Spectral Properties and Desmotropy of the Schiff Base of Diethylaminomalonate and Pyridoxal Hydrochloride

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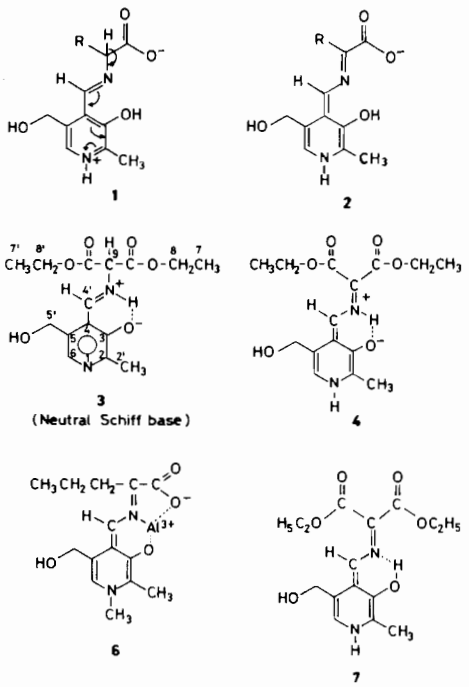
Abstract

Evidence is presented indicating that in aqueous solution, the product formed between diethyl aminomalonate and pyridoxal (vitamin B_6) is the Schiff base, and not the 1,4-dihydropyridine tautomer which exists in the solid state. The structure of the Schiff base is established unequivocally by its ^1H and ^{13}C NMR spectra. Reflectance spectroscopy shows that the solid dihydropyridine tautomer absorbs at 560 nm.

Introduction

Although the study of the interaction of pyridoxal with amino acids in non-enzymatic systems has been helpful in understanding the role of this coenzyme in enzymatic reactions, and the reactions in both enzymatic and model systems have generally been considered to parallel each other, a notable difference in the nature of the intermediates formed has been pointed out. Several B_6 enzymes have been found to form substrate complexes which absorb near 495 nm [1–4]. On the other hand the highest absorption in the visible region of the spectrum has been reported at 430 nm [5] for Schiff bases such as **1** formed in aqueous solution between pyridoxal and amino acids in nonenzymatic systems.

The evidence available from enzymatic studies indicates that the enzyme-substrate complexes which absorb near 495 nm may be considered due to a compound in which the α-carbon of the amino acid-coenzyme Schiff base has lost a proton [6]. Commonly written electronic shifts are indicated in **1**. The type of structure indicated by **2** was postulated by Metzler *et al.* [7] to be the intermediate moiety in nonenzymatic transamination. Structure **2** (a



'dihydropyridine' belonging to a class of compounds called pyridine methenes [8]) has been assigned on the basis of its ultraviolet–visible absorption spectrum, with bands between 450 and 500 nm in *N*-methanochloride Schiff bases [9] of *N*-methylpyridoxal, or in the Al(III) chelates of pyridoxal Schiff bases [10]. Up to the present time, its existence in solution has not been demonstrated by NMR measurements.

From a reaction mixture of diethylaminomalonate and pyridoxal Abbott and Bobrick [11] isolated a bright red compound, which was assigned a dihydropyridine structure on the basis of (1) an absorption maximum at 465 nm which was taken to indicate a longer π electron system than is present in normal Schiff bases, and (2) the absence of the α-H of the diethylaminomalonate in the ^1H NMR spectra when the red compound was dissolved in 5% DCl–D_2O.

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The purpose of this paper is to describe a study of the spectral properties of this red compound, further supporting its formulation as a dihydropyridine, and also to supply information which demonstrates that in solution the compound exists in the Schiff base form **3** instead of the dihydropyridine, **4**.

Experimental

Materials

Pyridoxal hydrochloride and diethylaminomalonate were purchased from Sigma Chemical Co. D_2O, NaOD, DCl and DMSO-D_6 were supplied by Aldrich Chemical Co. Other reagents were also of the highest purity obtainable.

Compound 4 was prepared by the method reported by Abbott and Bobrick [11]. The uncorrected melting point, which has not been reported previously, was found to be 205–207 °C.

