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N-Pyridoxylidenehydrazine-*N'*, *N'*-diacetic Acid. II.[1] Absorption Spectra and Molecular Species in Aqueous and Methanol Solutions

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The absorption spectra of *N*-pyridoxylidenehydrazine-*N'*, *N'*-diacetic acid (**1**) and related hydrazones in aqueous and methanol solutions of various acid-alkaline concentrations were measured. The pyridoxylidenehydrazines studied were **1**, *N*-pyridoxylidene-*N'*-methylhydrazine (**2**), *N*-pyridoxylidene-*N'*, *N'*-dimethylhydrazine (**3**), and *N*-pyridoxylidene-*N'*, *N'*-diphenylhydrazine (**4**). The spectra were sensitively dependent on the nature of the media. The intense absorptions in the region of 300—420 nm were ascribed to the π_1-band and the assignments of the band to the molecular species present in the solutions were made on the basis of the rules of the band shift established in the Schiff bases of pyridoxal.

We reported[1] that the ^{99m}Tc chelate of *N*-pyridoxylidenehydrazine-*N'*,*N'*-diacetic acid (**1**) was a good hepatobiliary tracer. However, as a radiopharmaceutical for hepatobiliary scintigraphy, it is not as satisfactory as the currently used ones such as the ^{99m}Tc chelates of *N*-(2,6-diethylphenylcarbamoylmethyl)iminodiacetic acid[2] and *N*-pyridoxylideneamino acids.[3] This was assumed to be due to the presence of polymeric forms of ^{99m}Tc.[1] In an effort to avoid the polymerization of ^{99m}Tc and to improve the yield of the ^{99m}Tc chelate, metal chelation of **1** and its related ligands have been studied.

The present work deals with an analysis of ultraviolet absorption spectra in aqueous and methanol solutions of compound **1** and related hydrazones, for the purpose of obtaining information on the molecular species present in solution. A study of this nature is an essential preliminary to the understanding of metal chelation of the compounds, which will be treated in the forthcoming paper. Compounds studied are the hydrazones of pyridoxal with hydrazine-*N*,*N*-diacetic acid (**1**), methylhydrazine (**2**), 1,1-dimethylhydrazine (**3**), and 1,1-diphenylhydrazine (**4**).

Experimental

Measurements

The absorption spectra were recorded at room temperature with a Shimadzu Model UV-240 spectrophotometer. All solutions were measured in a pair of calibrated silica cells. All spectral studies were carried out immediately after preparing samples.

Solutions for spectral measurements were prepared so as to contain 6.0×10^{-5}M concentration of the hydrazone under investigation, unless otherwise noted. The pH's of aqueous solutions were adjusted by using appropriate buffers. The desired acid-base species were obtained in methanol solutions by adding standard methanol solutions of $HClO_4$ or KOH.

Materials.

Spectrograde methanol and distilled water were used as solvents for spectral studies and proved to give reproducible results.

The hydrazones were prepared from pyridoxal and the appropriate hydrazines. *N*-Pyridoxylidenehydrazine-*N'*,*N'*-diacetic acid (**1**) and *N*-pyridoxylidene-*N'*,*N'*-dimethylhydrazine (**3**) were prepared as described previously.[1] Hydrazine-*N*,*N*-diacetic acid was prepared from hydrazine hydrate and monochloroacetic acid according to Bailey and Read.[4]

N-Pyridoxylidene-*N'*-methylhydrazine (**2**) : Aqueous neutral solutions of pyridoxal (737 nmol) and of an excess amount of methylhydrazine were mixed and refluxed for 1 hr. The solution was concentrated and the pale yellow precipitate was recrystallized from water. Pale yellow needles. Yield 65%. mp 174—177°C. Anal. Calcd for $C_9H_{13}N_3O_2$: C, 55.37 ; H, 6.71 ; N, 21.52. Found : C, 55.55 ; H, 6.68 ; N, 21.48. ^{1}H-NMR (CD_3OD) δ : 2.4 (3H, s, 2-CH_3), 2.97 (3H, s, N-CH_3), 4.66 (2H, s, 5-CH_2), 7.80 (1H, s, 6-H), 7.88 (1H, s, azomethine H).

