A Remarkable Blue Shift of Retinal Protonated Schiff Base due to Electrostatic Interaction of Positive Charges

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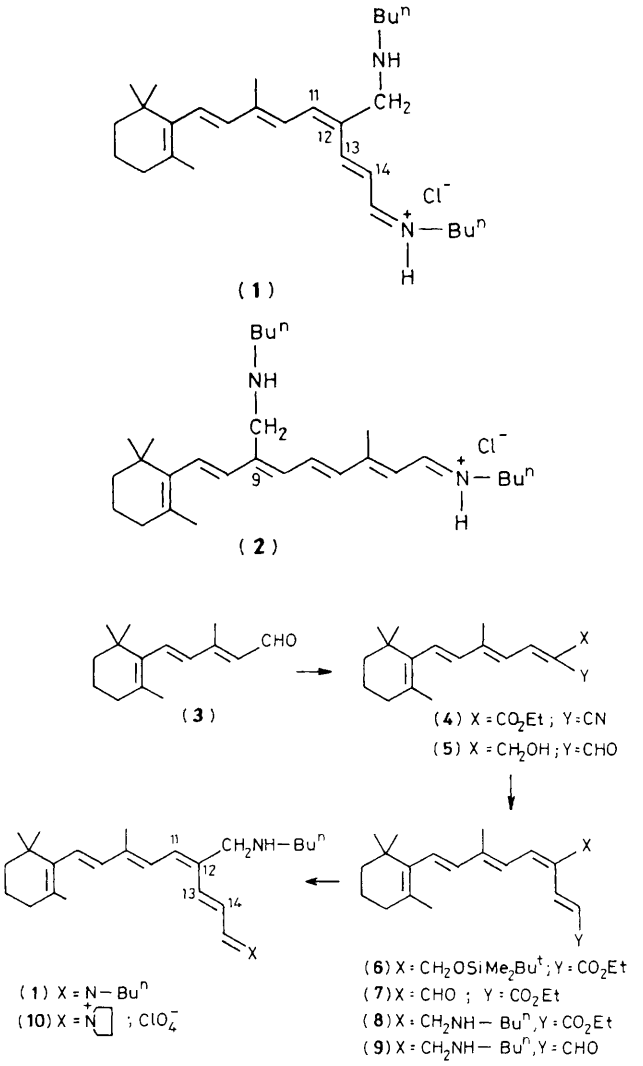
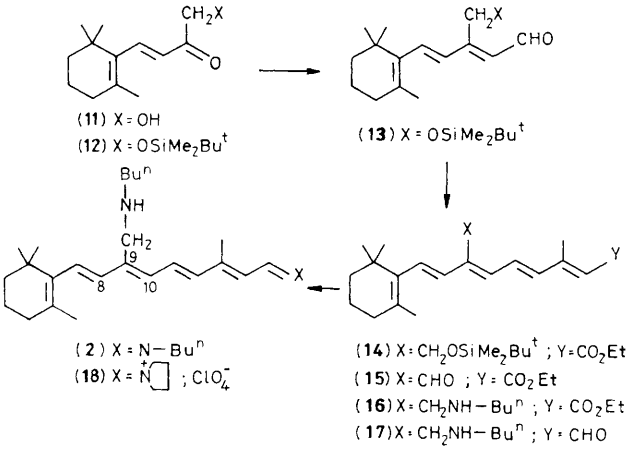
The absorption maxima of retinyl iminium polyene are more strongly affected by non-conjugated positive charge located in the vicinity of the β-ionyl moiety (**2**) than by those located in the vicinity of carbon atoms 12—14 (**1**).

