[Chem. Pharm. Bull. 25(10)2568—2575(1977)]

UDC 547.576.02.04 : 542.953.4

Molecular Species of Schiff Bases derived from *o*-Hydroxyaromatic Aldehydes. I. Spectral Assignments

Yoshiharu Karube and Yoshikazu Matsushima

Faculty of Pharmaceutical Sciences, Kyushu University 62[1)]

(Received February 8, 1977)

Schiff bases of 3-hydroxy-4-formylpyridine (**1**), pyridoxal (PL), pyridoxal-5′-phosphate (PLP), and related aldehydes with methyl valinate, *n*-butylamine, and various amino acids and their derivatives were prepared and their absorption spectra in various solvents and in acidic, neutral and alkaline methanol were measured. From the spectral analyses, the longest π bands of the eight possible molecular species of the Schiff base of **1** involved in acid-base equilibrium were determined as follows; anion, 368 nm; enolimine, 325 nm; ketoenamine, 420 nm; species with a phenolate and a pyridinium groups, 385 nm; species with a protonated azomethine and a phenol group, 365 nm; ketoenamine with a pyridinium group, 425 nm; enolimine with a pyridinium group, 330 nm; fully protonated species, 365 nm. Equilibria between equally protonated species were dependent on media and on the amine part. The wavelengths of the π bands were affected by media and slightly by the amine part. Corresponding species of Schiff bases of PL and PLP have the π band at the longer wavelength side of less than 10 nm. Infrared and proton magnetic resonance data of the Schiff bases are presented.

Keywords——Schiff base; UV absorption spectra; band assignment; 3-hydroxy-4-formylpyridine; pyridoxal; pyridoxal phosphate; enolimine-ketoenamine tautomerism; infrared spectra of imines

The chromophore of vitamin B_6 is sensitively dependent upon structural factors and functions as a built-in reporter group at the active sites of pyridoxal-5′-phosphate(PLP)-dependent enzymes. The correct interpretation of the spectrum of the coenzyme may assist the understanding of the structures of the active site. In PLP enzymes so far studied, the carbonyl group of the coenzyme is combined with the ε-amino group of lysine to form a Schiff base.[2)]

A number of PLP enzymes have an absorption band at 410—430 nm. In addition to the band, most PLP enzymes have a peak at 325—340 nm. Some enzymes display an absorption at 360—364 nm at high or at all pH values.[2)] These absorption bands can be related to specific species in acid-base equilibria of the Schiff base of PLP. The 410—430 nm bands are assigned to the Schiff base with a protonated azomethine nitrogen and a phenolate group, ketoenamine species, the 325—340 nm bands to that with a phenol group and without a proton on the azomethine nitrogen, enolimine species, and the 360—364 nm bands to that with phenolate group and unprotonated azomethine, anionic species.

The basis of this currently accepted assignment was provided by Heinert and Martell,[3)] who studied the spectra of the Schiff bases of 3-hydroxy-4-formylpyridine(**1**) and related aromatic aldehydes. The Schiff base of **1** constitutes the essential part of the chromophore of PLP Schiff base, as no profound effect on the spectra can be expected by the substituent groups at 2 and 5 position of PLP and by the amine part, if it is not conjugated with the azo-

1) Location: *Maidashi, Fukuoka, 812, Japan.*

2) E.E. Snell, P.M. Fasella, A.E. Braunstein, and A. Rossi-Fanelli (ed.), "Chemical and Biological Aspects of Pyridoxal Catalysis," Macmillan, New York, N.Y., 1963; E.E. Snell, A.E. Braunstein, E.S. Severin, and Yu. M. Torchinsky (ed.), "Pyridoxal Catalysis: Enzymes and Model Systems," Interscience, New York, N.Y., 1968; E.E. Snell and S.J. DiMari, "The Enzymes," 3rd ed., II., ed. by P.D. Boyer, Academic Press, New York, N.Y., 1970, Chapter 7; and references therein.