N-Pyridoxylidene-*N'*,*N'*-diphenylhydrazine (**4**) : 1,1-Diphenylhydrazine hydrochloride (737 nmol) was suspended in water, neutralized with aqueous NaOH, filtered to remove undissolved impurities, and mixed with an aqueous neutral solution of an equimolar pyridoxal. The solution was acidified with acetic acid and the green precipitate was recrystallized from ethanol. Pale green needles. mp 244—246°C. Anal. Calcd for $C_{20}H_{19}N_3O_2$: C, 72.05 ; H, 5.74 ; N, 12.60. Found : C, 71.96 ; H, 5.71 ; N, 12.54.

Pyridoxal hydrochloride and other chemicals were obtained from commercial sources. Nuclear magnetic resonance (NMR) spectra were obtained with a JEOL PS-100 NMR spectrometer.

Results and Discussion

The chromophores of pyridoxal and its Schiff bases are sensitively dependent upon structural factors. Extensive studies have been made on the assignments of their spectral bands to the specific species in solutions and the wavelength shift of the bands with the changes in the structural features of the species.[5] The interpretation of the spectra of the pyridoxal hydrazones in the present investigation was greatly assisted by the knowledge on the spectra of the Schiff bases of pyridoxal.

The absorption spectra of the four hydrazones in aqueous and methanol solutions of various acid-base concentrations were measured. Representative spectra are shown in Fig. 1—5. The spectra had an intense absorption band in the region of 300—420 nm.

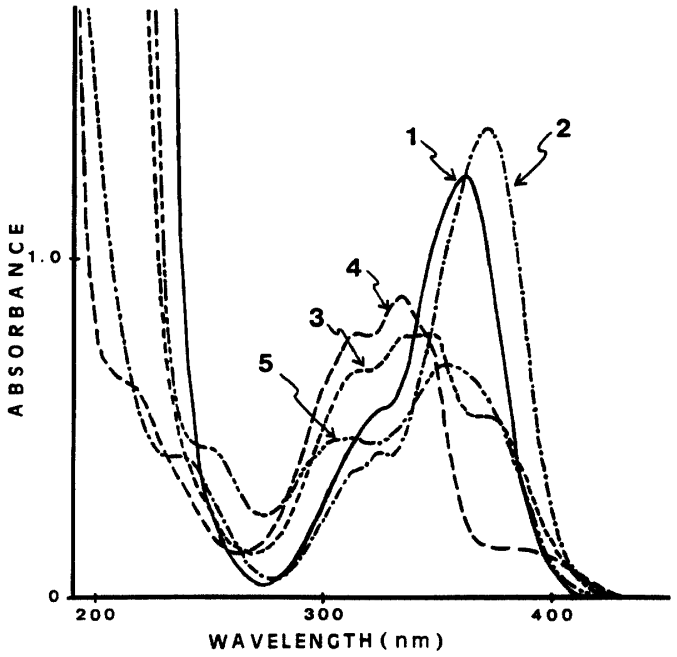


Fig. 1 Absorption spectra of **1** in aqueous solution.
1; pH, 3.0: 2; pH, 5.0: 3; pH, 7.01: 4; pH, 9.0: 5; pH, 11.01.

