Anion-Induced Wavelength Regulation of Absorption Maxima of Schiff Bases of Retinal†

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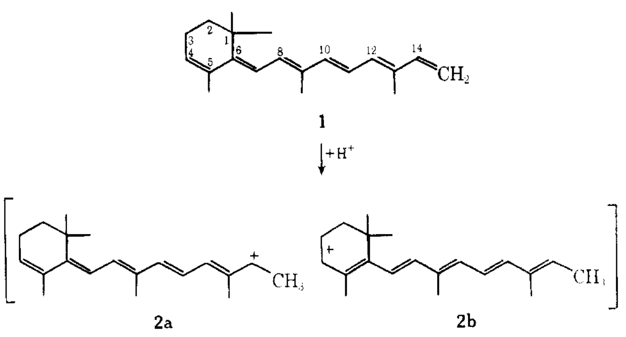
ABSTRACT: Ten pure salts of *N*-retinylidene-*n*-butylamine were prepared and their spectra recorded in $CHCl_3$, CCl_4, and benzene. In nonleveling solvents such as $CHCl_3$, CCl_4, and benzene there was an increase in λ_{max} which roughly followed the acid strength. There is a relationship between the light absorption energy and the ionic electrostatic interaction energy, $E_i = Z_C Z_A e^2/(r_c + r_a)$, of the salt. The ionic radii r_c (cation) and r_a (anion) are taken from crystal data. A plot of light absorption energy *vs.* E_i extrapolated to $E_i = 0$ gives a limiting value for the cation when the anion is removed to infinite distance. A quantitative fundamental relationship is derived for this phenomena: $\Delta E = \Delta E^0 + de^2/\epsilon d^2$. Values of ΔE^0 from this equation give an average of 582 nm in comparison to values of 588–590 nm obtained experimentally for the retinylic cation. It is concluded that the proximity of the anion regulates the λ_{max} of the cation. It is proposed that in the visual pigment, the protein can regulate the wavelength by moving the site of negative charge density by conformational change.

The two most important unsolved spectroscopic problems relating to visual pigments concern the bathochromic shift and wavelength regulation. The problem of the bathochromic shift can be appreciated when it is realized that retinal absorbs at *ca.* 367 nm in a hydrocarbon; yet when it is combined with a visual protein to form a visual pigment, the pigment may absorb at a wavelength as long as 575 nm. The question naturally arises, what molecular forces does the protein employ to induce the bathochromic shift? Actually, a more fundamental and important question is, what intrinsic structural property of retinal can be modified to allow this normally short wavelength absorber to absorb at these very long wavelengths? The second problem, that concerning wavelength regulation, can be appreciated when it is realized that in the human eye, although retinal is assumed to be the only small organic chromophoric molecule in the pigments, there are four visual pigments. This means that visual proteins have learned not only to shift the absorption band of the optical spectrum of retinal to long wavelengths, but in addition they have also learned how to regulate the wavelength shift to selected required values. It is obvious from these remarks that one cannot hope to understand wavelength regulation in a visual pigment unless it is understood in small organic chromophore molecules.

All evidence points to the conclusion that retinal is attached to a primary amino group associated with the visual protein. Some evidence has been presented to suggest that it is attached to lysine (Bownds, 1967; Akhtar *et al.*, 1967), whereas other evidence suggests it is attached to phosphatidylethanolamine (Kimbel *et al.*, 1970). In any event the proposed Schiff base linkage is commonly accepted, and this dictates that the bathochromic shift must be explained eventually in terms of a Schiff base of retinal.

In our laboratory, we have studied the ways in which large bathochromic shifts are induced. In a number of earlier articles (Blatz and Pippert, 1966, 1968; Blatz *et al.*, 1968a, 1968b) and in a recent article we have examined this problem in detail (Blatz *et al.*, 1971a). It was shown that retinol which absorbs at 325 nm could be converted easily into an electrochromic species, the retinylic cation, which absorbs at *ca.* 590 nm. A large number of related compounds were examined in this manner. Retinol belongs to a class of compounds called linear *even* alternant hydrocarbons. Species of this class are known to have an even number of atoms in their π-electron system and consequently have an even number of molecular orbitals in their LCAO solution; they also exhibit a high degree of bond order alternation. Also, compounds in this class are known to absorb at a relatively short wavelength and each additional double bond contributes approximately 25–30 nm to the λ_{max} of the compound. Under the influence of suitable experimental conditions, members of this major class are converted into another major class. This latter class, represented by the retinylic cation, is that of the linear conjugated *odd* alternant hydrocarbon. Species in this class have an odd number of atoms in their π system, an odd number of molecular orbitals in their LCAO solution, a low degree of bond order alternation, an absorption maximum at long wavelength, and each additional double bond has a value of 65–75 nm.

The conversion process and the properties of each class are exemplified by protonation of anhydroretinol (**1**). Compound **1** is a linear conjugated homoatomic even orbital polyene with pronounced bond alternation and a λ_{max} of 370 nm. When **1** is protonated at C_{15}, the product, the anhydroretinylic



1

2a

2b

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cation (**2a** and **2b**), becomes an odd orbital polyene with little bond order alternation and a λ_{max} of 620 nm. In **1**, an even orbital polyene, C_{15} has sp^2 hybridization; it contributes a p orbital to the π system and consequently, it is included in the π system. On the other hand, when **1** is protonated at C_{15}, that atom rehybridizes from sp^2 to sp^3 and no longer participates in the π system. Consequently, the even orbital system of **1** is converted into the odd orbital system of **2**. Many other examples of this kind of change are given in the above mentioned references (Blatz *et al.*, 1968b, 1971a).

