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Molecular Species of Schiff Bases derived from *o*-Hydroxyaromatic Aldehydes. III.[1] Schiff Bases of Pyridoxal and Its Analogs with Unsaturated Amino Acids

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The analyses of the electronic absorption spectra and the band assignments to the molecular species were carried out on the methanol solutions of the Schiff bases of pyridoxal (PL), pyridoxal N-methochloride (PLM), and related aromatic aldehydes with α,β-, β,γ-, and γ,δ-unsaturated amino acids and their methyl esters. The Schiff bases of PL with propargylglycine, allylglycine, and vinylglycine had spectra similar to those with a saturated amino acid, showing the unsaturated bond is not conjugated with the azomethine bond. In neutral and alkaline solutions of the Schiff bases of PLM with esters of alanine, propargylglycine, and allylglycine, 2—4% of the Schiff bases was deprotonated at the α-carbon atom, forming a quinoid species absorbing at 500 nm. With ester of vinylglycine, a species absorbing at 534 nm was formed, indicating the quinoid structure with an extended conjugation. It is suggested from the spectral analysis of the Schiff bases of methyl dehydrovalinate that in species protonated at the phenolate or the azomethine the molecule is not planar and the planarity is achieved through the metal chelation.

Keywords——enzyme model; Schiff base; UV-absorption spectra; band assignment; pyridoxal; pyridoxal N-methochloride; allylglycine; propargylglycine; vinylglycine; methyl dehydrovalinate

In the previous paper of this series,[3] we reported the absorption spectra of Schiff bases of pyridoxal and related compounds with amino acids and amines. Every absorption band was assigned to one of the species of the Schiff base by analyzing the solution spectra obtained under various conditions. These spectral assignments have led to definition of the eight

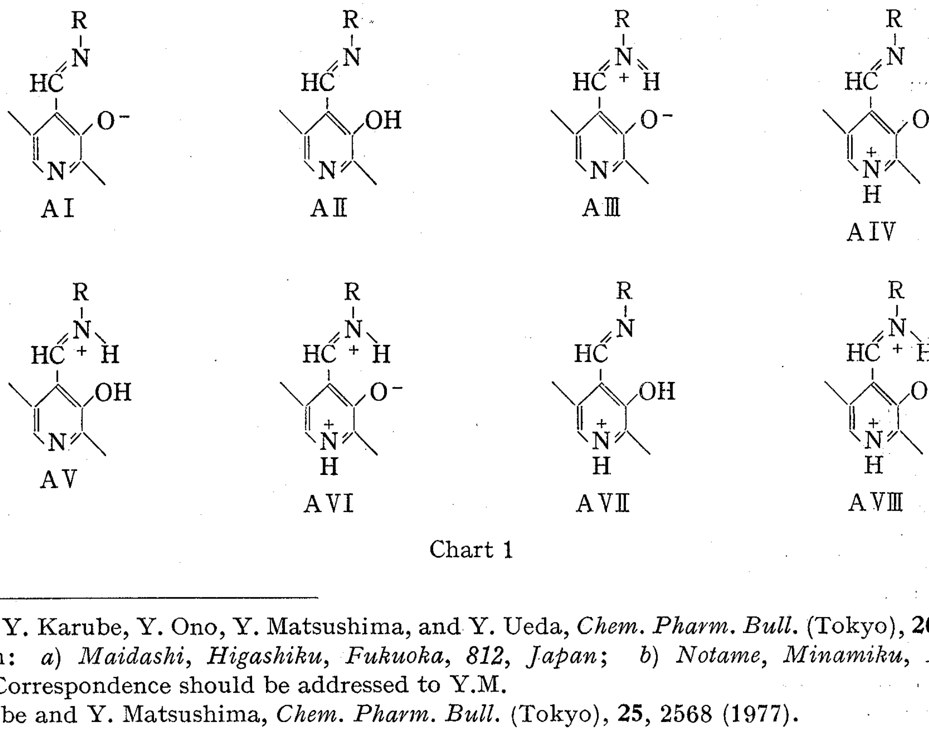

Chart 1

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[1] Part II: Y. Karube, Y. Ono, Y. Matsushima, and Y. Ueda, *Chem. Pharm. Bull.* (Tokyo), **26**, 2642 (1978).