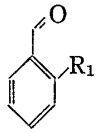
3) D. Heinert and A.E. Martell, *J. Am. Chem. Soc.*, **85**, 183, 188 (1963).

methine bond. A number of arguments for this conclusion have been presented from the spectral studies on solution equilibria of Schiff bases of pyridoxal(PL) and related compounds and on PLP enzymes and from other physicochemical studies.[2,4]

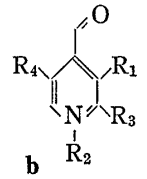
However, the state of protonation on the pyridine nitrogen in solution equilibria of the Schiff bases is ambiguous and the effect of azomethine protonation on the spectra seems unclear. Hence, we reinvestigated the solution spectra of the Schiff bases of **1** and related compounds including PL and PLP. Herein are the results of the spectral analysis as well as some comments on IR and PMR data. The attempt to correlate every spectral band to solute species has led to definitions of all possible molecular species, which are in some cases independent of but always complementary to conclusions from earlier studies.[2-4]

Results and Discussion

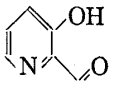
Schiff bases of the following aromatic aldehydes were prepared; 3-hydroxy-4-formylpyridine (**1**), benzaldehyde (**2**), anisaldehyde(*o*-methoxybenzaldehyde) (**3**), salicylaldehyde (*o*-hydroxybenzaldehyde) (**4**), 3-methoxy-4-formylpyridine (**5**), 1-methyl-3-hydroxy-4-formylpyridinium chloride (**6**), 1-methyl-3-methoxy-4-formylpyridinium iodide (**7**), 1-methylpyridoxal chloride (**8**), 3-hydroxy-2-formylpyridine (**9**), pyridoxal (PL), and pyridoxal-5′-phosphate (PLP). Structures of the aldehydes are shown in Chart 1.



a



b



c

	R_1	R_2	R_3	R_4
2: R_1=H				
3: R_1=OCH_3				
4: R_1=OH				
1:	OH	none	H	H
5:	OCH_3	none	H	H
6:	OH	CH_3	H	H
7:	OCH_3	CH_3	H	H
8:	OH	CH_3	CH_3	CH_2OH
PL:	OH	none	CH_3	CH_2OH
PLP:	OH	none	CH_3	$CH_2OPO_3H_2$

9

Chart 1

Schiff bases of *o*-hydroxyaldehydes were yellow with a exception of those derived from **9**, which were greenish yellow. Those derived from aldehedes without *o*-hydroxy group were colorless.

Various amines including amino acids and their derivatives were used in the preparations. The present paper is concerned principally with the spectral properties of the Schiff bases of methyl valinate (A) and *n*-butylamine (B). In the Schiff bases of these amines, the preparation and purification were easy and the solubilities were optimal for spectroscopic studies in various solvents. As shown below, the π bands of the molecular species of the Schiff bases were slightly affected by the amine part. In most cases, the π_1 bands were located at the longest wavelength in A and at the shortest wavelength in B in the corresponding molecular species.[5] For example, absorption maxima observed in neutral methanol at around 325 nm in the Schiff bases of **1** with A, methyl α-methylalaninate, methyl alaninate, potassium alani-

4) R.J. Johnson and D.E. Metzler, "Method in Enzymology," Vol. 18, Pt. A ed. by D.B. McCormick and L.D. Wright, Academic Press, New York, N.Y., 1970, pp. 433—471.

5) The molecular species of benzene and pyridine compounds of the type under investigation are characterized by two intense bands assigned to π-π^* transitions. The longer wavelength band has been designated as the π_1 band, whereas the shorter one as the π_2 band. See also ref. 7*f*, 8 and 9.

nate, and B were 325, 324, 323, 322, and 322 nm, respectively. Throughout this paper, the symbols such as **1A**, **2B** and PL-methyl tyrosinate are used to represent the Schiff bases derived from the aldehyde and amines indicated by the numbers, letters and names.