Compound **2** has a homoatomic π system composed of 11 carbon atoms; it is a pentaenylic cation and it absorbs at 620 nm. The question arises as to what would happen if the π system contains heteroatoms. Consider the following polymethine dye $(CH_3)_2\ddot{N}(CH{=}CH)_4CH{=}N(CH_3)_2$ (**3**). It too has 11 atoms in the π system, nine carbons bounded at either end by nitrogen. This compound is in fact a pentaenylic cation, and it also absorbs at 625 nm (Malhotra and Whiting, 1960). Evidently, replacement of the terminal π system carbon atoms by nitrogen atoms has little effect on the absorption maximum.

Instead of replacing both terminal carbons with nitrogens let only C_{15} be replaced with nitrogen. The resulting compound is exemplified by the Schiff base of retinal, *i.e.*, *N*-retinylidene-*n*-butylamine (**4**) shown in Figure 1. This compound is of the conjugated, linear, heteroatomic, even orbital class, and it should absorb close to the value for **1**, *i.e.*, 370 nm. In fact, it has a λ_{max} *ca.* 360 nm. Now let **4** be treated with acid in the same manner in which **1** was treated. On the basis of what happens to **1**, it could be predicted that the proton would in fact add to the nitrogen, and the nitrogen, in turn, could rehybridize from sp^2 to sp^3 resulting in a compound represented by resonance structures **5b** and **5c**. Inspection of **5b** and **5c** shows that the π system is now odd and similar to the π system in **2**. In fact, it is the retinylic cation, and therefore it can be concluded that this pentaenylic cation should absorb at 590 nm. If, indeed, protonated **4** did absorb at 590 nm, we would understand the chemical mechanism and the resulting molecular structure since we do understand the mechanism and structure in going from **1** to **2**. We are brought up short when it is realized that experimental observations do not agree with theory. Protonated **4** absorbs at 440 nm in methanol and not at 620 nm (Pitt *et al.*, 1955).

In summary it has been shown that odd homoatomic π systems absorb at *ca.* 600 nm; odd π systems terminated at both ends by nitrogen also absorb at *ca.* 600 nm. However, when a single nitrogen atoms terminates an odd π system only a fraction of the possible bathochromic shift occurs. In this paper we examine the spectroscopic properties of the conjugate acid of **4**.

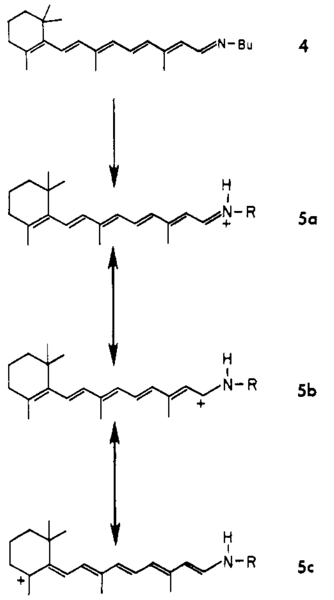

FIGURE 1: Structure of *N*-retinylidene-*n*-butylamine (**4**) and its conjugate acid **5**. **5a**, **5b**, and **5c** are major contributing resonance structures; **5a** is a major contributor when the anion is close and a minor contributor when the anion is distant. ($\mathbf{4} + H^+ \rightarrow \mathbf{5a}$.)

Experimental Procedures

A. Compound Preparation. 1. ALL-*trans*-*N*-RETINYLIDENE-*n*-BUTYLAMINE. The procedure followed in making the butylamine Schiff base of retinal was essentially the same as reported previously with only minor modifications (Erickson and Blatz, 1968; Blatz *et al.*, 1971b).

One gram of butylamine, freshly distilled under nitrogen, was added to a solution of 50 mg of all-*trans*-retinal in 5 ml of acetonitrile. This solution was allowed to stand at room temperature over a small amount of K_2CO_3 and a few beads of Linde 4A Molecular Sieve for approximately 3 hr. Before standing, the reaction flask was flushed with nitrogen and stoppered. Following the 3-hr reaction period, the solution was filtered through a small Büchner filter then transferred to a 25-ml round bottom flask in which it was evaporated to dryness using a rotary evaporator. The solid residue was redissolved in anhydrous ether and evaporated twice to assure complete removal of butylamine. This unrecrystallized residue was, in fact, found to have a sharp melting point at 63°, and recrystallization from a hydrocarbon solvent (at great loss of product) changed the melting point to 64°. Both the recrystallized and the unrecrystallized compound gave a simple absorption band at 363 nm in ethanol.

2. ALL-*trans*-*N*-RETINYLIDENE-*N*-BUTYLAMMONIUM SALTS. a. NRBA-HF,[1] NRBA-HCl, NBRA-HBR, and NBRA-HI. These salts were prepared by adding anhydrous acid to a solution of 30 mg of NRBA in 5 ml of anhydrous ether. The acid may be added either by bubbling the gaseous acid directly into the NRBA solution or by making a saturated solution of acid in ether which is added slowly to the NRBA solution until a voluminous precipitate results. If the former method is used, care must be taken not to add too much acid. Excessive acid results in decomposition of the product. Following the addition of acid, the flask containing the precipitated salt was flushed with nitrogen, stoppered, and allowed to stand overnight in the refrigerator to allow the fine salt precipitate to digest. The salt was filtered and dried with vacuum and stored under nitrogen in the refrigerator. These salts decompose at temperatures in excess of 120°. An analysis of NRBA-HBr gave: C, 65.47% (68.5%); H, 8.72% (9.0%); N, 3.96% (3.3%); Br, 19.62% (19.0%); the theoretical values are in parentheses. Although the other salts gave reasonably good elemental analyses for salts like these, the analysis of NRBA-HF indicated between four and five molecules of HF were attached to each molecule of Schiff base.