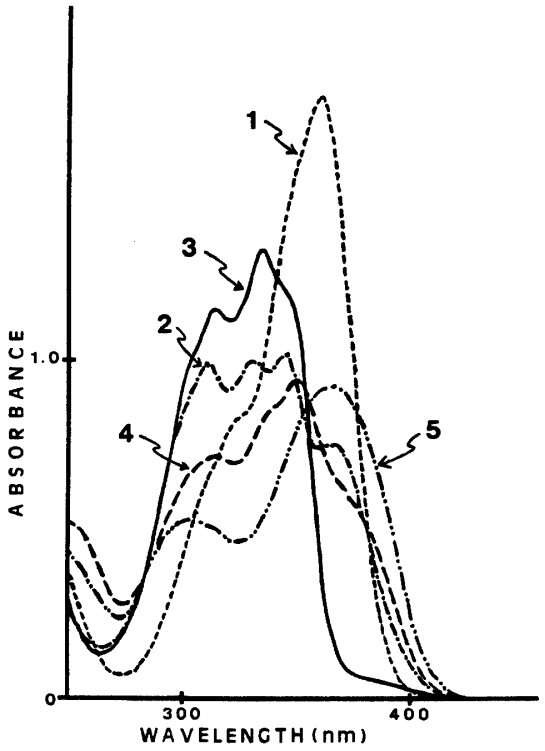


Fig. 2 Absorption spectra of **1** in methanol.
1; 6.2×10^{-3} M $HClO_4$: 2; 1.1×10^{-4} M $HClO_4$: 3; neutral: 4; 3.5×10^{-2} M KOH: 5; 1.5×10^{-1} M KOH.

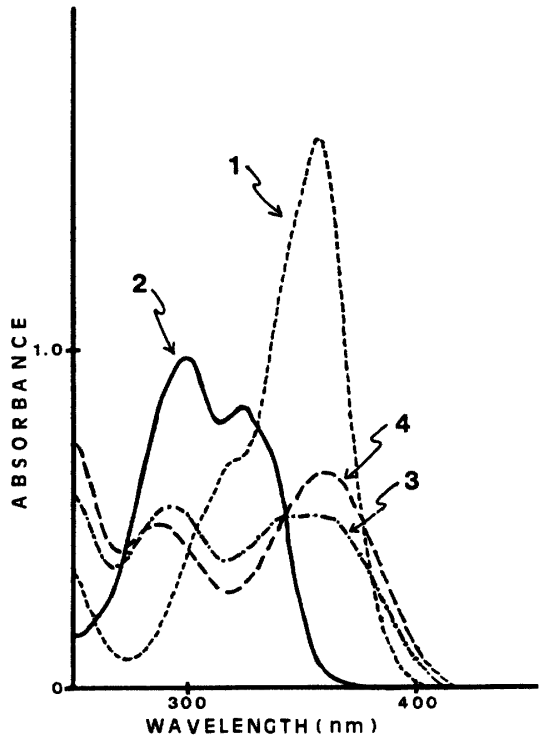


Fig. 3 Absorption spectra of **2** in methanol.
1; 6.2×10^{-3} M $HClO_4$: 2; neutral: 3; 5.0×10^{-3} M KOH: 4; 3.5×10^{-2} M KOH.

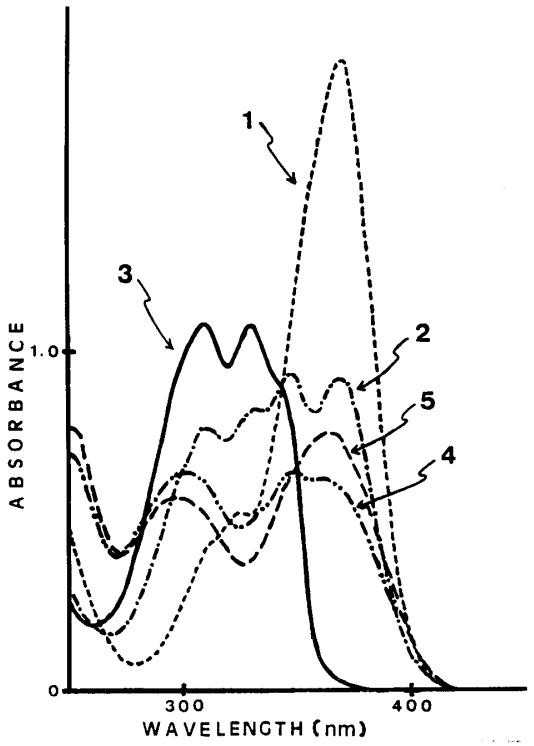


Fig. 4 Absorption spectra of 3 in methanol. 1; 6.2×10^{-3} M $HClO_4$: 2; 3.5×10^{-5} M $HClO_4$: 3; neutral: 4; 5.0×10^{-3} M KOH 5; 3.5×10^{-2} M KOH.

