Biotin and the Nucleophilicity of 2-Methoxy-2-imidazoline Toward the sp^2 Carbonyl Carbon

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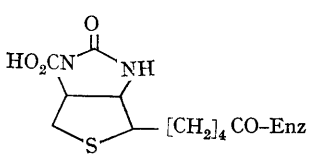
Summary A model for the biotin tautomer, 2-methoxy-2-imidazoline, possesses a nucleophilicity toward the sp^2 carbonyl carbon 10^{10}-fold greater than the previously considered 2-imidazolidone.

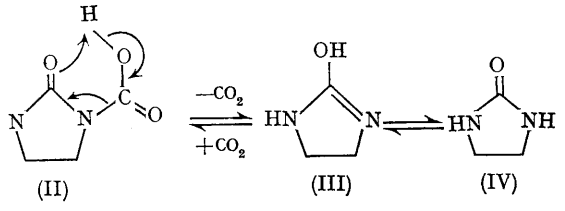
ALTHOUGH it has been conclusively shown[1] that the action of the coenzyme biotin in carbon dioxide transfer reactions involves two distinct steps with the intermediate formation of *N*-carboxybiotin (I) the detailed mechanism remains



(I)

to be clarified. One of the main problems encountered in the study of model systems of the coenzyme [*e.g.* non-enzymatically bound biotin or 2-imidazolidone (VI)] is the extremely low nucleophilicity of the ureido-group, making it difficult consequently to suggest any plausible scheme using these models which would show high reactivity in carboxylation.[2a]

Caplow[3] while studying the decarboxylation of *N*-carboxyimidazolidone noted that the reaction was far more rapid towards neutral pH; this, together with the



(II) (III) (IV)

large positive entropy of the reaction at low pH, suggested a ready unimolecular decarboxylation route (II) for the carbamate. It was later estimated[4] that the neutral species

was decarboxylated *ca.* 4000 times more rapidly than the corresponding anion. If this suggestion is, in fact, correct then by microscopic reversibility the carboxylation of 2-imidazolidone should occur by reaction with its tautomer, 2-hydroxy-2-imidazoline (III). Since the reactivities of such compounds as (III) with sp^2-hybridized carbon are unknown, we have investigated the reaction of 2-methoxy-2-imidazoline[5] with representative acetate esters (as models for ATP activated CO_2).

From the results listed in the Table it is seen that, although no reaction was detected between 2-imidazolidone (IV) and even the highly active acylating agent 1-acetyl-3-methylimidazolium chloride,[3] 2-methoxy-2-imidazoline reacts rapidly even with *p*-nitrophenyl acetate. Several concentrations of the amine (internally self-buffered by the presence of the amine hydrochloride) were used and in all cases plots of the observed rate constants against the free amine concentration were linear; such plots intersected the axis when [amine] = 0 at $k_{obs} = k_{HO^-}[HO^-]$, showing the absence of terms due to general acid or base catalysis. 2-Methylmercapto-2-imidazoline shows similar, if slightly enhanced, reactivity. But clearly the large positive deviation from the Brønsted relation (involving other amines) which is characteristic of imidazole and, to a lesser extent, 2-substituted imidazoles, does not occur with 2-methoxy-2-imidazoline (Table). In fact, the rate constant for the latter fits very well on a Brønsted plot (with slope *ca.* 1·0) encompassing other tertiary amines.[2b]

Second-order rate constants (l. mole^{-1} min.$^{-1}$) for acylation of 2-imidazolines

Nucleophile	pK_a	Substrate[a] PNPA	DNPA	Conditions[b]
2-Methoxy-2-imidazoline	9·14	2·07	24	A
	9·02	1·80		B
2-Methylmercapto-2-imidazoline	9·32	27·5	135	A
Imidoazole	6·95	20·5		B[6]
2-Methylimidazole	7·75	2·7		B[6]

[a] PNPA = 4-Nitrophenyl acetate; DNPA = 2,4-dinitrophenyl acetate.
[b] A = 30°, H_2O solvent, μ = 1·0 (KCl); B = 30°, 28·5% ethanol (water, μ = 0·5 (KCl).

Using these data an estimate can be made of the difference in reactivity of (III) and (IV) with a given acylating substrate. The pKa of 2-imidazolidone (IV) has been estimated as −1·05 in water (at 10°)[4] and −2·57 in anhydrous formic acid (biotin has approximately the same value)[7] so that $\Delta pK_{IV \rightarrow III} \approx 10$. If the Brønsted relation holds over this wide range (and there is precedent for this[7]), then $\Delta \log k_{IV \rightarrow III}$ must be of the order of 10 powers of 10. Similarly a rate constant of 5×10^4 l. mole^{-1} min.$^{-1}$ can be estimated[4] for the uncatalysed reaction of (III) with carbon dioxide at 10°.

Although the concentration of the enol component of biotin is low,[9] the immense reactivity difference between (III) and (IV) would appear to be consistent with a carboxylation route involving the enolized form. Any tendency to enolize biotin when bound to the enzyme[10] would of necessity greatly enhance its reactivity.

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[1] F. Lynen, *Biochem. J.*, 1967, **102**, 381.
[2] T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Benjamin, New York, 1966. (a) vol. II, ch. 11; (b) vol. I, ch. 1.
[3] M. Caplow, *J. Amer. Chem. Soc.*, 1965, **87**, 5774.
[4] M. Caplow and M. Yager, *J. Amer. Chem. Soc.*, 1967, **89**, 4513; M. Caplow, *ibid.*, 1968, **90**, 6795.
[5] Prepared by the method of G. I. Poos, J. Kleis, and C. K. Cain *J. Org. Chem.*, 1959, **24**, 645, and isolated as the hydrochloride, m.p. 90—91°. The pK_a listed by these authors is 5·8 (no conditions given).
[6] T. C. Bruice and G. L. Schmir, *J. Amer. Chem. Soc.*, 1958, **80**, 148.
[7] C. E. Bowen, E. Rauscher, and L. L. Ingraham, *Arch. Biochem. Biophys.*, 1968, **125**, 865.
[8] W. P. Jencks and M. Gilchrist, *J. Amer. Chem. Soc.*, 1968, **90**, 2622.
[9] J. A. Glasel, *Biochemistry*, 1966, **5**, 1851.
[10] W. Traub, *Science*, 1959, **129**, 210.