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[1] NRBA = *N*-retinylidene-*n*-butylamine (also compound **4**), $NRBAH^+$ = *N*-retinylidene-*n*-butylammonium cation, NRBA-HA = the HA salt of NRBA.

b. NRBA-trichloroacetic Acid. A Skelly F (petroleum ether) solution of dry trichloroacetic acid was added to the ether solution of NRBA. Only a color change resulted at first, but after cooling the solution in Dry Ice then storing it for 2 days in the freezer, large nicely formed crystals resulted. The product was dried and stored in the same manner as the NRBA-HA salts and had a melting point of 104–106°.

c. NRBA-HNO_3. A solution of anhydrous HNO_3 in Skelly F was added to an ether solution of NRBA. The resulting precipitate was handled in the same manner as in previous salt preparations. The product decomposes above 120°. Anhydrous HNO_3 was prepared by two different procedures. HNO_3 was distilled from a 50:50 mixture of fuming nitric acid and concentrated sulfuric acid. A few drops of this anhydrous material were added to Skelly F chilled in Dry Ice. By the second procedure, concentrated nitric acid was added to cold Skelly F over P_2O_5. The first procedure probably gives a more pure product.

d. NRBA-*p*-toluenesulfonic Acid. A small amount of ether solution of dry *p*-toluenesulfonic acid was added to an NRBA–ether solution (about 50 mg in 5 ml). The precipitate was allowed to digest overnight, filtered, and dried as previously described. The product melted at 111–113°.

e. NRBA-picric Acid. The same procedure as that followed in making NRBA-*p*-toluenesulfonic acid can be used to form NRBA-picric acid. The product decomposes above 120°.

f. NRBA-$HClO_4$. A similar procedure to that followed in making NRBA-HNO_3 is applicable to the formation of this salt. Care should be taken in handling the Skelly F–perchloric acid solution; heating, as during washing of P_2O_5 from the flask, causes the mixture to detonate. This salt also decomposes above 120°.

Certain general precautions apply to the preparation and use of NRBA and its salts. All reagents and solvents should be of high purity. Retinal was used as received from Distillation Products Industries. The butylamine was distilled under nitrogen (bp 67°). Gaseous HF and HCl were purchased from Matheson Coleman and Bell, and HBr and HI were purchased from Union Carbide. The purity of these gases is stated to be 99.9%, 99.0%, 99.8%, and 98.0%, respectively. All other acids used were reagent grade, except trichloroacetic acid which was purified by recrystallization, dried in a drying pistol, and stored in a desiccator over P_2O_5. NRBA and its salts should be prepared and handled in dim red light and stored in light tight containers in the freezer; under these conditions it may be stored for about 3 months.

B. Solvent Purification. In most instances solvent purifications were carried out according to procedures found in Weisberger *et al.* (1955). Unless otherwise noted, solvents were distilled through a 40-cm jacketed column packed with 2- to 3-mm glass beads and protected from atmospheric contamination during distillation by bubbling nitrogen continuously. The purified, halogenated hydrocarbon solvents were stored in brown bottles under nitrogen and over Molecular Sieve and kept in the refrigerator.

1. CARBON TETRACHLORIDE. Carbon tetrachloride was treated with three portions of alcoholic KOH then washed several times, until neutral, with water. The washed solvent was dried and distilled from P_2O_5.

2. CHLOROFORM. Several procedures were tried for purifying chloroform, but the procedure which gave the most acid-free product was as follows. Reagent grade $CHCl_3$ was washed twice with distilled water, then washed twice again with water that had been boiled to remove CO_2. Red and blue litmus indicated neutrality in the final wash. The washed $CHCl_3$ was dried for 16 hr over K_2CO_3 and distilled from P_2O_5 or Molecular Sieve.

3. 1,2-DICHLOROETHANE. The purification was reported previously (Blatz *et al.*, 1971a).

4. BENZENE. Benzene was purified by shaking with two portions of concentrated H_2SO_4, then washing once with distilled water, twice with dilute KOH, and twice again with distilled water. The washed benzene was dried over K_2CO_3 and distilled from over P_2O_5.

5. ACETONITRILE. Acetonitrile was distilled over P_2O_5.

6. METHANOL. Methanol was dried and purified by refluxing over magnesium for 16 hr, then distilling from the reflux pot through the 40-cm packed column. One should be cautious in starting the methanol–magnesium reflux since the reaction is quite exothermic.

7. ETHANOL. U. S. Industrial Chemicals Company absolute ethanol was used with further purification.

8. TETRAHYDROFURAN. THF was dried over $LiAlH_4$ for 40 hr then distilled through the 40-cm packed column.

Method of Calculation

The method of Pariser–Parr–Pople was used to calculate the charge distributions in the azomethine group and its cation. The PPP–SCF computer program was written by Dr. Jacob Kuhn of the University of Zürich, Zürich Switzerland, in Fortran IV and follows the procedure outlined by Nesbet (1955) and Roothaan (1951). The values of the parameters are: α, the coulomb; β, the resonance; and γ, the repulsion integrals (Brown and Penfold, 1957). The value of "*h*," the electronegativity parameter, is taken to be equal to 1.56 for the cation and equal to 0.125 for the neutral azomethine group.

