

A Study of π -Electron Delocalization in Model Compounds of Visual Pigment by UV and Carbon-13 NMR Spectra

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Abstract: Carbon-13 NMR and UV absorption spectra have been obtained for *all-trans-N*-retinylidene-*n*-butylamine and its acid-addition salts as an analogue of the Schiff's base linkage compound in visual pigment. Carbon-13 chemical shift changes on going from *all-trans-N*-retinylidene-*n*-butylamine to its acid-addition salts are observed. The π -electron density and π -bond order of polyene chain were calculated for these compounds by CNDO/2-MO method. The change in carbon-13 chemical shift was found to correlate quite well with that in π -electron density. It was deduced that the large red shift of UV absorption maximum by N protonation of *all-trans-N*-retinylidene-*n*-butylamine may be mainly due to the decrease of the degree of bond alternation and the delocalization of the positive charge in conjugated polyene chain. The effect of solvent (methanol and carbon tetrachloride) on the UV bathochromic shift and carbon-13 chemical shift was also discussed briefly.

In the process of visual excitation a visual pigment, rhodopsin, plays the role of a photoreceptor and the first step in the process is known as the photoisomerization of the rhodopsin visual chromophore, retinal, from the 11-*cis* to all-*trans* form.² The 11-*cis*-retinal which is the visual chromophore of the vertebrate has an absorption maximum at 380 nm. When this chromophore forms the visual pigment by combination with lipoprotein opsin, the position of its absorption maximum shifts to 500 nm, and thus the bathochromic shift is observed in the visual process.³ Many experimental results suggest that the retinal is attached by a Schiff's base linkage to the ϵ -amino group of a lysine residue in opsin,⁴ and the bathochromic shift has been regarded as a result of the formation of the protonated Schiff's base linkage of retinal with opsin. As the large bathochromic shift was not observed for the mere protonated Schiff's base compound,⁵ this shift may be induced by specific interactions between retinal and opsin. Many experimental studies have been carried out for model compounds in visual pigment and several theories have been proposed to explain the above spectral shifts.⁶⁻⁸ The proton nuclear magnetic resonance (NMR) spectra of stable isomers of retinal have been observed.⁹ Recently the carbon-13 NMR spectra of the several retinal isomers have also been observed,¹⁰ and this has enabled a study of the charge delocalization in all-*trans*-retinal Schiff's base to be undertaken.^{11,12}

In this paper, we will present the results of determination of the π -electron delocalization induced by N protonation in model compounds of visual pigment. In order to clarify the condition of visual pigment formation and the origins of the bathochromic shifts, it is necessary to use the pertinent model compounds which simplify the state of the binding site between retinal and opsin. In this study we will use the Schiff's base compound of retinal with *n*-butylamine, *N*-retinylidene-*n*-butylamine (NRB), as an analogue of the nonprotonated Schiff's base linkage compound and used its acid-addition salts, NRB·HCl, NRB·HI, NRB·HBr, NRB·TFA (trifluoroacetic acid), and NRB·TCA (trichloroacetic acid), as analogues of the protonated Schiff's base linkage compound in visual pigment. Recently, Shriver et. al.¹² have measured the carbon-13 NMR spectra of the propylamine Schiff's base of *all-trans*-retinal and its protonated species in CDCl₃ solution. It has been suggested that in the rhodopsin a Schiff's base linkage exists between the retinal chromophore and the protein opsin via an ϵ -amino group of lysine.⁴ Therefore, the Schiff's base with *n*-butylamine rather than that with propylamine may be a

more reasonable model for the rhodopsin. We will study the electronic state of these compounds by both experimental and theoretical methods. Experimentally, the UV and carbon-13 NMR spectra will be observed for these model compounds. We will calculate the π -electron densities and π -bond orders of polyene carbons by the CNDO/2-MO method and the chemical shift changes on going from NRB to NRB salts will be discussed. Finally, the correlation of the magnitude of the bathochromic shift and that of the electron delocalization will be discussed.