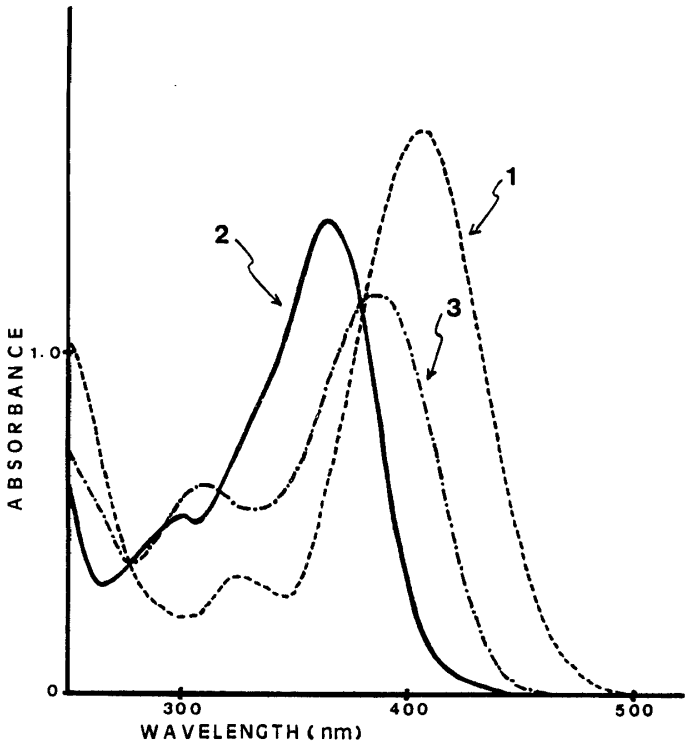
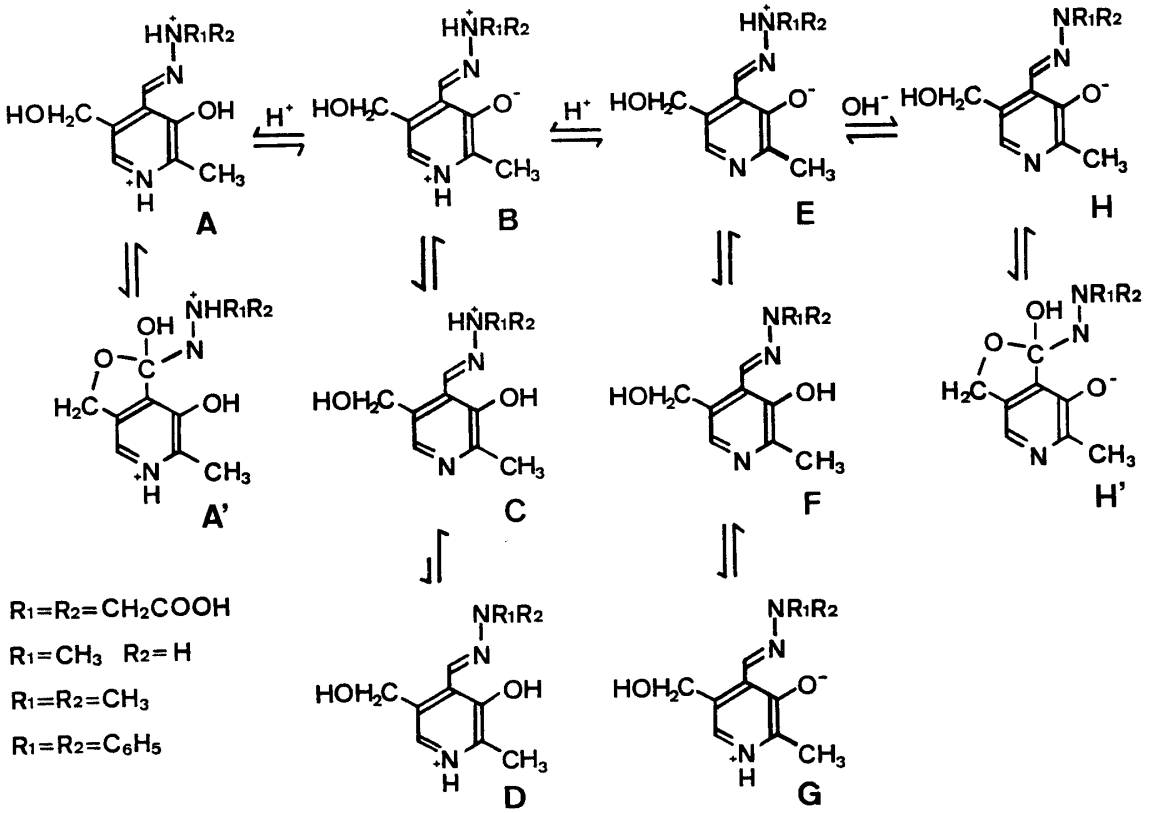