Results

A. Variation of λ_{max} of N-Retinylidene-n-butylamine with Selected Solvents: the Leveling Effect. The original work on Schiff bases of retinal, specifically *N*-retinylidenemethylamine (**6**), reported by Pitt *et al.* (1955), showed that the conjugate acid of **6** exhibited a λ_{max} of 440 nm in methanol, but also, most importantly, they showed that the conjugate acid of **6** absorbed at 455 nm in chloroform. The value of 440 nm has been taken as *the* λ_{max} for the conjugate acid of **6** and also for this entire class of compounds.

In an earlier paper, we made the observation that in certain solvents, the λ_{max} of a Schiff base is independent of the amount and kind of acid used, whereas in other solvents a dependency is found. Thus, in methanol the dichloroacetate, chloride, and perchlorate salts of **4** absorb at 445 ± 2 nm, whereas in 1,2-dichloroethane they absorb at 452, 465, and 490 nm, respectively (Erickson and Blatz, 1968). This work has been extended and in Table I we report λ_{max} values in methanol, ethanol, and tetrahydrofuran. The salts exhibit an identical λ_{max} in each solvent, although the values are not the same from solvent to solvent. (This is not strictly true for the iodide in tetrahydrofuran.) These solvents are referred to as leveling solvents in the same sense that water is said to level the acid strengths of HCl, HBr, and HI. Leveling solvents do not allow the character of the anion to be expressed.

However, the anion does affect the λ_{max} of NRBAH$^+$ (the conjugate acid of **4**) in a number of solvents such as benzene, chloroform, carbon tetrachloride, and 1,2-dichloroethane. We have studied the effect of selected solvents, and some of these

TABLE I: The Absorption Maxima of All-*trans*-*N*-retinylidene-*n*-butylammonium Salts in Leveling Solvents.

	λ_{max} (nm)					
Solvent	Fluoride[a]	Chloride	Bromide	Iodide	Trichloroacetate	*p*-Toluenesulfonate
Methanol	441	441	443	443	442	441
Ethanol		447	448	448	447	447
Tetrahydrofuran		430	432	441	430	432

[a] Not the monofluoride.

results will be given in the following article. The remainder of this paper is limited to the effect of the counter anion.

B. Variation of the λ_{max} *of N-Retinylidene-n-butylammonium (NRBAH⁺) Salts as a Function of the Counter Anion.* It has been shown that the λ_{max} of a given salt of **4** varies with the method of preparation (Blatz *et al.*, 1971b). Consequently in this work, the errors introduced by acidification features were avoided by making monoprotonated salts as given in the Experimental Section.

All solvents were carefully purified and stored under the best conditions to avoid decomposition or contamination. Solvents like CCl_4 and benzene are best to use in studying the anion effect because they can be expected to interfere little with ionic interactions since they do not hydrogen bond and they have a dipole moment of zero. These solvents, however, are poor for measuring the extinction values and half-bandwidth of NRBA-HA salts which are poorly soluble and tend to deprotonate in them. All of the NRBA-HA salts which were prepared are listed along with their absorption maxima in $CHCl_3$ and CCl_4 in Table II. The data serve to illustrate that solvent interaction is capable of producing not only a general shift in λ_{max} in going from CCl_4 to $CHCl_3$ but also a change in the order of λ_{max} of NRBA-HA salts. Table III gives spectral properties of six salts in benzene. The half-bandwidths in benzene are thought to be somewhat high due to the deprotonation problem in benzene that was mentioned earlier. $NRBAH^+$ halide salts have visible absorption spectra that are typical of NRBA-HA salts, whereas the $NRBAH^+$ picrate⁻ has a composite spectrum of the $NRBAH^+$ plus the absorption of the picrate anion. The spectra of $NRBAH^+$ Cl^-, $NRBAH^+$ Br^-, $NRBAH^+$ I^-, and $NRBAH^+$ picrate⁻ in $CHCl_3$, CCl_4, and benzene are shown in Figure 2. Resolution of the $NRBAH^+$ picrate⁻ spectrum on a DuPont 310 Curve Resolver indicates that the absorption of the picrate ion has little effect on the absorption of $NRBAH^+$.

It was previously noted that there is a relationship between the acid strength and the absorption maximum of the salt (Erickson and Blatz, 1968). The relative strengths of five proton acids in glacial acetic acid are as follows: $HClO_4 > HBr > H_2SO_4 > HCl > HNO_3$ (Audrieth and Kleinberg, 1953). The order of the maxima of NRBA-HA salts in $CHCl_3$ is: $HClO_4 > HBr > H_2SO_4 > HNO_3 > HCl$. The order of absorption maxima of these salts matches the relative strengths of the acids from which they are derived except for the inversion of HNO_3 and HCl. For close members in an acid strength series, the exact order is found to be dependent on the solvent and the reaction used to estimate the acidity. If small deviations are ignored there is no question that both solvents show a gross relationship between λ_{max} and acid strength.

TABLE II: Spectral Parameters of All-*trans*-*N*-retinylidene-*n*-butylammonium Salts in $CHCl_3$ and CCl_4.

Compound	λ_{max} (nm) $CHCl_3$	λ_{max} (nm) CCl_4	ϵ_{max}[a] $CHCl_3$	Half Band-width, $CHCl_3$ (cm^{-1})
NRBA	366	361		
NRBA-trichloroacetic acid	457	442	38,700	4804
NRBA-HCl	460	442	26,600	4700
NRBA-*p*-toluenesufonic acid	461	439	38,700	4787
NRBA-HNO_3	464	445		4639
NRBA-H_2SO_4	466	432		4598
NRBA-picric acid	468	464	45,400	
NRBA-HF[b]	468	447		
NRBA-HBr	468	451	40,560	4753
NRBA-HI	478	460	43,830	4732
NRBA-$HClO_4$	480	457		4580

[a] Extinction values listed are an average of two to three measurements. [b] Not the monofluoride.

