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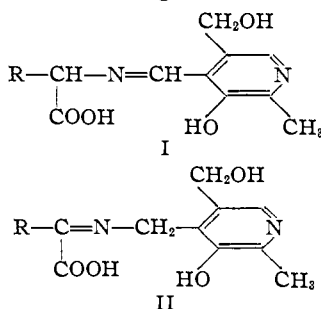
The Metal Complexes of Vitamin B₆ and Schiff's Base Derivatives^{1,2}

BY GUNTHER L. EICHHORN AND JAMES W. DAWES

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The studies of Snell and Metzler have demonstrated the catalytic effect of metal ions upon transamination reactions involving vitamin B₆, and they have led to the postulation that metal complexes of Schiff bases of the vitamin are formed as intermediates. In the present investigation the formation of such complexes has been confirmed through observations on the visible absorption spectra of transamination reaction solutions. It has been shown that solutions of copper or nickel ions in the presence of both pyridoxal and alanine exhibit spectra that are totally different from the spectra of the metal complexes of the components. Application of the method of continuous variation reveals that a 1:2:2 metal-pyridoxal-alanine complex is responsible for these spectra. Solutions containing copper or nickel ion and both pyridoxamine and pyruvic acid likewise yield absorption spectra that are significantly different from the spectra of the complexes of the components, thus indicating the formation of metal-pyridoxamine-pyruvic acid complexes. The spectra of such solutions change upon standing, until they become identical with those of the metal-pyridoxal-alanine complexes. Under these conditions, therefore, the equilibrium favors the transfer of the amino group from pyridoxamine to pyruvic acid.

The non-enzymatic transamination reactions with vitamin B₆ have been thoroughly investigated by Snell³ and Metzler and Snell.⁴⁻⁶ These workers have shown that pyridoxal is capable of removing an amino group from an amino acid, that pyridoxamine may transfer its amino group to a keto acid, and that these reactions are catalyzed by a variety of metal ions. It was postulated that the active intermediates in these reactions are the metal complexes of the following Schiff bases



The transfer of an amino group from an amino acid to pyridoxal was postulated to occur through initial formation of I, conversion of I to II, and subsequent hydrolysis of the latter. The amination of a keto acid by pyridoxamine would involve transformation of II into I.

In the present investigation spectrophotometric evidence is presented for the existence of the metal complexes of I and II in solution. The problem was approached through a preliminary examination of the absorption spectra of the copper and nickel complexes of pyridoxal, pyridoxamine, alanine and pyruvic acid, followed by an investigation of the spectra (and their changes with time) of mixtures of the metal ions with both pyridoxal and alanine, and with both pyridoxamine and pyruvic acid.

Experimental

Preparation of Solutions.—Standard solutions of pyridoxal, pyridoxamine, alanine and pyruvic acid were pre-

(1) Presented at the 124th meeting of the American Chemical Society, Chicago, Sept. 6-11, 1953.

(2) Taken in part from the M.S. thesis of J. W. Dawes, Louisiana State University, 1953.

(3) E. E. Snell, "Rapports des Symposiums, No. 5, Symposium sur le Métabolisme Microbien," IIe Congrès de Biochimie, Paris, July 21-27, 1952, p. 48-63.

(4) D. E. Metzler and E. E. Snell, *THIS JOURNAL*, **74**, 979 (1952).

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

(6) D. E. Metzler and E. E. Snell, *ibid.*, **198**, 363 (1952).

pared by diluting weighed quantities of these substances to the proper volume; the pyruvic acid was vacuum distilled immediately before use. A solution of nickel nitrate was standardized gravimetrically with dimethylglyoxime; copper nitrate solution was standardized by iodometric titration.

Absorption Spectra.—The solutions used in the determination of the absorption spectra were made 0.015 molar with respect to the total metal ion (the 1:2 solutions containing 0.030 molar coordinating agent). The acids were neutralized with sodium hydroxide in order to convert them into a form suitable for complex formation. All absorption measurements were made with a Beckman model DU spectrophotometer, using a 1-cm. Corex cell.

