

Models for Visual Pigments. The Effect of the Imidazolyl Group on the Absorption Maxima of the Retinal Schiff Base

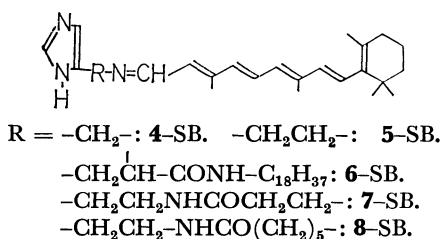
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The title effect has been investigated with the imidazolyl group either externally added or intramolecularly combined. *N*-Retinylidenebutylamine was protonated with imidazole derivatives under neutral conditions and was absorbed at longer wavelengths compared with carboxylic acids. The absorption peak with the imidazolyl group intramolecularly combined was highly affected by the structure, protonated *N*^α-retinylidene-L-histidine octadecylamide having an absorption maximum at 494 nm caused by the inductive effect and the polar imidazolyl group.

The absorption maxima of visual pigments bound to opsin by a Schiff-base linkage are observed at 500 nm, although the protonated products of synthetic analogues absorb at approximately 450 nm. As regards this bathochromic shift, a large number of interesting observations and theories have been reported: the polarizability of the micro environment,¹⁾ the electrostatic effect of polar or charged groups in the vicinity of the chromophore,²⁾ and the twist or distortion of the π -conjugated system.³⁾ However, there are still many unsolved problems. Recently two synthetic models were reported which absorb at longer wavelengths by means of protonation with trifluoroacetic acid⁴⁾ or in an aqueous hydrochloric acid solution.⁵⁾ Since native photochromic processes proceed under mild conditions, we examined the effects of amino acids as protonating agents.⁶⁾ In this paper we intend to deal especially with the imidazolyl group of the histidine residue. The imidazolyl group is known to be responsible for a buffering capacity;⁷⁾ hence, it may be able to protonate the Schiff-base linkage in a physiological pH range and to mediate a charge relay system between protonated Schiff-base nitrogens and counter anions. On the basis of these assumptions, we have examined the protonation of *N*-retinylidenebutylamine (Bu-SB) with the salt of *N*^α-benzyloxycarbonyl-L-histidine butylamide (**1**) plus *N*-benzyloxycarbonyl-L-alanine (Z- α -Ala), *N*^α-octadecanoyl-L-histidine (**2**), and 4,5-imidazoledicarboxylic acid monohexadecyl ester (**3**). As Schiff-base models with the imidazolyl group, the following were synthesized, and the absorption maxima of the protonated products were determined:



Experimental

all-trans-Retinal was prepared by the oxidation of vitamin-A with MnO₂ and was recrystallized from petroleum ether;⁸⁾ mp 59–60 °C, λ_{max} 367.5 nm, $E_1\%$ 1536 in petroleum ether. Isomerization to the *cis*-isomer was conducted by the irradi-

ation of *all-trans*-retinal (1% in petroleum ether) containing iodine (1% to retinal), using a 150 W xenon lamp with a UV filter fitted as the light source;⁹⁾ λ_{max} 364.5 nm, $E_1\%$ 1259 in petroleum ether.

Preparation of *N*^α-Benzyloxycarbonyl-L-histidinamide: A mixture of *N*^α,*N*^{1m}-bis(benzyloxycarbonyl)-L-histidine (Z-His-Z) 6.85 g, 15.5 mmol, the benzyloxycarbonyl group was abbreviated as Z) and dicyclohexylcarbodiimide (DCC) 3.5 g, 17 mmol in CH₂Cl₂ (50 ml) was stirred in an ice bath for 45 min, filtered, and washed with CH₂Cl₂. To the filtrate, alkylamine (31 mmol) in CHCl₃ (50 ml) was added, the mixture being stirred in an ice bath, allowed to stand overnight, and then refluxed for 30 min. While hot, the reaction mixture was filtered and washed with CHCl₃. The filtrate was dried over Na₂SO₄, concentrated to about 30 ml, and then cooled at -20 °C. The product thus precipitated was recrystallized from acetone or ethanol. **1**: yield, 62% mp; 143–145 °C. IR (KBr): 3300 (NH), 3150 (imidazole-NH), 1690 (carbamate C=O), 1650 cm⁻¹ (amide C=O). ¹H-NMR (CDCl₃): 0.9–1.5 (m, 7H, CH₃CH₂CH₂-), 3.0 (m, 4H NH-CH₂- and imi-CH₂-), 4.3 (q, 1H, α -CH), 5.2 (s, 2H, phenyl-CH₂-), 6.9, 7.8 (s, 2H, imidazole ring), 7.3 ppm (8H, phenyl, NH). Found: C, 62.68; H, 7.32; N, 16.01%. Calcd for C₁₈H₂₃N₄O₃: C, 62.97; H, 6.31; N, 16.33%. MS (*m/e*): 343 (M⁺, 11%). *N*^α-Benzyloxycarbonyl-L-histidine octadecylamide: yield, 36%; mp 151–154 °C.