C. Correlation between Anionic Radius and Light Absorption Energy of N-Retinylidene-n-butylammonium Cation. Although the acid strength correlation is satisfying, it is not of a fundamental nature and gives little insight into the reasons for the λ_{max} changes.

In a previous preliminary publication it was noted that a convenient calculated parameter which includes the ionic size is the ionic interaction energy $E_i = Z_C Z_A e^2/\epsilon d$, where Z_C and Z_A are the cationic and anionic charges, d is the distance between their centers of charge, ϵ is the dielectric constant, and e^2 is the charge on the electron (Blatz and Mohler, 1970). The electronic interaction energy, E_i, increases as the distance between the two charges (ions) decreases, and formation of this ionic bond will tend to isolate a fraction of the positive charge on nitrogen. This in turn will decrease delocalization of the charge over the π system. Since greater delocalization is consistent with a lower light excitation energy (ΔE), it can be rea-

TABLE III: Spectral Parameters of All-*trans*-*N*-retinylidene-*n*-butylammonium Salts in Benzene.

Compound	λ_{max} (nm)	ϵ_{max}	$\Delta\nu_{1/2}$ (cm^{-1})
NRBA	362		5071
NRBA-HCl	437	21,000	5569
NRBA-trichloroacetic acid	439		5042
NRBA-HBr	448	30,800	4987
NRBA-*p*-toluenesulfonic acid	449		4983
NRBA-HI	457	22,600	4846
NRBA-picric acid	458	43,600	

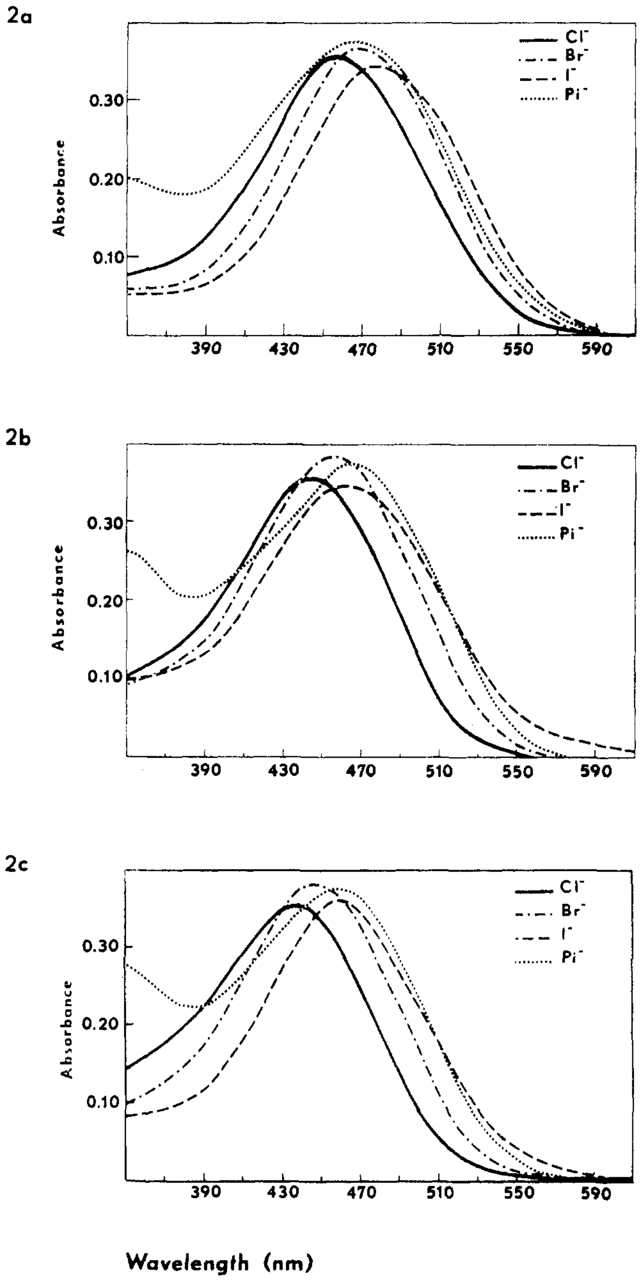


FIGURE 2: Absorption spectra of N-retinylidene-n-butylammonium chloride, bromide, iodide, and picrate in $CHCl_3$ (**2a**), CCl_4 (**2b**), and benzene (**2c**).

soned that ΔE should be indirectly proportional to the ionic distance.

To test this speculation, E_i must first be evaluated, and in a preliminary evaluation, Z_C and Z_A were both taken as unity; consequently, divalent anions were excluded. Furthermore, ϵ was also set at unity. This leaves only the radius of the cation and anion to be considered. Complex anions such as ClO_4^- and NO_3^- are not spherical and the effective solution ionic radius would not always be a simple function of size or volume. For this reason and for the fact that their different elemental composition would be expected to lead to varying solvent interactions, they were not used in the following treatment. The remaining series of halide anions would be expected to give the most accurate test of a possible correlation since they are monoatomic and singly charged, and their radii are known with considerable accuracy. The crystallographic radii used to construct the plots in Figure 3 are Cl^-, 1.81 Å; Br^-, 1.95 Å; I^-, 2.16 Å; and N^{3-}, 1.71 Å (Pauling, 1960). Two radii are available for the cation, the N^{3-} value of Pauling and the crystallographic value of NH_4^+, 1.43 Å. It was anticipated that the N^{3-} value, calculated on the basis of a full octet, along with the Pauling anion values would be the most consistent set of radii.

