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The Location of the Reactive Carbon in N^1-Methylnicotinamide

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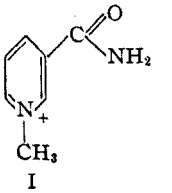
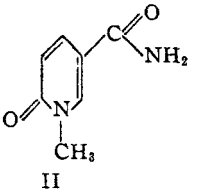
One uncertainty remaining in the structures of Coenzyme I and II as worked out by Warburg,[1] Karrer[2] and Schlenk,[3] involves the site of the reversible oxidation–reduction of the nicotinamide moiety. This uncertainty was recognized by Karrer in his first experiments using N^1-methylnicotinamide iodide as a model compound. Reduction occurs α to the ring nitrogen, but until now means have not been available to locate the reactive carbon in one of the two α-positions. From consideration of the properties of the quinine-oxidizing enzyme detailed below, the oxidation of N^1-methylnicotinamide by this enzyme to the corresponding 6-pyridone[4] enables us to locate the center of reactivity and therefore the site of oxidation–reduction in the 6-position of this quaternary nicotinamide ring. However, there is no direct evidence yet available about the arrangement obtaining in the pyridinonucleotide coenzymes themselves.

Oxidation by the quinine-oxidizing enzyme of rabbit liver is restricted to the unsaturated α-position of many pyridine, quinoline and pyrazine compounds.[5] This is referrable to the fact that only the pseudo-base forms are oxidized, as is evident from the parallelism between the rate of oxidation of various compounds and the ease of their pseudo-base formation.[6] In addition, the enzyme has been found to oxidize 1,2,2,5,5-pentamethyl - 6 - hydroxy - tetrahydropyrazine, which is itself a stable pseudo-base prepared by Dr. John G. Aston.[7] Consequently, if a compound is oxidized by the enzyme, it will be oxidized in the reactive α-position which is the site of unsaturation and of pseudo-base formation. Identification of an enzymic oxidation product will therefore locate the reactive carbon. In compounds such as pyridine, where both positions are similar, neither is "reactive" and no oxidation occurs.

The enzymic oxidation product of quinoline has previously been identified as carbostyril. We have also obtained 34 mg. of isocarbostyril, identified by mixed melting point, from enzymic oxidation of 40 mg. of isoquinoline. The reactivity in these compounds of carbon atoms 2 and 1, respectively, as shown by the enzyme reaction, thus agrees with the sites identified by other means.[8,9] Similarly, the product of the enzymic oxidation of N^1-methylnicotinamide chloride (I) has been identified as 1-methyl-3-carboxylamide-6-pyridone (II). Assay of the product in the enzyme mixture by its ultraviolet absorption at 259 and 290 mμ has shown it to be the sole product of the enzymic oxidation of N^1-methylnicotinamide chloride.[10]



I



II

This oxidation thus identifies the 6-carbon of N^1-methylnicotinamide as the reactive carbon. Because of the limits of enzyme specificity, the rate of oxidation of pure Coenzyme I by the enzyme is so slow as to preclude isolation of palpable amounts of the product for identification of the reactive carbon in this compound. However, N^1-methylnicotinamide also forms a reversibly oxidizable *o*-dihydro compound analogous in every way to the reduced coenzymes. The recent crystallization of this derivative is evidence that only one of the possible *o*-dihydro compounds is formed,[11] and it follows that this is the 1,6-dihydro derivative.

Summary

Oxidation of N^1-methylnicotinamide chloride to the corresponding 6-pyridone by the quinine-oxidizing enzyme shows that the 6-carbon is the reactive position of the quaternary nicotinamide ring.

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(1) Warburg, Christian and Griese, *Biochem. Z.*, **282**, 157 (1935).
(2) Karrer, Schwartzenbach, Benz and Solmssen, *Helv. Chim. Acta*, **19**, 811 (1936).
(3) Schlenk, *J. Biol. Chem.*, **146**, 619 (1942).
(4) Knox and Grossman, *J. Biol. Chem.*, **166**, 391 (1946).
(5) Knox, *J. Biol. Chem.*, **163**, 699 (1946).
(6) Sidgwick, Taylor and Baker, "The Organic Chemistry of Nitrogen," Oxford University Press, Oxford, 1937, pp. 526, 550, 556.
(7) Aston, THIS JOURNAL, **52**, 5259 (1930).
(8) Renshaw, Friedman and Gajewski, *ibid.*, **61**, 3322 (1939).
(9) Mills and Smith, *J. Chem. Soc.*, **121**, 2724 (1922).
(10) Knox and Grossman, *J. Biol. Chem.*, **168**, 363 (1947).
(11) Karrer and Manz, *Helv. Chim. Acta*, **29**, 1152 (1946); Karrer and Blumen, *ibid.*, **30**, 1157 (1947).