Electronic Absorption Spectra

Absorption spectra in ultraviolet and visible regions of **1A** and **1B** in various solvents are tabulated in Table I. Table II lists absorption bands of the various Schiff bases in acidic, neutral, and alkaline methanol. The Schiff bases were solvolyzed slowly in neutral and alkaline methanol. The decomposition was fast in acidic and aqueous media. In the Tables are presented the results on the initial spectra, which were taken during the solvolyses or decomposition were undetectable or were obtained by intrapolation.

Table I. Absorption Spectra of the Schiff Bases of 3-Hydroxy-4-formylpyridine with Methyl Valinate and *n*-Butylamine[a)]

Solvent	Schiff base with methyl valinate				Schiff base with *n*-butylamine				
Cyclohexane		334(0.43)	248(1.00)			330(0.43)	245(0.94)		
Dioxane		330(0.42)	251(0.88)			325(0.48)	249(0.82)		
Chloroform		332(0.42)	249(1.08)		422(0.01)	327(0.45)	246(1.00)		
Acetonitrily		327(0.41)	246(1.20)		418(0.02)	321(0.40)	242(0.96)		
Dimethylformamide		328(0.42)			420(0.02)	324(0.42)			
Acetic anhydride	420(0.03)	327(0.40)			416(0.19)	323(0.35)			
Acetic acid	420(0.06)	328(0.27)	250(0.52)		410(0.50)	329(0.24)	251(0.56)	264^{s}	272^{s}
Isopropanol		327(0.33)	247(0.82)		416(0.04)	324(0.40)	245(0.91)		
50% H_2O–methanol	406(0.07)	327(0.32)	280(0.18)	246(0.93)	410(0.38)	324(0.19)	263^{s} (0.51)		

a) Wavelengths are given in nm; numbers in parentheses give the absorbance for a 1-cm light path and 1×10^{-4}M. The superscript s indicates shoulder.

Table II. Absorption Spectra of Schiff Bases in Methanol[a)]

Aldehyde	Schiff base with methyl valinate		Schiff base with *n*-butylamine	
	Medium	Spectra	Medium	Spectra
1	Neutral	325 (0.40) 246 (1.04)	Neutral	416 (0.09) 322 (0.33) 245 (0.84)
	5×10^{-4}M KOH	368 (0.68)	2×10^{-3}M KOH	364 (0.67)
	5×10^{-4}M HCl	425 (0.06) 325 (0.32) 292 (0.27)	1×10^{-3}M HCl	418 (0.35) 327 (0.21) 262^{s} (0.47) 255 (0.53)
2	Neutral	280^{s} (0.22) 249 (1.82)	Neutral	280^{s} (0.17) 246 (1.55)
	1×10^{-3} M KOH	Same as the neutral spectrum	1×10^{-3}M KOH	Same as the neutral spectrum
	2×10^{-3}M HCl	280 (1.89)	2×10^{-3}M HCl	275 (1.58)
3	Neutral	312 (0.68) 255 (1.56)	Neutral	308 (0.64) 251 (1.50)
	1×10^{-3}M KOH	Same as the neutral spectrum	1×10^{-3} M KOH	Same as the neutral spectrum
	1×10^{-3}M HCl	358 (0.69) 283 (2.14)	2×10^{-3}M HCl	348 (0.61) 276 (1.95)
4	Neutral	321 (0.40) 258 (1.26)	Neutral	402 (0.20) 317 (0.33) 277 (0.50) 254 (1.08)
	1×10^{-1}M KOH	360 (0.65) 262 (0.93)	1×10^{-1}M KOH	354 (0.66) 258 (1.03)
	2.5×10^{-4} M HCl	420 (0.05) 317 (0.64) 258 (1.92)		
	2×10^{-3}M HCl	360 (0.51) 282 (1.98)	4×10^{-3}M HCl	352 (0.65) 275 (2.30)