[2] Location: *a*) *Maidashi, Higashiku, Fukuoka, 812, Japan*; *b*) *Notame, Minamiku, Fukuoka, 815, Japan.* Correspondence should be addressed to Y.M.

[3] Y. Karube and Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **25**, 2568 (1977).

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molecular species shown in Chart 1. Calculations of the theoretical energies of the π-π^* transitions by the molecular orbital (MO) method afforded the additional evidence for the assignments.[1]

It has been established that the coenzyme is combined as a Schiff base (aldimine)[4] with the ε-amino group of lysine of the apoprotein. Despite the common structural features of the chromophore, the spectral properties were diverse among the enzymes. This can be reasonably explained on the basis of the different spectral properties of the eight molecular species.

The coenzyme is converted to an aldimine with the amino group of a substrate in the enzyme-substrate complex. The aldimine then undergoes a variety of reactions depending upon the structures of the substrate and the nature of the enzyme protein.[5] The reactions are accompanied by spectral changes, some of which can not be accounted for by the eight molecular species of the simple aldimine. The elimination of functional groups of the substrate should lead to the structures with unsaturation at the amino acid side chain.

Herein we report the studies of the spectral properties of Schiff bases with unsaturated amino acids, which may contribute to understanding of the spectra of the pyridoxal enzymes in dynamic states.

Results and Discussion[4]

Though the Schiff bases derived from the eleven aromatic aldehydes described previously[3] were studied, the present paper is concerned principally with those derived from 4-formyl-3-hydroxypyridine (**1**), pyridoxal (PL), and pyridoxal N-methochloride (PLM).

Aldimine with an Unsaturated Side Chain

Table I lists the absorption spectra of the methanol solutions containing 1×10^{-4} M aldehyde and 1×10^{-3} M amines. Amines studied are allyglycine (2-amino-4-pentenoic acid: Alg), propargylglycine (2-amino-4-pentynoic acid; Prg), vinylglycine (2-amino-3-butenoic acid; Vng), and their methyl esters (AlgOMe, PrgOMe, and VngOMe, respectively), and allylamine (Allam). The spectral data of alanine (Ala) and methyl alaninate (AlaOMe) are included for the sake of the comparison.

Under the conditions employed, the formation of the aldimines required less than 30 min to reach equilibrium, where the conversion of the aldehydes to the aldimines was nearly complete. The data listed in Table I are those of the solutions after reaching the equilibrium.

The spectra of the Schiff bases of methyl vinylglycinate in neutral methanol was rather unstable and converted gradually to obscured spectra during the time course of a few hours. The spectral change was too rapid to obtain reliable data in alkaline and acidic media. No spectral change occurred for a period of several hours in the other unsaturated amines. The spectra of the most aldimines of **1** are not included in Table I, since they were analogous to those of PL.

The spectra of the aldimines with the unsaturated amines are essentially the same as those with saturated amines described earlier.[3,6] This indicates that the unsaturated bond

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[4] Throughout this paper, the following symbols and terms are used. Each molecular species of Schiff bases distinguished by the state of the protonation on the azomethine and pyridine nitrogens and the phenolate group is indicated by roman numerals as in Chart 1. The prime numerals represent the species in which the hydrogen of the pyridinium or the phenol group is replaced by methyl group. Specific Schiff base is expressed by hyphenating the symbols of the parent aldehyde and amine such as PL-AlaOMe and 1-Alg. The term "aldimine" indicates the Schiff bases of the aromatic aldehydes and amines, whose conjugate system does not extend beyond the azomethine group. B, C, and D indicate the quinoid structure derived by the α-deprotonation of aldimine, α,β-unsaturated aldimine, and β,γ-unsaturated quinoid structure, respectively. See ref. 1 and Chart 2—4.