Experimental Section

Materials. Vitamin A was a commercially available sample manufactured by Riken Vitamin Co. Ltd., Tokyo. The crystal of *all-trans*-retinal was prepared by oxidation of vitamin A with manganese dioxide in petroleum ether. The Schiff's base linkage compound of *all-trans*-retinal with *n*-butylamine was prepared as follows.¹³ *n*-Butylamine (2 cm³) was added to a solution of *all-trans*-retinal (2 g) in petroleum ether (50 cm³) and then this solution was kept at room temperature for several hours. The solution was filtered and the filtrate was completely evaporated. The precipitate was dissolved in petroleum ether (2-3 cm³) and the solution was cooled to -20 °C and kept at this temperature in the dark for 2 days. The pale yellow crystal of *all-trans*-NRB was obtained. NRB·HCl was prepared by an addition of gaseous hydrogen chloride to a solution of NRB (1 g) in petroleum ether (20 cm³). Other NRB salts, NRB·HBr, NRB·HI, NRB·TFA, and NRB·TCA, were prepared by an addition of corresponding acid solutions in petroleum ether to solution of NRB in petroleum ether. These salts which were immediately precipitated from solution by an addition of acids were filtered and dried *in vacuo*. This procedure resulted in a 1:1 (molar ratio) salt of acid to base. Great care was taken not to add too much acid, because excessive acid results in decomposition of the product.¹³

Methods. Ultraviolet absorption (UV) spectra were observed on a Beckman-25 spectrometer. Solvents used were spectral grade CCl₄ and CH₃OH and were manufactured by Tokyo Kasei Co., Tokyo. Carbon-13 NMR spectra were observed on a JEOL-PS-100 spectrometer linked with a PFT-100 Fourier transform system at 25.14 MHz, JEC-6 spectrum computer, deuterium field-frequency lock, and noise-modulated proton decoupling system. All measurements were carried out using natural abundance sample in CCl₄ and CD₃OD in the dark. Pulse recycle times in a series of pulsed NMR measurements were chosen to be at least five times the spin-lattice relaxation times of the sample in order to avoid the effect of saturation. The number of repetitions of 90° pulses is specified in each figure caption as the number of accumulations. Chemical shifts were expressed as downfield shifts from internal tetramethylsilane (TMS). The CNDO/2 method was used to calculate the π -electron densities and

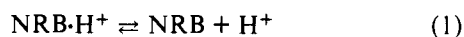
Table I. The UV Absorption Maximum (λ_{\max}) of *all-trans-N*-Retinylidene-*n*-butylamine and Its Acid-Addition Salts in CH_3OH and in CCl_4

Chromophore	Solvent	λ_{\max} , nm
NRB	CH_3OH	365
	CCl_4	366
NRB-TFA	CH_3OH	445
	CCl_4	450
NRB-TCA	CH_3OH	445
	CCl_4	445
NRB-HCl	CH_3OH	445
	CCl_4	445
NRB-HBr	CH_3OH	443
	CCl_4	450
NRB-HI	CH_3OH	440
	CCl_4	460

π -bond orders of polyene carbons in retinal molecule. Numerical calculation was carried out with the aid of the computer, HITAC 8700, at the Information Processing Center, Tokyo Institute of Technology.

Results

UV Absorption Spectra. Typical absorption spectra of NRB and its acid-addition salt are shown in Figure 1. In this figure, the spectra of NRB and NRB-HI in CH_3OH are shown. The absorption maximum (λ_{\max}) of NRB in CH_3OH (Figure 1A) appears at 365 nm. Besides this peak, a new absorption peak appears at 440 nm in the spectra of NRB-HI and the absorbance of this new peak increases gradually with increases of concentration of NRB-HI. At higher concentration the absorption of this new peak becomes larger than that of the peak at 365 nm as shown in Figure 1. The new peak at 440 nm may be reasonably assigned to the N-protonated Schiff's base linkage compound of retinal.¹³ The facts of simultaneous appearance of two peaks at 440 and 365 nm and the increase of ratio of molar absorption coefficients of these peaks with increase of salt concentration indicate the existence of an equilibrium reaction between the nonprotonated NRB and N-protonated NRB as follows



where $\text{NRB}\cdot\text{H}^+$ means the N-protonated NRB.

The UV spectra of other NRB acid-addition salts in CH_3OH were observed and similar results were obtained. Furthermore the UV spectra of these in CCl_4 were also observed. The results of UV spectra measurements are shown in Table I. It is obvious that the position of the N-protonated NRB's absorption depends on the kinds of acid of NRB salt and on the solvent used in the measurement. Considerable experimental evidence also indicates that the values of λ_{\max} of *N*-retinylidene-*n*-butylammonium cation and its C_{22} analogue are dependent on both the counteranion and the solvent.¹³ Generally speaking, the absorption maximum appears at almost the same or longer wavelength in CCl_4 than in CH_3OH . When the discussion is confined to the hydrohalide salts of NRB, an interesting change in the absorption maximum can be pointed out. In CCl_4 , the absorption maximum of the NRB salt shifts to longer wavelength with an increase in the size of the counteranion. An opposite shift is observed in CH_3OH . This contradiction in solvent effect cannot be explained at present.

