Stereospecific Irreversible Inhibition of Mammalian (*S*)-Ornithine Decarboxylase by (*R*)-(−)-Hex-5-yne-1,4-diamine

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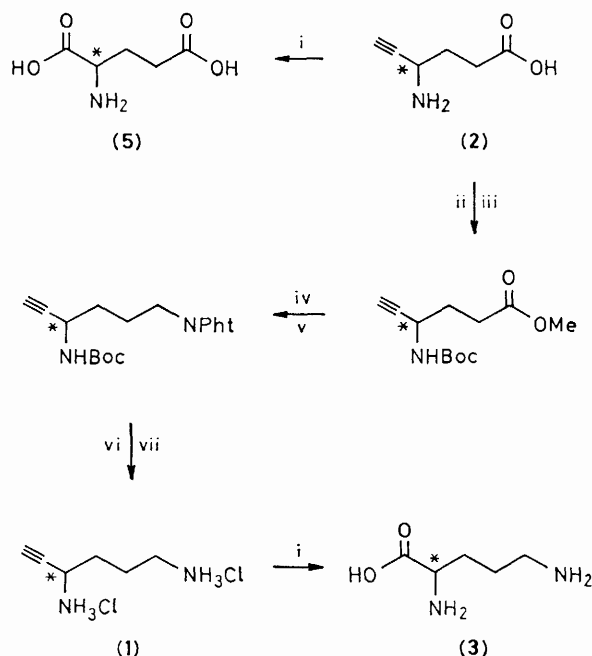
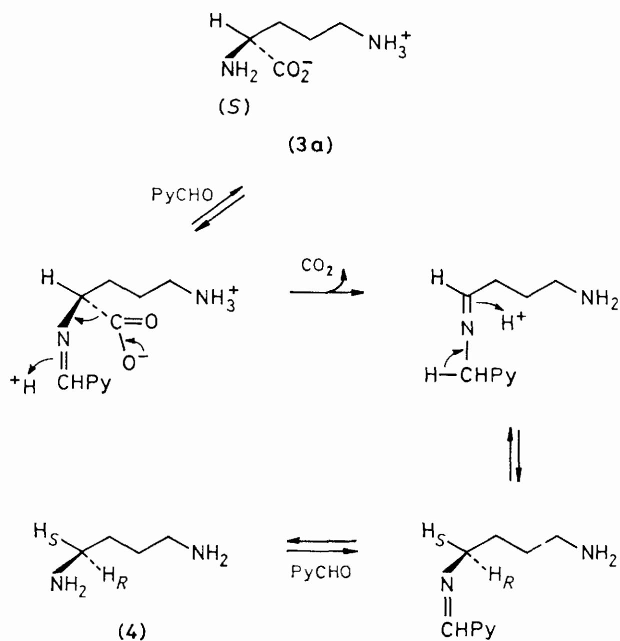
The synthesis of enantiomers of hex-5-yne-1,4-diamine, a potent irreversible inhibitor of (*S*)-ornithine decarboxylase (ODC, E.C. 4.1.1.17), is described; the stereospecificity of the inhibition by the (*R*)-isomer is consistent with a mechanism based on the microscopic reversibility principle of enzyme catalysis.

We reported previously that (*R,S*)-hex-5-yne-1,4-diamine (**1**) is an enzyme-activated irreversible inhibitor of (*S*)-ornithine decarboxylase (ODC), the enzyme catalysing the decarboxylation of (*S*)-(+)-ornithine (**3a**) to butane-1,4-diamine [putrescine (**4**)].[1] The mechanism of inactivation has been rationalized on the basis of the microscopic reversibility principle of enzyme catalysis. Based on thermodynamic considerations, this principle implies that, as in chemical reactions, all the steps of an enzyme-catalysed reaction are reversible. Therefore an enzyme must catalyse a reaction in both directions and the product must be able to serve as a substrate.[2] Thus, the hydrogen which is abstracted in a putrescine-analogue inhibitor is in the same position as the proton added in the natural putrescine produced after the decarboxylation step. Such a stereospecific abstraction of hydrogen has been demonstrated for the inactivation of bacterial (*S*)-glutamate decarboxylase (GAD, E.C. 4.1.1.15) and (*S*)-aromatic amino acid decarboxylase (AADC, E.C. 4.1.1.26) by (*R*)-(−)-4-aminohex-5-ynoic acid[3] and (*R*)-α-monofluoromethyl-dopamine,[4] respectively. In contrast, mammalian GAD has been shown to be inactivated only by (*S*)-(+)-4-aminohex-5-ynoic acid,[5] and (*S*)-histidine decarboxylase (E.C. 4.1.1.22) has been found to be inhibited more rapidly by (*S*)-α-mono-fluoromethylhistamine than by the (*R*)-isomer.[4] This is in



Scheme 1. i, RuO_2–$NaIO_4$–H_2O, 2 h, room temp.; ii, MeOH–HCl gas, room temp., 12 h; iii, Boc_2O–NEt_3–CH_2Cl_2, room temp., 12 h; iv, $LiAlH_4$–ether, −78 °C, 1 h; v, PPh_3–phthalimide–EtO_2CN=NCO_2Et, 12 h, tetrahydrofuran, room temp.;[8] vi, NH_2NH_2–EtOH, reflux, 12 h; vii, HCl 3 M, reflux, 1 h.

