CHEMISTRY LETTERS, pp. 1113-1116, 1974. Published by the Chemical Society of Japan

INTERACTION OF THIAMINE (VITAMIN B_1) WITH 1,4-DIHYDRONICOTINAMIDE. REDUCTION OF IMINO GROUPS [1)]

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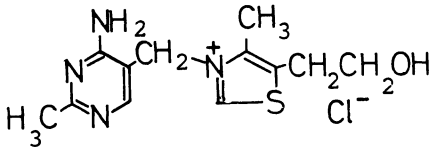
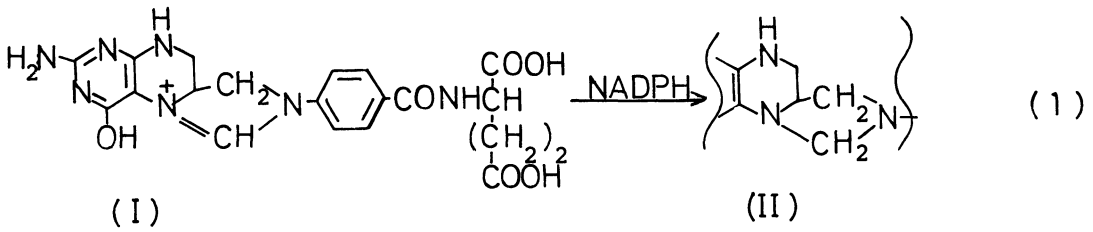
Thiamine (Vitamin B_1) and its analogue, 3-methylbenzothiazolium iodide serve as suitable imine substrates for reduction by 1-benzyl-1,4-dihydropyridine, and the reduction of the imino group to amine occurs easily at room temperature.

It has been established by Breslow[2)] that rapid deuterium exchange in the 2 position of a thiazolium salt proceeds in the absence of basic catalysts. This fact suggests that thiazolium salts would serve as an excellent electron-acceptor in the oxidation-reduction reaction, since the imino group of thiazolium salts must be fairly electron-deficient. In fact, the imino group of N^5,N^{10}-methenyl-tetrahydrofolic acid (I) is enzymatically reduced to N^5,N^{10}-methylene-tetrahydrofolic acid (II) by NADPH(eq.1).



Thiamine hydrochloride



(I) (II) (1)

Here we wish to report on a nonenzymatic reduction of the imino group of thiamine (Vitamin B_1) and its analogue, 3-methylbenzothiazolium iodide (III) by 1-benzyl-1,4-dihydronicotinamide (NBzNH, IV). Ultraviolet absorbance due to NBzNH was reduced with increasing time of incubation after mixing with thiamine as shown in Figure 1. A similar result was observed in the reduction of III by NBzNH. In contrast, NBzNH by itself was stable in the absence of thiamine. The reaction product of III was confirmed as follows: NBzNH (0.01 mole) in 50 ml. of acetonitrile and III (0.01 mole) in 50 ml. of an aqueous borate buffer solution (pH 8.5) were mixed and the clear solution was left at room temperature in the dark. After two