Aldehyde	Schiff base with methyl valinate					Schiff base with *n*-butylamine				
	Medium	Spectra				Medium	Spectra			
5	Neutral	312 (0.29)	275 (0.27)	286[s] (0.34)		Neutral	310 (0.48)	241 (0.98)		
	1×10^{-3}M KOH	Same as the neutral spectrum				1×10^{-3}M KOH	Same as the neutral spectrum			
	2×10^{-3}M HCl	324[s] (0.22)	314 (0.27)	289 (0.35)		5×10^{-4}M HCl	360 (0.075)	320 (0.085)	286 (0.46)	262[s] (0.35)
6	Neutral	385 (0.75)	235 (1.68)			Neutral	381 (0.87)	234 (2.10)		
	1×10^{-3}M KOH	Same as the neutral spectrum				1×10^{-3}M KOH	Same as the neutral spectrum			
	4×10^{-3}M HCl	428 (0.11)	330 (0.32)	260 (0.63)		2×10^{-3}M HCl	422 (0.56)	327 (0.23)	266[s] (0.52)	258 (0.59)
7	Neutral	290 (0.62)				Neutral	322[s] (0.03)	288 (0.60)		
	1×10^{-2}M KOH	290 (0.78)				3×10^{-3}M KOH	322[s] (0.05)	288 (0.58)		
	1×10^{-1}M HCl	364 (0.07)	292 (0.68)			4×10^{-3}M HCl	360 (0.04)	288 (0.56)		
8	Neutral	393 (0.95)	241 (1.90)			Neutral	385 (0.86)	238 (1.83)		
	1×10^{-3}M KOH	Same as the neutral spectrum				1×10^{-3}M KOH	Same as the neutral spectrum			
	2×10^{-2}M HCl	432 (0.37)	345 (0.40)	268 (0.67)		2×10^{-3}M HCl	427 (0.74)			
9	Neutral	398 (0.07)	319 (0.79)	244[s] (0.56)		Neutral	396 (0.33)	316 (0.66)	264[s] (0.28)	243 (0.59)
	1.5×10^{-2}M KOH	364 (0.90)	255 (0.93)			4×10^{-2}M KOH	357 (0.90)	254 (1.03)		
	2×10^{-3}M HCl	362[s] (0.33)	332 (0.50)			4×10^{-3}M HCl	351 (1.17)	257 (0.86)		
PL	Neutral	337 (0.42)	254 (0.92)			Neutral	400 (0.16)	336 (0.40)	284[s] (0.17)	251[s] (0.86)
	1×10^{-3}M KOH	382 (0.73)	235 (1.52)			2×10^{-2}M KOH	372 (0.82)	233 (1.76)		
	5×10^{-4}M HCl	426 (0.24)	341 (0.40)	266 (0.56)		2×10^{-3}M HCl	422 (0.82)	342 (0.24)	288[s] (0.30)	272[s] (0.38)
PLP	Neutral	340 (0.35)	254 (0.67)			Neutral	418 (0.24)	336 (0.39)	280[s] (0.15)	251[s] (0.82)
	1.6×10^{-1}M KOH	370 (0.51)	232[s] (1.48)			3.5×10^{-1}M KOH	356 (0.53)			
	2×10^{-3}M HCl	426 (0.25)	340 (0.32)	263 (0.47)		2×10^{-3}M HCl	422 (0.82)	342 (0.24)	285[s] (0.37)	270[s] (0.46)

a) See footnote on Table I.

All possible molecular species involved in acid-base equilibrium of the Schiff bases of **1** are presented in Chart 2.[6] The enolimine-ketoenamine tautomerism similar to that of II and III have been extensively studied in Schiff bases of acetylacetone, **4**, *o*-hydroxynaphthaldehydes and related aldehydes.[7] Through the analysis of solution spectra of **1**-valine, Heinert

6) In this paper, each molecular species is indicated by roman numerals as in Chart 2. The prime numerals such as II′ and IV′ represent the species in which the hydrogens of pyridinium or phenol group is replaced by methyl group.

