[CONTRIBUTION FROM THE BIOCHEMICAL INSTITUTE AND THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF TEXAS, AND THE CLAYTON FOUNDATION FOR RESEARCH]

Oxidative Deamination of Amino Acids by Pyridoxal and Metal Salts[1]

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The oxidative deamination of pyridoxamine to yield pyridoxal and NH_3, and of many α-amino acids to yield the corresponding α-keto acids and ammonia, is catalyzed in dilute aqueous solutions by pyridoxal and appropriate metal ions. 5-Desoxypyridoxal and 4-nitrosalicylaldehyde replace pyridoxal effectively, salicylaldehyde is far less effective. Of the metal ions tested, copper, cobalt, nickel and iron were effective; aluminum ions, which catalyze transamination reactions with pyridoxal, were ineffective in catalysis of these oxidative reactions. The evidence indicates that transamination is not an obligatory intermediate step in these oxidations. Of several amines other than pyridoxamine tested, only benzylamine was deaminated by this system. The biological significance, if any, of this chemical property of pyridoxal is not known.

Pfeiffer, *et al.*,[2] have reported that in the substituted bis-salicylaldimine chelates formed from salicylaldehyde, Cu^{++} or Ni^{++}, and amino acid esters, the latter component can undergo oxidative deamination in the presence, but not in the absence, of air. Martell and Calvin[3] have suggested investigation of such systems as possible models of the enzymatic oxidation of amines.

In previous studies of the reactions of aldehydes related to pyridoxal with amino acids, the oxidative deamination of glutamic acid by 4-nitrosalicylaldehyde and aluminum ions was noted.[4] Oxygen was not required; the hydrogen acceptor appeared to be the nitro group which was in part reduced completely to an amino group.[4] Because of the similarity in reactivity of pyridoxal and 4-nitrosalicylaldehyde toward amino acids,[4,5] the ability of pyridoxal and metal salts to catalyze oxidative deamination of various amino acids and amines was determined.

Results of such tests (Table I) showed that both pyridoxal and an appropriate metal ion were required for the deamination of glycine and pyridoxamine. Of the metal ions tested, copper was most effective, but iron, cobalt and nickel also were active. Aluminum ions, which catalyze transamination reactions between amino acids and pyridoxal,[5,6] were ineffective in catalyzing liberation of ammonia from glycine or pyridoxamine. Iron was considerably less effective in alkaline than in acid solution, probably due to its observed precipitation from the alkaline solutions. It was not necessary to add the metal ion in its more highly oxidized state to effect these oxidations; however, this result is not definitive inasmuch as oxidation by air may have occurred to supply the metal ion in its higher valence state.

5-Desoxypyridoxal and 4-nitrosalicylaldehyde effectively replace pyridoxal in catalyzing deamination of pyridoxamine and glycine (Table II). Salicylaldehyde did not promote deamination of pyridoxamine at *p*H 4.0, and was much less active than the other aldehydes in deamination of glycine at *p*H 9.6. The result again demonstrates the necessity for a strong electron-attracting group in the proper orientation on the ring for effective pyridoxal-like activity.[4,5]

TABLE I

EFFECT OF METAL SALTS AND PYRIDOXAL ON THE DEAMINATION OF PYRIDOXAMINE AND GLYCINE

All reactants were present at an initial concentration of 1 μM. per ml. Reaction mixtures were heated 30 minutes at 100°.

	μM. NH_3 formed per ml.			
Metal salt	Pyridoxamine		Glycine	
Pyridoxal present	*p*H 4	*p*H 9.6	*p*H4	*p*H 9.6
None	0.06	0.06	0.04	0.03
$CuSO_4$	.75	.43	.28	.51
$KAl(SO_4)_2$	.06	.07	.05	.05
$FeCl_3$	.44	.15	.12	.07
$CoCl_2$	.10	.38	.05	.28
$Ni(NO_3)_2$	.11	.17	.04	.10
Pyridoxal omitted				
$CuSO_4$	0.10			0.06

TABLE II

EFFECTIVENESS OF VARIOUS ALDEHYDES IN DEAMINATION OF PYRIDOXAMINE AND GLYCINE IN THE PRESENCE OF COPPER IONS

Reactants were at an initial concentration of 1 μM. per ml. Except where noted, reaction mixtures were heated for 30 minutes at 100°.