[5] H.C. Dunathan, *Adv. in Enzymol.*, 35, 79 (1971).

[6] Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **16**, 2046 (1968).

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Table I. Absorption Spectra of Schiff Bases in Methanol$^{a)}$

Schiff base	Medium		
	Neutral methanol	Alkaline methanol$^{b)}$	Acidic methanol$^{c)}$
PL-Ala	420^{s}(0.26, III), 338(0.39, II), 254(0.88, II)	372(0.68, I)$^{2)}$	422(0.26, VI), 338(0.36, VII)$^{1)}$
PLM-Ala	385(0.82, IV′), 340^{s}(0.32, VII′)	N$^{1)}$	429(0.34, VI′), 342(0.31, VII′), 268(0.50, VI′)$^{3)}$
PL-Alg	418(0.24, III), 336(0.40, II), 257(0.89, II)	374(0.72, I)$^{2)}$	418(0.26, VI), 336(0.39, VII), 257(0.91, VII)$^{2)}$
PLM-Alg	388(0.86, IV′)	N$^{1)}$	432(0.37, VI′), 342(0.36, VII′), 270(0.60, VI′)$^{4)}$
PL-Prg	398^{s}(0.18, III), 338(0.48, II), 258(1.03, II)	374(0.77, I)$^{2)}$	418(0.16, VI), 339(0.49, VII), 258(1.02, VII)$^{1)}$
PLM-Prg	388(0.86, IV′)	N$^{1)}$	432(0.26, VI′), 340(0.45, VII′), 268(0.79, VI′)$^{4)}$
PL-Vng	416(0.20, III), 338(0.41, II), 252(1.01, II)	375(0.65, I)$^{3)}$	426(0.22, VI), 338(0.40, VII), 268(0.68, VII)$^{4)}$
PLM-Vng	388(0.85, IV′)	N$^{1)}$	434(0.25, VI′), 342(0.33, VII′), 270(0.55, VI′)$^{4)}$
PL-Allam	390^{s}(0.10, III), 336(0.46, II), 252(0.92, II)	374(0.86, I)$^{2)}$	423(0.59, VI), 338(0.34, VII), 262^{s}(1.93, VI)$^{3)}$
PLM-Allam	386(0.86, IV′)	N$^{1)}$	429(0.57, VI′), 342(0.27, VII′), 264^{s}(0.46, VI′)$^{3)}$
PL-AlaOMe	402^{s}(0.15, III), 337(0.46, II), 253(0.99, II)	378(0.82, I)$^{2)}$	426(0.29, VI), 338(0.47, VII), 266(0.60, VI)$^{3)}$
PLM-AlaOMe	496(0.06, B), 389(0.55, IV′)	496(0.09, B), 389(0.55, IV′)$^{1)}$	432(0.22, VI′), 342(0.30, VII′), 270(0.42, VI′)$^{4)}$
PL-AlgOMe	400^{s}(0.19, III), 339(0.46, II), 254(1.04, II)	379(0.90, I)$^{2)}$	426(0.29, VI), 340(0.50, VII), 268(0.71, VI)$^{3)}$
PLM-AlgOMe	496(0.03, B), 392(0.58, IV′), 340(0.42, VII′)	502(0.04, B), 390(0.57, IV′)$^{1)}$	432(0.19, VI′), 340(0.33, VII′), 272(0.64, VI′)$^{5)}$
PL-PrgOMe	390^{s}(0.26, III), 338(0.47, II), 254(0.97, II)	382(0.93, I)$^{2)}$	430(0.16, VI), 338(0.52, VII), 268(0.69, VI)$^{3)}$
PLM-PrgOMe	496(0.02, B), 392(0.58, IV′)	504(0.06, B), 392(0.58, IV′)$^{1)}$	430(0.16, VI′), 342(0.35, VII′), 270(0.55, VI′)$^{4)}$
1-VngOMe	368(0.39, I), 336^{s}(0.33, II)		
PL-VngOMe	400^{s}(0.21, III), 342(0.35, II)		
PLM-VngOMe	534(0.07, D), 400(0.15, IV′), 334(0.90, VII′)		

a) Wavelengths are given in nm; numbers in parentheses give the absorbance for a 1.0-cm light path and 1×10^{-4} M. Abbreviations for the substances are given in the text. The Roman numerals indicate the assigned molecular species of the aldimines and B and D indicate the bands were assigned to the quinoid structures described in the text. Subscript s indicates shoulder.