Precipitation from the Schiff Base Complex Solutions.—Among the experimental difficulties encountered in this work is the precipitation of solids from the reaction mixtures. The time lapse between the initiation of the reaction and the emergence of the precipitate is greater for the nickel complexes; this circumstance is one of the factors that makes it easier to work with the nickel complexes than with those of copper. Nevertheless, precipitates were eventually found in all of the reaction mixtures. In order to elucidate the nature of these substances the supernatant liquids were filtered, and the spectra of the solutions were redetermined. This procedure was followed for the 1:1:1 and 1:2:2 copper-pyridoxal-alanine and the 1:2:2 nickel-pyridoxal-alanine solutions, since these contained the greatest quantity of precipitate. The precipitate from the 1:2:2 copper-pyridoxal-alanine solution was filtered on a fritted glass funnel, dried in air and analyzed.⁷

Anal. C, 19.85; H, 3.66; N, 7.19.

Continuous Variation Experiments.—0.03 molar nickel and pyridoxal-alanine solutions were used in the preparation of the samples. All solutions for use in the continuous variation studies were made up at the same time and allowed to remain standing overnight to diminish errors that might arise if the various samples were permitted to react for different lengths of time.

Results and Discussion

The System Nickel-Pyridoxal-Alanine.—The spectra of the complexes of the two organic components of the Schiff base are given in Fig. 1. It may be noted that the solution of nickel and pyridoxal exhibits the same absorption spectrum as uncoordinated nickel ion (except in the range approaching the ultraviolet region), thus indicating a very low degree of complex formation; the properties of the nickel-alanine solutions, on the other hand, clearly demonstrate complex formation.

These curves may now be compared with the spectra of solutions of nickel and pyridoxal and alanine together, plotted in Fig. 2. It is evident that the characteristics of the simple complexes have been completely eliminated; an entirely new spec-

(7) By Galbraith Laboratories, Knoxville, Tenn.

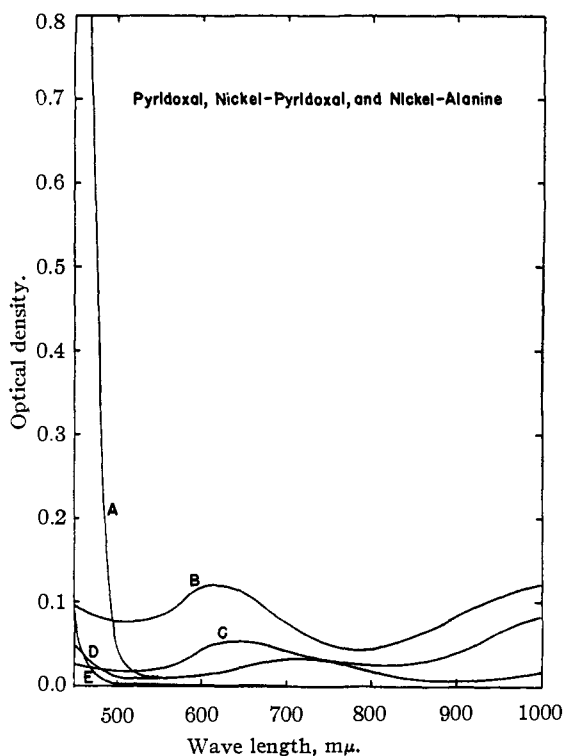


Fig. 1.—Absorption spectra of pyridoxal and the nickel complexes of pyridoxal and alanine: A, nickel-pyridoxal 1:2; B, nickel-alanine 1:2; C, nickel-alanine 1:1; D, nickel-pyridoxal 1:1; E, pyridoxal.

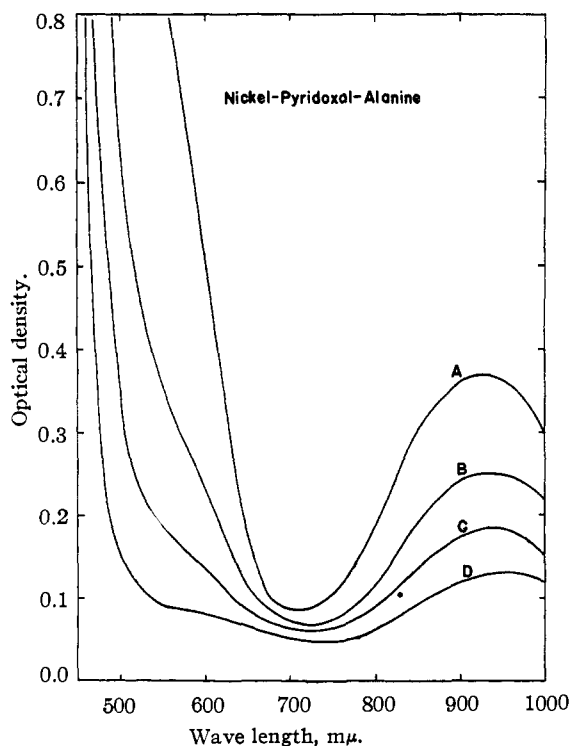
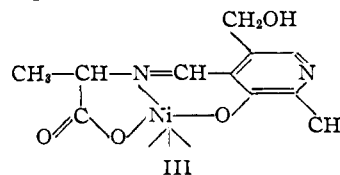