NMR Spectra

Nuclear magnetic resonance (NMR) spectra were obtained with a Varian XL-200. For ^1H NMR, 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt was employed as the internal reference. In this paper the terms p[H] and p[D] are defined as the negative logarithms of the hydrogen ion and deuterium ion concentrations.

Spectral Measurements

Infrared spectra were recorded with a Sargent-Welch 3-2000 spectrophotometer, in KBr pellet media. UV–Vis spectra were measured with a Cary Model 14 Spectrophotometer. Reflectance spectra were measured with the Cary Reflectance Attachment for the Model 14 and the absorption of the red solid was found to be located at 560 nm.

Results

The bright red compound, **4**, that separated out of the reaction mixture of pyridoxal hydrochloride and diethylaminomalonate at pH = 4.00 is too insoluble in D_2O for NMR measurements, however, when shaken with DMSO-D_6 it rapidly dissolved to give a yellow solution. Thus the half life of conversion of **4**–**3** must be on the order of one second or less.

Table I illustrates the signals observed for the ^1H NMR spectra when the compound is dissolved in DMSO-D_6, and when D_2O is added to the DMSO solution, respectively. After the addition of D_2O to the DMSO solution, only the signal due to the α-H of the diethylaminomalonate disappeared, as the result of deuterium exchange. The typical signals of the aldimine Schiff base have the same chemical shifts as those reported in previous work [12]. When **4** was treated with 5% DCl–D_2O it was rapidly dissolved with loss of color to give a yellow solution. The resulting spectrum showed the signals of the starting reagents, indicating rapid hydrolysis of **4** through **3′** to aldehyde and ester components (Table I).

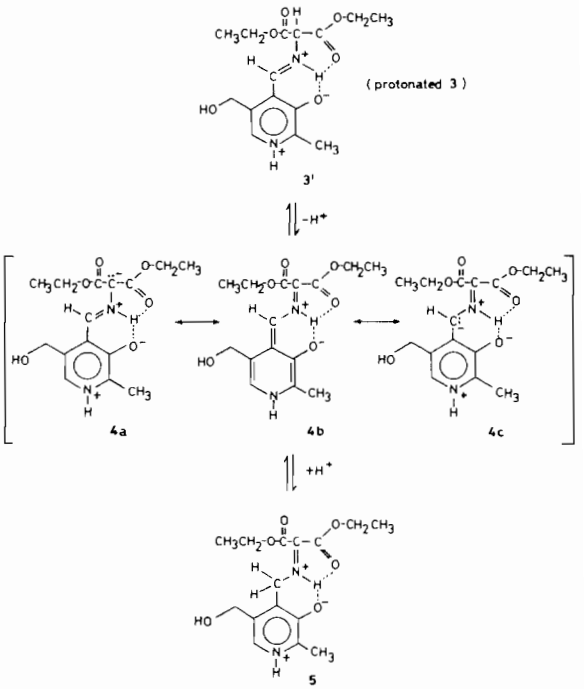

TABLE I. 200 MHz ^1H NMR Spectra of Aldimine **3** under Various Conditions

Assignment	**3** in DMSO-D_6		**3** in DMSO-D_6 + D_2O		**3** in 5% DCl/D_2O	
	δ(ppm)	Multiplicity	δ(ppm)	Multiplicity	δ(ppm)	Multiplicity
H-2′	2.38	(3H)s	2.38	(3H)s	2.69	(3H)s
H-4′	9.06	(1H)s	9.00	(1H)s	6.80	(1H)d
5′	4.55	(2H)d	4.55	(1H)d	5.35	(2H)q
H-6	7.97	(1H)s	7.97	(1H)s	8.23	(1H)s
H-7	1.16	(6H)m	1.16	(6H)m	1.65	(6H)t
H-8	4.25	(4H)m	4.25	(4H)m	4.40	(4H)q
H-9	5.37	(1H)s	–	–	–	–

Measurement of the ^{13}C NMR spectrum of **4** showed the typical signals of aldimine at 169.2 ppm in agreement with previous results [13] (Table II), and the signal of the α carbon of the diethylaminomalonate in the Schiff base at 72.4 ppm. The bound proton resonance test showed that the C-9 signals correspond to a methine group. Thus **4** is converted to Schiff base **3** in DMSO solution.

