but the presence of smaller amounts of the dipolar species VII cannot be excluded.

Discussion

Ultraviolet Spectra.—An analogy to the large bathochromic shift from V to VI is found in the case of the parent hydroxypyridinealdehydes. Conversion of the neutral species I into any form incorporating the mesomeric structure IV causes a red shift of the π_1-absorption bands of over 50 mμ. Differences between individual species of this type, such as the dipolar form II and the anion III, are negligible. In similar fashion, the conversion of the hydroxyaldehydes into the dipolar imine species VII, or into the imine anion, might be expected to result in a red shift of the π_1-transitions to approximately 365–385 mμ. The Schiff base species VI shows a remarkable additional shift of about 30 mμ over this value because of intramolecular hydrogen bonding and its unique electron delocalization.

While the above arguments support the assignment of the 404–425 mμ band of the hydroxy Schiff bases to the "keto-enamine" species VI and not to the dipolar species VII, the correctness of this assignment is further supported by the nature of the spectra in more polar solvents. Thus, dioxane or chloroform solutions of the *o*-hydroxypyridinealdehydes show only two absorption bands corresponding to the π_1- and π_2-transitions of the neutral, non-polar species I, which appear near 320 and 240 mμ, respectively (Table I). The characteristic π_1- and π_2-absorption bands associated with the neutral, dipolar species II, found near 380 and 270 mμ in aqueous solutions and in dioxane–water mixtures, are completely absent under these conditions. From this, the absence of the dipolar species of the Schiff bases (as, for example, VII in the case of the 3-hydroxypyridine-2-aldimines) from dioxane or chloroform solutions of the Schiff base may be inferred with reasonable certainty. Since hydration (hemiacetal formation) as well as hydrolytic cleavage are also excluded, the species VIII and IX as well as free hydroxyaldehyde are also absent and the Schiff base may be fully described by V and VI. Confirming evidence for this conclusion may be found in the spectrum of N-salicylidenevaline which is incapable of forming a dipolar species but exhibits four absorption bands, corresponding to two species, at wave lengths similar to the pyridine compounds.

Implications for the Chemistry of Pyridoxal.—The electronic absorption spectra of pyridoxal amino acid Schiff bases in aqueous solution have been studied by several investigators.[15,16] The possibility for the formation of tautomeric species has been recognized,[15] but no experimental evidence has thus far been obtained. The degree of validity of the results of this investigation for pyridoxal, and in particular for aqueous solutions, remains open. However, assignment of the 330 mμ absorptions to the dipolar species VII[16] appears inconsistent with the available information. Assignment to unreacted pyridoxal[15] or to the dipolar hemiacetal of the Schiff base[15] are definite possibilities, since the occurrence of the neutral, nonpolar species V in aqueous solution is unlikely. The presence of appreciable quantities of the pyridoxylideneimines in the "keto-enamine" tautomeric species VI, perhaps along with some proportion of the dipolar from VII, must be considered. If this should prove to be the case, a partial positive charge would be located at the imine nitrogen atom, which is in close proximity to the two carbon atoms involved in proton exchange during transamination and racemization reactions. If the positive nitrogen atom stabilizes the transition state in these reactions, their mechanism would not require the electron shift toward a positive charge at the pyridinium nitrogen formulated by Snell and co-workers.[17] Indeed, the occurrence of both transamination and racemization reactions at *p*H values up to 10 suggests such a possibility. Finally, the ease of transamination reactions in ethanol solution described by Matsuo[18] is readily understandable on the basis that the keto-enamine structure should be the predominant species in this solution.

(15) D. E. Metzler, *J. Am. Chem. Soc.*, **79**, 485 (1957).
(16) H. N. Christensen, *ibid.*, **80**, 99 (1958).
(17) D. E. Metzler, M. Ikawa and E. E. Snell, *ibid.*, **76**, 648 (1954).
(18) Y. Matsuo, *ibid.*, **79**, 2016 (1957).

[CONTRIBUTION FROM THE DEPARTMENTS OF CHEMISTRY OF CLARK UNIVERSITY, WORCESTER, MASS., AND OF THE ILLINOIS INSTITUTE OF TECHNOLOGY, CHICAGO, ILL.]

Pyridoxine and Pyridoxal Analogs. VII. Acid–Base Equilibria of Schiff Bases[1]

BY DIETRICH HEINERT[2] AND ARTHUR E. MARTELL[2]

RECEIVED AUGUST 1, 1962

The electronic absorption spectra of N-salicylidenevaline and N-(3-hydroxy-4-pyridylmethylene)-valine have been studied in dioxane and methanol solutions in the presence of varying amounts of acid or base. Analysis of the spectra resulted in species and band assignments which allow the formulation of the complete solution equilibria of these compounds, and equilibrium constants are reported where possible.