Fig. 2.—Absorption spectra of solutions containing initially nickel-pyridoxal-alanine: A, 1:2:2, after one day; B, 1:2:2, after 20 min.; C 1:1:1, after one day; D, 1:1:1, after 20 min.

trum with a peak at 940 $m\mu$ has appeared instead. The optical density of both solutions decreases upon standing without modification of any of the absorption characteristics, indicating that increasing quantities of the three-component complex are being formed in solution. The following structure may be postulated⁸ for the species responsible for the curves depicted in Fig. 2



The System Nickel-Pyridoxamine-Pyruvic Acid.—The absorption curves of solutions containing nickel ion and only pyridoxamine, and nickel and only pyruvic acid are plotted in Fig. 3. Since rapid precipitation occurs in a 1:2 solution of nickel and pyruvic acid, a spectrum for the latter could not be obtained.

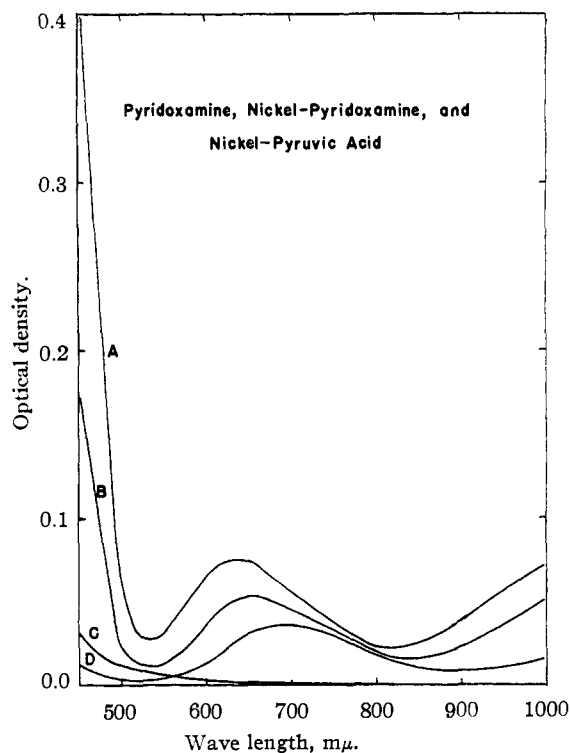


Fig. 3.—Absorption spectra of pyridoxamine, nickel-pyridoxamine and nickel-pyruvic acid: A, nickel-pyridoxamine 1:2; B, nickel-pyridoxamine 1:1; C, pyridoxamine; D, nickel-pyruvic acid.

The spectra of solutions containing all three components, nickel, pyridoxamine and pyruvic acid, are depicted in Fig. 4. At the outset of the reaction these solutions resemble those of the nickel-pyridoxamine complex. (No precipitation appears in the 1:2 solution, however, indicating that the insoluble 1:2 nickel-pyridoxamine complex is not present.) Upon standing the spectra of these solutions change until they become identical with those of

(8) After Snell and Metzler and Snell.

