reasons for this speculation are the distinct tetrahedral distortions in the basal planes of the square pyramids, which are quite common in this type of copper surrounding and the fact that Cu(I) is known to interact with π systems but not Cu(II). It is hoped that additional experimental observations which can be expected from our structure determinations of Cu(II) (*l*-tyrosine)$_2$ and the peptide glycyl-*l*-leucyl-*l*-tyrosine will throw more light on these questions.

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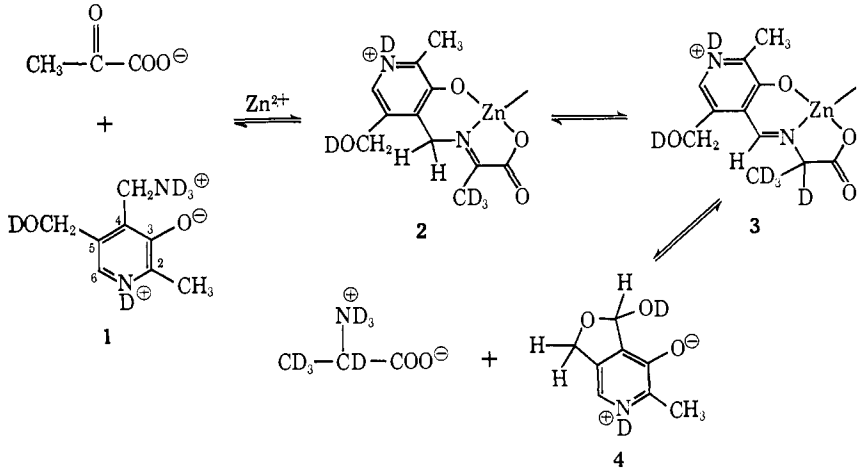
Detection and Identification of Intermediates and Products of a Nonenzymatic Transamination Reaction by Proton Resonance

Sir:

We are currently investigating by pmr the widely quoted mechanism[1] for transamination of α-amino acids and α-keto acids involving pyridoxal cofactors and metal ions[2] in aqueous solutions. Systems studied include 0.1 *M* pyridoxal–0.1 *M* alanine and 0.1 *M* pyridoxamine–0.1 *M* pyruvate in the presence and absence of 0.05 *M* Zn^{2+} over the pD range 1–13.[3] The purpose here is to demonstrate the direct detection and identification of the species implicated in that part of the over-all transamination sequence represented by the reaction pyridoxamine + pyruvate $\rightleftharpoons$ pyridoxal + alanine occurring at pD ~7. Previous pmr studies[4] serve to identify the signals and predominant forms of pyridoxamine (**1**) and pyridoxal (**4**) at this pD.

brief discussion of the spectra of solutions containing pyridoxamine and pyruvate (both at 0.1 *M*) in the absence of Zn^{2+}. The solutions do not transaminate rapidly at room temperature, but their spectra reveal formation of α-pyridoximinopyruvate (ketimine) at pD ~7–10. Ketimine formation results in labilization of the pyruvate methyl protons and substantial H–D exchange in D_2O solution. At pH 7.4 in H_2O solution a new signal appears at 50 cps corresponding to the condensed pyruvate methyl group.[5] In D_2O (pD 7.4) new signals are observed at 658, 276, and 116 cps, near to those of 6-H, 4-CH_2, and 2-CH_3 of free pyridoxamine, respectively, and are assigned to the corresponding protons of the ketimine. Extent of ketimine formation and ketimine chemical shifts are functions of pD; it suffices here to note that the former reaches a maximum of ~30% (by signal integration) at pD 9.8 and decreases to zero in strongly basic solutions (pD >11.5). Before transamination is observable, pmr spectra of D_2O solutions containing Zn^{2+} are rather similar to the metal-free solutions at a given pD except that the ketimine 2-CH_3 signal is broadened and shifted upfield (*cf.* Figure 2), indicating complexation of Zn^{2+} and slow exchange between coordinated and free ketimine, and the per cent solute in the ketimine form is more than 20% greater than in the metal-free solutions. All features of the pmr spectra *before* transamination are consistent with formation of the labile 1:1 and 1:2 Zn(II):ketimine complexes **2** with the concentration of the latter increasing with increasing pD.