Introduction

In the preceding paper[3] it was shown through an analysis of the electronic absorption spectra that the amino acid Schiff bases of *o*-hydroxypyridinealdehydes and of *o*-hydroxybenzaldehydes in neutral, non-aqueous solution constitute tautomeric equilibrium mixtures. This investigation has now been extended to a study of the absorption spectra of these compounds in non-aqueous solution under conditions of varying *p*H. The objective of this work was to establish the nature of the reactions which take place, to measure these reactions quantitatively, and to provide evidence for the origin of certain band groups in the π_1- and π_2-transitions of specific species. The elucidation of these objectives is desirable because the various forms in which these Schiff bases exist, depending on hydrogen ion concentration and the solvent medium, would be expected to vary considerably in their involvement in the transamination and similar reactions. It was also hoped to gain at least qualitative information on the relative proton affinities of the various species of these compounds, even though the exact determination of acid dissociation constants would not be feasible because

(1) This investigation was supported by research grants A-1307 and A-5217 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

(2) Department of Chemistry, Illinois Institute of Technology, Chicago 16, Ill.

(3) D. Heinert and A. E. Martell, *J. Am. Chem. Soc.*, **85**, 183 (1963).

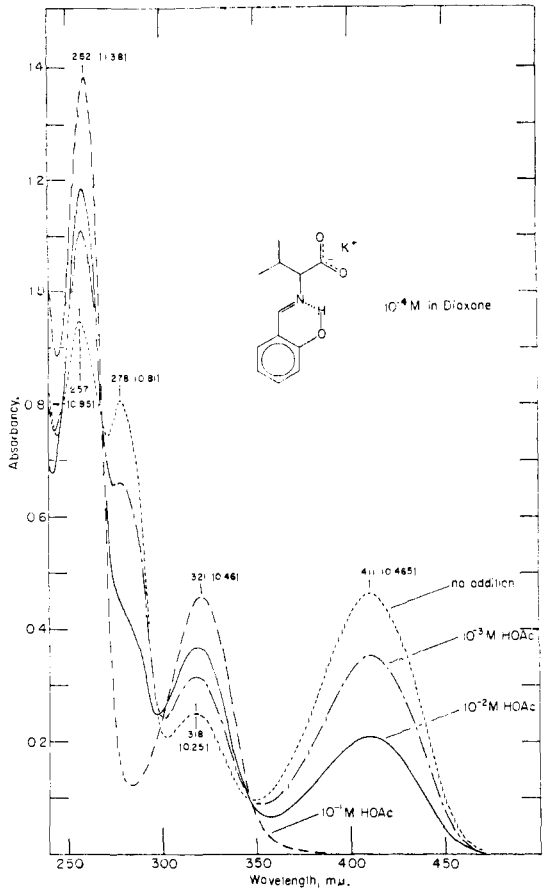

Fig. 1.—Electronic absorption spectra of N-salicylidenevaline 10^{-4} mole/l., in neutral and acidic dioxane solutions.

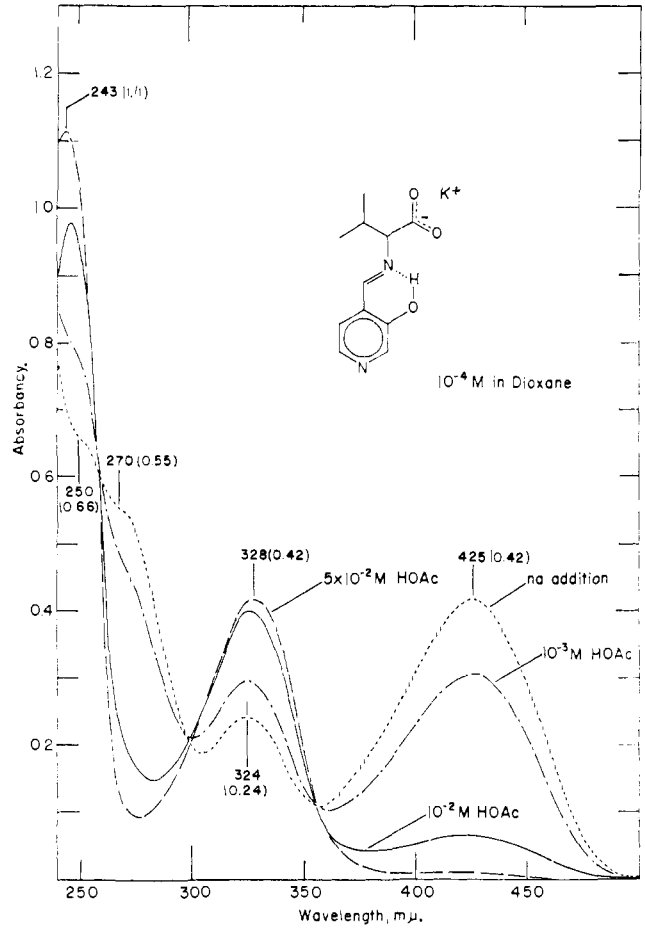

Fig. 2.—Electronic absorption spectra of N-(3-hydroxy-4-pyridylmethylene)-valine, 10^{-4} mole/l., in neutral and acidic dioxane solutions.

of the inadequate definition of *p*H in 100% dioxane or alcohol solutions.

Experimental

Measurements.—The electronic absorption spectra in the visible and ultraviolet regions were recorded with a Cary model 14 spectrophotometer at 20°. All solutions were measured in a pair of 10-mm. stoppered quartz cells at a concentration of 10^{-4} mole/liter of the Schiff base.