b) N indicates the spectrum was the same as the neutral spectrum. Concentrations of KOH in metanol were 2 mM (1), 4 mM (2), and 5 mM (3).

c) Concentrations of HCl in methanol were 0.1 mM (1), 0.3 mM (2), 0.5 mM (3), 1.0 mM (4), and 2.0 mM (5).

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in the side chain did not migrate to a position of conjugation with the azomethine and the pyridine moieties. The spectral properties of the aldimines are expected to be hardly affected by the insulated unsaturated bond.

The band assignments included in Table I were made according to the procedure described previously.[3]

Quinoid Species

A weak and broad absorption at around 496 nm was present in the spectrum of PLM–AlaOMe, but not in that of PL–AlaOMe. This may be due to the presence of a quinoid intermediate (B), in which the α-carbon of the amino acid ester in the aldimine is deprotonated (Chart 2). The formation of the quinoid in nonenzymatic reaction was first reported by Schirch and Slotter,[7] who found a species absorbing at 480 nm in the reaction of PLM with diethyl aminomalonate in ethanol. They fail to observe a similar species with amino acid esters other than diethyl aminomalonate.

We reported that a species absorbing at 488 nm was formed in the reaction of pyridoxamine, ethyl pyruvate, and Al(III) ion,[8] and in that of PL, AlaOEt, and Al(III) ion.[9] The 488-nm species was identified as the Al(III) chelate of the quinoid. The quinoid structure was shown to be greatly stabilized by the chelation with metal ions, especially with Al(III) ion.

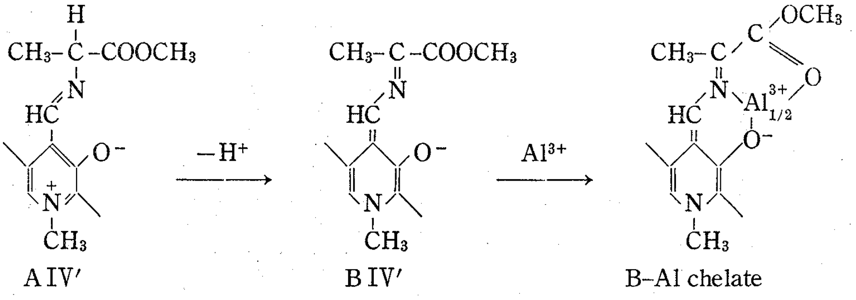

Chart 2

No report has, so far, described explicitly the formation of the quinoid with a simple amino acid or its ester and without metal ion of chelating ability.

In the reaction of PLM (1×10^{-4} M), AlaOEt (1×10^{-3} M), and Al(III) ion (5×10^{-4} M), the Al(III) chelate of the quinoid with an absorption at 500 nm was formed.[10] The absorbance in its maximum was 2.30.

The amount of the quinoid species formed in the metal ion free solution would be estimated, if the following two assumptions are considered proper; one is that PLM is completely converted to the Al(III) chelate of the quinoid under the conditions and the other is that the molar absorptivity is the same in the chelated and unchelated forms of the quinoid.

The results show that 2—4% of the Schiff base was deprotonated to form the quinoid species in alkaline and neutral methanol solutions of PLM and the amino acid esters under the investigation. Only in the concentrated methanol solution of PLM and alanine, a weak absorption of the quinoid species became observable at 508 nm. The conversion to the quinoid calculated on the same basis was 0.26% in neutral methanol.