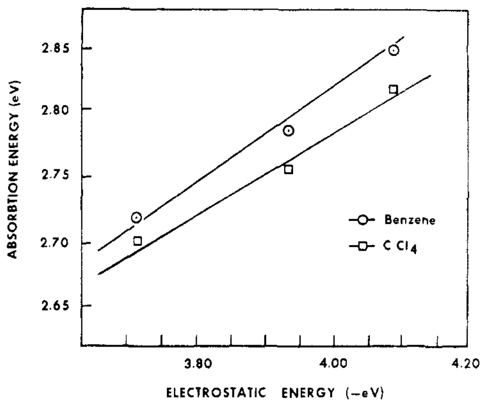


FIGURE 3: Light absorption energy ΔE in electron volts for the three halide salts plotted *vs.* $E_i = Z_CZ_Al^2/\epsilon(r_c + r_a)$. $Z_C = +1$, $Z_A = -1$, $\epsilon = 1$, $r_c = 1.71$ Å, and r_a is the Pauling radius of the respective anion.

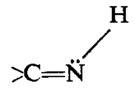
Figure 3 indicates a linear correspondence between ΔE and E_i. If the anion is removed to an infinitely large distance E_i will vanish, and although it is impossible to accomplish this in simple solution, the predicted λ_{max} of the cation can be gained from extrapolation of the lines in Figure 3. Consequently, the following empirical relationship is deduced

$$\Delta E = \Delta E^0 + \frac{mZ_CZ_Ae^2}{\epsilon(r_c + r_a)}$$

Here ΔE^0 is the y intercept at $E_i = 0$. From consideration of the resonance structures in Figure 1 and the experimental results from acidification of anhydroretinol, it is reasonable to predict that ΔE^0 will be equal to 2.10 eV or 590 nm—the limiting wavelength of the retinylic cation. Actually, when the curves obtained when CCl_4 and benzene are solvents are extrapolated to $E_i = 0$, the values of ΔE^0 are 780 and 765 nm, respectively. These values are close to that of the retinylic cation regardless of the above assumptions. From the above empirical relationship, two conclusions are indicated: (1) the anion polarizes the π system in such a way that the resulting fractional charge on nitrogen is some function of the ionic distance, and (2) the anions interact with the fractional charge on nitrogen also as a function of the ionic distance.

D. Spectroscopic Model of N-Retinylideneakylammonium Salts. First, it is necessary to define the anion polarization effect which can be thought of in terms of the utilization of the unit charge on nitrogen. If Z_i is the fractional charge on nitrogen engaged in ionic bonding, then Z_p is the fractional charge remaining for π-electron polarization, and these two quantities must equal unity. For simplicity, the azomethine group ($>C=N<$) was chosen for study, and the charge problem gives rise to two boundry conditions. In case I, the total charge is used in ion bond formation, $Z_i = 1$, so that there is no residual fractional charge left for π-bond polarization, $Z_p = 0$. In this case, as the negative charge reaches its limiting minimum distance, d_0, with respect to nitrogen, the resulting species shows no bond polarization, and this is equivalent to

TABLE IV: Electron Densities on Nitrogen for Various Values of the Coulomb Integral for Neutral and Charged Azomethine.

Charge State	α_N (eV)	α (eV)	Q_N Ground	Z_p[b]	Q_N Excited	Z_p[b]
	−22.569[a]	−22.248	0.941	−0.059	1.000	+0.000
	−23.691	−22.248	1.062	+0.062	1.000	+0.000
$\overset{+}{>C=N<}$	−32.957	−28.948	1.329	+0.329	1.000	+0.000
	−36.351	−28.948	1.615	+0.615	1.000	+0.000

[a] From Brown and Penfold (1957). [b] Fractional positive charge engaged in bond polarization.

calculating the unprotonated azomethine. In the alternative boundary condition, case II, the anion is at infinite distance resulting in no ionic bond formation, $Z_i = 0$, and full π-bond polarization, $Z_p = 1$. When the anion is in between these two distances, Z_i and Z_p will have fractional values.

Ground-state polarization of the azomethine bond was studied quantum mechanically by Brown and Penfold (1957). They found that as the coulomb integral of nitrogen is made more negative with respect to carbon, the electrons are withdrawn toward nitrogen. In the MO calculations given here the parameters of Brown and Penfold were used in a Pariser–Parr–Pople type calculation of the electron density for both the ground and first excited states. The results in Table IV show that for the ground state there is a redistribution of electrons toward nitrogen as the coulomb integral of nitrogen becomes more negative. It also shows that Z_i decreases as bond polarization increases. This is in accordance with expectations; however, the effect on excited-state electron distribution is most interesting. If the results of these calculations are to be believed, the charge state of nitrogen has no effect on the electron distribution in the excited state of the azomethine. Consequently, the conclusion is that ground-state polarization changes as a function of anion distance but excited-state polarization does not.

