Solvolysis of Pyridoxal Hydrochloride in Alcohols

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HPLC, ^{1}H-NMR, MS and product analysis showed that pyridoxal hydrochloride was solvolyzed in methanol to form pyridoxal monomethylacetal. The reaction followed first order kinetics with the rate constant of 1.45×10^{-4} s^{-1} at 40 °C. The rate was not appreciably enhanced in the presence of an excess amount of HCl. The reaction was greatly retarded by addition of an equimolar amount of KOH. The results showed that the pyridinium-phenol species of pyridoxal hemiacetal is reactive. The reaction is responsible for the "aging" of alcoholic solutions of pyridoxal, which has caused poor reproducibility of the kinetic data for the formation of Schiff bases with amino acids.

Keywords pyridoxal; solvolysis; methanol; acetal; aldimine

Pyridoxal phosphate, a biologically active form of vitamin B_6, is an essential cofactor to many enzymes which catalyze amino acid reactions. The catalytic activities have been shown to be duplicated by pyridoxal in the absence of specific apoprotein. Studies on these non-enzymatic model reactions have greatly improved our understanding of its catalytic role.[1)]

According to the established formulation of a general mechanism that explains the role of pyridoxal in the catalysis of both model and enzymatic reactions, the initial step of the reactions is formation of the Schiff base (aldimine) between pyridoxal and an amino acid. In aqueous solution, the formation of the aldimine of pyridoxal is incomplete even in the presence of one hundred fold excess of an amino acid. On the other hand, in alcoholic media the formation is almost complete at equimolar concentration. Many model reactions were carried out in alcoholic media, since these systems were considered to mimic the enzymes better.[1,2)]

A kinetic study on the formation of the aldimine in methanol was reported in 1968 by one of the present authors.[3)] Freshly prepared methanol solution of pyridoxal was used in the study. On standing of the solution, however, the reproducibility of the kinetic data invariably became poor without any appreciable change in the absorption spectrum of the solution. The formation of pyridoxal acetal with the solvent was suspected.

Recent development of HPLC offered a facile method to solve the problem. The present paper is concerned with the "aging" of alcoholic solutions of pyridoxal.

Results and Discussion

The UV absorption spectrum of a methanol solution of pyridoxal hydrochloride has peaks at 290 and 230 nm, which have been assigned to the π–π^* transition bands of internal hemiacetal species of pyridoxal (I_A).[4)] No appreciable change of the spectrum was observed after standing of the solution for several hours at 40 °C. When a solution of ethyl alaninate was added to a freshly prepared pyridoxal solution, the spectra of the mixture showed gradual appearance of the bands at 335 and 250 nm, which are assignable to the enol-imine species of the aldimine.[3,4)] The spectral changes were slower and the spectra became obscured, when ethyl alaninate was added after standing of the pyridoxal solution for several hours.

The methanol solution of pyridoxal was analyzed by means of HPLC. Two peaks were separated and their areas varied with the standing time. One of the peaks was identified as pyridoxal from the chromatographic behavior. Since the formation of an acetal in the solution was suspected, pyridoxal monomethylacetal (II) was prepared. The retention times of the other peak were the same as those of II under various chromatographic conditions.

The product of the solvation was isolated from the solution and it was identified as pyridoxal monomethylacetal from the mass spectra shown in Fig. 1. The product contained a small amount of pyridoxal, as indicated by the fragment at m/z 167 (M^+).

^{1}H-NMR spectra of pyridoxal hydrochloride and pyridoxal monomethylacetal in tetradeuteromethanol (CD_3OD) are shown in Fig. 2. The spectral changes with time of pyridoxal hydrochloride can be explained in terms of the conversion of pyridoxal into pyridoxal trideutero (d_3)-monomethylacetal. The spectrum of pyridoxal monomethylacetal was not changed on standing of the solution at room temperature, but was converted to that of pyridoxal d_3-monomethylacetal by addition of HCl.

