24 g) in anhydrous dioxan (30 ml) was added dropwise to a stirred suspension of lithium aluminium deuteride (630 mg) in dioxan (20 min) during 20 min. The mixture was heated under reflux for 2 h, excess of deuteride was decomposed with ethyl acetate, water was then added, and the solids were filtered off. The filtrate was evaporated and the residue partitioned between chloroform and water. The product from the organic solvent was purified by column chromatography under pressure (100 kP cm^{-2}), on silica gel (Merck type H) using chloroform–methanol (9 : 1 v/v) as eluant. Crystallisation from benzene–n-hexane gave 1-*benzyl*-[methylene-$^2H_2$]-4-*hydroxymethylimidazole* (2.37 g), m.p. 79—80° (m.p. of protio-sample 79—80°), δ 5.00 (2 H, s, PhCH_2), 6.80 and 7.44 (each 1 H, s, imidazole), and 7.25 (5 H, m, phenyl-H) [no signal at 4.55 (CH_2OH of unlabelled alcohol)], *m/e* 190 (M^+).

1-*Benzyl*[methylene-$^2H_2$]-5-*hydroxymethylimidazole* (1.41 g) was prepared analogously from methyl 1-benzylimidazole-5-carboxylate (2.16 g) and had m.p. 139—141° (from $CHCl_3$–ether) (lit.,[29] m.p. of protio-compound 139—140°), *m/e* 190 (M^+).

(c) *Debenzylation of* 1-*benzyl*[methylene-$^2H_2$]-4-*hydroxymethylimidazole*. A solution of 1-benzyl[*methylene*-$^2H_2$]-4-hydroxymethylimidazole (2.85 g) in freshly distilled liquid ammonia was treated with small pieces of sodium metal until a permanent blue colour was obtained. This was discharged by addition of solid ammonium chloride, the solvent was evaporated, and the residue was extracted with dioxan to give material which was recrystallised from acetonitrile yielding [*methylene*-$^2H_2$]-4(5)-hydroxymethylimidazole (1.08 g), m.p. 92—94° (lit.,[26] m.p. of protio-material 93—94°), *m/e* 100 (M^+); no signal at δ 4.40 (CH_2 of unlabelled material).

2-*Acetamido*-3-[*imidazol*-4(5)*yl*]*acrylic Acid* (38) *and the* [3-^{2}H]-, *and*]3-^{3}H]-*Labelled Forms* (39) *and* (40).—To a solution of 4-formylimidazole (480 mg) in acetic anhydride (3 ml) was added anhydrous sodium acetate (820 mg) and when this had dissolved, *N*-acetyglycine (1.17 g) was added. The mixture was heated at 100 °C for 30 min, then cooled and mixed with ice–water (10 ml). Recrystallisation of the precipitate from benzene gave 2-*methyl*-4-[N-*acetylimidazol*-4(5)-*ylmethylene*]-5-*oxazolone* (35), m.p. 148—150° (690 mg) (Found: C, 54.8; H, 4.3; N, 18.9. $C_{10}H_9N_3O_3$ requires C, 54.8; H, 4.15; N, 19.15%), δ(CD_3SOCD_3) 2.40 (3 H, s, MeC=N–), 2.67 (3 H, s, MeCO), 7.14 (1 H, s, HC=C), and 8.20 and 8.32 (each 1 H, d, *J* 2 Hz, imidazole), *m/e* 219 (M^+).

The [3-^{2}H]-, and [3-^{3}H]-labelled forms were made as above from the corresponding labelled aldehydes; the [*formyl*-^{2}H]aldehyde contained 82 ± 3% ^{2}H-species. The ^{2}H-product (36) had m.p. 147—149°, *m/e* 220 (M^+), δ as for the protio-system above but with 0.18 H at 7.14, corresponding to 82 ± 3% ^{2}H-species.

The [*formyl*-^{3}H]aldehyde (34) of specific activity 3.6 × 10^3 μCi $mmol^{-1}$ (200 mg) and unlabelled aldehyde (280 mg) were mixed and treated with *N*-acetylglycine (1.17 g) to give the ^{3}H-product (37) (640 mg).

The foregoing unlabelled oxazolone (330 mg) was heated under reflux with water (10 ml) for 30 min, then evaporated and the residue was crystallised from water to give the imidazolylacrylic acid (38) (294 mg), m.p. (capillary) 280—282° (decomp.) (lit.,[30] 280°) (Found: C, 49.0; H, 4.6; N, 21.7. $C_8H_9N_3O_3$ requires, C, 49.2; N, 4.65; N, 21.5%), δ(CD_3SOCD_3) 2.04 (3 H, s, NAc), 7.0 (1 H, s, HC=C), and 7.41 and 7.78 (each 1 H, s, imidazole), *m/e* 195 (M^+).

The ^{2}H-product (39) prepared in the same way showed *m/e* 196 (M^+) and n.m.r. as above save for 0.15 H at δ 7.0 corresponding to 85 ± 3% ^{2}H-species.

