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The Mechanism of Decarboxylation of Some 5-Aminoimidazole-4-carboxylic Acids and the Influence of Transition Metals

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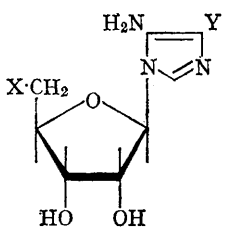
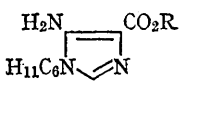
The aminoimidazolecarboxylic acid ribotide (I) (Carboxy-AIR), a central intermediate in the biosynthesis *de novo* of purine nucleotides, is formed by a reversible enzymic carboxylation of the aminoimidazole ribotide (II) (AIR) with hydrogen carbonate (0·3M and pH 8·3 for maximum yield) and these are the only substrates required.[1] Carboxy-AIR is briefly reported to be unstable in acid solution, and decomposed to AIR in 0·25N-sulphuric acid at room temperature after 2—3 hours.[1] We have now examined this decarboxylation in more detail both with (I), the corresponding nucleoside (III), and especially with the more readily available model compound (IV; R=H).[2]

The kinetics of the spontaneous decarboxylation of the amino-acids were measured by following the change in absorption at 260 mμ or (at low pH-values) 280 mμ over the pH range 1—9 at various temperatures between 40—60°. The efficacy of the method was confirmed by preliminary experiments in which evolved carbon dioxide was measured directly. The curves plot the first-order rate constants as a function of pH at 50° for the three compounds studied. The main results obtained in this study to date may be briefly summarised as follows. (*a*) In the case of the model compound (IV;R=H) the rate of decarboxylation increases with decrease in pH in two main steps corresponding to measured (at 20° by potentiometric titration and spectrophotometry) dissociation constants of pK 3·1 and 7·26. The rate varies with the 1-substituent but the variation between the nucleoside (III) and nucleotide (I) is especially noteworthy. (*b*) The rate of decarboxylation increases with increase in the basic constituents (acetate, phosphate, or glycine) of the buffer solutions at constant pH; *e.g.* at pH 5·50



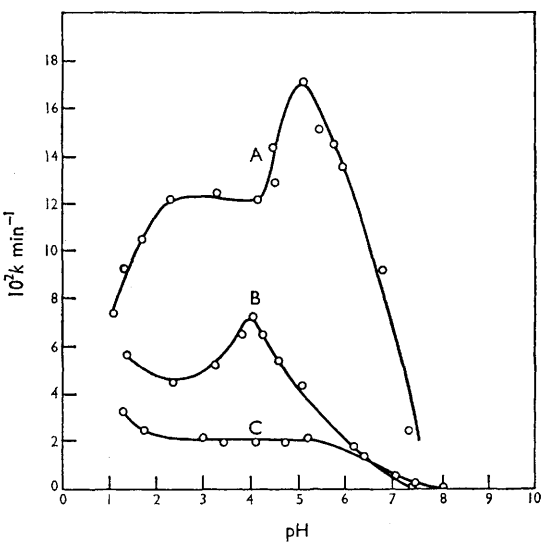


(IV)

(I) X=O·PO_3H_2, Y=CO_2H
(II) X=O·PO_3H_2, Y=H
(III) X=OH, Y=CO_2H

[1] L. N. Lukens and J. M. Buchanan, *J. Biol. Chem.*, 1959, **234**, 1799.
[2] G. Shaw and D. V. Wilson, *J. Chem. Soc.*, 1962, 2937.

and constant ionic strength ($I = 0{\cdot}10$) changes in the concentration of the sodium acetate (0·02—0·10 moles/l.) buffer resulted in the rate changing from $6{\cdot}2 \times 10^{-2}$ to $17{\cdot}6 \times 10^{-2}$ min.$^{-1}$ Similar results were obtained in phosphate buffers at pH 6·0 and 7·0, and in glycine buffers at pH 3·0. (*c*) The rate of decarboxylation is strongly reduced by bivalent (especially transition) metal ions in the pH range >4·5 and at the same time hyperchromic and bathochromic shifts in the spectra of the imidazoles are produced. At constant ionic strength ($I = 0{\cdot}07$) pH 4·50, 40°, and a nucleotide (I) concentration of 2×10^{-5}M, the rate varied from $4{\cdot}9 \times 10^{-2}$ min.$^{-1}$ in the absence of bivalent metal ions to $0{\cdot}00875 \times 10^{-2}$ min.$^{-1}$ in the presence of 0·011M-$NiCl_2$ which produced the greatest effect of the transition-metal ions (Mn, Co, Ni) studied. At low pH-values there is no change in the spectra with added nickel and this coincides with an absence of any effect on the rate of decarboxylation. (*d*) Nickel ions also had a striking effect on the enzymic decarboxylation of Carboxy-AIR (I) at 25° and pH 8·2 under the conditions outlined in the Table; the enzyme reaction is completely inhibited by 10^{-4}M-$NiCl_2$.