The pmr spectrum of the pyridoxamine:pyruvate:Zn^{2+} solution after transamination is shown in Figure 1b. In order to identify reaction products a complete pmr study of D_2O solutions of pyridoxal and alanine



In the pyridoxamine–pyruvate–zinc system transamination in D_2O solution occurs rapidly but can be conveniently followed by pmr. Spectra taken before and after reaction (~20 min) are given in Figure 1; those recorded at intermediate times are superpositions of the two shown. Interpretation of Figure 1a requires (0.1 *M*), both metal-free and with Zn^{2+} added, has been carried out. In the metal-free solutions formation of N-pyridoxylidenealanine (aldimine) was detectable at pD $\geq$7 by the appearance of the characteristic[6] low-field azomethine proton signal at ~760 cps. In addition, two 6-H and 2-CH_3 signals and two alanine methyl doublets and CH quartets were observed. Features due to free pyridoxal, which exists mainly in the dipolar hemiacetal form **4** at pD 4.4–9,[4] and free alanine were readily identified; those remaining must

(1) D. E. Metzler, M. Ikawa, and E. E. Snell, *J. Am. Chem. Soc.*, **76**, 648 (1954).

(2) For extended discussions of the transamination reaction, *cf.* T. C. Bruice and S. J. Benkovic, "Bioorganic Mechanisms," Vol. II, W. A. Benjamin, Inc., New York, N. Y., 1966, Chapter 8; B. M. Guirard and E. E. Snell, "Comprehensive Biochemistry," Vol. 15, M. Florkin and E. H. Stotz, Ed., Elsevier Publishing Co., New York, N. Y., 1964, Chapter V.

(3) pD = pH + 0.40: P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, **64**, 188 (1960).

(4) O. A. Gansow and R. H. Holm, *Tetrahedron*, **24**, 4477 (1968).

(5) All chemical shifts refer to 100 Mc and a *t*-butyl alcohol internal standard; the chemical shift of this signal is close to those observed for α-oximinopropronic acid and acetaldoximine.

(6) M. J. O'Connor, R. E. Ernst, J. E. Schoenborn, and R. H. Holm, *J. Am. Chem. Soc.*, **90**, 1744 (1968).