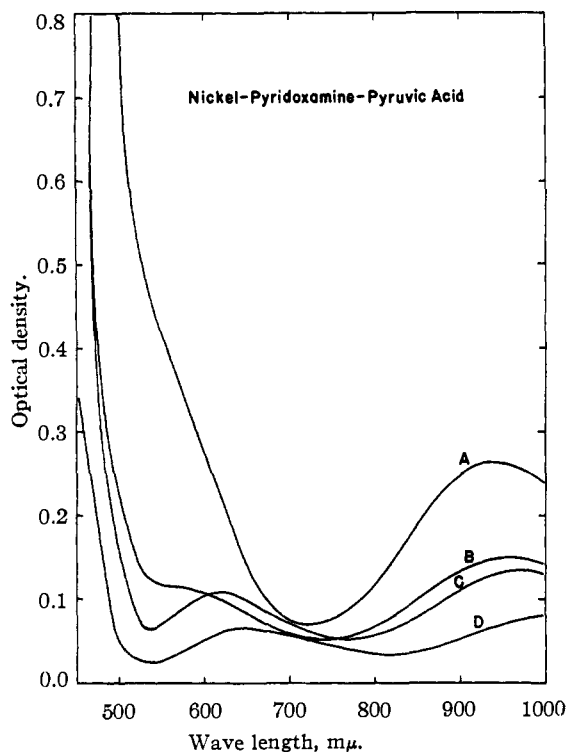
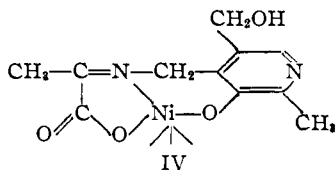


Fig. 4.—Absorption spectra of solutions containing initially nickel-pyridoxamine-pyruvic acid: A, 1:2:2, after 18 hr.; B, 1:1:1, after 18 hr.; C, 1:2:2, after 20 min.; D, 1:1:1, after 20 min.

Fig. 2. These data may be interpreted by assigning the following structure to the species initially present in solution



and by assuming the gradual conversion of IV into III.

The Reaction with Copper.—The changes that take place in the absorption of the transaminating systems involving copper ion are not nearly so spectacular as those that have been described for the nickel system, because all of the simple copper complexes exhibit considerable absorption prior to Schiff base formation. Nevertheless, the changes are significant, and can be interpreted in the same manner as corresponding transformations in the nickel systems. Moreover, the spectrum of the copper-Schiff base complex may be compared with that of the copper complex of salicylal-glycine, which has been measured, and further evidence for the structure of the complex may thus be introduced.

Inspection of Figs. 5 and 6 reveals that the spectra of solutions of the three component system copper-pyridoxal-alanine bear the same relationship to the spectra of the simple complexes as the spectra of copper-salicylaldehyde-glycine solutions to those of the copper complexes of salicylaldehyde

and glycine.⁹ In the solutions containing two moles of the organic molecules to one of copper, for example, Schiff base formation in both cases results in a shift in the peak from 625 mμ for the amino acid complex to 650 mμ for the complex of the Schiff base, and the optical density is doubled. Such similarity suggests that the copper-pyridoxal-alanine complex involves the same functional groups as the copper-salicylal-glycine complex, and thus furnishes additional evidence for structure III.

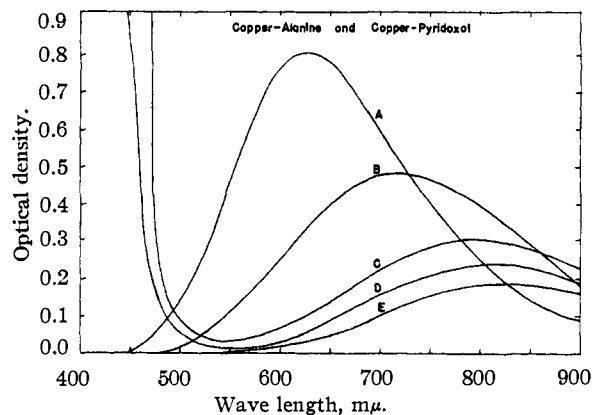


Fig. 5.—A, copper-alanine 1:2; B, copper-alanine 1:1; C, copper-pyridoxal 1:2; D, copper-pyridoxal 1:1; E, copper(II) ion.