FIGURE

Variation of first-order rate constant with pH for some 5-aminoimidazole-4-carboxylic acids at 50°.

Buffer salt concentration 10^{-2}M. Total ionic strength 0·10.

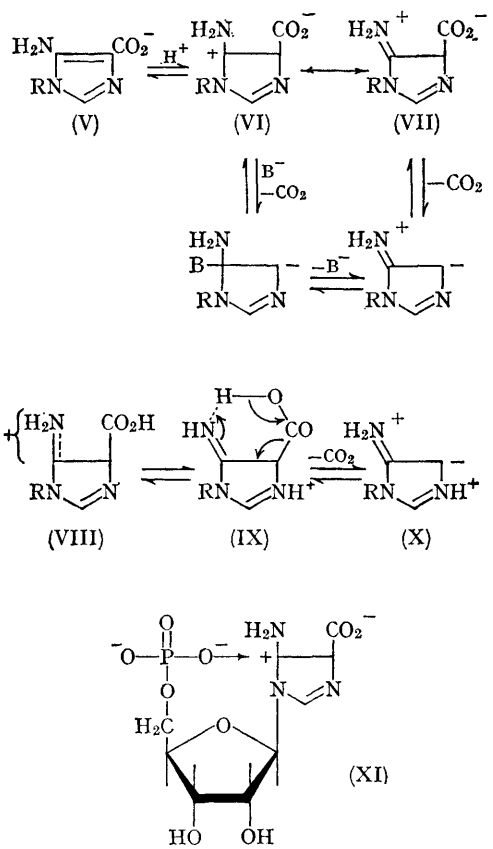
A. 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5′-phosphate (I).
B. 5-Amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid (III).
C. 5-Amino-1-cyclohexylimidazole-4-carboxylic acid (IV; R=H).

TABLE

Effect of nickel ions on the enzymic decarboxylation of 5-amino-1-β-D-ribofuranosylimidazole-4-carboxylic acid 5′-phosphate (I)

$NiCl_2$ (moles per litre)	Initial Rate (absorption units per min. at 260 mμ)
—	$1{\cdot}4 \times 10^{-2}$
10^{-6}	$2{\cdot}25 \times 10^{-3}$
10^{-5}	$1{\cdot}50 \times 10^{-3}$
10^{-4}	0

T = 25°; Nucleotide concentration $2{\cdot}5 \times 10^{-5}$M. Boric acid buffer pH 8·2, ionic strength 0·10. Constant enzyme concentration.



The experimental results indicate that the unstable species in these reactions are the mono- and di-protonated forms of the stable anion (V)

and that the rate of decarboxylation is further increased by general nucleophilic catalysis. We suggest that the results are consistent with the following reaction scheme.

Protonation of the stable anion (V) will include attack at the α-carbon atom leading to dipolar forms (VI) and (VII) which would be expected not only to decarboxylate readily but also to undergo nucleophilic catalysis in the manner outlined.

Further protonation of the dipoles (VI) and (VII) leads to the carboxylic acid (VIII) which can assume several forms including the bonded ketimine (IX) which may readily decarboxylate *via* (X). The general positions assigned to the protons are confirmed by the similarity of the ultraviolet absorption spectrum of the methyl ester (IV;R=Me) (λ_{max_1} 243—248 mμ, λ_{max_2} 268 mμ at pH 1—3) to that of the acid (IV;R=H) (λ_{max_1} 240—245 mμ, λ_{max_2} 267—268 mμ at pH 1·41; λ_{max} 253 mμ at pH 8·56) at low pH-values only. The enhanced rate of decarboxylation of the phosphate (I) near pH 5—6 may now be regarded as the result of perhaps an intramolecular type of nucleophilic catalysis as illustrated (XI). This effect is in fact most noticeable for the nucleotide in the region of secondary dissociation (pK 6) for a sugar phosphate, and the difference in rates between the nucleoside and nucleotide has largely disappeared at pH < 1 where phosphate dissociation is suppressed.

Inhibition of the decarboxylation by nickel ions and the accompanying shifts in the ultraviolet absorption spectra are consistent with complex-salt formation especially of the mono-protonated anion; the structure of the complex is under investigation. Similarly, inhibition of the enzyme-controlled decarboxylation by nickel ions is also presumably due to a modification of the substrate by complex formation rather than to a direct effect on the enzyme itself.

The general results and conclusions outlined here we hope may be of value in the assessment of the nature of the mechanism of the enzyme-induced decarboxylation of (I) which is under investigation.

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