Materials.—N-(3-Hydroxy-4-pyridylmethylene)-valine and N-salicylidenevaline monopotassium salts were prepared as described earlier.[4] Reagent grade anhydrous acetic acid, potassium hydroxide and gaseous hydrogen chloride were used for preparation of standard acid and base stock solutions. The purification of the solvents was carried out by the methods described in the preceding paper.[3]

Results

Schiff Base Spectra in Acidified Dioxane Solution.—The initial measurements in the present investigation were carried out with the relatively simple compound N-salicylidenevaline. In Fig. 1 are compared the absorption spectra of this compound obtained in neutral dioxane solution and in dioxane solutions containing 10^{-3}, 10^{-2} and 10^{-1} mole/liter of glacial acetic acid. According to previous work[3] the absorption bands at 411 and 278 mμ in neutral dioxane solution are due to the π_1- and π_2-transitions[5] of the ketoenamine species III_A of the Schiff base, while the bands at 318 and 257 mμ are associated with the π_1- and π_2-transitions of the tautomeric enol-imine species II_A. In the presence of increasing amounts of acid in dioxane solutions of the Schiff base, the 411 and 278 mμ absorptions are seen to decrease, while at the same time the extinctions of the 318 and 257 mμ bands increase with formation of well-defined isosbestic points. Finally, in 10^{-1} molar acetic acid, only two bands—or one species—absorbing at 321 and 262 mμ remain. Under the conditions chosen only one structure of the product formed in acidic solution is conceivable which is also consistent with the observed absorption characteristics: that of

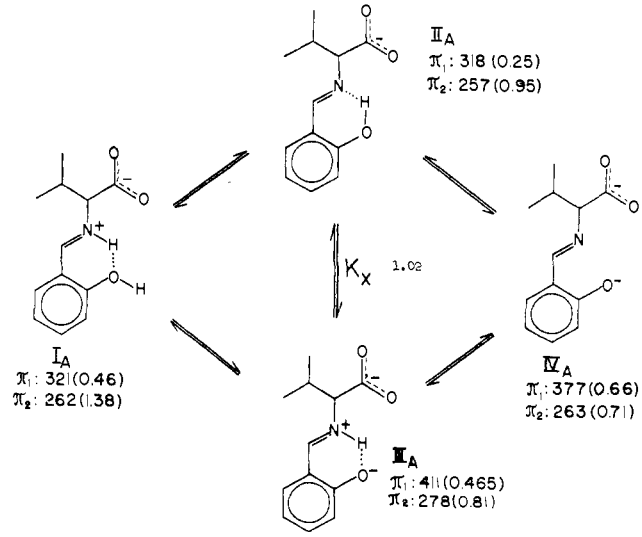

Plate I.—Solution equilibria of N-salicylidenevaline: I, acidic; II, III, neutral dioxane solutions; IV, basic methanol solution. Absorption maxima of each species are given in mμ, numbers in parentheses denote absorbancy.

(4) D. Heinert and A. E. Martell, *J. Am. Chem. Soc.*, **84**, 3257 (1962).

(5) Throughout this paper the abbreviations π_1 and π_2 are used for π–π_1^*- and π–π_2^*-transitions, respectively. The correlation between π_1- and π_2-bands and other band-designation systems has been pointed out previously *J. Am. Chem. Soc.*, previous paper, footnote 8).

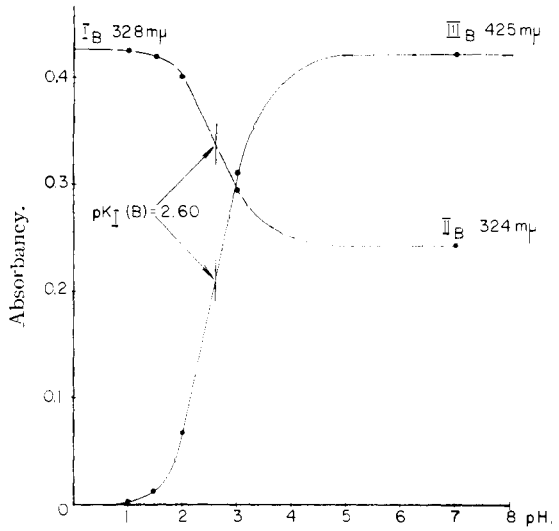

Fig. 3.—Variation of absorbancy for various absorption bands of N-(3-hydroxy-4-pyridylmethylene)-valine as a function of acetic acid concentration in dioxane.