N^α-Octadecanoyl-L-histidine (**2**) was prepared with octadecanoyl chloride; yield, 45%; mp 165–180 °C.¹⁰⁾ The 4,5-imidazoledicarboxylic acid monohexadecyl ester (**3**) was obtained by condensation with 4,5-bis(chloroformyl)imidazole¹¹⁾ and hexadecyl alcohol; yield, 71%; mp 167–170 °C. In the IR of the salt containing **1** and Z- α -Ala (mp 125–126 °C), the absorption at 1710 cm⁻¹ (COOH) no longer exists, and the salt peak is observed at 2000–2750 cm⁻¹. ¹H-NMR and elemental analyses made it possible to identify the structure. 4-(Aminomethyl)imidazole (**4**) was obtained starting with D-fructose *via* 4-(hydroxymethyl)imidazole, oxidation with HNO₃ to aldehyde, conversion to oxime, and reduction with H₂ (5% Pd on charcoal) in methanol saturated with HCl.¹²⁾

L-Histidine Octadecylamide (6**):** To *N*^α-Z-histidine octadecylamide (2.35 g, 4.4 mmol) in anhydrous acetic acid (5.0 ml), a 30% solution of hydrogen bromide in acetic acid (5.7 ml) was added with vigorous stirring at room temperature. The reaction mixture was stirred for 1 h and then poured into anhydrous ether (100 ml). **6**·HBr was filtered, thoroughly washed with ether, and then dissolved in ethanol (10 ml). The solution was basified with saturated aqueous Na₂CO₃. The product was extracted with ethanol and recrystallized from ethanol-ether, **6**: yield, 42%; mp 110–115 °C. Found: C, 70.79; H, 11.53; N, 13.73%. Calcd for C₂₄H₄₀N₄O: C,

70.93; H, 11.33; N, 13.79%.

Preparation of *N*-(ω -Aminoalkanyl)histamine (7, 8): A mixture of *Z*- β -Ala (3.94 g, 17.7 mmol) and DCC (4 g, 19.5 mmol) in a 1 : 2 mixture of dimethylformamide-acetonitrile (20 ml) was stirred in an ice bath for 45 min; the reaction mixture was then filtered and washed with dimethylformamide-acetonitrile (30 ml). To the filtrate, histamine dihydrochloride (3 g, 16.1 mmol) and NaOH (0.7 g) in H₂O (15 ml) were added in one portion; the mixture was vigorously stirred for 5 h, and then filtered. The filtrate was concentrated to about 40 ml and poured into cold 10⁻¹ M hydrochloric acid (250 ml, 1 M = 1 mol dm⁻³). The insoluble by-product (DCC urea, *Z*- β -Ala-DCC) was filtered off. The filtrate was basified with saturated aqueous Na₂CO₃ and cooled in a refrigerator overnight. The product thus precipitated was filtered, washed with H₂O, and then recrystallized from ethanol-ethyl acetate. *N*-(*N*-Benzyloxycarbonyl- β -alanyl)histamine: yield, 62%; mp 137–139 °C. *N*-[ω -(Benzyloxyamino)hexanoyl]-histamine: yield, 82%; mp 111–114 °C. Deblocking of the ω -amino group were conducted in a manner similar to that used for **6**. **7**: yield, 52%; mp 67–68 °C. Found: C, 52.89; H, 8.38; N, 29.94%. Calcd for C₈H₁₄N₄O: C, 52.75; H, 7.69; N, 30.77%. **8**: yield, 67%; mp 44–46 °C. Found: C, 59.04; H, 9.50; N, 24.32%. Calcd for C₁₁H₂₀N₄O: C, 58.93; H, 8.93; N, 25.00%.