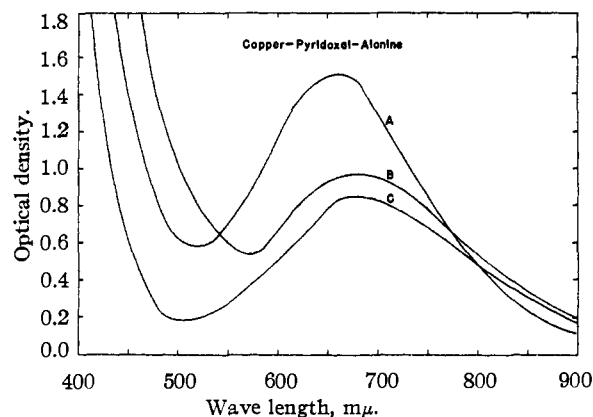


Fig. 6.—Absorption spectra of solutions containing initially copper-pyridoxal-alanine; A, 1:2:2, after 20 min.; B, 1:1:1, after one day; C, 1:1:1, after 20 min.

A comparison of curves B and C in Fig. 6 leads to the conclusion that, as in the nickel reaction, the concentration of the Schiff base complex increases with time. Once more we observe that there is no change in the peak of the solution that initially contained pyridoxal and alanine, indicating that this Schiff base, once formed, is stable, and does not tend to isomerize to pyridoxamine-pyruvic acid (structure IV).

Examination of curves C and D in Fig. 7 reveals the change in absorption of copper pyridoxamine upon the addition of pyruvic acid. The species responsible for curve C presumably has structure IV. Upon standing, the spectrum of the copper-pyridoxamine-pyruvic acid solution changes from

(9) G. L. Eichhorn and N. D. Marchand, *THIS JOURNAL*, **76**, in press (1954).

C to B; the latter closely resembles spectrum B in Fig. 6. This observation leads to the interpretation that structure IV is also unstable when nickel is replaced by copper, and is therefore converted into III.¹⁰

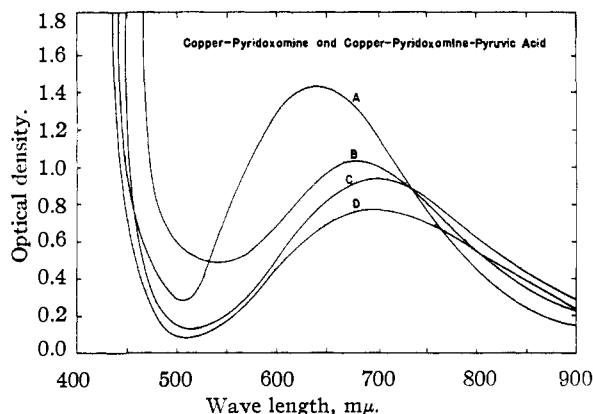


Fig. 7.—Absorption spectra of solutions containing initially copper-pyridoxamine or copper-pyridoxamine-pyruvic acid: A, copper-pyridoxamine 1:2; B, copper-pyridoxamine-pyruvic acid 1:1:1, after 3 hr.; C, copper-pyridoxamine-pyruvic acid 1:1:1, after 1/2 hr.; D, copper-pyridoxamine 1:1.

The Nature of the Precipitates.—The absorption spectrum of the 1:2:2 nickel-pyridoxal-alanine solution suffered a decrease in absorption intensity throughout the visible range, after the precipitate that had formed on standing had been filtered, but there was no change in the character of the spectrum; the precipitated substance, therefore, probably has approximately the same composition as the complex in solution. Similar results were obtained for the 1:1:1 copper-pyridoxal-alanine; the 1:2:2 solution, however, exhibited an entirely different type of spectrum. Analysis of the precipitate yields a carbon-hydrogen-nitrogen ratio that corresponds to the ratio of these elements in alanine, but the actual percentages of these elements are much lower than in a simple copper alanine complex; the precipitate may consist of a copper-alanine complex in which the copper atoms are linked to each other by means of hydroxy bridges.

Continuous Variation.—*Y*-Values (the difference between the observed optical density and the optical density calculated on the assumption that no reaction occurs between the components¹¹) have been plotted in Fig. 8 for solutions containing the same total molar concentration of nickel and pyridoxal-alanine,¹² but varying ratios of the two. At both 950 and 580 $m\mu$ the *Y*-values pass through a maximum at a point corresponding to a solution that contains two moles of pyridoxal-alanine to one of nickel ion.

(10) The appearance of precipitates in the copper containing solutions made it impossible to obtain spectra of the 1:1 Schiff base complex after 3 hours, and the 1:2 complex could not be studied at all for this reason.

(11) W. C. Vosburgh and G. R. Cooper, *THIS JOURNAL*, **63**, 437 (1941).