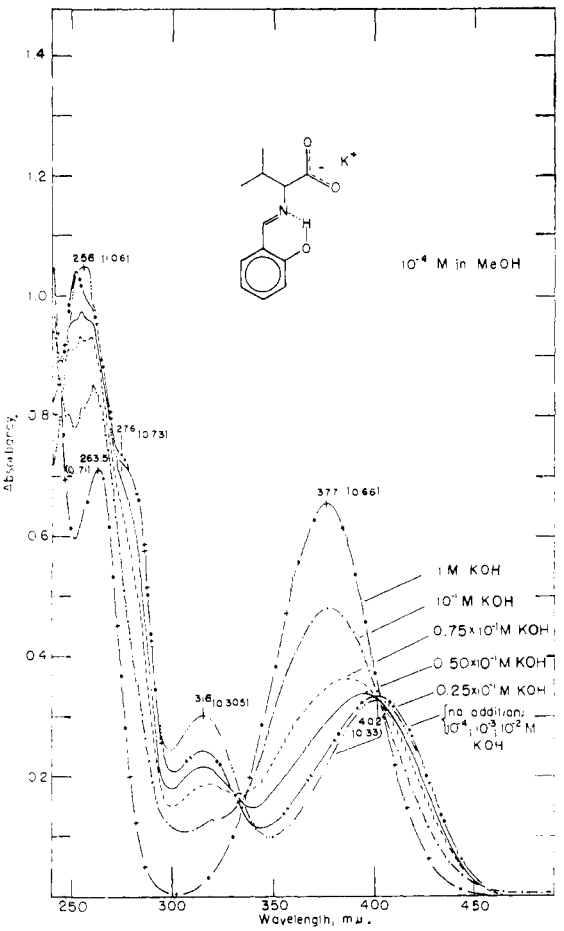

Fig. 4.—Electronic absorption spectra of N-salicylidenevaline, 10^{-4} mole/l., in neutral and alkaline methanol solutions.

the immonium cation[6] I_A. It is evident that both species II_A and III_A are converted to this common cation by the addition of a proton. However, the influence of protonation on the absorption of species II_A differs markedly from that on species III_A. In the latter, annihilation of the phenolate anion removes the unique resonance system of this compound, which links it to the vinylogous amides and is responsible for

(6) Throughout this paper the ionization state of each species is expressed without regard to the amino acid carboxylate group.

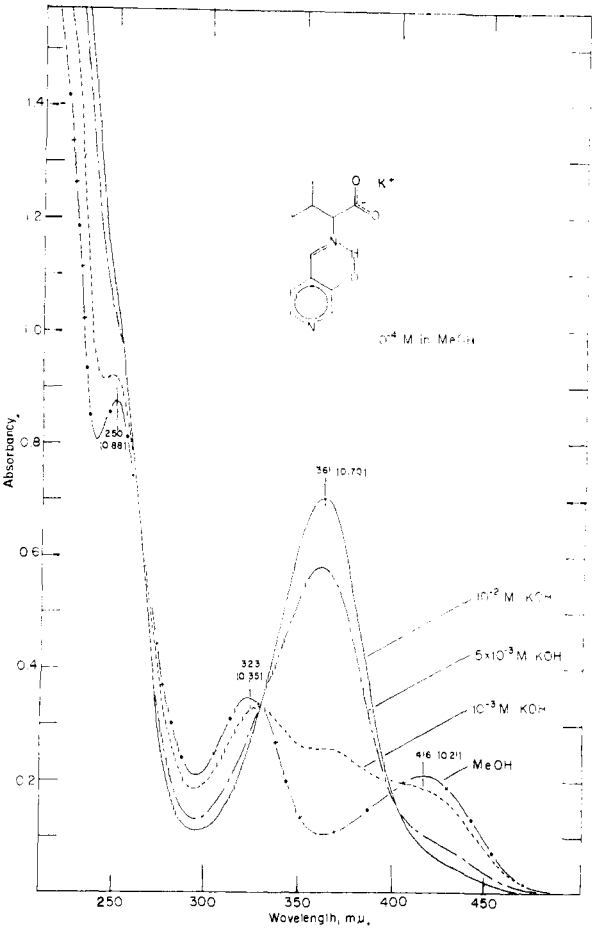

Fig. 5.—Electronic absorption spectra of N-(3-hydroxy-4-pyridylmethylene)-valine, 10^{-4} mole/l., in neutral and alkaline methanol solutions.

the yellow coloration of the Schiff bases.[3] Proton addition to the imine grouping in II_A on the other hand, is not expected to result in a remarkable shift of the absorption bands.[7] Thus, the similarity of the ab-

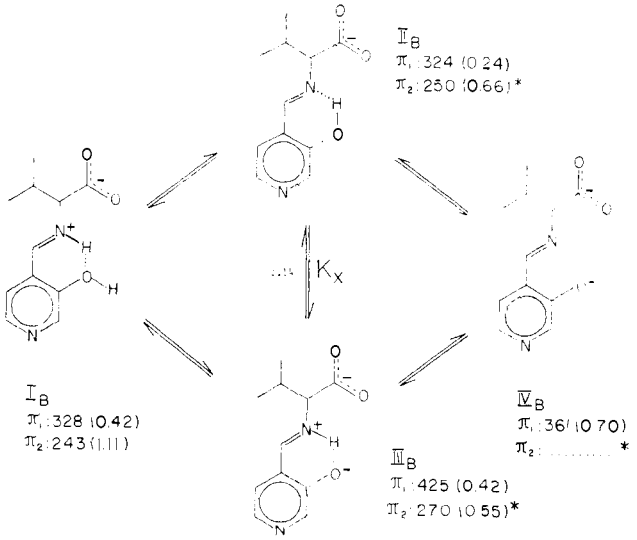

Plate II.—Solution equilibria of N-(3-hydroxy-4-pyridylmethylene)-valine: I, acidic; II, III, neutral dioxane solutions; IV, basic methanol solution. Absorption maxima of each species are given in mμ; numbers in parentheses denote absorbancy; asterisk denotes shoulder.