The Schiff base was prepared by mixing solution of retinal (10⁻² M) and imidazole derivatives (10⁻² M) in absolute ethanol containing 3A molecular sieve 1/16 in. pellets at room temperature for 8 h in the dark. In the case of **4** and **6**, the reaction time was prolonged to 24 h. The absorption spectra were recorded on a Hitachi 200-10 spectrophotometer, using quartz cells 1 mm and 10 mm in path length. The pH of the protonating solution (90% aqueous ethanol) was measured on a Horiba M-7E pH meter. The calibration curve for the protonated *N*-retinylidenebutylamine (Bu-SBH⁺) satisfied the Lambert Law over the concentration range from 1.4 × 10⁻⁵ to 2.2 × 10⁻⁴ M.

Results and Discussion

Effect of Imidazole Derivatives on the Protonation of *N*-Retinylidenebutylamine (Bu-SB). Figure 1 shows the degree of protonation plotted against the pH values of the solution, in which the pH values were controlled by the addition of *Z*- α -Ala (10⁻² M) to **1** (2.5 × 10⁻³ M) in 90% aqueous ethanol. The basic nitrogen of imidazole ring (N³, p*K*_a 14.5) is, of course, neutralized with *Z*- α -Ala in a polar solvent, and some association may take place between **1** and *Z*- α -Ala. However, the absorption peak at 440 nm appeared even under neutral conditions, which indicates that undissociated imine hydrogen

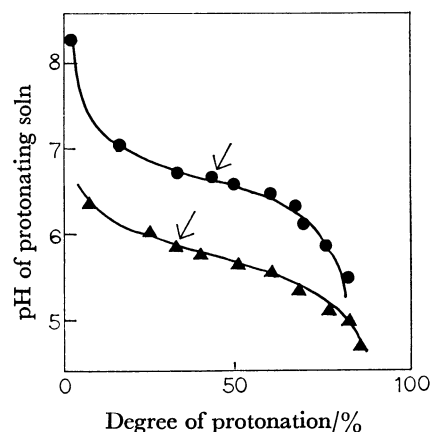


Fig. 1. Protonation of Bu-SB with amino acids.

●: With a mixture of **1** and *Z*- α -Ala, ▲: with *Z*- α -Ala only.

Bu-SB, 5 × 10⁻⁴ M. **1**, 2.5 × 10⁻³ M. Solvent, 90% aqueous ethanol. The arrows indicated the equimolar addition of *Z*- α -Ala to Bu-SB.

(N¹-H of imidazole ring) protonated the Schiff-base linkage.

In a nonpolar solvent such as chloroform, in which carboxylic acids with no steric hindrance at the α -position (*Z*- β -Ala, hexanoic acid) remain almost in undissociated or dimeric state, the extent of protonation increased upon the addition of **1** (5 × 10⁻³ M) to the

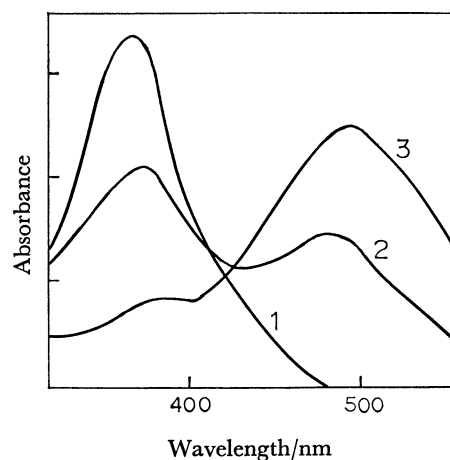


Fig. 2. Absorption spectra of **6**-SB

1: **6**-SB, 2: **6**-SBH⁺, 3: **6**-SBH₂⁺. Concentration, 3.3 × 10⁻⁵ M; solvent, chloroform.

TABLE 1. ABSORPTION MAXIMA OF IMIDAZOLE DERIVATIVES

$\lambda_{\max}/\text{nm}$	SBH ⁺ a)			SBH ₂ ⁺ b)		
	$\lambda_{\max}/\text{nm}$	$\Delta\lambda^c/\text{nm}$	Absorbance	$\lambda_{\max}/\text{nm}$	$\Delta\lambda^c/\text{nm}$	Absorbance
4 -SB 368	468	100	0.458	482	114	0.695
5 -SB 366	467.5	101.5	0.595	479	113	0.747
6 -SB 370	482	112	0.305	494	124	0.651
7 -SB 367	464	97	0.505	470.5	103.5	0.779
8 -SB 367	464	97	0.577	466.5	99.5	0.790
Bu-SB 366	460	94	0.848			

a) Monoprotonated Schiff base. b) Diprotonated Schiff base. c) The magnitude of shift against the Schiff base. Concentration, 3.3 × 10⁻⁵ M; solvent, chloroform.

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