A methanol solution of pyridoxal hydrochloride was allowed to stand at 40 °C. Pyridoxal and pyridoxal monomethylacetal in the solution were determined by HPLC at intervals. The results (Fig. 3) indicated that the reaction shown in Chart 1 proceeded smoothly without any detectable by-product until almost complete conversion of pyridoxal to the product at 7 h. The reaction followed first-order kinetics and the pseudo-first-order rate constant was calculated to be 1.45×10^{-4} s^{-1}.

Methanol solutions of pyridoxal monomethylacetal and ethyl alaninate were mixed. No significant spectral change was observed. The facts clearly show that the monomethylacetal formation is responsible for the "aging" phenomenon of methanol solutions of pyridoxal.

Kinetic studies on the reaction were carried out in the presence of varying amounts of HCl and KOH and at 30 °C, 40 °C and 50 °C. The results are summarized in Table I. Shielding of the reaction solution from light and conducting the reaction under an N_2-atmosphere did not change the results. The reaction was greatly retarded by addition of an equimolar amount of KOH but the rate was not appreciably enhanced in the presence of an excess amount of HCl. The results show that the pyridinium species of pyridoxal hemiacetal (I_A) is reactive and an

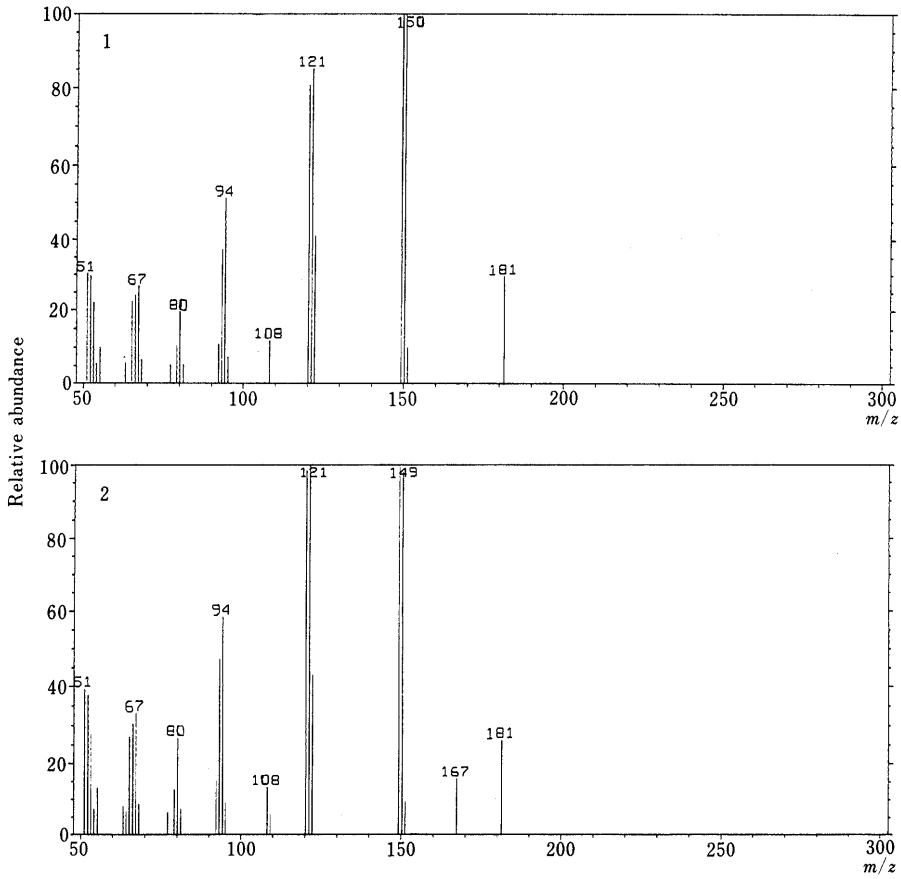

Fig. 1. Mass Spectra of (1) Pyridoxal Monomethylacetal and (2) the Solvation Product of Pyridoxal in Methanol

