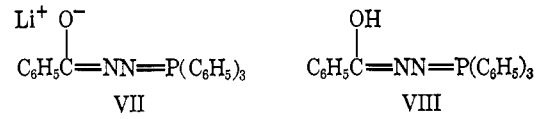
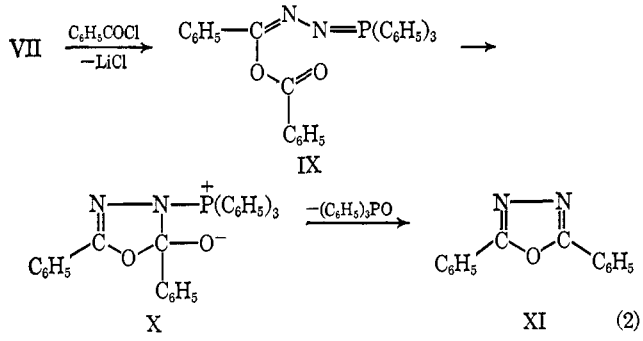


VII VIII

eq 2, 94% yield, mp 138°, lit.[6a] mp 138°) and triphenylphosphine oxide.[6b] Reaction of I with other acid chlorides yields unsymmetrical 2,5-disubstituted 1,3,4-

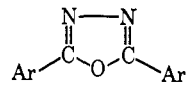


IX

X XI (2)

oxadiazoles (Table I). The new oxadiazole synthesis is general, efficient, and the mildest yet reported. Formation of the 1,3,4-oxadiazoles must involve intramolecular ring closure of intermediate α-acyloxybenzylidenetriphenylphosphazines such as IX. Such intermediates are producible by reaction of hydrazinotriphenylphosphonium bromide[1,2] with 2 equiv of an acyl halide in the presence of excess triethylamine. There results indeed therefrom (eq 1b, 1c, and 2) a rapid and effective method of generating symmetrical 2,5-disubstituted 1,3,4-oxadiazoles (Table II).

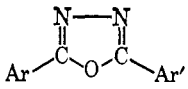
Table I. 1,3,4-Oxadiazoles Derived from I and Aroyl Chlorides



Ar	Ar′	Yield,[a] %
C_6H_5	C_6H_5	>64
C_6H_5	p-$O_2NC_6H_4$	94
C_6H_5	3,5-$(O_2N)_2C_6H_3$	79
C_6H_5	p-BrC_6H_4	70

[a] Procedure 1; no attempts were made to maximize yields.

Table II. 1,3,4-Oxadiazoles Derived from Hydrazinotriphenylphosphiniminium Bromide and Aroyl Chlorides



Ar	Yield,[a] %
C_6H_5	93
p-BrC_6H_4	76
p-$O_2NC_6H_4$	69
m-$O_2NC_6H_4$	67

[a] Procedure 2, no attempts were made to maximize yields.

The cyclizations of the intermediate α-acyloxybenzylidenetriphenylphosphazines (eq 2) are the first examples of reaction of phosphorus–nitrogen ylides phosphazines) with carbonyl groups of esters with expulsion of triphenylphosphine oxide.[7] The rapid ring-closure processes are apparently related to favorable intramolecular probability factors and in particular to the delocalization in 1,3,4-oxadiazoles.

The following procedures are representative for preparing unsymmetrical (procedure 1) and symmetrical (procedure 2) 1,3,4-oxadiazoles.

(1) p-Nitrobenzoyl chloride (0.19 g, 1.0×10^{-3} mol) was added to N-benzoylamidotriphenylphosphinimine (I, 0.40 g, 1.0×10^{-3} mol) and triethylamine (0.20 g, 2.0×10^{-3} mol) in benzene (30 ml). After 24 hr the benzene was removed, and the residue was washed with water and ethanol to yield 2-p-nitrophenyl-5-phenyl-1,3,4-oxadiazole (0.25 g, 94% yield, mp 210–211°, lit.[8] mp 206.5–208°).

(2) m-Nitrobenzoyl chloride (0.74 g, 4.0×10^{-3} mol) and then triethylamine (1.0 g, 1.0×10^{-2} mol) were added to a stirred slurry of hydrazinotriphenylphosphinium bromide (0.75 g, 2.0×10^{-3} mol) in ether (30 ml). After 30 hr at 25° the solid was filtered, washed with water, and boiled with ethanol to give 2,5-bis(m-nitrophenyl)-1,3,4-oxadiazole as residue (0.45 g, 67% yield, mp 228–229°, no depression by an authentic sample[9]).

The utility of alkylation of salts of N-acylamidotriphenylphosphinimines and of base-catalyzed acylation of N-thioacylamidotriphenylphosphinimines and of N-acylamidinotriphenylphosphinimines will be reported subsequently.

Acknowledgment. This research was supported in part by the National Science Foundation.

(6) (a) R. Stolle, *J. Prakt. Chem.*, **69**, 157 (1904). (b) A related reaction is described by T. L. Bieber and E. H. Eisman, *J. Org. Chem.*, **27**, 678 (1962).