Carbon-13 NMR Spectra of NRB and Its Salts. All peaks in the carbon-13 NMR spectra of NRB and N-protonated NRB have been assigned by using a variety of techniques including spin-lattice relaxation time measurements, proton-coupling and proton-decoupling experiments, application of the NMR shift reagent, and by comparison to the carbon-13 NMR spectra of related retinal isomers, as shown in a previous

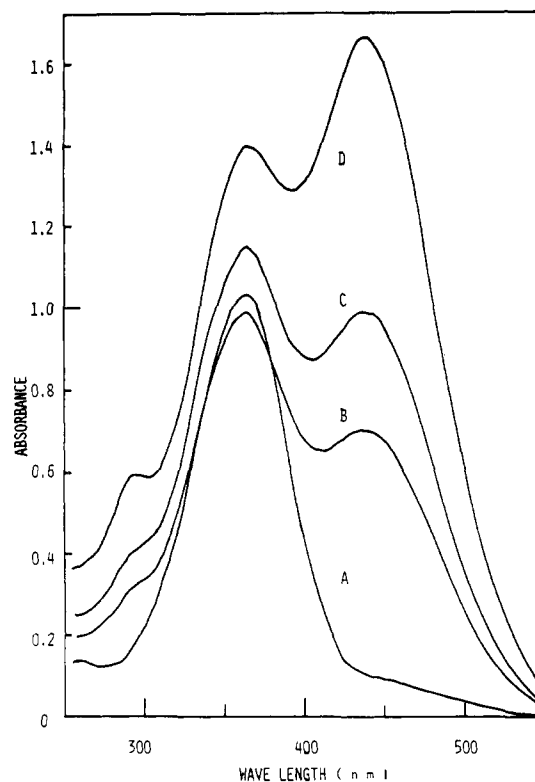
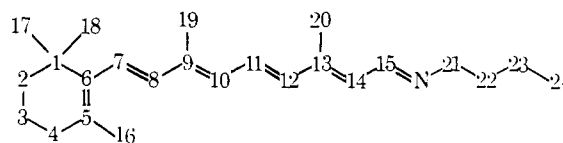


Figure 1. UV absorption spectra of *all-trans-N*-retinylidene-*n*-butylamine (NRB) and its HI salt in CH_3OH . (a) NRB (1.9×10^{-5} mol/L), (b) NRB-HI (3.5×10^{-5} mol/L), (c) NRB-HI (4.3×10^{-5} mol/L), (d) NRB-HI (6.0×10^{-5} mol/L).

paper.^{10,11} The carbon-13 FT NMR spectrum of NRB-HCl in CD_3OD consists of only one set of peaks, indicating that only one isomer with respect to the protonated species was present. As mentioned in a previous section, there may be equilibrium reaction between the nonprotonated and N-protonated NRB in the solution of NRB-HCl and this chemical exchange reaction must be rapid enough for only one set of carbon-13 resonance peaks to be observed. In the rapid-exchange limit, the resonant position of a given nucleus is given by average frequency δ_{av} as follows

$$\delta_{\text{av}} = \delta_p P + \delta_n (1 - P) \quad (2)$$

where δ_p is the frequency of an N-protonated NRB, δ_n is the frequency of a nonprotonated NRB, and P is the probability that the NRB molecule is found in an N-protonated state. As shown in the UV spectra of the acid-addition salt of NRB, the probability P increases with an increase of the salt concentration. The salt concentration of carbon-13 NMR sample is approximately 1 mol/L, whereas that of the UV sample is approximately 10^{-5} mol/L. Thus the probability P can be safely assumed to be almost unity, that is, the carbon-13 NMR spectrum can be considered to be exclusively that of N-protonated NRB-HCl. In Figure 2 are shown the results of assignment of the conjugated polyene carbons in the form of a stick spectra. The numbering of the carbons in NRB is given by the following fashion



all-trans-N-retinylidene-*n*-butylamine (NRB)