The esters of allylglycine and propargylglycine formed the quinoid species with PLM in the similar manner to methyl alaninate. On the other hand, methyl vinylglycinate showed the long wavelength absorption at 534 nm. Deprotonation at the α-carbon atom of this

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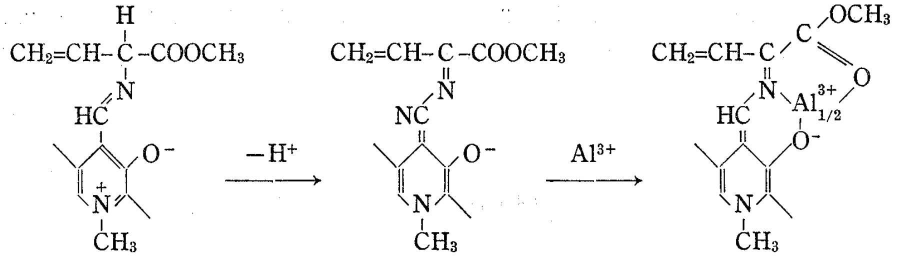
7) L. Schirch and R.A. Slotter, *Biochemistry*, **5**, 3175 (1966).
8) S. Matsumoto and Y. Matsushima, *J. Am. Chem. Soc.*, **94**, 7211 (1972).
9) S. Matsumoto and Y. Matsushima, *J. Am. Chem. Soc.*, **96**, 5228 (1974).
10) Y. Karube and Y. Matsushima, *Chem. Pharm. Bull.* (Tokyo), **26**, 2066 (1978).

amino acid ester in the aldimine with PLM would result in the structure with an extended conjugation system (Chart 3). The structure D is postulated for one of the intermediates in pyridoxal catalyzed β,γ-elimination and γ-replacement reactions of amino acids.[11]



AIV′ DIV′ D–Al chelate

Chart 3

Hence the 534-nm absorption should be assigned to this structure, possibly DIV′.

In the replacement reaction of the γ-substituent of methionine catalyzed by PLM and Al(III) ion, we observed an absorption at 550 nm and assigned it to the Al(III) chelate of D.[12] The addition of Al(III) ion to the methanol solution of PLM and methyl vinylglycinate resulted in the intensification of the 534-nm absorption and the red shift to 550 nm. This result supports the previous assignment.[12]

Schiff Base of Dehydrovaline Ester

The Schiff base of pyridoxal phosphate with α,β-unsaturated amino acid has been assumed as one of the unstable or metastable intermediates in the enzyme catalyzed α,β-elimination and β-replacement reactions of amino acids.[11] Transient absorptions observed in enzymatic reactions at 455—470 nm have been ascribed to this intermediate structure.[13]

We reported the transient absorption at 467 nm ascribable to the Al(III) chelate of the Schiff bases of PLM and α-aminoacrylic acid in the Al(III) chelate mediated nonenzymatic β-replacement reaction.[14]

Table II. Absorption Spectra of the Schiff Bases of Methyl Dehydrovalinate in Methanol[a]

Schiff base	Medium	Absorption bands and assignments
PL-DhvOMe	10 mM KOH	404(1.36, CI)
	2.0 mM KOH	404(1.00, CI)
	Neutral	352(0.74, CII), 328(0.88), 296(1.18)
	0.1 mM HCl	376^{s}(0.68, CVII), 353(0.95, CII), 344(0.96), 329(0.97), 299(0.16)
	2 mM HCl	376^{s}(1.00, CVII), 356^{s}(1.28, CII), 344(1.31), 300^{s}(1.05)
PLM-DhvOMe	4 mM KOH	412(1.02, CIV′)
	Neutral	422^{s}(0.49, CVI′), 381(0.66, CVII′), 360(0.66), 345(0.68), 330^{s}(0.64)
	0.5 mM HCl	377(0.78, CVII′), 360(0.95), 345(0.97), 330^{s}(0.90), 310(0.86)
9-DhvOMe	20 mM KOH	380(0.88, CI)
	Neutral	356^{s}(0.89, CVII), 342(1.02, CII)
	20 mM HCl	365(0.75, CV)

a) See footnote[a] on Table I. Structures of the assigned species are given in Chart 4.