The chromophore of rhodopsin, the visual pigment, consists of 11-*cis*-retinal[1] bound to the ε-amino terminal of a lysine residue of the apoprotein opsin *via* a protonated Schiff base linkage.[2,3] The 11-*cis*-retinal protonated Schiff base formed from n-butylamine absorbs at 440 nm in methanol,[4] whereas the maxima of visual pigments from various sources have maxima as far to the red as 580 nm. These red shifts (in cm^{-1}) from 440 nm which are due to the effects of the protein environment have been called 'opsin shifts.'[5]

Bacteriorhodopsin, the major constituent of the purple membrane of *Halobacterium halobium*, has all-*trans*-retinal as its chromophoric group linked to a lysine.[6] This pigment has



Scheme 1



Scheme 2

its absorption maxima at 560 nm. Recently, Nakanishi[5,7] has proposed the external point charge model, which places in addition to a counter-anion near the Schiff base iminium nitrogen, a second negative charge. In bovine rhodopsin, where the opsin shift is 2730 cm^{-1}, the second negative charge is located in the vicinity of carbon atoms 12—14 of the chromophore. In bacteriorhodopsin (opsin shift 4870 cm^{-1}), the second negative charge is located in the vicinity of the ionone moiety.

In order to gain information on the electrostatic interactions between non-conjugated charges and the retinyl-iminium polyene, we studied the effect of positive charges on the absorption maxima of compounds (**1**) and (**2**). In compound (**1**) (by protonation of the amino-group), a positive charge is located *ca.* 3 Å from C-12 and C-14, while in compound (**2**), it is *ca.* 3 Å from C-8 and C-10 (by protonation). The different locations of the positive charges along the polyene shed light on the sensitivity of the polyene to charges in its different parts.

Compound (**1**) was prepared from the aldehyde (**3**) (Scheme 1) by condensation with the sodium salt of ethyl cyanoacetate at −78 °C to give the cyano-ester (**4**) as the only isomer. Reduction with di-isobutylaluminium hydride in hexane at −78 °C afforded the hydroxy-aldehyde (**5**). The alcohol group was protected with t-butyl(dimethyl)silyl chloride, and then the aldehyde group was condensed with the sodium salt of triethyl phosphonoacetate at 25 °C to give, after separation of isomers, compound (**6**). Deprotection of the alcohol group with Bu_4NF in tetrahydrofuran (THF) and oxidation with MnO_2 afforded the aldehyde-ester (**7**): ^1H n.m.r. ($CDCl_3$) δ 1.07 (6H, s), 1.31 (3H, t, $CO_2CH_2\mathit{Me}$), 1.76 (3H, s, 18-Me), 2.17 (3H, s, 19-Me), 4.25 (2H, q, $CO_2C\mathit{H}_2Me$), 6.33 (1H, d, *J* 16 Hz, 8-H), 6.67 (1H, d, *J* 16 Hz, 7-H), 6.74 (1H, d, *J* 12 Hz, 10-H), 7.00 (1H, d, *J* 16 Hz, 13-H), 7.44 (1H, d, *J* 12 Hz, 11-H), 7.66 (1H, d, *J* 16 Hz, 14-H), and 10.62 (1H, s, CHO); u.v. λ_{max} (EtOH) 388 nm (ε 30 000). Reductive amination of (**7**), using Bu^nNH_2 and $NaBH_4$, gave the amino-ester (**8**), which then was transformed to the aldehyde (**9**) by reduction with di-isobutylaluminium hydride followed by oxidation with MnO_2: ^1H n.m.r. ($CDCl_3$) δ 1.04 (6H, s), 1.73 (3H, s, 18-Me), 2.04 (3H, s, 19-Me), 2.61 (2H, t, $C\mathit{H}_2CH_2NH$), 3.51 (2H, s, $C\mathit{H}_2NH$), 6.04—7.08 (5H, m), 7.65 (1H, d, *J* 16 Hz, 13-H), and 10.2 (1H, d, *J* 8 Hz, CHO); u.v. λ_{max} (EtOH) 377 nm (ε 32 000). The aldehyde was condensed with Bu^nNH_2 and pyrrolidine perchlorate, to give compounds (**1**) and (**10**) respectively.

Compound (**2**) was synthesized from β-ionone by bromination of its C-9-Me group using 5,5-dibromo-2,2-dimethyl-4,6-dioxo-1,3-dioxan.[8] The bromo-group was subjected to nucleophilic attack of sodium formate in MeCN (reflux; 30 min) and the resulting formate was hydrolysed with ammonium hydroxide in MeOH (30 min; 25 °C) to give the alcohol (**11**). Protection of the alcohol group with t-butyl(dimethyl)silyl chloride in dimethylformamide (DMF) with imidazole afforded the protected hydroxy-ketone (**12**).