When compound **4** was dissolved in methanol (10^{-4} M) a yellow solution was formed. The same result was observed when the compound was dissolved in neutral aqueous medium. In both solvents three absorption bands were observed with maxima at 460, 350, and 260 nm. The absorption maximum at 460 nm in the visible region corresponds to the normal absorption range of aldimine Schiff bases and is responsible for their typical yellow color. The reflectance spectrum of **4** showed absorption maxima at 350 and 560 nm, the latter corresponding to the intense red color of the compound.

The infrared spectrum (Table III) of **4** was obtained in KBr pellets and is consistent with the band positions expected for the dihydropyridine structure **4**.

TABLE II. 200 MHz ^{13}C NMR Spectra of Aldimine **3** in DMSO-D_6

Assignment	(ppm)	Carbon type
C-2	148.8	quaternary
C-2′	18.8	primary methyl
C-3	153.6	quaternary
C-4	119.6	quaternary
C-4′	169.2	methine
C-5	133.8	quaternary
C-5′	62.2	methylene
C-6	138.4	methine
C-7	13.8, 14.8	methyl
C-8	58.4, 58.8	methylene
C-9	72.4	methine

Discussion

The so-called key step in pyridoxal catalysis involving diethylaminomalonate is the prototropic shift resulting in the interconversion of **3′** and **5**. The original mechanism proposed by Metzler and Snell [7] included a dihydropyridine-like structure, **4**, as the key intermediate in all pyridoxal-catalyzed reactions. In the course of our on-going investigation of pyridoxal phosphate-catalyzed elimination, it was of interest to carry out NMR studies of intermediates such as **4**. Of particular interest was the isolation of a 1,4-dihydropyridine formed from diethylaminomalonate and pyridoxal [10]. Both ^{1}H and ^{13}C NMR spectra (Tables I and II) indicate that it is the Schiff base which is formed in solution from diethylaminomalonate and pyridoxal and that it has an aldimine structure, **3**, instead of a dihydropyridine, 4. The 460 nm signal band in the visible region is in agreement with the yellow range of the spectrum where aldimine Schiff bases absorb. Compound **4** should absorb above 500 nm, as has been observed for the quinonoid intermediate **6** between 2-aminobutanoic acid and *N*-methanopyridoxalchloride [13], which absorbs at 514 nm. When 2-aminobutanoic acid is replaced by 2-aminobutenoic acid the absorption shifts to 550 nm, in accordance with its more extended conjugation [14].

Although the dihydropyridine type structure **4b** is indicated as a form of **4a** and **4c**, this concept is subject to some question (because of the tetrahedral nature of the heterocyclic nitrogen in **4b**) depending on its degree of resonance contribution to the overall structure of the intermediate, as has recently been pointed out [14]. The valence bond structure of **4b**, taken literally, indicates that the heterocylic nitrogen is tetrahedral, while in **4a** and **4c** it is trigonal. The only alternative to the conclusion that **4b** is a separate species, which is not in resonance with **4a** and **4c**, is that the hydrogen bound to the hetero-

TABLE III. Principal Infrared Bands in **4**

Frequency (cm^{-1})	Intensity	Assignment
3420	medium, broad	O–H stretch (hydrogen bonded, phenolic and primary alcohol)
3250	weak, broad	N–H stretch
2980	weak, broad	probably CH_3, CH_2 modes
1690	strong	C=O stretch (conjugated ester)
1640, 1620	weak, broad	C=N stretch aldimine
1510, 1495, 1480	medium, broad	N–H bend (azomethine)
1390	medium	O–H bend (phenolic)
1330, 1255	medium	C–O stretch (phenolic)
1160	strong	C–N stretch (conjugated heterocycle)
1090	strong	C–O stretch (alcohol or ester)
1030	weak	C–O stretch primary alcohol
980, 860, 770	medium	C–C, C–N stretch, C–H rocking

cyclic nitrogen is slightly displaced from the plane of the pyridine ring, and that **4b** is a minor resonance contributor to the overall structure represented by these three formulas.