7) *a*) G.O. Dudek and R.H. Holm, *J. Am. Chem. Soc.*, **84**, 2691 (1962); *b*) D.G. Anderson and G. Wettermark, *ibid.*, **87**, 1433 (1965); *c*) J.W. Ledbetter, Jr., *J. Phys. Chem.*, **70**, 2245 (1966); *d*) G.O. Dudek and E.P. Dudek, *J. Am. Chem. Soc.*, **88**, 2407 (1966); *e*) R.S. Becker and W.F. Richey, *ibid.*, **89**, 1298 (1967); *f*) Y. Matsushima and A.E. Martell, *ibid.*, **89**, 1322 (1967); *g*) T.L. Fisher and D.E. Metzler, *ibid.*, **91**, 5323 (1969); *h*) R. Herscovitch, J.J. Charette, and E. de Hoffmann, *ibid.*, **95**, 5135 (1973).

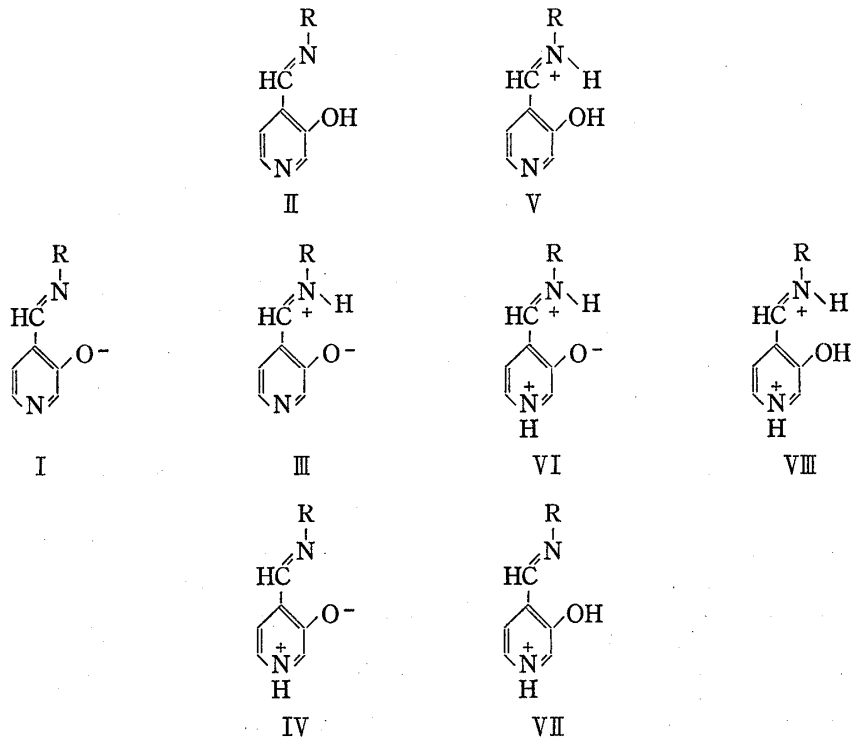


Chart 2

and Martell[3] established the tautomerism between II and III. The band assignments to the species I, II, III, IV and V were given, but the species VI, VII, and VIII were neglected.

Neutral and Anionic Species

In nonpolar solvents, **1**A developed only two bands at around 330 and 250 nm. As **1**A should be present as a nonpolar enolimine species, II, in these solvents, the two bands can be assigned to this species. In the spectrum in neutral methanol, there are the corresponding two bands, which shows **1**A exists primarily as II. A slight blue shift of the π_1 band in polar media is noted. This may be caused by the stabilization of the ground state due to hydrogen bonding with the solvents.

Besides the two bands ascribable to II, **1**B had a weak absorption at around 420 nm, which has been assigned to the π_1 band of the ketoenamine species, III.[3,7] It was shown in the Schiff bases of PL that the equilibrium between II and III depends greatly on the properties of amines.[8] The equilibrium is also dependent on the media. Polar solvents seem to displace the equilibrium to the formation of III, probably because of more polar nature of III rather than II.

Dipolar neutral species, IV, may not be present significantly in methanol solutions. It has been established that 3-hydroxypyridine derivatives exist as dipolar species corresponding to IV in aqueous media, whereas in methanol they exist as nonpolar species.[9] That the pyridine nitrogen of PL Schiff bases does not bear a proton in neutral methanol can be derived from pmr data.[10] In neutral water, the Schiff bases should exist primarily as IV, though the rapid decomposition made impossible to obtain reliable spectra in this medium. The peak at 406—410 nm in 50% H_2O–methanol may be produced by the superposition of the π_1 bands of III and IV.

8) Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **16**, 2046 (1968).
9) K. Nakamoto and A.E. Martell, *J. Am. Chem. Soc.*, **81**, 5857, 5863 (1959).
10) Y. Matsushima and T. Hino, *Chem. Pharm. Bull.* (Tokyo), **16**, 2277 (1968). See also the latter part of this paper.

Schiff bases of **6** should exist solely as the species with a phenolate group and a methylated pyridinium nitrogen in neutral methanol. The absorption bands at 385 nm (**6**A) and 381 nm (**6**B) can be assigned to the π_1 band of IV'.[6] Since spectral characteristics are expected to be similar in species with a protonated and methylated pyridinium nitrogen, IV should have the π_1 band at about 385 nm. The same argument can be made on the species with OH and OCH_3 groups.[3] The absorption band at 312—310 nm of **5**A and **5**B in **neutral** methanol can be ascribed to II'.

In alkaline methanol, the Schiff bases of **1**,**4**,**9**,PL, and PLP absorbed at 360—370 nm. This band is assigned to the anionic species, I, in conformity with the previous assignments.[3,7f,8]

Acidic Species

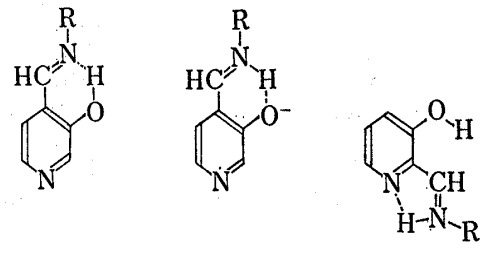
In the Schiff bases of **3** and **7**, a red shift of more than 40 nm was observed on acidification of the solvent. Since the azomethine nitrogen is the only basic group in the molecules, the considerable red shift should be ascribed to the protonation of the nitrogen. In a number of Schiff bases of *p*- and *m*-substituted benzaldehydes, the conjugate acids have been reported to absorb at longer wavelengths than the Schiff bases and the parent aldehydes.[11] It has been established that a similar but more profound bathochromic shift is caused by protonation of the Schiff base of retinal, a visual pigment with a conjugated azomethine.[12]

These facts indicate that the π_1 band of V should be at considerably longer wavelength region than that of II. Therefore, the previous assignments[3] to V and corresponding species of **4**-valine seem unlikely and the failure to observe the long wavelength absorption in acidic media can be explained if it is concluded that the reported spectra are those of hydrolysis products.

In mildly acidic methanol ([HCl]; 5×10^{-4}—1×10^{-3} M). the Schiff bases of **1** should be present as monopositive species, *i.e.* V, VI, and VII. The bands at 425 nm (**1**A) and 418 nm (**1**B) are assigned to the π_1 band of VI, whereas those at 325 nm (**1**A) and 327 nm (**1**B) to VII. These assignments are supported by the fact that very similar spectra were observed in the Schiff bases of **6**, the possible species of which in acidic methanol are VI' and VII'.

Replacement of A by B in the Schiff bases is seen to shift the equilibrium toward the formation of VI. A similar shift was observed in the equilibrium between II and III. Protonation of II and III on the pyridine nitrogen do not significantly affect the wavelengths of the π bands and the equilibrium between them.

The species V seems to be absent or a minor species in acidic methanol. The possible reason for the fact may be that the structures monoprotonated at the phenolate-azomethine region as II, III, VI, and VII are stabilized by the intramolecular hydrogen bond as shown in Chart 3 **d**, **e** and offer hindrance to further addition of a prototon in the vicinity. Thus the protonation to II and III occurs at the pyridine nitrogen.



d **e** **f**

Chart 3

The 362- and 351-nm bands of **9**A and **9**B in acidic methanol, however, can be assigned to the species with a protonated azomethine and a phenol group (Chart 3 **f**). In these Schiff bases, intramolecular hydrogen bond can be formed between the azomethine and pyridine nitrogens and the protonation on both the azomethine and phenolate become feasible in mildly acidic conditions. That **5**B had an absorption at 360 nm in acidic methanol gives

11) G.M. Santerre, C.J. Hansrote, Jr., and T.I. Crowell, *J. Am. Chem. Soc.*, **80**, 1254 (1958); R.L. Reeves and W.F. Smith, *ibid.*, **85**, 724 (1963).