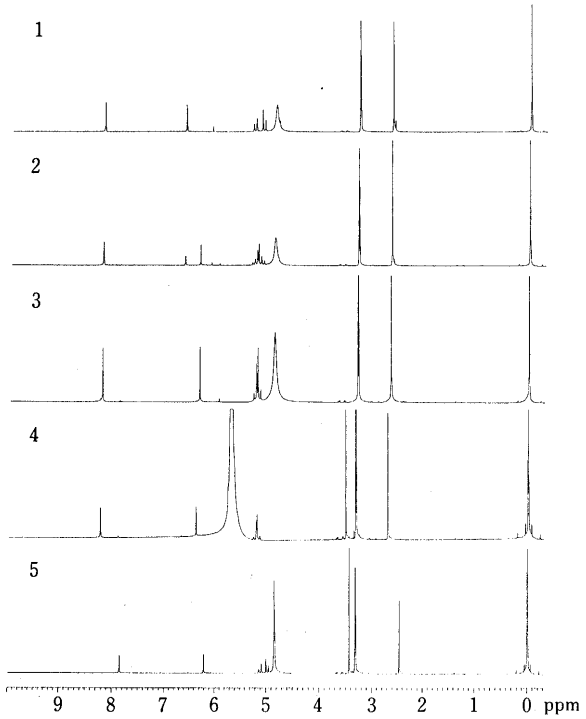

Fig. 2. ^{1}H-NMR Spectra of Pyridoxal Hydrochloride and Pyridoxal Monomethylacetal

1—3, Pyridoxal hydrochloride in CD_3OD (standing times after the preparation of the solution were 0, 20 h and 6 d for 1, 2 and 3, respectively). 4, Pyridoxal monomethylacetal in CD_3OD–HCl. 5, Pyridoxal monomethylacetal in CD_3OD.

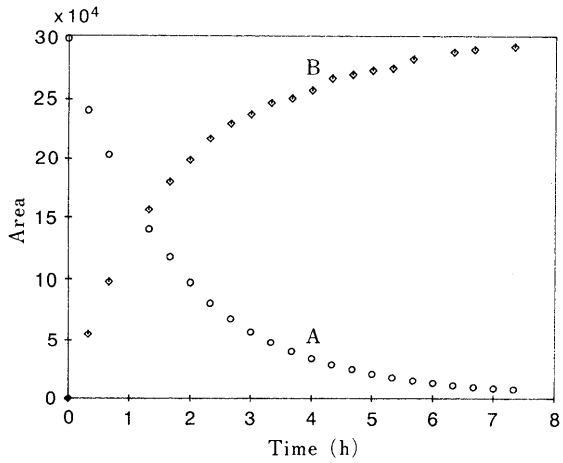

Fig. 3. Concentration Changes of Pyridoxal and Pyridoxal Monomethylacetal in a Methanol Solution of Pyridoxal Maintained at 40 °C

Peak areas of pyridoxal (A) and pyridoxal monomethylacetal (B) on the chromatograms were plotted against time.

excess amount of acid does not have a large catalytic effect.

N-Methylpyridoxal chloride (III) was prepared. In methanol, III was solvolyzed to form its monomethylacetal (IV) and the reaction also followed first-order kinetics. The rate constants measured are listed in Table I. The reaction was retarded by KOH but not accelerated in the presence of excess acid. *N*-Methylpyridoxal chloride should be present as the pyridinium-phenol species (III_A) in metha-

TABLE I. The First-Order Rate Constant ($\times 10^{-4}\,s^{-1}$)[a]