	μM. NH_3 formed per ml.		
	Pyridoxamine		Glycine
Aldehyde	*p*H 4	*p*H 9.6	*p*H 9.6
None	0.10[a]	0.50[a]	0.06
Pyridoxal	.75[b]	.43[b]	.51
5-Desoxypyridoxal	.68		.55
4-Nitrosalicylaldehyde	.39		.55
Salicylaldehyde	.11		.26
None (heated one hour)	.47		

[a] The corresponding figures for pyridoxal formation under these conditions were 0.07 and 0.62 μM. per ml. at *p*H 4.0 and 9.6, respectively. [b] A total (pyridoxal added plus pyridoxal formed) of 1.49 and 1.08 μM. per ml. of pyridoxal was present after reaction at *p*H 4.0 and 9.6, respectively.

That pyridoxamine is oxidized to pyridoxal by air in the presence of copper and iron salts has been mentioned previously.[6] This process appears to be autocatalytic, since it is greatly speeded at *p*H 4.0 by the addition of pyridoxal (Table I) and since in the absence of *added* pyridoxal the amount of ammonia formed in one hour at this *p*H substantially exceeds twice that formed in one-half hour (Table II). At *p*H 9.6 the deamination of pyrid-

(1) A preliminary abstract has appeared (M. Ikawa and E. E. Snell, *Federation Proc.*, **13**, 235 (1954)).

(2) P. Pfeiffer, W. Offermann and H. Werner, *J. prakt. Chem.*, **159** 313 (1941).

(3) A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952, p. 399.

(4) M. Ikawa and E. E. Snell, THIS JOURNAL, **76**, 653 (1954).

(5) D. E. Metzler, M. Ikawa and E. E. Snell, *ibid.*, **76**, 648 (1954).

(6) D. E. Metzler and E. E. Snell, *ibid.*, **74**, 979 (1952).

oxamine is faster than at *p*H 4.0 and has reached maximum within the 30-minute reaction time so that added pyridoxal has no apparent effect on the extent of the reaction. Side reactions of an uncharacterized nature occur at the alkaline *p*H since pyridoxal appears in amounts smaller than ammonia, the discrepancy being larger in those reaction mixtures containing added pyridoxal (Table II).

In Table III the susceptibility of several amino acids to oxidation in air in the presence of copper ions and with or without added pyridoxal is compared. In all instances except histidine, oxidation by copper ions alone at these concentrations is very small, and is increased greatly in all cases by the addition of pyridoxal.

If oxidative deamination were the only reaction occurring under these conditions, ammonia and keto acids should appear in equimolar amounts, and the concentration of pyridoxal should remain unchanged. Experimentally, ammonia formation is almost invariably greater than keto acid formation, a result not unexpected in view of the instability of many α-keto acids to heat, and the already known promotion of aldehyde, NH_3 and CO_2 formation from α-amino acids by carbonyl compounds.[7] No attempt to measure aldehyde formation in these reaction mixtures was made.

The variable disappearance of some pyridoxal during the reaction with amino acids also is noted in Table III. The products formed are not known in most instances. During reaction with glycine, serine and threonine, however, some loss of pyridoxal through formation of β-pyridoxylserine[8] would be expected. Under the reaction conditions, most amino acids undergo transamination with pyridoxal to some extent[6] with the formation of pyridoxamine; to the extent that the latter escapes oxidation deamination (*cf.* Table II) its formation also would result in a net loss of pyridoxal. Finally, small amounts of the carboxylic acid corresponding to pyridoxal, 4-pyridoxic acid have been observed in some of these reaction mixtures.

Formation of the expected keto acid was demonstrated in the case of glycine, alanine, valine and glutamic acid. Serine (and threonine to a lesser extent[8]) deaminates by a non-oxidative mechanism under these conditions to yield pyruvate[9] (or α-ketobutyrate from threonine). However, glyoxylic acid rather than α-ketobutyric acid was the principal product from threonine, and undoubtedly arose from glycine, formation of which by the cleavage of threonine is catalyzed by pyridoxal and metal ions.[8]

Since pyridoxal does transaminate with many amino acids to yield α-keto acids and pyridoxamine, a portion of the oxidative deamination of amino acids observed here undoubtedly occurs *via* this reaction followed by oxidative deamination of the pyridoxamine formed. Several facts, however, indicate that an alternative mechanism not involving transamination is possible. These are (a) the observed catalysis of oxidation of pyridoxamine itself by pyridoxal, (b) the chemical transamination between pyridoxamine and amino acids[6] has a *p*H optimum near 4–5 and little or no transamination occurs at *p*H 9.6, whereas certain of the amino acids are oxidized optimally at the latter *p*H, and (c) glycine and valine, which undergo chemical transamination very slowly, are readily oxidized under these same conditions.