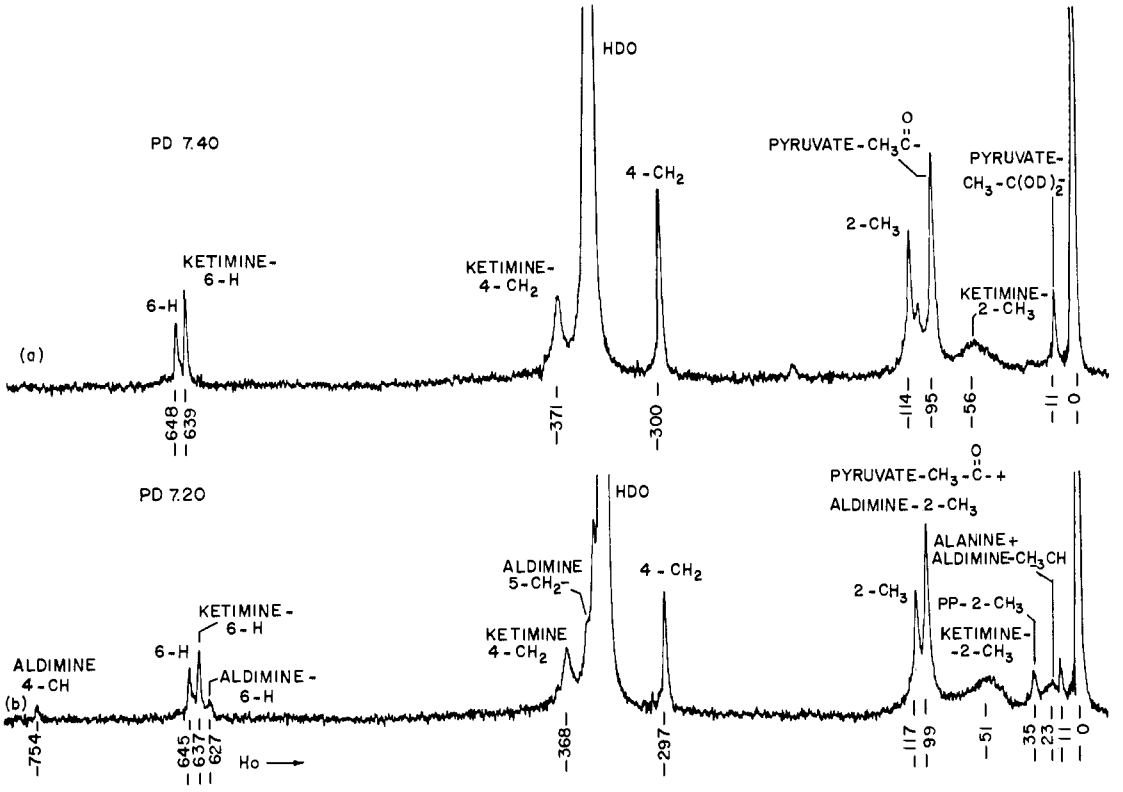


Figure 1. 100-Mcps pmr spectra of a solution initially containing 0.1 *M* pyridoxamine, 0.1 *M* sodium pyruvate, and 0.05 *M* Zn^{2+} at 33°: (a) before transamination, illustrating formation of ketimine species; (b) after transamination, revealing the presence of ketimine and aldimine species. Signals labeled 6-H, 4-CH_2, and 2-CH_3 refer to free pyridoxamine; PP = N-pyridoxylidenepyridoxamine. Chemical shifts are relative to a *t*-butyl alcohol internal standard.

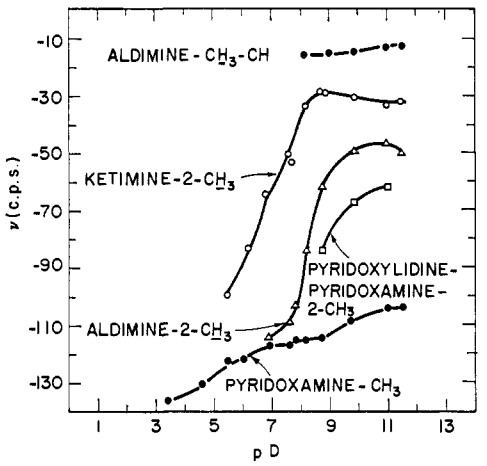


Figure 2. 100-Mcps chemical shifts as a function of pD in the methyl region of transaminated solutions at 33° initially containing 0.1 *M* pyridoxamine, 0.1 *M* sodium pyruvate, and 0.05 *M* Zn^{2+}. Chemical shifts are relative to a *t*-butyl alcohol internal standard.

arise from the aldimine, whose chemical shifts and extent of formation, like those of the ketimine, are pD dependent. Spectra of 0.05 *M* Zn^{2+} solutions were similar to the metal-free solutions except that aldimine formation was first detectable at pD 4.2 and at pD 9.8 was complete, but only 25% complete in the metal-free case. Further, the observed broadening and upfield shifting of the aldimine 2-CH_3 signal with increasing pD (*cf.* Figure 2) indicates formation of labile 1:1 and 1:2 Zn(II):aldimine complexes **3** with the latter predominating above pD ~8.5. Similar studies were performed on solutions containing pyridoxal, pyridoxamine, and Zn^{2+}.