Solvent	Temp (°C)	Pal·HCl +4HCl	+HCl		+KOH	+2KOH	+3KOH	+6KOH	Pal-NCH_3·Cl +5HCl	+2HCl	+HCl		+KOH	+2KOH	+5KOH
MeOH	50	6.21	5.87	4.95	0.48	0.26	0.18	0.011							
	40	1.78	1.72	1.45	0.20	0.11	0.086	0.011	1.41	1.59	1.53	1.41	0.014	0.006	<0.003
	30	0.51[b]		0.47	0.067			<0.003[c]							
EtOH	40	1.18	1.23	1.03	0.14	0.014	<0.003	<0.003							

a) Values are the first-order rate constants in ($\times 10^{-4}\,s^{-1}$) unit for the conversion of pyridoxal hydrochloride (Pal·HCl) and *N*-methylpyridoxal chloride (Pal-NCH_3·Cl) to their monomethyl- or monoethyl-acetal in MeOH or EtOH in the presence of equimolar HCl or KOH as indicated. b) In the presence of 10-fold molar excess of HCl. c) In the presence of 10-fold molar excess of KOH.

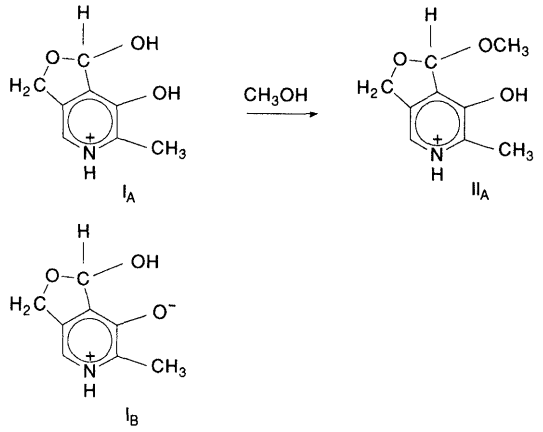

Chart 1

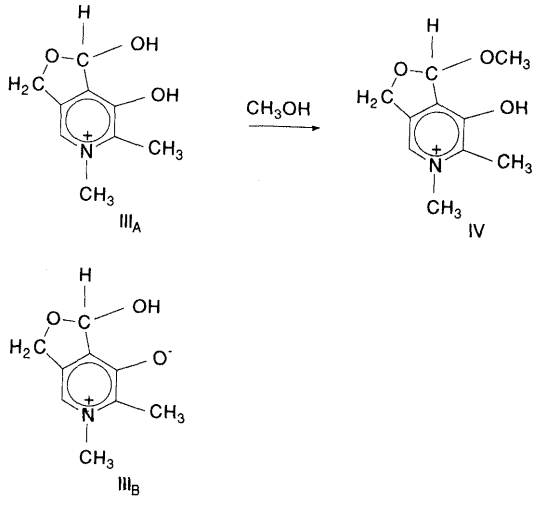

Chart 2

nol, and the addition of equimolar KOH should cause the dipolar pyridinium-phenolate species (III_B) to become predominant. The results indicate that the pyridinium-phenol species (III_A) is reactive while the pyridinium-phenolate species (III_B) is not. Similarly, the dipolar species of pyridoxal hemiacetal (I_B) must be inert.

Pyridoxal hydrochloride underwent similar solvolysis in other alcoholic solvents. The pseudo-first-order rate constants were 1.03, 1.01, 1.06 and $0.86\times10^{-4}\,s^{-1}$ at 40 °C in ethanol, 1-propanol, 2-propanol and 1-butanol, respectively. They were comparable to each other but slower than that in methanol ($1.45\times10^{-4}\,s^{-1}$).

Experimental

General Procedures UV spectra were recorded on a Shimadzu UV-240 UV-visible recording spectrophotometer and mass spectra (MS) were measured with a JEOL JMS-DX303. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM-GX270 spectrometer. Chemical shifts are expressed in ppm on the δ scale from tetramethylsilane as an internal standard. The following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet. Melting points were determined with a Yanaco micro melting point apparatus. In the kinetic study, the reaction mixtures, placed in glass stoppered tubes, were shaken in a thermostated bath. A portion of the mixtures was withdrawn at intervals and analyzed by HPLC.