In only a few cases tabulated is the total keto acid formed more than might result through complete reduction of the added metal ion. However, separate trials have shown that with lower cupric ion concentrations, oxidation continues much beyond this point (*e.g.*, cupric sulfate, glycine and pyridoxal at concentrations of 0.2, 1.0 and 1.0 μM. per ml., respectively, heated together for 30 minutes at 100° yielded 0.36 μM. of ammonia per ml., or 3.6 times that which could be formed by reduction of Cu^{++} present), *i.e.*, there is a true catalysis of the oxidation by the metal ion as well as by pyridoxal. We view the oxidative step as occurring through the same type of amine–pyridoxal–metal

TABLE III

DEAMINATION OF AMINO ACIDS BY PYRIDOXAL AND COPPER IONS

All reactants were present at a concentration of 1 μM. per ml. Reaction mixtures were heated 30 minutes at 100°.

Amino acid	*p*H	μM. NH_3 formed per ml. No pyridoxal	μM. NH_3 formed per ml. With pyridoxal	Pyridoxal lost, μM./ml.	Keto acid formed[a]	Keto acid concn. μM./ml.
None	4		0.00	0.04		
	9.6		.00	.04		
Glycine	4	0.03	.28	.14	Glyoxylic	0.25
	9.6	.06	.51	.22		.41
Proline	4		.04			
	9.6		.02			
Alanine	4	.02	.45	.13	Pyruvic	.30
	9.6	.06	.44	.20		.67
Glutamic acid	4	.03	.68	.20	α-Ketoglutaric	.57
	9.6	.09	.60	.15		.57
Phenylalanine	4	.02	.74	.11	Phenylpyruvic	.59
	9.6	.06	.58	.21		.12
Valine	4	.01	.14	.01	Dimethylpyruvic	.15
	9.6	.04	.40	.08		.35
Tryptophan	4	.03	.45			
	9.6	.05	.35			
Histidine	4	.06	.56			
	9.6	.24	.40			
Lysine	4	.05	.72			
	9.6	.03	.54			
Arginine	4	.06	.72			
	9.6	.08	.54			
Serine	4	.06	.59	.13	Pyruvic	.48
	9.6	.06	.53	.28		.29
Threonine	4	.02	.31	.12	Glyoxylic	.22
	9.6	.04	.43	.31		.42

[a] Keto acids were identified chromatographically as their 2,4-dinitrophenylhydrazones (see Experimental). In some cases, more than a single keto acid is formed, *e.g.*, serine yields also small amounts of glyoxylic acid *via* the cleavage reaction (*cf.* threonine), and threonine yields small amounts of α-ketobutyrate *via* the dehydration reaction (see D. E. Metzler, J. B. Longenecker and E. E. Snell, THIS JOURNAL, **76**, 639 (1954)). In such cases, the total keto acid was determined and recorded.

(7) An extensive listing of carbonyl compounds active and inactive in degrading α-amino acids to aldehydes is given by A. Schonberg, R. Moubasher and A. Mustafa (*J. Chem. Soc.*, 176 (1948)). A mechanism involving formation of a Schiff base, decarboxylation, a prototropic shift of the double bond, and hydrolysis of the new azomethine linkage is proposed for the reaction.

(8) D. E. Metzler, J. B. Longenecker and E. E. Snell, THIS JOURNAL, **76**, 639 (1954).

(9) D. E. Metzler and E. E. Snell, *J. Biol. Chem.*, **198**, 353 (1952).

ion chelate complex previously postulated as intermediate in other pyridoxal-catalyzed reactions of amino acids.[5,10,11] Catalysis of other pyridoxal-dependent reactions by aluminum ions[5] contrasts with their inactivity in this system, and suggests that alternate reduction of the metal ion by the amino acid and its reoxidation by oxygen may be a feature of these reactions. However, as noted previously, aluminum ions catalyze oxidative deamination of glutamate by 4-nitrosalicyladehyde,[4] and since a reversible oxido–reduction of the aluminum ion seems unlikely, the factor of primary importance in these oxidative systems may be fixation of the hydrogen acceptor in close proximity to the pyridoxal–amino acid–metal chelate system. In this connection, the role of iron and copper complexes as reversible oxygen carriers in natural systems, and the similar chemical behavior of cobalt-containing chelates of salicylaldehyde,[12] suggests that valence changes in the metal ion during these reactions may not be obligatory.

Of several amines (pyridoxamine, benzylamine, phenylethylamine, tyramine, histamine, β-alanine, γ-aminobutyric acid) and diamines (ethylenediamine, putrescine) tested, only pyridoxamine was oxidized under conditions used for α-amino acids. When the concentration of reactants was increased ten-fold, ammonia was formed slowly from benzylamine (which may be considered as analogous in some respects to pyridoxamine), and the odor of benzaldehyde could be detected. Even at these concentrations, the other amines tested were not oxidized.