(12) For purposes of this continuous variation study the three-component system has been simplified to a two-component system by assuming the formation of a Schiff base between pyridoxal and alanine.

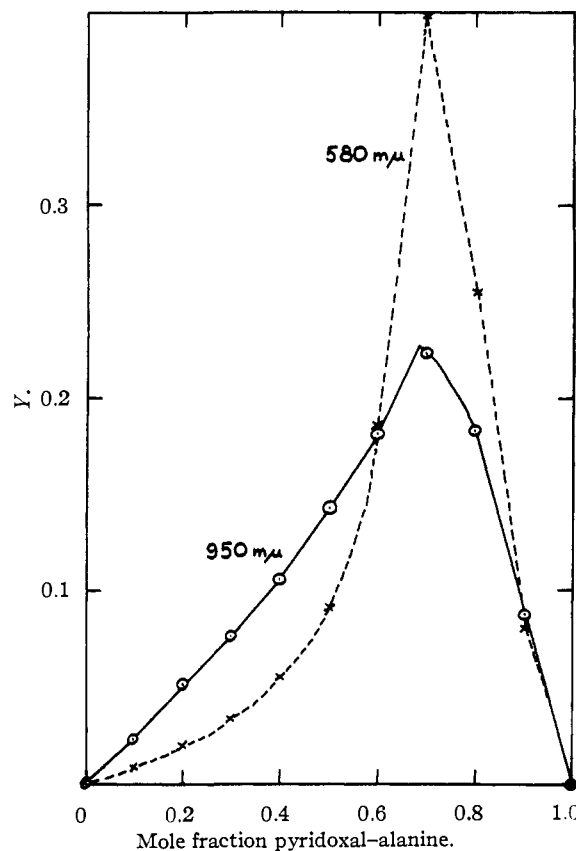


Fig. 8.—Continuous variation of nickel(II) with pyridoxal-alanine; total concentration of all solutions 0.03 *M*.

Conclusions

The experiments that have been described demonstrate that the same end product is formed in solution when copper or nickel ions are treated with either pyridoxal and alanine or pyridoxamine and pyruvic acid. This product differs spectroscopically from the metal complexes of pyridoxal, pyridoxamine, alanine and pyruvic acid. The stable complex in these solutions must therefore involve three components: the metal ion, pyridoxal and alanine, or the metal ion, pyridoxamine and pyruvic acid. That the three components are present in the form of a Schiff base complex would be anticipated from a knowledge of the great tendency of an amino function and a carbonyl function to combine in this manner, whenever they are brought together. The fact that the same three-component complex is produced, regardless of the starting materials used, appears extremely difficult to explain without assigning the structure of a Schiff base complex to the substance.

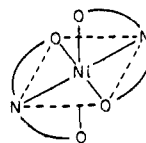
Evidence that the final complex in the solution conforms to the structure III, rather than structure IV, comes from the fact that the absorption characteristics of this complex may be observed very rapidly in the solution that initially contained pyridoxal and alanine but only after considerable elapse of time in the solution that contained pyridoxamine and pyruvic acid.

If structure III is correct, the functional groups coordinated to the metal are the same as those in

a complex of the salicylaldehyde-glycine Schiff base. The similarity of the spectra of these two complexes (in the copper reaction) constitutes additional evidence for structure III, and makes coordination through other functional groups of the vitamin (e.g., the ring nitrogen) unlikely.

From the continuous variation study it may be concluded that two molecules of Schiff base are coordinated to each nickel ion. Two of the oxygen donors and the nitrogen donor of each molecule may be bound as shown in the following

formulation



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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CARNEGIE INSTITUTE OF TECHNOLOGY]

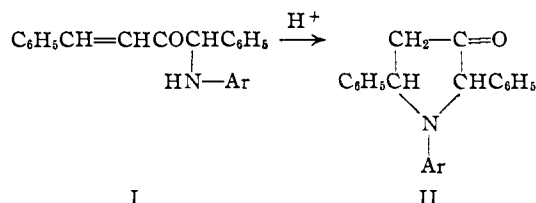
The Cyclization of Arylaminoethyl Styryl Ketones to 1-Aryl-5-phenyl-3-pyrrolidones