(7) (a) Phosphoranes do not react with esters other than formates (slowly) with expulsion of triphenylphosphine oxide (H. Pommer and G. Wittig, German Patent 1,047,763 (Dec 31, 1958): see *Chem. Abstr.*, **52**, 16411*h* (1958)). (b) Reactions of phosphoranes with azides to give 1,2,3-triazoles involve intermediates structurally related to X (G. R. Harvey, *J. Org. Chem.*, **31**, 1587 (1966)).

(8) R. Huisgen, J. Sauer, H. J. Sturm, and J. H. Markgraf, *Ber.*, **93**, 2112 (1960).

(9) A. Siegrist, E. Maeder, and M. Duennenberger, Swiss Patent 383,985 (Jan 29, 1965); *Chem. Abstr.*, **62**, 14867*b* (1965).

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A Variation of the Square-Pyramidal Copper(II) Surrounding. A Possible Copper Interaction with Tyrosine[1]

Sir:

In a series of investigations on the crystal structures of metal complexes of amino acids and peptides, we determined the structure of the copper chelate of glycyl-*l*-leucyl-*l*-tyrosine. The complex can be prepared by mixing equimolar amounts of $CuSO_4$, $Ba(OH)_2$, and the peptide. After separation of $BaSO_4$, large blue crystals of the complex can be obtained by equilibration with ether of the aqueous (pH 6–7) chelate solution. The crystals decompose very rapidly (within 1 min) on exposure to the atmosphere. The crystals are stable in solution when ether is added to the mother liquor.

(1) This research was supported by Grant GM 10514 from the National Institutes of Health.

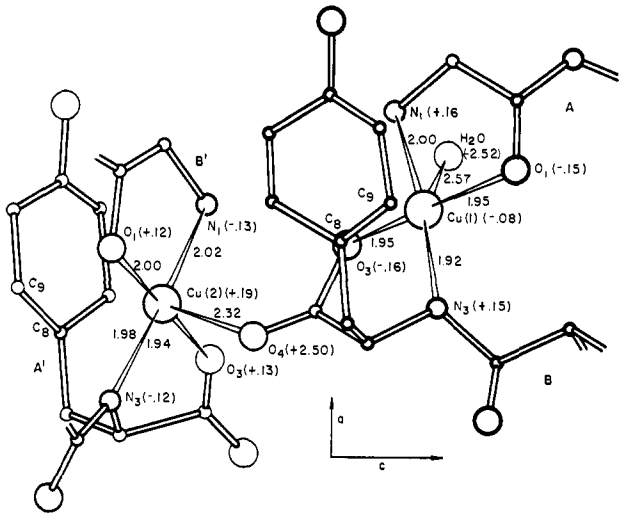

Figure 1.

The X-ray diffraction data were taken with crystals in this environment while sealed in thin-walled glass capillaries. The compound crystallizes in the orthorhombic space group $P2_12_12_1$ with cell dimensions: $a = 9.136 \pm 0.002$ Å, $b = 25.76 \pm 0.02$ Å, and $c = 21.05 \pm 0.01$ Å. The contents of the asymmetric unit were obtained from the complete structure determination and proved to be $Cu_2(C_{17}H_{25}N_3O_5)_2 \cdot 8H_2O \cdot C_2H_5OC_2H_5$. The final R value for 2043 observed reflections was 10.3%. The details of the crystal structure determination and description will be published elsewhere.[2] However, the surrounding of the Cu(II) ions (Figure 1) is novel.

The base of the square-pyramidal surrounding for both copper ions is formed by the terminal nitrogen and the carbonyl oxygen of the glycyl residues and a carboxyl oxygen and the deprotonized amide nitrogen of the tyrosyl residues. These four ligand atoms form very flattened tetrahedrons with the Cu atoms slightly displaced toward the fifth ligand atom. The number in parentheses behind the identification of the atoms is the deviation (in Å) of those atoms from the least-squares planes through $O_1N_1O_3$ and N_3 of peptide molecules A and B, and A′ and B′, respectively. The fifth interactions toward the top of the two square pyramids are weaker as is commonly observed. The copper–ligand distances are shown in Figure 1 (standard deviations for these distances as calculated from the inverse of the least-squares matrix are between 0.01 and 0.02 Å). They compare well with the average values given by Freeman.[3] The largest deviations occur for Cu(2)–N_3(A′) (average,[3] 1.92 Å) and Cu(2)–O_1(B′) (average,[3] 1.95 Å). Pertinent angles in the square pyramids are shown in Table I.

The square-pyramidal surrounding of the Cu(II), so far described, has been observed many times in copper complexes of peptides and other chelating agents. The novelty of the present results is the location of the benzene rings of the tyrosine residues below the base of the pyramid forming the surrounding of both copper ions. The planes of the benzene rings are approximately parallel to the bases of the pyramids (18 and 21° for the surrounding around Cu(1) and Cu(2), respectively). The closest approaches for Cu(1) are Cu(1)–C_9(B), 3.17 Å, and Cu(1)–C_8(B), 3.21 Å; and for Cu(2) they are Cu(2)–C_9(A′), 3.27 Å, and Cu(2)–C_8(A′), 3.34 Å.