The reactions described herein are in net result the same as those carried out by the amino acid and the amine oxidases, all of which so far studied contain a riboflavin derivative as prosthetic group.[13] Some of these enzymes, *e.g.*, the *ophio*-L-amino acid oxidase and especially diamine oxide, are strongly inhibited by carbonyl reagents, indicating the probable involvement in their action of a carbonyl compound.[13] Microbiological assays of suitable hydrolysates of rattlesnake venom and of concentrates of *ophio*-L-amino acid oxidase prepared therefrom as described by Singer and Kearney[14] have failed, however, to reveal the presence of pyridoxal in amounts that would indicate a functional role for it in this enzyme. Whether pyridoxal plays such a role in any of the amine oxidases, or whether its activity in catalyzing amine oxidation is a chemical property which nature failed to utilize, remains to be established.

Experimental

Quantitative Procedures.—Pyridoxal was determined spectrophotometrically, and keto acids colorimetrically after conversion to their 2,4-dinitrophenylhydrazones by methods described previously.[6] Reactions at *p*H 4.0 were carried out in 0.05 *M* acetate buffer in sealed tubes; those at *p*H 9.6 in the presence of 0.05 *M* sodium bicarbonate and 0.025 *M* sodium carbonate. In the latter case, 2-ml. aliquots of the reaction mixtures were connected to the acid-containing absorption tubes throughout the heating period to prevent losses of ammonia at the high *p*H used. Ammonia was determined by aeration from alkalinized samples into acid followed by Nesslerization.[9] Other experimental details are given with the tables.

(10) J. Baddiley, *Nature*, **170**, 711 (1952).

(11) Pfeiffer (ref. 2) postulated oxidation of amino acid esters through the analogous chelates formed with salicylaldehyde by a mechanism not involving valence changes in the metal ion.

(12) A. E. Martell and M. Calvin, "Chemistry of Metal Chelate Compounds," Prentice-Hall, Inc., New York, N. Y., 1952, p. 337.

(13) H. A. Krebs, in J. B. Sumner and K. Myrback, "The Enzymes," Vol. 2, pt. 1, Academic Press, Inc., New York, N. Y., 1951, p. 499; E. A. Zeller, *ibid.*, p. 536. Riboflavin was tested as a possible hydrogen acceptor in the presence and absence of pyridoxal in reaction mixtures containing amino compounds not oxidized by the copper–pyridoxal system. No significant deamination was observed except with ethylenediamine, where addition of riboflavin promoted ammonia production even in the absence of pyridoxal.

(14) T. P. Singer and E. B. Kearney, *Arch. Biochem. Biophys.*, **29**, 190 (1950).

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Antiamebic Agents. III.[1] Basic Derivatives of Chloro-8-quinolinols

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Fifteen new compounds related to the amebacidal agent, 5-chloro-7-diethylaminomethyl-8-quinolinol (I), have been synthesized by means of the Mannich reaction. Observations have been made concerning the instability of some compounds of this type, and the nature of certain by-products has been determined. In selecting the compounds to be prepared for testing against amebiasis, consideration was given to the probable influence of the groups introduced upon each of the two characteristic types of infection, namely, intestinal and extra-intestinal amebiasis. Two of the compounds appeared to be as effective as I against intestinal infections in dogs.

Poorly absorbed drugs such as Diodoquin (5,7-diiodo-8-quinolinol) are known to be clinically effective against intestinal amebiasis,[2] whereas certain drugs of the antimalarial type such as Atabrine, chloroquine and Camoquin, which are absorbed systemically, have been indicated to be clinically effective against hepatic amebiasis.[2,3] It was with this knowledge in mind that 5-chloro-7-diethylaminomethyl-8-quinolinol (I) was synthesized,[4] and it was considered that an intestinal amebacide, 5-chloro-8-quinolinol,[5] might be con-

(1) Previous publication, W. H. Edgerton and J. H. Burckhalter, THIS JOURNAL, **74**, 5209 (1952).

(2) H. H. Anderson and B. L. Hansen, *Pharmacological Revs.*, **2**, 402 (1950).

(3) P. E. Thompson and J. W. Reinertson, *Am. J. Trop. Med.*, **31**, 715 (1951).

(4) J. H. Burckhalter and W. H. Edgerton, THIS JOURNAL, **73**, 4837 (1951).

(5) Office of Publication Board Reports, Dept. of Commerce, Washington, D. C., PB 95022, 1948, pp. 85–95.