$C_7H_{11})_2$ and a tabulation of bonding parameters (2 pages). Ordering information is given on any current masthead page.

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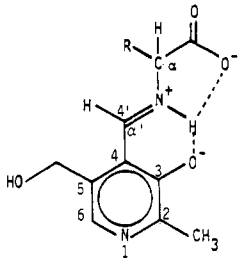
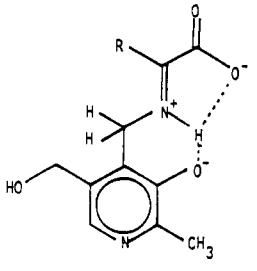
NMR Evidence for the Delocalized α,α' Carbanion of Pyridoxal and Pyridoxamine Schiff Bases as the Intermediate in Vitamin B_6 Catalyzed Transamination

Sir:

The mechanism of enzymatic and nonenzymatic vitamin B_6 catalyzed transamination of α-amino and α-keto acids proposed by Metzler et al.[1] involves the formation and interconversion of aldimine and ketimine Schiff bases **1** and **2**. The

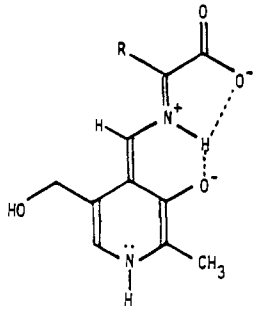


1, Monoprotonated pyridoxal Schiff base (HL^-)



2, pyridoxamine Schiff base, monoprotonated form

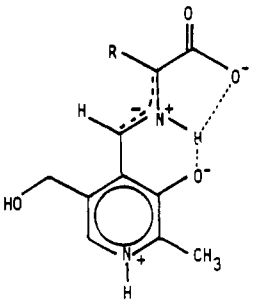
intermediate obtained by dissociation of the α proton of the amino acid moiety of the aldimine was first suggested by Metzler et al. to be the dihydropyridine type tautomer **3**. This



3, "Snell-Braunstein" intermediate

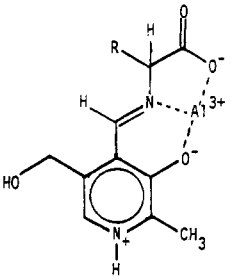
intermediate was later suggested by Schirch and Jenkins[2] as the absorbing species with a maximum near 505 nm in pyridoxal enzyme-substrate complexes. Analogous dihydropyridine intermediates derived from vitamin B_6 analogues have been described by Maley and Bruice.[3]

More recently NMR evidence for a deprotonated intermediate of the pyridoxal-alanine Schiff base, stabilized by complexation with Al(III), was reported by Abbott and Martell,[4] and the analogous monoprotonated Schiff base was suggested as a possible intermediate in vitamin B_6 catalyzed transamination in the absence of metal ions. Subsequently it was pointed out[5] that the diprotonated Schiff base is the more likely precursor of the carbanionic intermediate **4**.



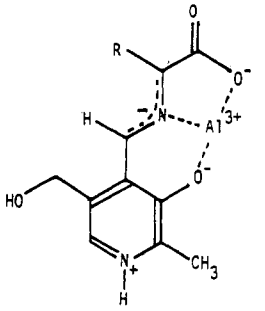
4, carbanion intermediate

In the present research, the kinetics of formation of the aldimine-Al(III) chelate **5** from pyridoxal and alanine, and also from pyridoxamine and pyruvate, has been followed by NMR. These systems are found to lead to the formation of



5, protonated Schiff base-Al(III) chelate

an equilibrium mixture consisting primarily of the 1:1 Al(III):Schiff base (aldimine) chelate and a smaller amount of the 1:2 aldimine chelate. The presence of these Schiff base complexes in solution was found to be accompanied by the formation of the new species **6** having an NMR spectrum



6, α-deprotonated intermediate (αDI)

resembling that of the aldimine, but differing in having a more negative charge. It is apparently an aldimine-type deprotonated Al(III) chelate of the type described earlier.[4] This compound forms as transamination proceeds, finally approaching a steady concentration that seems to be a constant fraction of the parent Schiff base formed.