Table I. Pertinent Angles (Degrees) in the Square Pyramids[a]

	Cu(1)	Cu(2)
O_1–Cu–N_1	82.7	82.3
O_3–Cu–N_3	84.9	85.2
O_t–Cu–O_1	89.8	94.9
O_t–Cu–N_1	78.7	97.9
O_t–Cu–N_3	114.4	99.5
O_t–Cu–O_3	84.0	88.3

[a] O_t is H_2O and O_4 for the Cu(1) and Cu(2) angles, respectively. The esd's for the angles are between 0.6 and 0.8°.

The possibility of an interaction with hydrogen in the sixth position in square-pyramidal surroundings of Cu(II) has been reviewed and discussed by Bonamico, *et al.*,[4] and more recently by Freeman.[5] Freeman concluded that it was unlikely that these represented bonding interactions, using as arguments the very small bond order calculated from the formula $D(n) = D(1) - 0.6 \log n$ (Pauling[6]) and the fact that the sum of the van der Waals radii are smaller than the observed Cu···H distances.

In the present case to decide if the close contacts between Cu and C constitute bonds, the same formula of Pauling also yields a small bond order, but the sum of the van der Waals radii, estimated to be between 3.5 and 4.1 Å, is significantly larger than the observed contacts (3.17–3.34 Å). For that matter a similar close contact, *i.e.*, the Cu–N distance of 3.38 Å in the structure of copper phthalocyanine,[7] has been thought to be responsible for the large Hall effect observed in this structure compared to the metal-free ligand.[8]

One can argue that the location of the tyrosyl groups is a result of the most efficient crystal packing. They are both in the same *gauche* position: $X_A = 54°$ and $X_B = 59°$. The angle X has been defined[9] to specify the location of C_γ with respect to the peptide chain. In this respect it should be noted that the *trans* position ($X \approx 180°$) is the only configuration observed for a tyrosine residue[9] previous to the present structure determination. The *trans* position would also not interfere with the H bonding of the anti-parallel pleated sheet structure which is observed in the structure.[2] The other *gauche* position ($X \approx 300$) would cause interference with this H bonding.

It is possible to speculate that the observed close contact of the tyrosyl residues with the Cu(II) ions has bearing on the mechanism of the enzyme tyrosinase. Although this enzyme contains univalent copper when isolated, the participation of divalent copper in the mechanism of the enzyme is quite likely.[10] Other

(2) (a) W. A. Franks and D. van der Helm, to be submitted for publication; (b) W. A. Franks, Thesis University of Oklahoma, 1968.

(3) H. C. Freeman in "The Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N. Y., 1966, pp 77–114.

(4) M. Bonamico, G. Dessy, A. Mugnoli, A. Vaciago, and L. Zambonelli, *Acta Cryst.*, **19**, 886 (1965).

(5) H. C. Freeman, *Advan. Protein Chem.*, **22**, 357 (1967).

(6) L. Pauling, "Nature of the Chemical Bond," 3rd ed, Cornell University Press, Ithaca, N. Y., 1960, Chapter 7.

(7) J. M. Robertson, *J. Chem. Soc.*, 615 (1935); J. M. Robertson and I. Woodward, *ibid.*, 219 (1937).

(8) S. E. Harrison and J. M. Assour, *J. Chem. Phys.*, **40**, 365 (1964).

(9) G. N. Ramachandran and A. V. Lakshminarayanan, *Biopolymers*, **4**, 495 (1966).

(10) H. S. Mason in "The Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N. Y., 1966, p 340.

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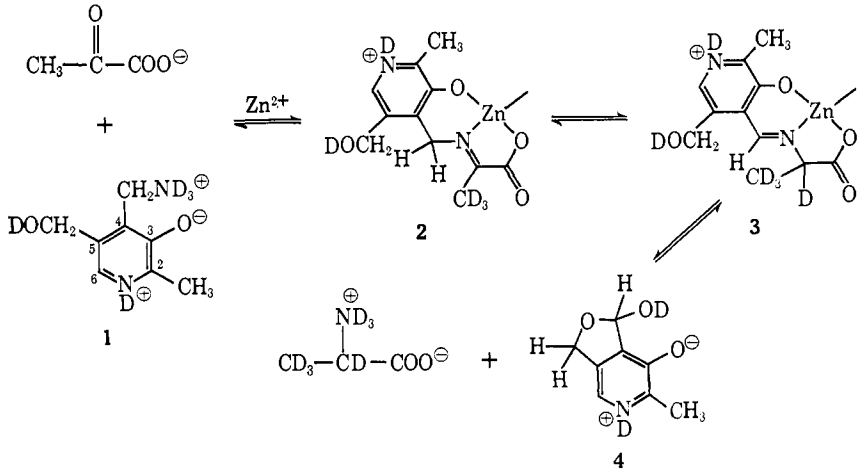
Detection and Identification of Intermediates and Products of a Nonenzymatic Transamination Reaction by Proton Resonance

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



(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

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

(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).