By PHILIP L. SOUTHWICK AND HAROLD L. DIMOND¹

RECEIVED MAY 29, 1954

A series of five arylaminoethyl styryl ketones has been obtained by treatment of iodomethyl styryl ketone with aniline, *p*-chloroaniline, *p*-toluidine, *p*-anisidine and ethyl *p*-aminobenzoate. Four of the arylaminoethyl styryl ketones were cyclized to give 5-phenyl-3-pyrrolidones with the phenyl, *p*-chlorophenyl, *p*-tolyl or *p*-methoxyphenyl groups in the 1-position. 1,2-Diphenylpyrrolidine and 1,5-diphenyl-3-hydroxypyrrrolidine have been obtained by reduction of 1,5-diphenyl-3-pyrrolidone. Anilinoethyl styryl ketone has been converted into the sodium salt of 1-anilino-4-phenyl-2-butanone-4-sulfonic acid by addition of sodium bisulfite.

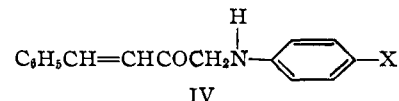
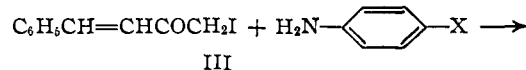
It has recently been shown² that 1-aryl-2,5-diphenyl-3-pyrrolidones (II) can be prepared by means of the acid-catalyzed cyclization of unsaturated arylamino ketones of the type described by formula I, compounds which can be obtained from benzyl styryl ketone by bromination with N-bro-



mosuccinimide,³ followed by treatment of the resulting 1,4-diphenyl-1-bromo-3-butene-2-one with aromatic amines.² By application to benzalacetone of a sequence of reactions similar to that applied to benzyl styryl ketone it was planned to prepare the less highly substituted 1-aryl-5-phenyl-3-pyrrolidones (V). To achieve this objective it was necessary to develop the new cyclization procedures to be described in the present paper.

The conversion of benzalacetone into iodomethyl styryl ketone (III) *via* 4-phenyl-1,3,4-tribromo-2-butanone already has been described.^{3,4} Iodomethyl styryl ketone (III) reacts readily with aromatic primary amines to give the yellow, crystalline arylaminoethyl styryl ketones (IV). The

amines used were aniline and four *para*-substituted anilines



(X = H, Cl, CH₃, OCH₃, CO₂C₂H₅)

p-chloroaniline, *p*-toluidine, *p*-anisidine and ethyl *p*-aminobenzoate. Ammonia and aliphatic primary amines reacted without yielding any well-defined products other than the hydroiodides of the amines used.

The cyclization of the arylaminoethyl styryl ketones (IV) to 1-aryl-5-phenyl-3-pyrrolidones (V) was not successfully accomplished by heating with 20% aqueous alcoholic sulfuric acid, a procedure which was highly successful with the amino ketones (I) derived from benzyl styryl ketone, for in place of the expected pyrrolidones dark tars were formed. Experiments with a variety of acids (acetic, hydrochloric, hydrobromic, sulfuric), using many variations in solvent, concentration of acid, time and reaction temperature, led either to extensive tar formation and, at best, only a trace of the cyclized product, or to recovery of unchanged starting material. Alkaline catalysis with sodium hydroxide also was ineffective. Heating the anilino ketone (IV, X = H) in boiling xylene did not produce ring closure. In one experiment the anisidino ketone (IV, X = OCH₃), when refluxed for 24 hours in neutral aqueous ethanol at a concentration of 1 g. in 20 ml. of solution, yielded enough of the corresponding pyrrolidone to permit purification of the product, but again an excessive amount of tar was formed and the purification was very difficult. It should be noted that in this case the tar did not

(1) Institute Fellow in Organic Chemistry, 1951-1953. This paper is based on the Ph.D. Thesis of Harold L. Dimond, Carnegie Institute of Technology, March, 1953.

(2) P. L. Southwick, D. I. Sapper and L. A. Pursglove, *THIS JOURNAL*, **72**, 4940 (1950).

(3) P. L. Southwick, L. A. Pursglove and P. Numerof, *ibid.*, **72**, 1604 (1950).

(4) Bromination of benzalacetone with N-bromosuccinimide is not a successful method for preparation of bromomethyl styryl ketone, a compound which has yet to be reported. See P. L. Southwick, L. A. Pursglove and P. Numerof, *ibid.*, **72**, 1600 (1950).