12) S. Ball, F.D. Collins, P.D. Dalvi, and R.A. Morton, *Biochem. J.*, **45**, 304 (1949); R.H. Callender, A. Doukas, R. Crouch, and K. Nakanishi, *Biochemistry*, **15**, 1621 (1976).

further support for the assignment. Since the π_1 band of the corresponding species were located at slightly shorter wavelength region in the Schiff bases of **9** than in those of **1**, V_{1A} and V_{1B} must have the π_1 band at about 365 nm.

From the argument given above, the further protonation to the species bearing two protons, *i.e.* V, VI, and VII, is expected to be so greatly hindered that the fully protonated species, VIII, should be present only in strongly acidic media. Under the conditions the solvolysis of the Schiff bases was too rapid to obtain a reliable spectrum. As **7A** and **7B** absorbed at 360—364 nm in acidic methanol, the π_1 band of VIII was expected to be in this region.

The spectrum of **1B** was measured in 0.5 M HCl by means of the rapid scan and stopped-flow techniques. A significant absorption was detected at around 365 nm, which was ascribable to VIII. The 365-nm species decomposed completely in 20 msec.

Infrared Spectra

Infrared (IR) spectra of crystalline and liquid Schiff bases were measured in KBr disc and in CCl_4 solutions, respectively. Results were essentially the same as the previous investigation.[13] One of the most characteristic features in the IR spectra is the absorption at 1630—1650 cm^{-1}, which is assigned either the stretching frequencies of C=N or amide I band of the conjugated amide. These were obseved as a clearly defined absorption in the Schiff bases of amino acid esters. In those of amino acids, the imine frequencies were overlapped by the carbonyl absorption.[13] The amine part do not affect significantly on the imine frequencies. The frequencies seemed to be hardly affected by the ortho substituents of the aldehydes.

Table III. Infrared Sprectra of Schiff Bases[a]

Schiff base	Solvent	O–H st.		Ester C=O st.		Imine st.	C=C conj. Amide st.
1A	KBr		2700 m. br.	1745 s		1640 s	
1-Methyl alaninate	CCl_4		2700 m. br.	1745 s		1640 s	
1-Methyl tyrosinate	KBr	3120 s	2700 m. br.	1740 s		1640 s	1514 s
1-Ethyl tyrosinate	KBr	3140 s	2700 m. br.	1740 s		1640 s	1513 s
1B	CCl_4		2700 m. br.			1641 s	
2A	CCl_4			1750 s	1735 s	1641 s	
3A	CCl_4			1747 s	1735 s	1634 s	
4A	CCl_4		2700 m. br.	1752 s	1742 s	1635 s	
8-Methyl alaninate	KBr	3400 s	2600 m. br.	1740 s		1633 s	
9A	KBr		2700 m. br.	1725 s		1630 s	
PL-A	KBr	3285 s	2650 m. br.	1746 s		1631 s	1510 w
PL-Methyl tyrosinate	KBr	3250 s	2700 m. br.	1747 s		1632 s	1518 s

a) The frequencies of ν_{max} are expressed in cm^{-1}. The intensities are indicated as s, strong; m, medium; w, weak. In broad bands indicated by br., ν_{max} values are to be considered within an accuracy of ±10 nm.

The band at 1500—1518 cm^{-1}, attributed to a C=C or C=N stretching vibration of the conjugated amide[13] was absent in some Schiff bases of *o*-hydroxyaldehydes. This may be due to the predominance of the enolimine species.