It was found that, for a 1:1:1 ratio of pyridoxal, alanine, and Al(III) in D_2O at 10.0 °C and pD 5.0, the aldimine chelate concentration reaches a maximum and then levels off, as indicated by the NMR spectra in Figure 1. As the reaction progresses, the Al(III) complex of the α-deprotonated intermediate (αDI) appears somewhat more slowly than the aldimine chelate and the concentration increases and levels off, finally achieving a constant concentration of about 10% of that

(1) Metzler, D. E.; Ikawa, M.; Snell, E. E. *J. Am. Chem. Soc.* **1954**, *76*, 648.
(2) Schirch, L. V.; Jenkins, W. T. *J. Biol. Chem.* **1964**, *239*, 3801.
(3) Maley, J. R.; Bruice, T. C. *J. Am. Chem. Soc.* **1968**, *90*, 2843.
(4) Abbott, E. H.; Martell, A. E. *J. Am. Chem. Soc.* **1973**, *95*, 5014.
(5) Martell, A. E. *Adv. Enzymol.* **1982**, *53*, 163.

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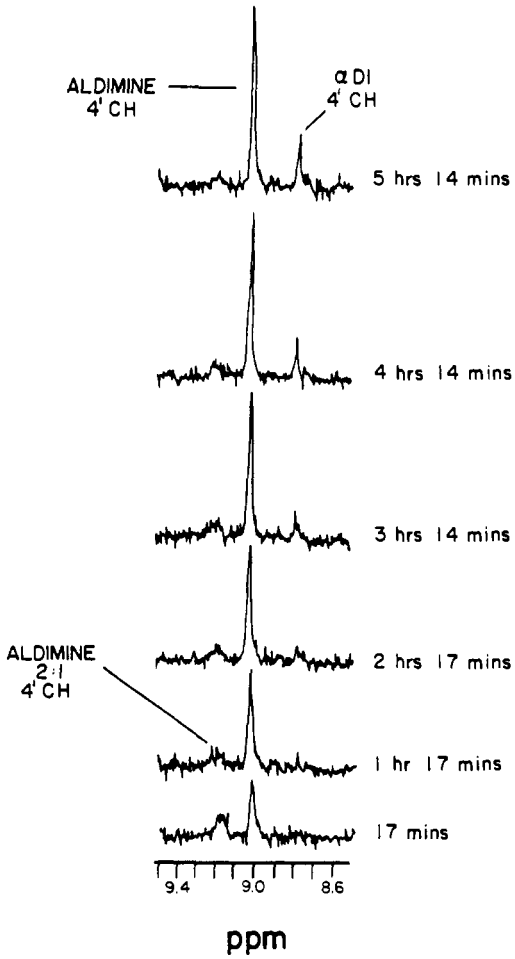

Figure 1. Growth of the 4'-CH proton resonances of αDI and the 1:1 and 1:2 Al(III):aldimine chelates in 0.1 M D_2O solution containing a 1:1:1 molar ratio of pyridoxal:alanine:Al(III) at pD 5.0 and 10.0 °C.

of the Al(III) aldimine complex.

Because the reverse transamination reaction is much more rapid, measurements of the reaction between pyridoxamine and pyruvate at about pD 5 in D_2O were made at 0 °C. Figure 2 shows the variation of integrated intensities of 4'-CH protons with time, indicating that formation of the same αDI Al(III) chelate occurs more rapidly initially than does formation of the aldimine. The concentration of the intermediate increases to its maximum value in about 2h and then decreases and levels off, while the concentration of the aldimine–Al(III) chelate continues to build.