If we assume that the polarization of the charged azomethine linkage can be simply imposed on a conjugated polyene, the conclusions noted above can be applied to the *N*-retinylidenealkylammonium cations. Thus, the energy of the anion-perturbed excited-state E_x is equal to the energy of the anion-unperturbed excited-state $E_x{}^0$,

$$E_x = E_x{}^0 \qquad (1)$$

The ground state of the perturbed species E_G will be equal to the energy of unperturbed ground state $E_G{}^0$ plus the energy of the perturbation. The perturbation energy is $E_i = Z_C Z_A e^2/\epsilon d$ (the quantities were defined previously) and thus:

$$E_G = E_G{}^0 + Z_C Z_A e^2/\epsilon d \qquad (2)$$

If 2 is subtracted from 1, we receive:

$$\Delta E = \Delta E^0 - Z_C Z_A e^2/\epsilon d \qquad (3)$$

In eq 3, ΔE, Z_C, and d are variables. However, from above, Z_C should be replaced by Z_i, the fractional charge directed toward ionic reaction. If Z_C is taken equal to unity, then $Z_i = Z_C d_0/d$ or simply d_0/d, where d_0 is the minimum distance of approach of a negative charge. Equation 3 becomes:

$$\Delta E = \Delta E^0 + d_0 e^2/\epsilon d^2 \qquad (4)$$

In our preliminary report (Blatz and Mohler, 1970), ΔE values of three salts were plotted *vs.* E_i values (calculated from Pauling radii) and the curves were extrapolated to $E_i = 0$ in order to obtain ΔE^0. This, of course, is equivalent to plotting ΔE *vs.* $1/d$ and extrapolating to find ΔE^0 values. Table V gives ΔE^0 values found from $1/d$ plots for four solvents—benzene, CCl_4, CH_2Cl_2, and $CHCl_3$. There is a spread of 114 nm in the ΔE^0 values which have an average of 818 nm. This average λ_{max} is quite long compared to the 590-nm value of the retinylic cation. In light of the above derivation, the observed scatter in ΔE^0 in such a plot would be expected, since in a $1/d$ plot, the slope contains a variable—the charge on the cation.

However, eq 4 indicates that ΔE should be a linear function of $1/d^2$, and consequently, this relationship was evaluated by the least-squares method. Again, values for d are taken from Pauling radii. Results in terms of ΔE^0 and the slope are given in Table V. It is obvious that two things happen: (1) the spread between ΔE^0 values is reduced from 114 to 17 nm, and (2) the average ΔE^0 value in four solvents is changed from 818 to 582 nm compared with the experimental values of 588–590 nm for the retinylic cation. It can only be concluded that the model derived from fundamental considerations enjoys quantitative fit with experimental evidence.

The quantity d_0, the minimum approach of a negative charge, can be calculated from eq 4 when the slope is used from Table V, $\Delta E^0 = 582$ nm and $\Delta E = 365$ nm—λ_{max} of the uncharged Schiff base. At this wavelength $Z_i = 1$ and d_0/d must be unity. The resulting d_0 values are also given in Table V. The values of 2.63 and 2.54 Å in benzene and CCl_4 are taken to be base values since these solvents should have the

TABLE V: The Values ΔE^0 of the Unperturbed Cation Calculated from the Models for Different Solvents. The Minimum Charge Penetration Distance d_0 and the Calculated Dielectric ϵ Are Also Given.

	Calcd for $1/d$	Calculated for $1/d^2$					
Solvent	ΔE^0 (nm)	ΔE^0 (nm)	Slope	d_0	ϵ[a]	ϵ'[b]	Dielectric Constant[c]
C_6H_6	880	585	8.80	2.63	4.30	4.22	2.284
CCl_4	760	571	7.84	2.54	4.69	4.73	2.238
CH_2Cl_2	804	582	7.43	2.44	4.71	4.99	9.08
$CHCl_3$	823	588	7.26	2.38	4.72	5.11	4.806

[a] ϵ = dielectric calculated from d_0. [b] ϵ' = dielectric calculated from constant d_0 = 2.58. [c] Literature values.

TABLE VI: Values of λ_{max} Calculated from Equation 4 for Given Distances between Centers of Charge.

	λ_{max} (nm) in Solvents		
d^a (Å)	C_6H_6	CCl_4	$CHCl_3$
3.50	429	441	459
4.50	486	485	502
5.50	514	510	528
6.50	533	526	544
7.50	545	537	554
8.50	553	544	561
9.50	559	549	566
10.50	564	553	570

[a] Center-to-center distances between charges.

minimum effect on the ion pair. The average value of 2.58 Å does not agree with the cationic radius of 1.71 Å, but it does agree with the distance equal to 0.5 the distance between the sum of the ionic and covalent distance, 2.63 Å or possibly 0.5 the distance between the Van der Waal's radii and the covalent distance of 2.52 Å (Braude and Waight, 1954).

In any event d_0 can be used to calculate Z_i and hence Z_p, since $Z_i = d_0/d$. Thus, the value of Z_i for NRBA-HCl in CCl_4 is 2.54/3.52 or 0.72. It can be shown that Z_i^2 is proportional to the fractional change in ΔE; consequently, $Z_i^2 = (0.72)^2 = 0.52$, and for the above salt in CCl_4 whose λ_{max} is 442 nm, the fractional change in ΔE is $(2.805 - 2.102)/(3.397 - 2.102) = 0.54$, where $\Delta E_{590} = 2.102$ eV, $\Delta E_{442} = 2.805$ eV and $\Delta E_{365} = 3.397$ eV. Z_i^2 for NRBA-HI in $CHCl_3$ is 0.39, and the fractional change in $\Delta E = 0.38$. This appears to indicate that the model successfully predicts the fraction of positive charge used in ionic bonding and acts as a further test of the model. If Z_i is known, Z_p can be calculated. Z_p, the fractional charge engaged in bond polarization, can be related to the value of the coulomb integral α_N necessary to calculate a given ΔE. Consequently, α_N may be related to a real value of the effective charge used in bond polarization.