Hydrolysis of all the ^{3}H-oxazolone above, without purification, gave the [^{3}H]*imidazolylacryclic acid* (40) (540 mg), specific activity 1.4 × 10^3 μCi $mmol^{-1}$.

N-*Acetyl*-(αS,βS)-[3-$^2H_1$]*histidine* (41) *in Admixture with the* (αR,βR)-*Isomer* (43).—A solution of the foregoing ^{2}H-acrylic acid (39) (300 mg) in water–acetic acid (1 : 4, 20 ml) was shaken at 20 °C and 760 mmHg with hydrogen and 10% palladium–charcoal (30 mg) until uptake ceased. The filtered solution was evaporated and the residue crystallised

from water to yield the mixture of acids (41) and (43), identified by comparison with authentic unlabelled material [20] (Found, for unlabelled material: C, 44.3; H, 6.0; N, 19.7. $C_8H_{11}N_3O_3{\cdot}H_2O$ requires C, 44.6: H, 6.1; N, 19.5% *m/e* 198 (M^+ for ^{2}H-sample), $[\alpha]_D^{20}$ 0.0° (*c*, 1.0 in H_2O), δ(CF_3CO_2D) 2.26 (3 H, s, NAc), 3.62 and 5.05 (each 1 H, d, *J* 5.5 Hz, CHD·CH), 7.45 and 8.61 (each 1 H, s, imidazole), and 3.46 (weak signal from CH_2CH species corresponding to 78 ± 3% $^2H_1$-species).

(αS,βS)-[β-$^3H_1$]*Histidine* (45).—The foregoing ^{3}H-imidazolylacrylic acid (40) (520 mg) was hydrogenated as above to yield the racemate (42) and (44), specific activity 1.39 × 10^3 μCi mmol^{-1} (491 mg). This was diluted with unlabelled *N*-acetyl-(α*RS*)-histidine and the product (860 mg) was treated as for earlier resolutions with hog kidney acylase-I (40 mg, Sigma, 600 units mg^{-1}). Ion-exchange separation of the products gave (αS,βS)-[β-$^3H_1$]*histidine* (45) which crystallised from aqueous ethanol (137 mg), specific activity 7.7 × 10^2 μCi mmol^{-1}. The other fraction contained *N*-acetyl-(α*R*,β*R*)-[β-$^3H_1$]histidine (44) (see below).

(αS,βR)-[β-$^3H_1$]*Histidine* (47).—Part (360 mg) of the *N*-acetyl-(α*R*,β*R*)-[β-$^3H_1$]histidine (44) from the preceding experiment was heated at 100 °C for 30 min with acetic anhydride (0.5 ml) and acetic acid (5 ml). The solvent was then evaporated off and the product, now a mixture of the (α*R*,β*R*)- and (α*S*,β*R*)-derivatives (44) and (46), was resolved with acylase-I. The hydrolysed product was isolated by ion-exchange as previously but could not be crystallised, and attempted purification caused losses; the remainder was therefore diluted with unlabelled (α*S*)-histidine (102 mg) and recrystallised twice from aqueous ethanol to give (αS,βR)-[β-$^3H_1$]*histidine* (47), specific activity 1.38 × 10^2 μCi mmol^{-1}.

Unlabelled Histaminol [*as* (19)] (cf. *ref.* 26).—A solution of 1,4-dihydroxybutan-2-one [31] [prepared [31] from butyne-1,4-diol (86 g)] in water (*ca.* 800 ml), and 40% aqueous formaldehyde (225 ml) were added to a stirred solution at 80 °C of copper sulphate (500 g) in water (2 l) and 35% aqueous ammonia (1.2 l). After the mixture had been stirred at 80 °C for 2 h, it was cooled to 0—2 °C, and the brown solid collected and washed with ice–water until neutral. The solid was then suspended in water (800 ml), adjusted to pH 5 with 2N-hydrochloric acid and treated with a stream of hydrogen sulphide until precipitation of copper sulphide was complete. This was filtered off, washed with hot water (800 ml), and the aqueous solution evaporated to *ca.* 200 ml. After adjustment with potassium carbonate to pH 8, the filtrates were evaporated and the residue was dissolved in anhydrous ethanol (250 ml) before evaporation to dryness; this treatment with ethanol was repeated twice. The final residue was extracted with chloroform–ethanol (4 : 1, 3 × 400 ml) and the oil from these extracts was filtered in the minimum volume of chloroform–methanol (4 : 1) through silica gel (300 g). Evaporation of the percolate and distillation of the residue (b.p. 144–146° at 0.05 mmHg) gave histaminol (31.6 g), *m/e* 112 (M^+) which crystallised from dry chloroform, m.p. 87—89° (lit.,[22] 92°). The corresponding picrate had m.p. 142—144° (*cf.* samples obtained in earlier experiments above).

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