It is noticeable that the chemical shifts in the conjugated polyene carbons of NRB-HCl in CD_3OD are distributed in a

Table II. The Carbon-13 Chemical Shifts of Conjugated Polyene Chain Carbons in Acid-Addition Salts of *all-trans-N*-Retinylidene-*n*-butylamine^a

Chromophore	Solvent	5	6	7	8	9	10	11	12	13	14	15	N
NRB·HCl	CD ₃ OD	2.2	-0.1	6.4	-0.0	8.1	-0.1	11.0	-3.8	19.7	-9.3	5.8	
NRB·HBr	CD ₃ OD	2.2	-0.1	6.4	-0.1	8.1	-0.1	11.1	-3.8	19.8	-9.2	5.8	
NRB·HI	CD ₃ OD	2.3	-0.3	7.0	-0.8	8.3	-0.5	11.1	-5.1	22.8	-10.9	8.1	
NRB·TFA	CD ₃ OD	2.2	-0.1	6.3	-0.0	8.1	-0.1	11.1	-3.7	19.7	-9.1	5.8	
NRB·TCA	CCl ₄	2.3	-0.8	7.0	-0.8	7.8	-0.6	10.9	-5.3	21.4	-10.1	8.5	
	CD ₃ OD	2.2	0.0	6.4	0.1	8.2	0.0	11.3	-3.6	20.0	-9.2	5.8	
NRB·TCA	CCl ₄	2.3	-0.9	7.1	-0.8	7.7	-0.6	10.9	-5.3	21.2	-10.9	8.3	

^a Chemical shifts of each carbon were expressed as downfield (plus) and upfield (minus) shifts from corresponding carbon of *all-trans-N*-retinylidene-*n*-butylamine.

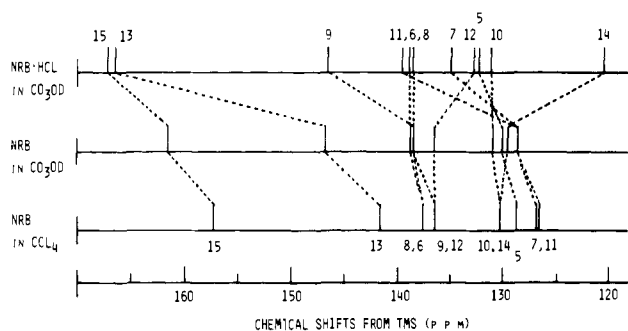


Figure 2. Correlation diagram for carbon-13 chemical shifts of unsaturated carbons in NRB in CCl₄ and in CD₃OD and NRB·HCl in CD₃OD.

relatively wide range of approximately 46 ppm, whereas those of NRB in these solvents are in a range of approximately 33 ppm. Those of NRB in CCl₄ are in the range of 30 ppm and those of *all-trans*-retinal are in a wide range of 60 ppm.¹¹ These interesting results will be discussed in terms of the distribution of the π electron in the following section. The solubilities of NRB·HCl and NRB·HBr in CCl₄ and that of NRB·HI in CD₃OD are so poor that we cannot observe the carbon-13 NMR spectra of these salts.

The chemical shifts of the conjugated polyene carbons in NRB salts relative to NRB are shown in Table II. The plus and minus signs mean respectively the downfield and upfield shifts by N protonation. For conjugated polyene carbons, it is clearly recognized that the peaks assigned to the odd-numbered carbon shifts in the direction of downfield and the peaks assigned to the even-numbered carbons remain in the same position or shift to upfield. The downfield shifts of odd-numbered carbons are pronounced. The experimental result that the chemical shifts in the polyene chain can be classified into two types according to whether the numbering on the carbon is even or odd may be explained by the changes in the bond alternation and localization of π electrons in the polyene chain when NRB forms the N-protonated Schiff's base linkage.

The chemical shift data depend little on the kinds of acids but notably depend on the kinds of solvents. Particularly, the relative chemical shift of C(15) in CCl₄ is about 8 ppm, whereas that in CD₃OD is about 5.8 ppm. It is expected that the order of delocalization of the positive charge on the polyene chain depends on the strength of the cation-anion attraction at the protonated N atom. If the counteranion is farther away from the N atom, the positive charge is more loosely attracted to the N atom and then the positive charge is more delocalized on the polyene chain. In CD₃OD, the electronegative hydroxyl group of methanol molecule can interact with the N-protonated NRB cation, whereas there is no interactive group in the CCl₄ molecule. The positive charge induced by N protonation may be more strongly attracted to the N atom in CD₃OD than in CCl₄. Thus, the magnitude of the downfield shifts of odd-numbered carbons in CD₃OD becomes smaller than those in CCl₄.