HPLC The HPLC system consisted of a Shimadzu LC-6A pump, a Rheodyne 7125 injection valve, a Hitachi L-4000 UV detector, a Hitachi D-2500 Chromato-Integrator and a stainless steel column (25 cm × 4 mm i.d.) packed with Hitachi gel #3056 (silica-ODS). The system was operated at room temperature. The samples were eluted with a mobile phase of methanol containing 0.5 mM perchloric acid at a flow-rate of 1 ml/min, and monitored at 280 nm.

Materials Pyridoxal hydrochloride was obtained from Sigma and Wako. Other chemicals were of reagent grade. Pyridoxal monomethylacetal (II), *N*-methylpyridoxal chloride (III) and *N*-methylpyridoxal monomethylacetal iodide (IV) were prepared according to the methods described by Heyl *et al.*[5] with some modifications. The compounds were purified by chromatography on silica gel columns and recrystallization, and characterized by means of elemental analyses and absorption, MS, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The NMR spectra were measured in hexadeuterodimethylsulfoxide (DMSO-d_6) or a mixture of DMSO-d_6 and deuterium oxide (D_2O). The spectral data corresponded to those in CD_3OD shown in Fig. 2. Spectral data which have not been reported so far are described below.

Pyridoxal Monomethylacetal (II) ^{1}H-NMR (DMSO-d_6) δ: 2.37 (3H, s, CH_3), 3.32 (3H, s, $-OCH_3$), 4.94 (1H, d, J=13 Hz, $-CH_2-$), 5.05 (1H, d, J=13 Hz, $-CH_2-$), 6.19 (1H, d, J=2 Hz, CH), 7.93 (1H, s, aromatic-H), 9.71 (1H, s, OH). ^{13}C-NMR (DMSO-d_6) δ: 18.75 (q, CH_3), 54.02 (q, $-OCH_3$), 69.69 (t, $-CH_2-$), 104.6 (d, CH), 130.56 (s, pyridine-2C), 132.18 (d, pyridine-6C), 135.04, 145.45, 145.99 (s, pyridine-3,4,5C). MS m/z 181 (M^+).

***N*-Methylpyridoxal Chloride (III)** ^{1}H-NMR (DMSO-d_6, D_2O) δ: 2.63 (3H, s, CH_3), 4.23 (3H, s, NCH_3), 5.08 (1H, d, J=14 Hz, $-CH_2-$), 5.21 (1H, d, J=14 Hz, $-CH_2-$), 6.63 (1H, s, CH), 8.46 (1H, s, aromatic-H). ^{13}C-NMR (DMSO-d_6, D_2O) δ: 13.57 (q, CH_3), 47.22 (q, N–CH_3), 69.66 (t, $-CH_2-$), 98.75 (d, CH), 131.32 (d, pyridine-6C), 138.36, 140.00, 146.17, 149.31 (s, pyridine-2,3,4,5C). MS m/z 182 (M^+-Cl).

***N*-Methylpyridoxal Monomethylacetal Iodide (IV)** ^{1}H-NMR (DMSO-d_6) δ: 2.39 (3H, s, CH_3), 3.28 (3H, s, $-OCH_3$), 4.82 (3H, s, N–CH_3), 4.85 (1H, d, J=13 Hz, $-CH_2-$), 4.99 (1H, d, J=13 Hz, $-CH_2-$), 5.99 (1H, s, CH), 7.34 (1H, s, aromatic-H). ^{13}C-NMR (DMSO-d_6, D_2O) δ: 12.06 (q, CH_3), 45.60 (q, N–CH_3), 53.75 (q, $-OCH_3$), 69.88 (t, $-CH_2-$), 106.22 (d, CH), 114.24 (d, pyridine-6C), 132.58, 136.77, 146.75, 162.07 (s, pyridine-2,3,4,5C). MS m/z 196 (M^+-I).

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