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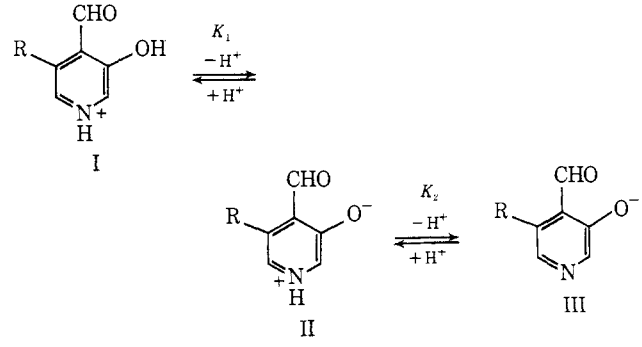
Reduction of Pyridoxal Phosphate (and Analogs) by 1,4-Dihydropyridine

Sir:

Though in enzymatic reactions nicotinamide–adenine dinucleotide (NADH) serves as a reducing agent for aldehydes, *via* direct hydrogen transfer, rather extensive search has led to no aldehyde substrate reducible by 1,4-dihydropyridine in simple model systems operating in aqueous solution at ambient temperature.[1] For horse liver alcohol dehydrogenase the Zn^{II} species present at each of the two NADH-containing active sites has been suggested to facilitate aldehyde reduction by polarization of the carbonyl group through direct interaction with the carbonyl oxygen.[2] Though Creighton and Sigman[3] have quite recently described a Zn^{II}-dependent reduction of 1,10-phenanthroline-2-carboxaldehyde by *N*-propyl-1,4-dihydronicotinamide (NPrNH), this system, being reported only in acetonitrile, is apparently restricted to aprotic solvents. Apparently searches for aldehyde substrates have never been directed to pyridoxal phosphate and analogs. This is rather surprising considering the fact that, as cofactors, NADH and pyridoxal phosphate cohabit in the same cell milieu. We report herein that pyridoxal phosphate (PLP), pyridoxal (PL), and 3-hydroxypyridine-4-aldehyde (PCHO) serve as suitable aldehyde substrates for reduction by the 1,4-dihydropyridines, NPrNH, and 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine (Hantzsch ester). Also, we find the reductions to be facilitated by metal ions in aqueous solution. Kinetic studies were carried out spectrophotometrically under N_2 in Thunberg cuvettes employing two media: (a) neat boiling methanol (NPrNH at 354 nm, Hantzsch ester at 372 nm); and (b) 52.1 wt % aqueous methanol at 30°, μ = 0.01 with KCl (NPrNH at 362 nm, Hantzsch ester at 372 nm). Tlc and nmr studies established that the products of reaction of PCHO and NPrNH were *N*-propylnicotinamide and the carbinol formed by reduction of the aldehyde. In refluxing methanol without buffer, the predominant species of pyridine aldehydes present will be II and III. We may assume that the rate of reduction of II $\gg$ III. The second-order rate constants (M^{-1} min^{-1}) for reduction of species II are as follows: 16 [PCHO][NPrNH]; 0.74 [PCHO][Hantzsch ester]; 0.28 [PL][Hantzsch ester]. The nmr spectrum of the product from reaction of PL and Hantzsch ester in refluxing methanol-*d* had singlet peaks of almost identical integral intensities at 4.63 and 4.82 ppm (δ). This result establishes direct transfer of a hydrogen from the dihydropyridine to the 4-aldehyde of PL. In the buffered aqueous methanol solutions at pH 8.34 the following



apparent second-order rate constants were obtained employing Hantzsch ester: PCHO, no reaction; PL, 0.22; PLP, 0.48 M^{-1} min^{-1}. From the pH dependence of the reaction of PLP with NPrNH the rate constants for the ionic forms of PLP corresponding to I, II, and III could be estimated at 17, 2.7, and $\sim 10^{-1}$ M^{-1} min^{-1}, respectively. The order of reactivity of $PLP_I > PLP_{II} > PLP_{III}$ > pyridine-4-aldehyde = 0 is that previously noted for imine formation[4] and finds similar explanation.

One would anticipate enhancement in the rate of reduction of PLP, PL, and PCHO upon complexation by metal ions, much as in the transamination reaction.[5] This was found to be the case. Employing 52.1 wt % methanol–water (30°) buffered by EDTA (pH 7.05–7.10) at 0.02 *M* with metal ion at 0.015 *M*, the following order of catalysis was observed in reduction of PLP by Hantzsch ester: $Ni^{2+}(k_{rel} = 7.2) > Co^{2+}(k_{rel} = 3.4) \geq Zn^{2+}(k_{rel} = 2.8) > Mn^{2+}(k_{rel} = 1.3) = Mg^{2+}(k_{rel} = 1.2)$, and no metal ion ($k_{rel}$ = 1.0). Considering that [EDTA] slightly exceeded [metal ion] in these experiments, the metal ion enhancement of rate is appreciable.

At present, it would appear as though the only aldehydes susceptible to 1,4-dihydropyridine reductions in aqueous solutions at ambient temperatures are PLP and its analogs. Also, the metal ion promotion of these reactions apparently represents the only case of metal ion catalysis of aldehyde reduction by a 1,4-dihydropyridine in aqueous solution.

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(2) For a review see A. S. Mildvan, *Enzymes*, 2, 446 (1970).
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Nicandrenone, an Insecticidal Plant Steroid Derivative with Ring D Aromatic

Sir:

In 1951, the isolation of a substance termed "nicandrin" from the Peruvian weed *Nicandra physalodes* was reported.[1] It was later found to possess strong insect repellent and mild insecticidal properties, and as it was

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