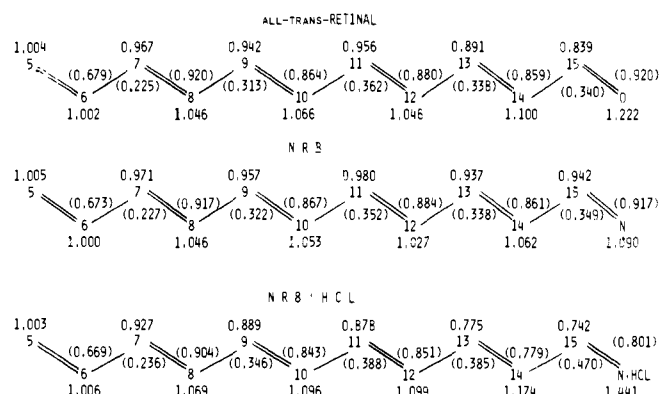


Figure 3. π -electron densities and bond orders (in parentheses) of conjugated polyene chain in (a) *all-trans*-retinal, (b) NRB, and (c) NRB·HCl.

Discussion

π -Electron Densities and π -Bond Orders of Polyene Chain.

To understand clearly the origin of chemical shift differences in polyene carbons of NRB and its acid-addition salts, it is necessary to know the electronic structure of polyene carbons of these compounds. Generally the carbon-13 chemical shifts of any specified carbon atom, A, in a molecule may be approximated as follows^{14,15}

$$\delta^A = \delta_d^A + \delta_p^A + \sum_{B(\neq A)} \delta^B + \delta_{ring}^A \quad (3)$$

where δ_d^A and δ_p^A are the diamagnetic and paramagnetic contributions on the specified carbon atom A, respectively, $\sum \delta^B$ is sum of the contributions from the neighboring atoms bonded to the atom A, and δ_{ring}^A is the contribution from the ring current. Usually the effects of $\sum \delta^B$ and δ_{ring}^A in the carbon-13 chemical shifts are known to be very small compared with the remaining terms. Karplus and Pople¹⁶ have deduced that the carbon-13 chemical shifts of conjugated π -electron systems are generally dominated by the contribution from the paramagnetic term and that they are related to the π -electron density and π -bond order. According to the theoretical calculation,¹⁶ when a 2p electron is added, the increase in δ_d^A is only about 10% of the entire observed range of the carbon-13 chemical shifts. The differences in carbon-13 chemical shifts among the same class of carbon atoms are mostly due to the difference in δ_p^A . In this paper, to investigate the correlation between the electronic structure and the carbon-13 chemical shifts of conjugated polyene carbons, the π -electron density and π -bond order of polyene carbons of *all-trans*-retinal, NRB, and NRB·HCl were calculated by using the CNDO/2-MO method. The data of x-ray analysis¹⁷ were taken as the intramolecular coordinates of these compounds. In Figure 3 are shown the results of calculation of the π -electron density and π -bond order. On going from *all-trans*-retinal to NRB π -electron density on odd-numbered carbons increases but

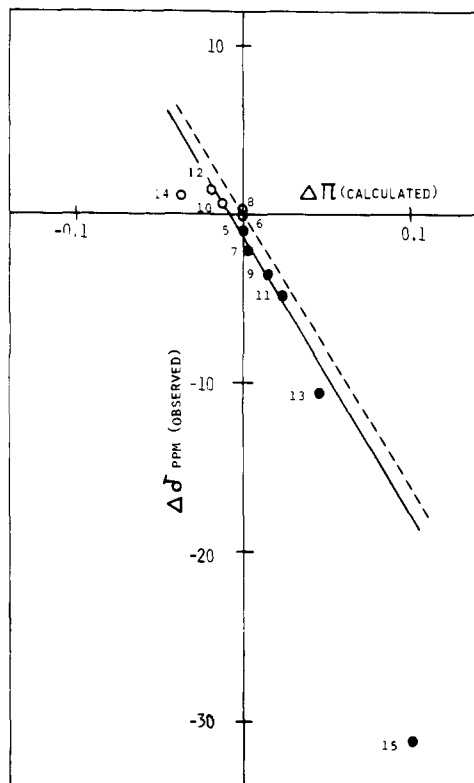


Figure 4. The plot of the chemical shift differences $\Delta\delta$ vs. π -electron differences $\Delta\pi$. Each value of $\Delta\delta$ and $\Delta\pi$ is derived by subtraction of chemical shifts and π -electron density of *all-trans*-retinal from those of NRB, respectively. A broken line represents the theoretically expected correlation.