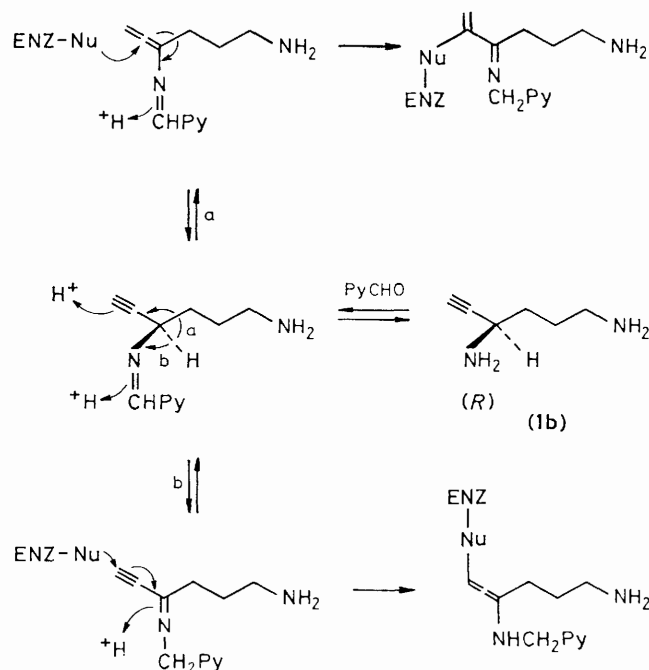


Scheme 2. Py = pyridoxal phosphate ring system.

spite of the fact that these decarboxylases have been demonstrated to introduce in the reaction product a *pro-R*-hydrogen with retention of configuration,[5,6] in agreement with the general mechanism proposed by Dunathan for the pyridoxal phosphate (PLP) dependent α-amino acid decarboxylases.[7]

These contradictory results, which were tentatively explained by a possible transaminase activity for these enzymes,[5] prompted us to determine the stereospecificity of the inhibition of ODC by (**1**).

The optically pure enantiomers, required for this study, were eventually obtained by an independent synthesis from



Scheme 3. Py = pyridoxal phosphate ring system.

the easily accessible (*S*)- and (*R*)-4-aminohex-5-ynoic acids[3] {(**2a**), $[\alpha]_D^{26}$ +35.6°, *c* 1.03, H_2O; (**2b**), $[\alpha]_D^{26}$ −37.6°, *c* 1.00, H_2O, m.p. 220 °C} as described in Scheme 1. The dihydrochloride of (**1**) (**a** or **b**) was crystallized from ethanol–ether {m.p. 165 °C, (**1a**), $[\alpha]_D^{26}$ +20°, *c* 0.37, H_2O; (**1b**), $[\alpha]_D^{26}$ −20.2°, *c* 0.41, H_2O}.† The overall yield from (**2**) (**a** or **b**) was 40%. The *S*- and *R*-configuration had been assigned respectively to (**2a**) and (**2b**) on the basis of their chemical conversion into 2-(*S*)-(+)- and 2-(*R*)-(−)-glutamic acid (**5**) by oxidation of the triple bond with RuO_2–$NaIO_4$.[9] Oxidation of (**1a**) into 2-(*S*)-(+)-ornithine under similar conditions confirmed the absence of epimerisation during the transformation. The enantiomeric purity of the various isomers was assessed after separation by a gas chromatographic assay using Chirasil-Val,[10] a chiral polysiloxane-type stationary phase; the isomers of (**1**) were *N*-acylated with trifluoroacetic anhydride and the isomers of (**2**) were esterified with ethanol–HCl gas and *N*-acylated with pentafluoropropionic anhydride. The enantiomeric excess was higher than 99.5% for the isomers of (**1**) and (**2**).[11]

When the enantiomers (**1a**) and (**1b**) were incubated with an ODC preparation obtained from the livers of rats which had been injected with thioacetamide,[1] time-dependent inactivation of the enzyme occurred only with the (*R*)-isomer (**1b**). The kinetic constants K_I (apparent dissociation constant) and $t_{\frac{1}{2}}$ (time of half inactivation of the enzyme extrapolated at infinite concentration of inhibitor) have been calculated according to the method of Kitz and Wilson.[1,12] They are K_I = 1.1 μM, $t_{\frac{1}{2}}$ = 8.8 min. The (*S*)-isomer (**1a**) displayed only a competitive inhibitory activity towards ODC and the apparent dissociation constant, measured from a Dixon[13] plot is K_I = 100 μM. These results are in agreement with the kinetic constants of the racemic mixture[1] for the irreversible inhibition of ODC (K_I = 2.3 μM, $t_{\frac{1}{2}}$ = 9.8 min).

In view of the difficulty of obtaining a pure preparation of mammalian ornithine decarboxylase and of the extreme

† N.m.r. and i.r. spectra and elemental analyses were in agreement with the proposed structure.

instability of this purified enzyme[14] no attempt has been made to determine the stereochemistry of the decarboxylation of ornithine by this enzyme. However, on the assumption that the decarboxylation of (*S*)-ornithine by ODC occurs with retention of configuration[7] (Scheme 2), the stereospecific inhibition of this enzyme by (*R*)-hex-5-yne-1,4-diamine (**1b**) would be consistent with the mechanism previously proposed. The diamine (**1b**) could replace putrescine in the active site, and the prop-1-ynylic proton, which has the same configuration as the *pro-R*-hydrogen of putrescine, could be abstracted, leading to the formation of a prop-1-ynylic anion which induced irreversible inactivation of ODC *via* either path a or b as indicated in Scheme 3.

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