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[11] L. Davis and D.E. Metzler, "The Enzymes," Vol. 7, 3rd ed., P.D. Boyer Ed., Academic Press, N.Y., 1972, Chapter 2.

[12] Y. Karube and Y. Matsushima, *J. Am. Chem. Soc.*, **99**, 3756 (1977).

[13] M.K. Goldberg and L. Baldwin, *Biochemistry*, **6**, 2113 (1967); M.A. Becker, N.M. Kredich, and G.M. Tomkins, *J. Biol. Chem.*, **244**, 2418 (1969); S. Guggenheim and M. Flavin, *ibid.*, **246**, 3562 (1971); M. Tokushige and A. Nakazawa, *J. Biochem.*, **72**, 713 (1972).

[14] Y. Karube and Y. Matsushima, *J. Am. Chem. Soc.*, **98**, 3725 (1976).

The preparation of the α,β-unsaturated Schiff bases was attempted in order to obtain the spectra in its unchelated forms. Since α,β-unsaturated amino acid is unknown, methyl ester of dehydrovaline (methyl 2-amino-3-methyl-2-butenoate; DhvOMe) was employed for the present study. The Schiff bases with PL, 2-formyl-3-hydroxypyridine (**9**), and 4- and 5-nitrosalicylaldehydes were successfully prepared according to the general procedure.[15] That with PLM was hygroscopic and decomposed readily in air and that with 1 was contaminated with a minute amount of impurities.

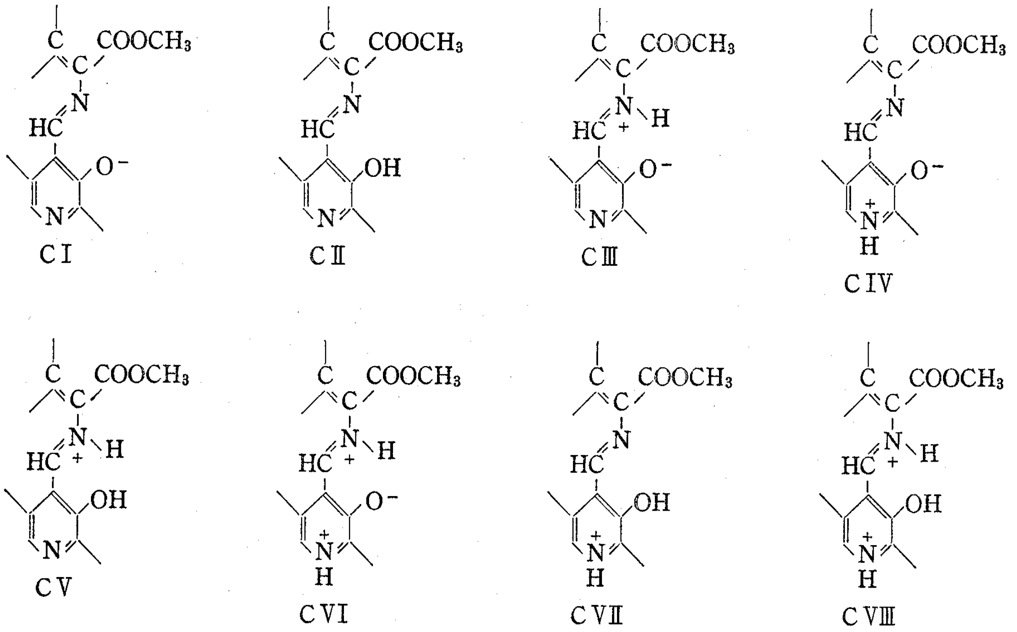

Chart 4

Spectral data in methanol are summarized in Table II. An intense absorption at 404 nm observed in alkaline solution of PL–DhvOMe can be ascribed to the anionic species, CI (Chart 4). There was no other appreciable absorption. The spectrum of PLM–DhvOMe in alkaline methanol was similar, but the peak of the visible band was at 412 nm, which was ascribable to CIV′.