Plots of chemical shift *vs.* pD for each signal in all of the solutions described above has permitted certain identification of all signals in the spectra of transaminated solutions having pD 5–10. As an example, a plot of chemical shift in the methyl region is given in Figure 2. Signal assignments in the transaminated solution having pD 7.2 are indicated in Figure 1b. Several salient factors pertinent to the mechanism of nonenzymatic transamination at pD 4–9.5 emerge from this work: (i) ketimine and aldimine complexes are definitely produced in a sequential fashion in the reaction; (ii) pyruvate methyl protons of **2** and the free ketimine exchange rapidly with solvent at a rate comparable to or exceeding that of transamination; (iii) alanine methyl and CH protons of **3** and free aldimine do not exchange with solvent; (iv) ketimine $\rightleftharpoons$ aldimine conversion (**2** $\rightleftharpoons$ **3**) does not occur as a consequence of rapid, complete exchange of *both* 4-CH_2 protons of **2** with solvent accompanied by protonation of an intermediate[1,2] to yield **3**, since the latter is not deuterated at the azomethine carbon. Observation i is completely consistent with the proposed mechanism,[1] whereas factors ii and iv are not anticipated by it. The origin of (iv) presumably rests in the chelate ring conformations of **2** which confer a selective lability on one of the two methylene protons. The significance of (iii) in connection with the racemization of amino acids effected by pyridoxal and metal ions is being investigated and the results of this work together with the full details of the pmr study of transamination will be presented shortly.

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The Racemization of Sulfonium Salts. II. The Racemization of Substituted Benzylethylmethylsulfonium Perchlorates[1,2]

Sir:

Previously we reported that *t*-butylethylmethylsulfonium perchlorate racemizes faster than it undergoes solvolysis. The excess of loss of optical activity over solvolysis ($k_\alpha - k_t$) was interpreted as racemization involving an inversion of the sulfonium salt. Recently Mislow and Scartazzini reported[3] a study of 1-adamantylethylmethylsulfonium perchlorate in which they tested and confirmed our conclusion with regard to the inversion of sulfonium salts.

We now wish to report that similar behavior is observed with benzylethylmethylsulfonium perchlorate (I), *p*-nitrobenzylethylmethylsulfonium perchlorate (II), and phenacylethylmethylsulfonium perchlorate (III) and to present evidence that the racemization of *p*-methoxybenzylethylmethylsulfonium perchlorate (IV) is best interpreted by a different mechanism.

All the active salts were obtained by resolution of the corresponding (−)-dibenzoylhydrogentartrate salts followed by replacement of the dibenzoylhydrogentartrate ion by perchlorate. The salts had the following physical properties:[4] I, mp 34.5°, $[\alpha]^{25}D$ −8.20° (*c* 3.0, methanol); II, mp 83.5–85°, $[\alpha]^{25}D$ +7.6° (*c* 0.9, methanol); III, mp 144–144.5°, $[\alpha]^{25}D$ −10.7° (*c* 0.88, methanol); IV, mp 79–81°, $[\alpha]^{25}D$ −11.8° (*c* 0.62, methanol).

The relative rate constants for loss of optical activity, k_α, of I:II:III[5a,b] are 1:0.99:0.6 in solvent methanol at 70°. Compound I racemizes 38 times faster than it undergoes methanolysis. For methanolysis the relative rate constants,[5a,c] k_t, of I:II and I:III are 1:0.2 and 1:0.03 at 70 and 90°, respectively. These results are consistent with a scheme in which racemization is independent of solvolysis and racemization involves pyramidal inversion about the sulfur atom. The data show that electron-withdrawing groups have a negligible effect upon the inversion of sulfonium salts.

Markedly different behavior is exhibited by *p*-methoxybenzylethylmethylsulfonium perchlorate (IV). For methanolysis of IV the first-order rate constants, k_t, were 3.94×10^{-6} sec^{-1} and 203×10^{-6} sec^{-1} at 25 and 50°, respectively. At the same temperatures the polarimetric rate constants, k_α, were 6.59×10^{-6} sec^{-1} and 308×10^{-6} sec^{-1}, respectively. The ratio k_α/k_t is 1.5 at 50°. Compound IV undergoes solvolysis over three powers of ten faster than I in methanol at 50°. Racemization of IV ($k_\alpha - k_t$) is faster than I by a factor of ~15.