In this transamination system (pyridoxal–alanine and pyridoxamine–pyruvic acid) the aldimine is much more stable than the ketimine, and the reaction system reaches an equilibrium consisting primarily of aldimine, regardless of which pair of compounds is used as the initial reactants. Thus it is seen that the changes of concentration of αDI in the course of achieving transamination, the relative rates of αDI and aldimine formation, and the fact that the same compound is formed in the forward and reverse reactions provide compelling evidence that αDI is the intermediate for both the forward (PL + ALA) and reverse (PM + PY) transamination reactions. The buildup of αDI concentration initially in advance of the increasing concentrations of aldimine, in the reaction between pyridoxamine and pyruvate, and the subsequent decrease in concentration to a lower value show it to be a mandatory intermediate for the transamination process leading from ketimine to aldimine.

A unique characteristic of the system described is the fact that the deprotonated intermediate is formed in concentrations

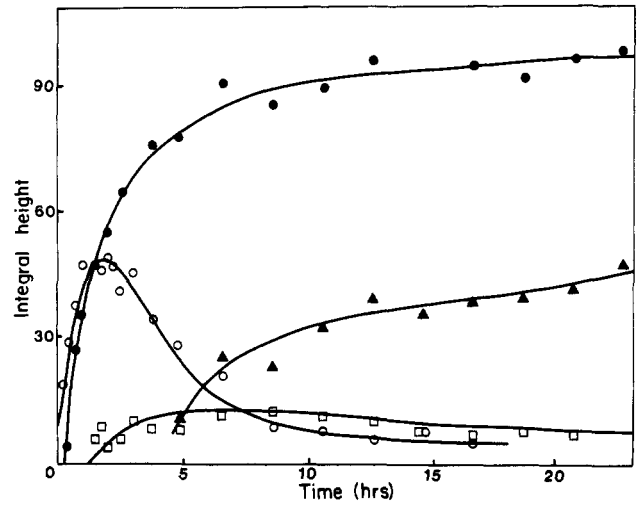

Figure 2. Integral height of 4-CH proton NMR resonances of a 1:1:1 molar ratio of pyridoxamine:pyruvate:Al(III) vs. time (t = 0 °C, pD 5.0, 0.10 M components): (●) 1:1 aldimine complex; (▲) 2:1 aldimine complex; (○) 1:1 αDI complex; (□) 2:1 αDI complex.

that are high enough to be measured by NMR. This phenomenon is considered to be due mainly to the ability of the highly charged Al(III) ion to stabilize the adjacent negative charge of the carbanion intermediate. In the absence of metal ions, the proton coordinated to the azomethine nitrogen of the aldimine or the ketimine may serve a similar function. In such cases, however, the single positive charge of the proton would produce much less coulombic stabilization of the α-deprotonated form, with the result that it would be present at concentrations much lower than are detectable by proton NMR under the reaction conditions employed. The α- (or α'-) deprotonated species formed from either the aldimine (or the ketimine) is therefore suggested as the common intermediate for transamination, and for related reactions such as α- and α'-proton exchange, racemization, and elimination of electronegative substituents at the β position of the amino acid moiety. It has been pointed out that a carbanion[5] of the type represented by **4**, which is indicated here as being formed by α- and α'-proton dissociation, may also be formed by C–C bond dissociation, thus functioning as the intermediate in α-decarboxylation and dealdolation reactions.

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Role of Peroxo vs. Alkylperoxo Titanium Porphyrin Complexes in the Epoxidation of Olefins

Sir:

We have recently reported on the stereoelective epoxidation of olefins with alkyl hydroperoxides catalyzed by molybdenum porphyrins[1] and proposed that the active species is a *cis*-hydroxy(alkylperoxo)(porphyrinato)molybdenum(IV) complex,[2] reminiscent of the intermediate suggested by Sheldon[3-5]

(1) Ledon, H. J.; Durbut, P.; Varescon, F. *J. Am. Chem. Soc.* **1981**, *103*, 3601-3603.

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