In the Schiff bases of the *o*-hydroxyaldehydes, medium intense and poorly resolved bands were observed at 3200—2500 cm^{-1}, which was ascribed to intramolecularly hydrogen bonded O–H frequencies. PL Schiff bases showed a strong absorption at 3285 cm^{-1}, which lacked in the Schiff bases of **1**, **2**, and **3**. This may be ascribed to intermolecular hydrogen bond.

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

Proton Magnetic Resonance Spectra

The prepared Schiff bases were studied by means of proton magnetic resonance (PMR) spectroscopy. The results were in general agreement with those of the previous investigations.[10,14] Hence, detailed data are not presented.

The α-proton of A at 3.28[15] as a doublet in CD_3OD showed a considerable downfield shifts in Schiff bases. The chemical shifts were 4.00 (**1**A), 3.76 (**2**A), 3.70 (**3**A) 3.86 (**4**A), 4.40 (**9**A), 4.00 (PL–A), and 3.90 (PLP–A). The labilization of the α-proton by Schiff base formation is clearly seen. Downfield shift of the β-proton, which was observed at 1.98 in a multiplet, was less eminent. Its chemical shifts were 2.34 (**2**A,**3**A,**4**A), 2.40 (**1**A,**9**A), and 2.38 (PL–A). Other protons in A were much less affected by the Schiff base formation.

The downfield shifts of α-protons similar to A were observed in other amino acids and their esters. The same phenomenon was noted in the α-protons of B; 2.63 t (B), 3.69 t (**1**B) in CD_3OD. In the spectrum of **1**B in $CDCl_3$ was detected coupling between α-protons of B (3.66 t) and the azomethine proton (8.32 s) with $J=1.2$ Hz. Methyl signal of α-methylalanine ester at 1.33 s was shifted to 1.60 s upon Schiff base formation with **1** in CD_3OD.

The signals of the protons at pyridine ring of **1** shifted upfield on Schiff base formation. The chemical shifts of 2- and 6-protons in CD_3OD were 8.39 s, 8.22 d (**1**), 8.30 s, 8.12 d (**1**A), 8.21 s, 8.02 d (**1**B), and 8.14 s, 7.78 d (**1**-valine), respectively. These signals shifted downfield on acidification of the medium.

Experimental

Schiff bases were prepared according to the method of Heinert and Martell.[13] Materials used for the preparation were obtained from commercial sources, except for **1**,[16] **5**,[16] **6**,[17] **7**,[18] **8**,[19] and **9**,[16] which were synthesized by the methods cited.

The electronic absorption spectra were recorded at room temperature with a Union-Giken Model SM-202 spectrophotometer. For the measurements of rapidly changing spectra, a Union-Giken Model RA-1300 rapid reaction analyzer was used. Infrared and PMR spectra were taken on a JASCO DS-701G and a JEOL PS-100 spectrophotometer, respectively.

Acknowledgment This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, for which we are grateful. Y.K. thanks Japan Securities Scholarship Foundation for a Predoctoral Fellowship. In the interpretation of the spectra, we are greatly assisted by unpublished experimental results elaborated in this laboratory by Dr. Shigenobu Matsumoto, Mmes. Akemi Ikei, Mayumi Kai and Rieko Gondo and Miss Ritsuko Takemoto, to whom we are grateful.

14) O.A. Gansow and R.H. Holm, *Tetrahedron*, **24**, 4477 (1968); N.M.D. Brown and D.C. Nonhebel, *ibid.*, **24**, 5655 (1968).
15) Chemical shifts are expressed by δ values (ppm) from tetramethylsilane as an internal standard. Abbreviations; s, singlet; d, doublet; t, triplet.
16) D. Heinert and A.E. Martell, *J. Am. Chem. Soc.*, **81**, 3933 (1959).
17) J.R. Maley and T.C. Bruice, *Arch. Biochem. Biophys.*, **136**, 187 (1970).
18) **7** was prepared by the treatment of **5** with CH_3I in dry benzene.
19) D. Heyl, E. Luz, S.A. Harris, and K. Folkers, *J. Am. Chem. Soc.*, **73**, 3430 (1951).