those on even-numbered carbons decreases, whereas on going from NRB to NRB·HCl opposite phenomena are observed. For π -bond order, there are no remarkable differences between *all-trans*-retinal and NRB, but on going from NRB to NRB·HCl π -bond orders of double bonds decrease and those of single bonds increase, clearly showing the formation of N-protonated Schiff's base linkage causes the decrease of the degree of the bond alternation. The changes are slightly larger in the vicinity of the polyene chain end. Spiesscke and Schneider¹⁸ showed the simple linear correlation between the carbon-13 chemical shifts, δ , and the local excess π electron, $\Delta\pi$, in aromatic ring carbon systems, such as

$$\delta = c\Delta\pi \quad (4)$$

where the constant c is found empirically to have a value about 160 ppm per electron. In Figure 4 are shown the plots of observed carbon-13 chemical shift differences $\Delta\delta$ vs. calculated π -electron density differences ($\Delta\pi$) between NRB and *all-trans*-retinal, where $\Delta\delta$ and $\Delta\pi$ are values derived by subtraction of chemical shifts and π -electron density of *all-trans*-retinal from those of NRB, respectively. The good linearity is obtained, except for C(14) and C(15). A broken line shown in the figure represents the correlation derived from eq 4. The abscissa and the slope of the experimental straight line (solid) are in good agreement with those of the broken line. These results show that the chemical shift differences of polyene carbons between *all-trans*-retinal and NRB are mainly related to the differences of π -electron density. When the terminal atom of the polyene chain changes from oxygen to nitrogen, total π -electron density of conjugated polyene carbons of NRB increases as a result of the decrease of electronegativity at the terminal atom. This increase of π -electron density results in the increase of π -electron density of odd-numbered carbons of NRB and this causes the upfield shift

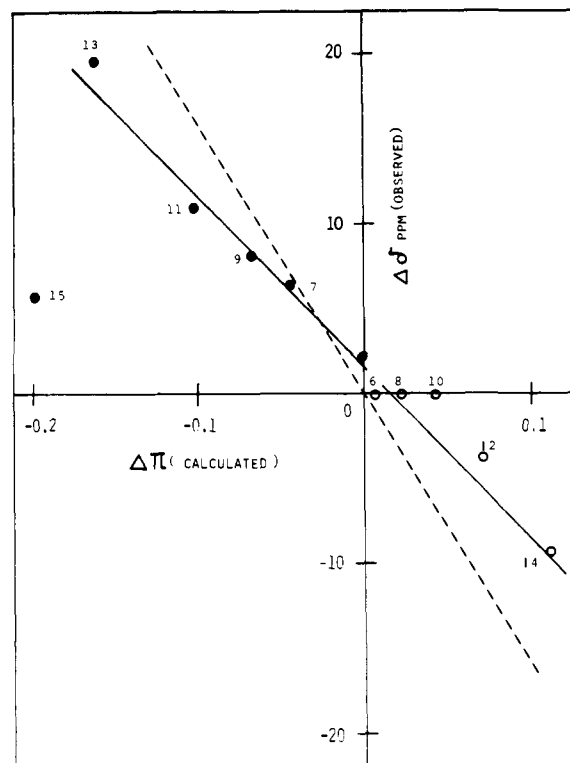


Figure 5. The plot of the chemical shift differences $\Delta\delta$ vs. π -electron differences $\Delta\pi$. Each value of $\Delta\delta$ and $\Delta\pi$ is derived by the subtraction of chemical shifts and π -electron density of NRB from those of NRB·HCl, respectively. A broken line represents the theoretically expected correlation.