(7) For example, the π_1-bands of pyridine, 256 mμ (2.8), remain unchanged upon protonation to the pyridinium ion, λ_{max} 256 mμ (5.7), but the oscillator strength increases considerably. This compares with a shift of +3 mμ of

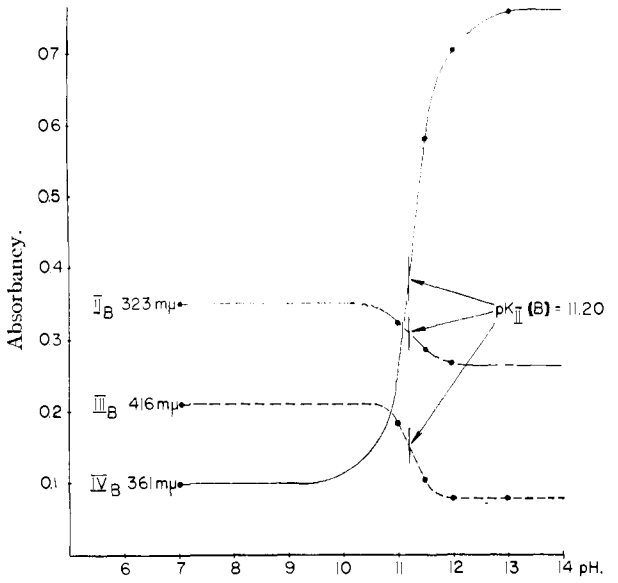

Fig. 6.—Variation of absorbancy for various absorption bands of N-(3-hydroxy-4-pyridylmethylene)-valine as a function of KOH concentration in methanol.

sorption of species I_A and II_A, resulting in very small red shifts of +3 mμ and +5 mμ of the π_1- and π_2-bands upon acidification, becomes understandable. The over-all results of acidification lend support to the previous assignments of species II_A and III_A. Parallel decreases in intensity of the 411/278 mμ group of absorption bands confirms their equilibrium relationship as indicated on Plate I.

Although protonation of the carboxylate group may also take place partially in strongly acidic solutions, no appreciable effect on the absorption of any species would be expected, and none was observed.

The results of acidification of the dioxane solutions of the more complicated compound N-(3-hydroxy-4-pyridylmethylene)-valine are shown in Fig. 2. The absorption bands present in neutral dioxane solution were previously assigned[3] as shown in Plate II, to the enol-imine species II_B and the keto-enamine species III_B. Upon acidification, the species III_B disappears while the absorption bands attributed to species II_B increase in extinction and shift slightly in position, as observed in the case of N-salicylidenevaline. This result is interpreted as the formation of a single acid form I_B from both monoprotonated species. Again, as in the first example, protonation of the imine group or the pyridine nitrogen in II_B would not be expected to result in more than a slight shift of the absorption maxima.[7,8] These observations confirm the previous band assignments of species II_B and III_B. The similarity of the results obtained with the benzenoid and the pyridinoid series is worthy of note since it indicates that presence of the pyridine nitrogen is not a requirement for the occurrence of these transformations.

Equilibrium Constants.—Approximate values of the over-all first ionization constants, K_I, of both systems were obtained by the graphical method described previously.[3] In this investigation plots of the change in absorbancy of various bands as a function of acetic acid concentration in dioxane were used to calculate apparent pK values. An example of such an absorbancy plot as a function of acid concentration is given for N-(3-hydroxy-4-pyridylmethylene)-valine in Fig. 3. The resulting pK_I value obtained for the benzenoid

the π_1-band upon conversion of II_A into I_A, and increase in absorbancy from 0.25 to 0.46.

(8) K. Nakamoto and A. E. Martell, *J. Am. Chem. Soc.*, **81**, 5857, 5863 (1959).