Compound (**12**) was converted into the aldehyde (**13**) and then into (**14**), using standard Horner–Emmons reactions. The

Table 1. λ_{max}/nm of protonated schiff bases.

Chromophore	pH	λ_{max}	Solvent	Δν/cm^{-1}
(**2**)	2[a]	410	EtOH	1660[b]
(**2**)	2[a]	420	$CHCl_3$	2070[b]
(**18**)	8	450	EtOH	—
(**18**)	2[a]	420	EtOH	1590[c]
(**18**)	8	475	$CHCl_3$	—
(**18**)	2[a]	435	$CHCl_3$	1940[c]

[a] Acidified with HCl. [b] Δν Between (**2**) and all-*trans*-retinal protonated Schiff base in the same solvent. [c] Δν for (**18**) between pH 8 and pH 2.

all-*trans*-isomer of (**14**) was separated, using flash chromatography:[9] ^{1}H n.m.r. ($CDCl_3$) δ 0.12 (6H, s, $SiMe_2$), 0.90 (9H, s, $SiBu^t$), 1.02 (6H, s), 1.31 (3H, t, CO_2CH_2Me), 1.71 (3H, s, 18-Me), 2.33 (3H, s, 20-Me), 4.15 (2H, q, CO_2CH_2Me), 4.54 (2H, s, CH_2OSi), 5.65 (1H, s, 14-H), 6.1—6.3 (4H, m), and 6.9 (1H, dd, J_1 16, J_2 12 Hz, 11-H); u.v. λ_{max} (EtOH) 359 nm (ϵ 38 000). Compound (**14**) was converted into (**16**) by the method described above for the transformation of (**6**) to (**8**). The desired amino-aldehyde (**17**)† was obtained by reduction with di-isobutylaluminium hydride and oxidation with MnO_2. Compound (**17**) was condensed with n-butylamine and pyrrolidine perchlorate, to give compounds (**2**) and (**18**), respectively (Scheme 2).

The absorption maxima of (**18**) at pH 8, in EtOH, was very similar to the absorption maxima of the pyrrolidine perchlorate salt of all-*trans*-retinal in the same solvent (450 nm), showing that the presence of nitrogen atom at C-9 has no effect on the absorbance. Nevertheless, on protonation of the amino-group at C-9 (at pH 2), a blue shift to 420 nm ($\Delta\nu$ 1590 cm^{-1}) was obtained (Table 1). A similar blue shift ($\Delta\nu$ 1660 cm^{-1}) was observed with compound (**2**). At pH 2, when both the Schiff base and the amino-group were protonated, it absorbed at 410 nm in EtOH, while the protonated Schiff base of all-*trans*-retinal absorbed at 440 nm. In the nonpolar solvent $CHCl_3$, the blue shifts were even larger: protonation of (**18**) shifted the absorption maxima from 475 to 435 nm ($\Delta\nu$ 1940 cm^{-1}). Protonated (**2**) in $CHCl_3$ absorbed at 420 nm, while the protonated Schiff base of all-*trans*-retinal absorbed at 460 nm ($\Delta\nu$ 2070 cm^{-1}). Compounds (**1**) (436 nm, EtOH; 456 nm, $CHCl_3$) and (**10**) (446 nm, EtOH; 467 nm; $CHCl_3$) showed very similar absorption maxima to that of 11-*cis*-retinal–Bu^nNH_2 protonated Schiff base (at pH 2) and pyrrolidine perchlorate salt, respectively, both in EtOH and $CHCl_3$. The absorption maxima of (**10**) was changed by acidification from pH 8 to pH 2 by only 3 nm.

The experiments described above show that non-conjugated charges can affect the absorption maxima of retinyl iminium polyene.[10] A positive charge, located *ca.* 3 Å from C-9, causes a remarkable blue shift[11] of 1940 cm^{-1}. The blue shift is probably caused by destabilization of the excited state, and is stronger in non-polar solvents.

The results should encourage the synthesis of the corresponding analogues of retinal containing a negatively charged group in the vicinity of C-9. Spectroscopic information on such compounds would permit the evaluation of the external point charge model[5,7] for explaining the red shift in various rhodopsins.

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† This compound was very unstable and therefore was characterized as its Bu^nNH_2 Schiff base.

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