of these carbons.¹¹ In Figure 5 are shown the plots of observed chemical shifts vs. π -electron density differences of NRB·HCl relative to NRB. The good linearity between $\Delta\delta$ and $\Delta\pi$ and good conformity with eq 4 are observed, except for C(15). The latter conformity shows that the positive charge at the N atom generated by protonation is distributed onto the polyene carbons, especially onto the odd-numbered carbons, and this delocalization of positive charge results in the lower field shift for these carbons (also see Figure 2). The experimental correlation shown by the solid line, however, slightly deviates from the broken line derived from eq 4. This small deviation should originate from the facts that the carbon-13 chemical shifts of NRB·HCl are affected by the species of solvent. Unfortunately such solvent effect could not be taken into consideration in the CNDO/2-MO calculation. We may safely say, as a first approximation, that the change in carbon-13 chemical shifts of conjugated polyene chain of NRB and its salts can be directly related to that in π -electron density. Recently, Shriver et al.¹² have also measured the carbon-13 NMR spectra of the propylamine Schiff's base of *all-trans*-retinal and its protonated species in CDCl₃ solution. Their results show that the most important contribution to the chemical shift is the charge density and the relative chemical shifts on going from propylamine Schiff's base to its protonated species can be qualitatively interpreted in terms of a charge density-chemical shift correlation by means of the Karplus-Pople relation.¹⁶ Our results of relative chemical shifts (both in sign and magnitude) presented here are compatible with those of Shriver et al. However, they have not discussed the correlation between the bathochromic shift and the electron delocalization. They also have not paid any attention to the change of the bond alternation.

Correlation between UV Bathochromic Shift and Carbon-13 Chemical Shift. It is well known that the absorption maximum shows a hypsochromic (blue) shift with a magnitude of about

Table III. The Sum of the Odd-Numbered Carbon's Chemical Shifts, Relative to *all-trans-N*-Retinylidene-*n*-butylamine, $\Sigma\Delta\delta$, and the UV Absorption Maximum, λ_{\max} , of Acid-Addition Salts of *all-trans-N*-Retinylidene-*n*-butylamine

Chromophore	Solvent	$\Sigma\Delta\delta$, ppm	λ_{\max} , nm
NRB-HCl	CD ₃ OD	47.4	445
NRB-HBr	CD ₃ OD	47.6	443
NRB-HI	CCl ₄	51.5	460
NRB-TFA	CD ₃ OD	47.4	445
	CCl ₄	49.4	450
NRB-TCA	CD ₃ OD	48.1	445
	CCl ₄	49.2	445

20 nm on going from *all-trans*-retinal to NRB, while there is a large bathochromic (red) shift of about 80 nm by N protonation on NRB. These phenomena have been explained by the concept that π electrons of conjugated polyene carbons are delocalized by the collapse of the bond alternation.⁷ Schaffer et al.,⁸ however, assumed that there is no collapse of the bond alternation between the *all-trans*-retinal and NRB for consideration of the spectroscopy of them. The latter assumption is supported by x-ray¹⁷ and resonance-enhanced Raman spectra.¹⁹ As shown in the previous section, the π -bond order shows no remarkable difference between *all-trans*-retinal and NRB, but on going from NRB to NRB-HCl the decrease of the degree of the bond alternation is remarkable and the localization of π electron (delocalization of the positive charge) of NRB-HCl is slightly larger than that of *all-trans*-retinal or NRB. Therefore, the blue shift with a magnitude of about 20 nm may be due mainly to the delocalization of π electron, while the large red shift of about 80 nm by N protonation on NRB may be due mainly to the decrease of the degree of the bond alternation and the delocalization of the positive charge. It is expected that the sum of the carbon-13 chemical shifts of odd-numbered polyene carbons, except for C(15), in NRB salt relative to those of NRB may represent the magnitude of positive charge delocalized on these carbons, since these carbons show the large lower field shifts by N protonation. The sum of the odd-numbered carbon's chemical shifts ($\Sigma\Delta\delta$) and the absorption maximum (λ_{\max}) of NRB salts are tabulated in Table III. In CCl₄, it is obvious, from a comparison between $\Sigma\Delta\delta$ and λ_{\max} , that the absorption maximum shifts to longer wavelength with increasing the value of $\Sigma\Delta\delta$. Thus, this

bathochromic shift can be related to the magnitude of positive charge delocalization on the conjugated polyene carbons; the latter may be related to the degree of influence of counteranion in CCl₄. In CD₃OD, on the other hand, the variations of $\Sigma\Delta\delta$ and λ_{\max} are small and nearly constant. This result may be due to the solvent effect of methanol, which is lowering the influence of counteranion.

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