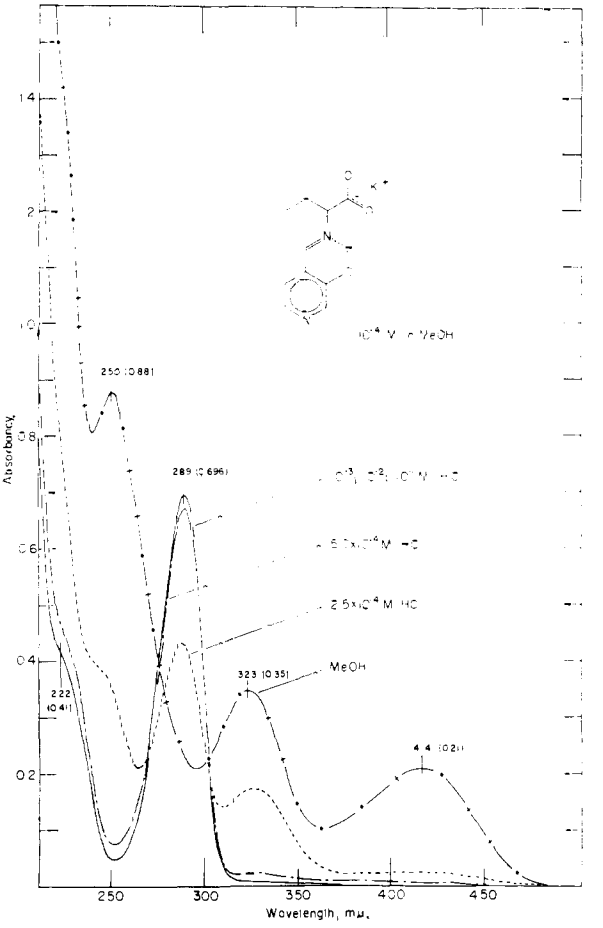

Fig. 7.—Electronic absorption spectra of N-(3-hydroxy-4-pyridylmethylene)-valine, 10^{-4} mole/l., in neutral and acidic methanol solutions.

series, $pK_I(A) = 2.10$, represents the conversion of species I_A to the equilibrium mixture of II_A and III_A. The pyridinoid compounds gave a $pK_I(B)$ of 2.60 for the over-all conversion of I_B to II_B and III_B. Individual equilibrium constants for conversion of species I to III, $pK_a(A)$ and $pK_a(B)$, as well as conversion of species I to species II, $pK_b(A)$ and $pK_b(B)$ of both compounds, were obtained from the relationship[8]

$$K_x = K_a/K_b$$

and

$$K_I = K_a + K_b$$

Utilization of the K_x values in dioxane solution reported earlier[3] gave

$$K_x(A) = 1.02;\ K_I(A) = 8.0 \times 10^{-3}$$

and

$$K_x(B) = 1.14;\ K_I(B) = 2.51 \times 10^{-3}$$

Thus the following individual equilibrium constants were calculated

$$pK_a(A) = 2.39;\ pK_b(A) = 2.40$$
$$pK_a(B) = 2.88;\ pK_b(B) = 2.93$$

Schiff Base Spectra in Alkaline Methanol.—The nature of *o*-hydroxyaldehyde Schiff bases of the type under consideration in neutral methanol solutions has already been discussed.[3] In the case of N-salicylidenevaline, formation of the neutral hemiacetal is the main side reaction. In the pyridinealdehyde Schiff bases, for example N-(3-hydroxy-4-pyridylmethylene)-valine, dipolar forms VII and dipolar hemiacetal forms VI may be present in addition to the neutral hemiacetal (not shown) and species II_B and III_B.

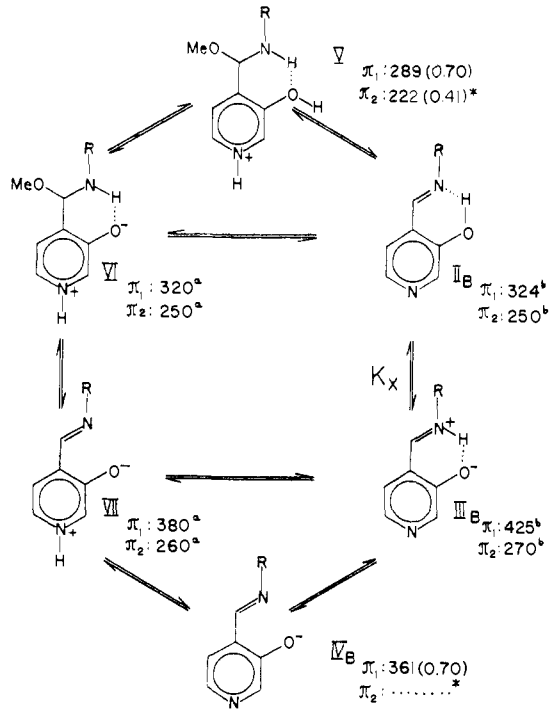

Plate III.—Solution equilibria of N-(3-hydroxy-4-pyridylmethylene)-valine in methanol solution: V, acidic; II, III, VI, VII, neutral; IV, basic; *, shoulder; [a] estimated; [b] dioxane data, *cf.* Plate II. Absorbancy and wave lengths in methanol are expected to differ slightly from these values; R = $-CH[CH(CH_3)_2]CO_2K$.

In spite of these complications, measurements in alkaline methanol solution should meet with little difficulty, since all species present in neutral solution should be converted to a common anion IV and acid-catalyzed hemiacetal formation would not take place. Therefore, the spectra of the two Schiff bases were measured in methanol solutions containing increasing concentrations of base. The results, illustrated in Figs. 4 and 5, show only one strong absorption band, at 361–377 mμ, assigned to the π_1-transitions of the species IV_B and IV_A, respectively. The corresponding band in the 250 mμ region is assigned to the π_2-transitions.