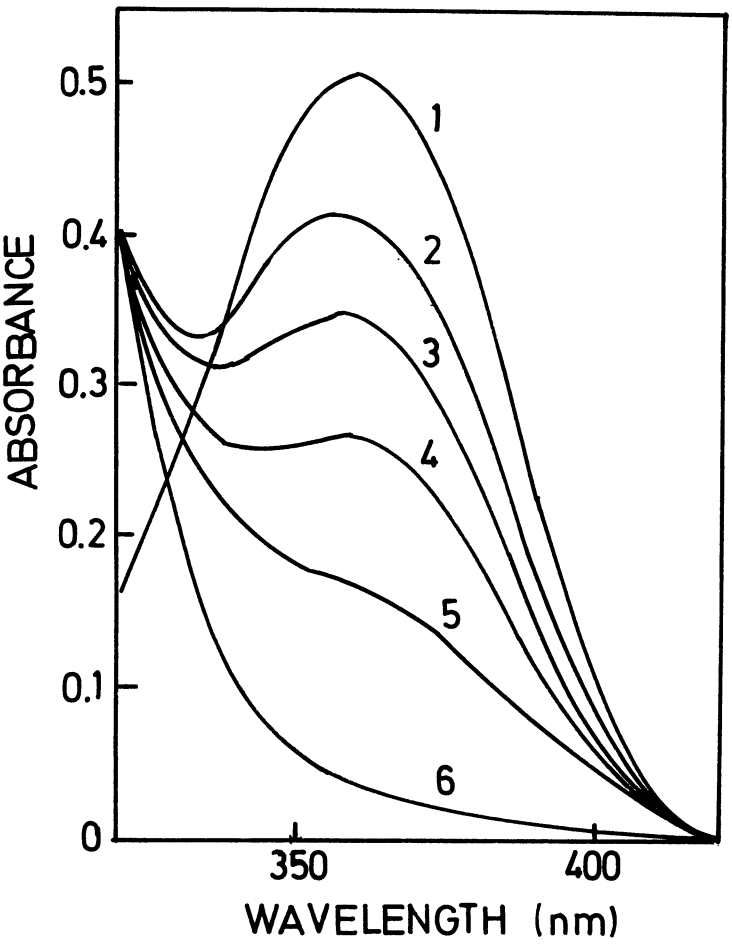
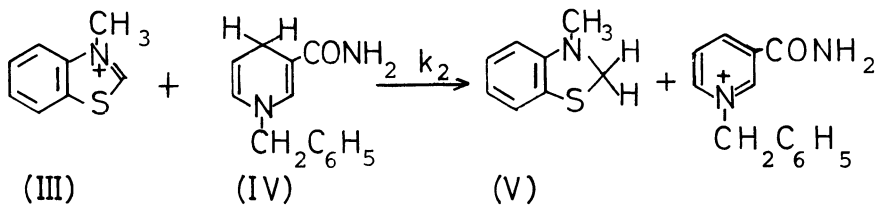


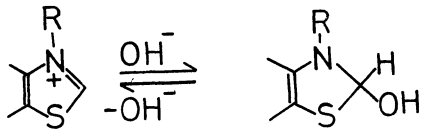
Figure 1.
The reduction of thiamine by 1-benzyl-1,4-dihydronicotinamide (NBzNH, IV). 30°, μ = 0.1, pH = 10.40, 2.5 vol% acetonitrile; [Thiamine] = 1.25×10^{-3}M, [NBzNH] = 7.0×10^{-5}M. 1, before mixing (NBzNH); 2, after mixing with thiamine; 3, 10 min; 4, 31 min; 5, 60 min; 6, 135 min.

reduction by NBzNH.

days acetonitrile was removed in vacuo, and the reddish oily layer separated from the aqueous layer was analysed by glc (Silicon column, 220°) and tlc (Silica-gel, chloroform). The product possessed almost the same retention time and R_f value (0.86) as those of 3-methyl-benzothiazoline (V, R_f = 0.88) prepared by Zn-HCl reduction of III.[3] Distillation in vacuo (bp 142-144°/15mm; lit.[4] 133°/11mm) provided a light-yellow oil. An NMR spectrum($CDCl_3$) of the compound was identical with that of V: 1.64 ppm, 3H, N-CH_3; 4.45 ppm, 2H, CH_2; 6.2-7.1 ppm, 4.2H, aromatic. These results clearly prove that the imino group of III was reduced to the tertiary amine group(eq.2). The second-order rate constants, k_2 for the reduction of III were determined by monitoring the decrease of NBzNH absorbance (λmax 360 nm). They were 7.4 and ca. 0.1 $M^{-1}min^{-1}$ at pH 8.28 and 10.40, respectively (30°, μ = 0.1). The diminished reactivity of III at the higher pH is attributable to the decrease of the benzothiazolium fraction, since it is reported that thiazolium salts form 2-hydroxy-thiazolines at high pH's(eq.3)[4-6] which would be inactive for the