The spectra of the Schiff bases in neutral and acidic methanol had a very broad absorption ranging 260—380 nm with several peaks and shoulders. Such an overlap of bands has not been met in the study of Schiff bases of saturated amino acids.[3] Similar broad absorptions in the same region were reported for the Schiff bases of aniline with salicylaldehyde[16] and 4-nitrosalicylaldehyde.[17] The two aromatic rings are supposed not to be coplanar in the Schiff bases.[18]

Hence it is probable that the molecules are nonplanar for the Schiff bases of DhvOMe. It is more likely so for the species protonated in the azomethine-phenolate region (CII, CIII, CV, CVI, CVII, CVIII), since the broad and overlapped bands were observed in neutral and acidic media.

The nonplanarity may be responsible for the situation that the magnitude of the bathochromic shift accompanying the α,β-unsaturation was not as much as one might expect from the length of the conjugate system.

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15) D. Heinert and A.E. Martell, *J. Am. Chem. Soc.*, **81**, 3933 (1959).
16) J.W. Ledbetter, Jr., *J. Phys. Chem.*, **70**, 2245 (1966).
17) Y. Karube and Y. Matsushima, *Yakugaku Zasshi*, **97**, 1322 (1977).
18) N. Ebara, *Bull. Chem. Soc. Jpn.*, 33, 534 (1960).

Unequivocal band assignments would be impossible, since the overlapped absorption band cannot be clearly resolved into its components. The peaks appeared in the broad absorption and the tentative assignments to the species are included in Table II.

A weak absorption at around 470 nm appeared on addition of Al(III) ion to the methanol solution of the Schiff bases. The band can be ascribed to the Al(III) chelate of the Schiff bases of DhvOMe and is closely related to the band observed at 467 nm in the Al(III) mediated reaction reported previously.[14]

It has been found in the aldimines of amino acid, A, the quinoid, B, and the β,γ-unsaturated quinoid, D, that upon the conversion of the phenolate species (I, IV) to the Al(III) chelate the wavelengths of the π-π^* bands do not shift significantly.[19] These may indicate that the unchelated Schiff base as well as the chelated ones are planar molecule.

In the α,β-unsaturated Schiff base, B, the magnitude of the shift accompanied by the Al(III) chelation was about 60 nm. The large shift may support the assumption that the unchelated molecule is not planar and the planarity of the whole molecule is achieved through the metal chelation.

Experimental

The preparation of the *o*-hydroxyaldehydes and the measurement of spectra were described in the previous paper.[3] Allylglycine[20] propargylglycine,[21] and vinylglycine[22] were synthesized by the method described in the cited literature. The esterification of the unsaturated amino acids were carried out under a N_2 atmosphere. Methyl dehydrovalinate was prepared according to the method of Shin *et al.*[23] Other amino acids and allylamine were obtained from commercial sources.

The Schiff bases of DhvOMe were prepared under a N_2 atmosphere according to the general procedure.[3,15]

Acknowledgement Financial support from the Ministry of Education, Science and Culture is acknowledged. Y.K. thanks Miyata Senji Foundation for a Fellowship.

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19) See ref. 3 and the literature cited therein.

20) H.L. Goering, S.J. Cristol, and K. Dittmer, *J. Am. Chem. Soc.*, **70**, 3310 (1948).

21) H. Gershon, J. Shapira, J.S. Meek, and K. Dittmer, *J. Am. Chem. Soc.*, **76**, 3484 (1954); A.C.A. Jansen, R.J.M. Weustink, K.E.T. Kerling, and H. Havinga, *Rec. Trav. Chim. Pays-Bas*, **88**, 819 (1969).

22) J.E. Baldwin, S.B. Haber, C. Hoskins, and L.I. Kruse, *J. Org. Chem.*, **42**, 1329 (1977); P. Friis, P. Helboe, and P.O. Larsen, *Acta. Chem. Scand. Ser. B*, **28**, 312 (1974).

23) C. Shin, M. Masaki, and M. Ohta, *J. Org. Chem.*, **32**, 1860 (1967).

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