Information on the relative electron interactions of protons and metal ions may be found in the electronic spectral shifts observed for multidentate ligands containing nitrogen and oxygen donors. Thus the frequencies of the absorption maxima of chelating agents containing phenolic, conjugated carbonyl, *o*-hydroxyazo, or similar groups are much lower for the anion than for the corresponding structures in which a proton is attached to one of the oxygen or nitrogen donor groups. When the proton is replaced by a metal ion, the frequencies of the absorption maxima vary between the extremes of the protonated and dissociated ligands in such a manner that the frequencies increase with the stabilities of the metal chelates. Thus far, no case where the frequency of the metal chelate absorption maxima is higher than that of the corresponding proton complex has been observed. This trend in behavior, previously pointed out by Martell [15], is what one might expect from first principles since the binding of an electron pair (of the donor) through the 1s orbital of hydrogen would be expected to be much stronger than the binding of the electron pair through hybrid 4p-4s-3d orbitals of the first row transition metals, or through the appropriate orbitals of other metal ions.

Most of the IR bands of the solid indicated in Table III are characteristic of what would be expected for an aldimine such as **3**, or a dihydropyridine structure **4**. Although both structures have many functional groups in common, the IR spectra are of some assistance in distinguishing between **3** and **4**. The most important structural feature of compound **4** which distinguishes it from ordinary Schiff bases is the presence of the extended conjugation which is expected to affect the chemical shift of the most unambiguously assignable infrared band: the ester carbonyl frequency. The IR spectrum in KBr shows a very strong peak at 1690 cm^{-1} which is about 50 cm^{-1} lower than a normal saturated ester carbonyl. Structure **4** as drawn shows an imminium hydrogen bonded to a phenolate. This configuration is supported by the presence of a very weak broad band at 2350 cm^{-1} [16]. Broad bands at 3420 and 2980 cm^{-1} indicate hydrogen bonded OH and N–H stretching frequencies. On top of the latter are small spikes involving aliphatic CH and =C–H stretching. Because of the fact that this molecule possesses very many functional groups, the finger print region does not provide unambiguous information on the dihydrogen structure. However, there is a medium peak at 1620 cm^{-1}, with at least one similar neighbor at 1640 cm^{-1} which is in the region of N=C stretch as well as of C=C skeletal stretch modes. The several peaks at 1510, 1495 and 1480 cm^{-1} could be due to the azomethine N–H bond and various aromatic modes. The peaks at 1390, 1330, 1255 cm^{-1} probably belong to C–O phenolic and alcoholic, or ester vibrations, although the peaks at 1160, 1090, 1030 cm^{-1} are more likely to involve the C–N ring, C–O ester and C–O alcohol stretching vibrations respectively. There are a few other peaks at 980, 860 and 770 cm^{-1} which are consistent with the presence of C–N, C–C stretch and C–H rocking vibrations.

The reflectance spectrum has an absorption at 560 nm, which is in the energy range expected for the extended conjugated system such as that indicated by formula **4**. In fact it is difficult to see how anything but a quinonoid-type structure of this kind can account for such a long wave length absorption. Since the red compound does not exist in aqueous or DMSO solutions, it seems that some property of the solid state must stabilize the product formed by tautomerization of **3**. The required conditions could be provided, for example, by close proximity in the solid state of the α C–H of one molecule and the polar heterocyclic nitrogen of another, thus facilitating the proton transfer needed to form **4**. The observed color does not require that the proton transfer be complete, since an appreciable concentration of the conjugated quinonoid structure is all that would be necessary to account for the observations. Formation of **3** from **4** when the latter is dissolved would readily occur by rapid transfer of a proton from atom 1 to atom 9 (see **3** for numbering).

It appears that the zwitterionic structure (*i.e.*, the ketoenamine structure) of **3** requires the stabilizing effect of a polar solvent. In the absence of strong solvation, it is known that such species exists in the tautomeric phenolic form. It is also considered likely that the red dihydropyridine-like compound **4** exists in the tautomeric form, indicated by **7**, in solvents of low polarity.

It should be pointed out, in conclusion, that the isolation and identification of the two desmotropes, dihydropyridine **4** and its tautomer, does *not prove* that pyridoxal transamination reactions proceed through **2**. Mechanisms not involving **2** can be written for all reactions of PAL-catalyzed transformations. Such proof (*i.e.*, reactions proceeding through formation of **2**) has not yet been demonstrated.

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