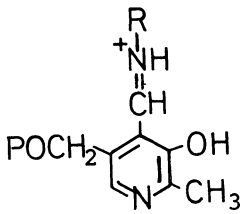


(III) (IV) (V) (2)

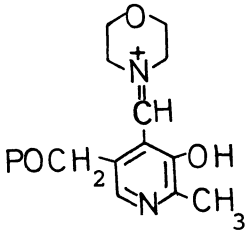


(3)

The reduction of carbonyl compounds by 1,4-dihydronicotinamide has been limited to the extremely polar groups such as hexachloroacetone[7] and trifluoroacetophenone.[8] Recently Creighton and Sigman[9] have described a Zn(II)-dependent reduction of 1,10-phenanthroline-2-carboxaldehyde by 1-propyl-1,4-dihydronicotinamide (NPrNH). Pyridoxal 5'-phosphate, a cofactor, is also established to undergo reduction by NPrNH at room temperature.[10] Since 1,10-phenanthroline-2-carboxaldehyde and pyridine-4-carboxaldehyde can not serve as substrates, it may be concluded that a metal-chelation and a hydrogen-bonding with carbonyl oxygens facilitate the reduction of aldehydes. In this work, reduction of the imino groups is probably due to the cationic charge on the nitrogen, since there is no evidence that a neutral imine (or a Shiff base) serves as the substrate. This difference may be understood by comparing a relative reactivity of imines. From studies of semicarbazone formation from pyridoxal 5'-phosphate in the presence of excess morpholine, Cordes and Jencks[11] have concluded that the most reactive species of pyridoxal phosphate imine toward transamination is protonated (4a) and that the morpholine imine (4b) serves as a useful model of the protonated imine.



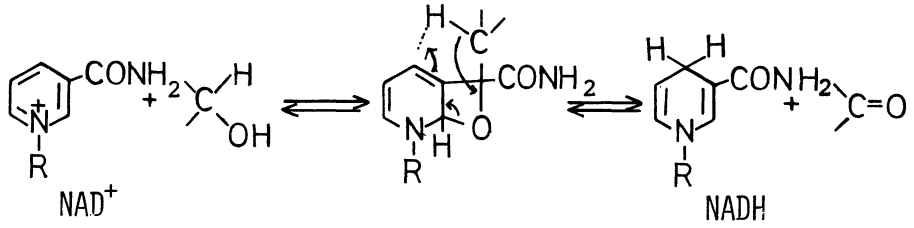
(4a)



(4b)

These results imply that the protonated imine would be the most susceptible to the reduction by dihydronicotinamide.

Hamilton[12] proposed that the interconversion of aldehydes and alcohols coupled to the oxidation-reduction of NAD^+ proceeds by the following scheme(eq.5).



(5)

Though this mechanism involving the covalent intermediate is favorable to the froward direction, the reverse reaction requires nucleophilic attack of an enamine part of NADH on the carbonyl oxygen, not on the sp^2 carbon. If the mechanism of eq.5 were correct, the reduction of carbonyl compounds by NADH should be highly unfavorable energetically. In fact, nonenzymatic NADH-catalyzed reduction of aldehydes may, depending on the carbonyl function, be quite facile in aqueous solutions. Application of this mechanism to the reduction of imino groups is hardly conceivable since it is difficult to imagine how the nitrogen of the thiazolium salts could form the covalent intermediate with NADH. In the case of the present work, therefore, it would be reasonable to propose some hydrogen transfer mechanism without the covalent intermediate.

At present, it would appear as though thiamine and its analogue are the only imines susceptible to 1,4-dihydronicotinamide reductions at room temperature. Since a stronger nature of protonated imines as electron-withdrawing substituents than that of carbonyl groups has generally been observed,[13] a variety of protonated imino groups may serve as substrates for 1,4-dihydronicotinamide reductions and this combination may be important in enzymatic reactions as well as in reactions in vitro.

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(Received July 5, 1974)