It is interesting to note the large shift of the π_1-absorption band of the anions[6] relative to the π_1-bands of the species II and III. The bathochromic shift of 50 ± 10 mμ upon conversion of II into IV corresponds to the shift created in removal of the proton from the simple hydroxyaldehydes[3] leading to the mesomeric anions. The large hypsochromic shift of 50 ± 15 mμ, on the other hand, which is observed if one considers the conversion of III into IV (Plates I and II), is understandable in view of the combined effects of removal of the hydrogen bond and of the merocyanine type resonance system present in III.[3]

A limited amount of information on the relative acidity of the proton in species II and III of the benzenoid *vs.* the pyridinoid series may be gained from the measurements shown in Figs. 4 and 5. Assuming complete dissociation of KOH and disregarding solvent effects, the absorbancy values of various bands from each series, plotted *versus* *p*H, resulted in the determination of apparent over-all second ionization constants pK_{II} for each compound; $pK_{II}(A) = 13.0$ for the benzenoid compounds corresponds to the conversion of II_A and III_A into the common anion IV_A. Its value was found to be almost two log units lower for the pyridine compounds, for which the apparent $pK_{II}(B)$ was found to be 11.20 in methanol. The simultaneous determination of pK_{II} values from three different absorption bands is illustrated in Fig. 6. Differences in the pK_{II} values in dioxane or water solution are expected to parallel these results, although the magnitudes of the dissociation constants (and the differences between them) would vary considerably with the solvent.

Schiff Base Spectra in Acidic Methanol.—The results of the measurement of N-(3-hydroxy-4-pyridylmethylene)-valine in methanol solutions containing increasing concentrations of acid are shown in Fig. 7. If these results are compared with those obtained for the same compound in dioxane solution (Fig. 2) a profound difference is apparent. Thus, not only the species absorbing at 414 mμ, but also those absorbing at 323 mμ in methanol solution, gradually disappear when acid is added, while an entirely new compound, absorbing at 289 and 222 mμ, is produced. Since this species will be completely protonated, but its highest absorption is at shorter wave length than that of species I_B, it is concluded that these absorptions are due to the π_1- and π_2-transitions of the hemiacetal cation V, and that this species is present exclusively in highly acidic methanol solution. With this result, and those given above for alkaline solutions, acid-base equilibria of the *o*-hydroxypyridinealdehyde Schiff bases in methanol may now be formulated as shown in Plate III.

Discussion

Significance of These Results.—The present investigation shows that, under the experimental conditions employed, the complete analysis of the absorption spectra of *o*-hydroxypyridinealdehyde Schiff bases is feasible. It is expected that these results, summarized in Plates I, II and III, will facilitate the analysis of more complicated systems of this type, such as those undergoing transamination reactions under a variety of conditions.

Implications for the Chemistry of Pyridoxal.—The solution equilibria of pyridoxal Schiff bases in aqueous or alcohol solution, along with the corresponding band assignments, are expected to be similar to those of the 3-hydroxypyridine-4-aldimines shown in Plate III. One additional complication arises from the possible formation of the neutral and dipolar hemiacetals by intramolecular ring closure of the 5-hydroxymethyl group in pyridoxal. However, the absorption characteristics of the internal hemiacetals would be identical, or very similar, to those of the hemiacetals formed with alcohol solvents.

The absorption spectra of a pyridoxal amino acid Schiff base in neutral and alkaline methanol solution were described by Matsuo,[9] but species assignments were not given. Comparison of the spectra given in this paper with those obtained by Matsuo[9] shows that these spectra are very similar to those of N-(3-hydroxy-4-pyridylmethylene)-valine under similar experimental conditions. Thus, assignments of equilibrium solution species of pyridoxal based on the published spectra may readily be made on the basis of the results reported in this paper.

The absorption spectra of pyridoxal Schiff bases in aqueous solution have been reported by Metzler.[10] The probable implications of the present investigation for the species assignments in neutral solution have already been discussed.[3] In alkaline aqueous solution Metzler observed band shifts associated with the formation of a Schiff base anion—results which were quite parallel to those obtained in this investigation for

(9) Y. Matsuo, *J. Am. Chem. Soc.*, **79**, 2016 (1957).
(10) D. E. Metzler, *ibid.*, **79**, 485 (1957).

alkaline methanol solution (Plates I and II). In acidic aqueous solution, however, hydrolysis of the pyridoxal Schiff bases was found to increase to a considerable extent,[10] notwithstanding the application of a 10^4 molar excess of the amino acid over pyridoxal in order to retard such hydrolysis. Thus, the observation of cationic pyridoxal species analogous to I_A, I_B and V has not been possible.

The initial dissociation constants for salicylidenevaline, and for 3-hydroxy-4-pyridylidenevaline, are somewhat larger than one might predict. The ease of removal of the first proton seems to be an indication of the strength of hydrogen bond formed in II_B–III_B and II_A–III_A mixtures.