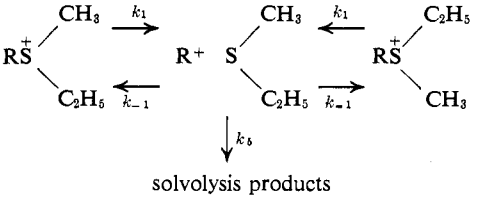
The increase in rate of racemization of IV relative to I is unexpected if pyramidal inversion is to account for the racemization of both sulfonium salts. It is difficult to rationalize the accelerative effect of the *p*-methoxy group in view of the fact that electron-withdrawing substituents have no significant effect upon the rate of inversion of sulfonium salts.

Two other processes might account for the racemization of IV. The first of the alternative reactions involves nucleophilic displacement on the primary benzylic carbon by ethyl sulfide, produced on the solvolysis of the sulfonium salts, as shown in eq 1.

$$\underset{\text{active}}{RCH_2\overset{+}{S}(CH_3)C_2H_5} + CH_3SC_2H_5 \xrightarrow{k_2} \underset{\text{racemic}}{RCH_2\overset{+}{S}(CH_3)C_2H_5} + CH_3SC_2H_5 \quad (1)$$

The addition of methyl ethyl sulfide speeds up the rate of loss of optical activity. The second-order rate constant, k_2, for reaction by eq 1 is 2.45×10^{-5} l. mol^{-1} sec^{-1} at 25°. Using this rate constant and the titrimetric and polarimetric rate constants the following conclusions can be reached. After 50% loss of optical activity, 34% of the sulfonium salt has undergone solvolysis. Of the unsolvolyzed salt 24.3% must be racemic. Only ~2% of the unsolvolyzed salt could have been racemized by the scheme shown in eq 1. At 50°, k_2 is 7.4×10^{-4} l. mol^{-1} sec^{-1}. After 50% loss of optical activity, 36.6% of the sulfonium salt has been solvolyzed and 21% of the unreacted salt is racemic; ~1.3% of the unreacted salt could have been racemized by the nucleophilic displacement reaction.[6] Hence, the principal pathway for racemization of IV must be some process other than that shown in eq 1.

Racemization of IV could also occur by carbon–sulfur bond heterolysis to yield an ion–neutral molecule pair which could react to give solvolysis products or return to racemic sulfonium salt as shown in eq 2.



(2)

While this reaction sequence does not account for the racemization of other sulfonium salts previously studied, it is a reasonable process in this case since the highly stabilized *p*-methoxybenzyl cation would be formed on bond heterolysis. This scheme would account for the acceleration of both the solvolysis and racemization reactions by the *p*-methoxy group.

We prepared *p*-methoxy-*m*-nitrobenzylethylmethylsulfonium perchlorate (V) to distinguish between racemization by pyramidal inversion and by the scheme shown in eq 2. Compound V was prepared from 4-hydroxy-3-nitrobenzyl alcohol[7] and had mp 137–138° and $[\alpha]^{27}D$ +10.6 (*c* 2.93, acetone).

(1) A portion of this material was presented before the Division of Organic Chemistry at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, Abstract S20.
(2) For part I: D. Darwish and G. Tourigny, *J. Am. Chem. Soc.*, **88**, 4303 (1966).
(3) R. Scartazzini and K. Mislow, *Tetrahedron Letters*, 2719 (1967).
(4) All new compounds gave satisfactory analyses.
(5) (a) The sulfonium salts were 0.02–0.04 *M*. (b) The racemization of III was studied with 0.003 *M* $HClO_4$ added to avoid complications due to ylide formation. (c) The solvolyses of I and II were carried out in the presence of *ca.* 0.08 *M* 2,6-lutidine.
(6) Similar behavior is observed for I and II. After 50% racemization *ca.* 0.1 and 0.02% of the racemization of I and II, respectively, could have resulted from the reaction shown in eq 1.
(7) J. B. Fishman, *J. Am. Chem. Soc.*, **42**, 2292 (1920).