Fig. 5 Absorption spectra of 4 in methanol. 1; 6.2×10^{-3} M $HClO_4$: 2; neutral: 3; 5.0×10^{-3} M KOH.



1 $R_1=R_2=CH_2COOH$

2 $R_1=CH_3$ $R_2=H$

3 $R_1=R_2=CH_3$

4 $R_1=R_2=C_6H_5$

Chart 1

The band is ascribed to that due to the π—π_1* transition,[5] which is found at 256 nm in pyridine[6] and is shifted to 398 nm in pyridoxal.[5a] The band has been designated as the π_1-band.[5a,b]

Possible species present in solution of the pyridoxal hydrazones are shown in Chart 1. No significant effect is expected on the spectra by the dissociation of the carboxylic acid groups of compound **1**. On the other hand, protonation of the pyridine nitrogen, hydrazone nitrogen, and phenolate oxygen showed profound effects on the spectra of the hydrazones.

The absorption peak at 360 nm in the spectrum of **1** in strongly acidic methanol (Fig. 2) is assigned to the π_1-band of the fully protonated species, A. The shoulder at around 325 nm can be ascribed to the intramolecular hemiacetal species, A′. The magnitude of the blue shift is consistent with the reported data.[5] The spectrum in weakly acidic methanol had peaks at 311, 331, 345, and 366 nm. The 366-nm and 314-nm peaks are assigned to the π_1-band of species B and C, respectively. It has been well established[5,7] that the dissociation of the phenol group produces a red shift, whereas that of the pyridinium proton a blue shift for the π_1-band of the 3-hydroxypyridine derivatives. The spectrum in neutral methanol had two peaks at 314 and 336 nm and a shoulder at 347 nm. They correspond to the three shorter wavelength peaks of the weakly acidic spectrum. The 336-nm peak is assigned to the π_1-band of species F, which is red shifted by the deprotonation of the hydrazine nitrogen of species C. The 347-nm absorption may arise from species E, since the deprotonation of the pyridinium nitrogen in the 366-nm species should give the absorption in this region. The spectrum in alkaline methanol showed the absorption maxima at 305 and 365 nm. The 365-nm peak is assgined to the π_1-band of the alkaline species, H, and the 305-nm band to the intramolecular hemiacetal species, H′.

By the analogous consideration described above, the absorption spectra of **1** in aqueous solution and those of **2**, **3**, and **4** in methanol solutions were analysed. The results are summarized in Table I. The band of the dipolar neutral species B was intense in the spectra of aqueous solution of **1**, while it was a weak band in those of methanol solution. These indicated that the equilibrium between species B and C of **1** in weakly acidic

TABLE. I Wavelengths of the π_1-Band of the Molecular Species of the Hydrazones

hydrazone	solvent	species[a]									
		A	A′	B	C	D	E	F	G	H	H′
1	Water	362	325	371	317	—	342	338	383	354	310
1	MeOH	360	325	366	314	—	347	336	—	365	305
2	MeOH	357	319	—	229	—	342	323	—	360	289
3	MeOH	369	325	—	310	—	343	331	—	363	297
4	MeOH	405	324	—	300	—	—	364	—	386	308

a) Values are the wavelengths of the band in nm.

media is shifted toward the formation of C in methanol. The spectra of **2**, **3**, and **4** in methanol lacked the band of species B. The fact may reflect the polar nature of **1**. The absorption assignable to the dipolar species G was observed in water but was invisible in methanol solutions.

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