The large difference in the last dissociation constants (in methanol) of the pyridinoid and benzenoid Schiff bases is considered significant. The fact that the dissociation of the pyridine derivative is nearly one hundred times (two pK units) greater than that of salicylidenevaline shows that the equilibrium concentration of IV_B would be much greater than that of IV_A under the same solution conditions in the range where species II and III are the more stable forms. Thus the formation of IV, and its metal chelates, would be greatly favored when an electron-withdrawing group such as the pyridine nitrogen atom is present in the Schiff base. It is seen, therefore, that the requirement of an electron-withdrawing substituent on the aromatic ring, pointed out earlier by Snell,[11] has a direct bearing on the equilibrium formation of the reactive Schiff base species in solution.

The acid dissociation constants used in this paper are only apparent constants, since ionization of the acetic acid added to the system certainly cannot be complete. Since the dielectric constants of methanol and especially dioxane, are considerably lower than that of water, one can assume that the acetic acid molecules (or a mixture of acetic acid molecules and ion pairs formed between acetate ion and the protonated solvent) comprise the acidic reactant. Thus the constants given are actually acid–base equilibrium constants with acetic acid as one of the reacting species. The dissociation constants would therefore be similar to aqueous dissociation constants, in which acetic acid molecules (or ion pairs) replace the hydronium ion.

(11) E. E. Snell, *Physiol. Revs.*, **33**, 509 (1953).

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY, TEMPE, ARIZ.]

Purine Nucleosides. III. Methylation Studies of Certain Naturally Occurring Purine Nucleosides[1]

BY JESSE W. JONES AND ROLAND K. ROBINS

RECEIVED JUNE 29, 1962

1-Methyladenosine (V) and 2′-deoxy-1-methyladenosine (VI) have been isolated and characterized as products from the methylation of adenosine and 2′-deoxyadenosine, respectively. Guanosine and 2′-deoxyguanosine provided 7-methylguanosine and 2′-deoxy-7-methylguanosine upon methylation in neutral media. These latter compounds were isolated and characterized in the form of an unusual internal nucleoside zwitterion (XIV, XV). Xanthosine and inosine under similar conditions gave 7-methylxanthosine (XVI) and 7-methylinosine (XXI), which were also isolated in the betaine form. At pH 8.5 inosine was methylated to give 1-methylinosine (XIV). 1-Methyladenosine and 2′-deoxy-1-methyladenosine rearranged in aqueous sodium hydroxide to give N^6-methyladenosine (VIII) and 2′-deoxy-N^6-methyladenosine (IX), respectively. These studies are discussed in view of previously reported alkylations of purine nucleosides. The possible biochemical significance of the present work is considered.

A substantial number of N-methylpurines have been isolated from various biological sources and identified in recent years.[2] A significant increase in 1-methylhypoxanthine, 8-hydroxy-7-methylguanine and 7-methylguanine has been noted in the urine of patients with leukemia.[3] It seems quite possible that the simple N-methylpurines might arise as degradation products of methylated purine nucleosides or nucleotides hydrolyzed enzymatically *in vivo* or cleaved during isolation procedures.[4] Several of these N-methylpurines have been isolated directly from nucleic acid.[5-9] Littlefield and Dunn[10] identified N^6-methyladenosine (6-N-methylamino-9-β-D-ribofuranosylpurine) and N^6,-N^6-dimethyladenosine (6-N,N-dimethylamino-9-β-D-ribofuranosylpurine) as degradation products of ribonucleic acid from various microbial, plant and mammalian sources. This identification was based on chromatographic and ultraviolet absorption data and was greatly aided by the fact that both 6-N-methylamino-9-β-D-ribofuranosylpurine and 6-N,N-dimethylamino-9-β-D-ribofuranosylpurine were readily available from previous syntheses.[11,12] 2′-Deoxy-N^6-methyladenosine (6-N-methylamino-9-β-D-2′-deoxyribofuranosylpurine) has been isolated[13] from the DNA of a strain of *Escherichia coli*. At least three different N-methylguanosine derivatives have been detected from various sources of ribonucleic acid.[14] Identification of these purine nucleosides was based on chromatographic and spectroscopic comparison with samples prepared by enzymatic means. It is quite clear that various N-methylpurine ribosides and deoxyribosides of established structure, available in pure crystalline form, are needed for present biochemical studies and would be of considerable assistance in the isolation and identification of various purine nucleosides as minor constituents of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). The preparation of such N-

(1) Supported by research grants CY-4008(C3) and CY-4008(C4) from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) See J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **84**, 1914 (1962), for a list of pertinent references.

(3) R. W. Park, J. F. Holland and A. Jenkins, *Cancer Research*, **22**, 469 (1962).

(4) D. B. Dunn and J. D. Smith, in "Proc. Intern. Congr. Biochem., 4th Congr.," Vol. VII, Vienna, 1958, p. 72, Pergamon Press, Inc., New York, N. Y., 1959.

(5) D. B. Dunn and J. D. Smith, *Nature*, **175**, 336 (1955).

(6) J. W. Littlefield and D. B. Dunn, *ibid.*, **181**, 254 (1958).

(7) M. Adler, B. Weissmann and A. B. Gutman, *J. Biol. Chem.*, **230**, 717 (1958).

(8) D. B. Dunn, *Biochim. et Biophys. Acta*, **46**, 198 (1961).

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