Table V also shows two calculated values of the effective solution dielectric. One value ϵ is calculated allowing d_0 to vary and another ϵ' allowing d_0 to be constant at 2.58 Å. It is noted that ϵ' advances as the experimental wavelength increases.

The slopes and intercepts obtained from the ΔE *vs.* $1/d^2$ plots in C_6H_6, CCl_4, and $CHCl_3$ and reported in Table V were used to calculate the λ_{max} as a function of increasing separation between the centers of charge. The results of these calculations are presented in Table VI. To attain the λ_{max} of rhodopsin, a center-to-center separation of about 4.50 to 5.00 Å is required. This means that if 3.52 Å is the ionic distance between the two centers, there is at most 1.50 Å of void. This void is scarcely large enough to accommodate the simple molecule, water.

E. Leveling Solvents. It is now enlightening to examine the action of leveling solvents with the aid of the proposed theory. In a solvent such as methanol $NRBAH^+$ salts would not be expected to exist as an ion pair. Indeed, the halide anions are known to be soluble in methanol, and most certainly the cation $NRBAH^+$ would also be soluble. Consequently, the salt should be fully dissociated in methanol, and at first, one might suspect that the cation would absorb at 590 nm. However, the electron-rich oxygen of methanol may interact with the cation to isolate a fractional positive charge on the nitrogen by means of a dipole–ion bond. Thus, the charge on nitrogen will interact with as many methanol oxygen atoms as it is energetically and sterically possible. The net effect is that this interaction is strong enough to cause the cation to absorb at *ca.* 442 nm in methanol. This is the same wavelength observed for the HCl salt in CCl_4. This means that the summation of the alcohol effect is equivalent to the chloride with its unit negative charge and with its negative center 3.52 Å away from the positive center. It also means that the methanol molecules are able to localize *ca.* 0.72 of a positive charge on nitrogen.

One can imagine the nitrogen atom of the Schiff base surrounded by methanol molecules giving rise to an oriented solvent cage. The methanol molecules are tightly held by virtue of a high dipole–ion interaction energy. At the same time, the anion is shielded at great distances by the high dielectric of methanol so that small changes in anionic radius will no longer influence the charge on the cation. Consequently, the cation is now insensitive to the anion.

Even though a series of salts of NRBA gives the same λ_{max} in one alcohol, the series gives another unique λ_{max} in another alcohol. Thus, in ethanol the salts absorb at 447 nm. The change in λ_{max} with a change in alcohol can be explained by change in dielectric constant or a steric effect.

Tetrahydrofuran is a more difficult case to analyze. The λ_{max} values of various salts except that of HI are the same, *ca.* 431 nm, and of lower value than when determined in methanol. Tetrahydrofuran would not be expected to solvate the anions enough to cause dissociation; consequently, the salt would be in the form of an ion pair. However, the electron-rich nucleophilic oxygen would interact in a dipole–ion type bond and aid in further localization of charge on nitrogen. The strong dipole–ion interaction apparently compensates for small change in anionic distances.

Discussion

We must conclude that an odd, homoatomic polyene absorbs at a much longer wavelength than its even counterpart of approximately the same length. This is a known inherent property. In the case of an even polyene hydrocarbon, the conversion can be accomplished by protonation. In the process, the terminal unsaturated carbon is converted from sp^2 to sp^3. This simple conversion process is prevented by the proximity of the anion in the case of a protonated Schiff base. The anion fixes or localizes a considerable fraction of the positive charge on nitrogen, leaving a lesser fraction of the charge to engage in polarization of the azomethine bond. This hypothesis may now be examined in context with a visual pigment.

It is clear that no water or other nucleophilic agent can be near the nitrogen in the visual pigment. If an electron-rich center, such as the oxygen of water, is near the nitrogen, it will engage in a dipole–ion or ion–ion interaction and decrease Z_p, the fractional charge engaged in bond polarization. This specifically excludes these kinds of groups from being near the nitrogen. Furthermore an unattached anion, such as chloride, cannot be near the nitrogen. This will engage in an ion–ion interaction with nitrogen resulting in a small value of Z_p. One way to visualize how the protein can control the charge separation is to assume that the anion is attached to the protein chain as in a phosphate-esterified serine. Then, as the peptide chain moves with respect to the positive center the distance between these centers changes.

The above simple model accounts for the λ_{max} by considering the distance between two charge groups: the conjugate acid and its specific counter anion. This is equivalent to a 1:1 electrolyte. However, it must be remembered that the protein, by virtue of its ionic groups, is itself a polyelectrolyte. This electrically neutral molecule contains a large number of positive and negative centers, and instead of analyzing the charge state as a 1:1 electrolyte, it should be analyzed as a polyelectrolyte. For this purpose, the positive charge of the conjugate acid can be visualized to be located at some fixed point in the electrostatic field. With respect to the conjugate acid the electrostatic field will have one excess negative charge. In comparison to the order in a crystal of an ionic compound, the protein may be considered as a random matrix of fixed charges. The center of negative charge density with respect to the conjugate acid will be a contribution of the nearest negative groups, and this negative charge density does not have to be located at the same point as the negative group. The distance from the cation to the center of negative charge density of the electrostatic field is equal to the $r_c + r_a$ term. This means that with respect to the conjugate acid each protein conformation will have a certain center of negative charge density, and consequently, the resulting change in electrostatic interaction energy will induce a change in excitation energy.

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Mössbauer Parameters of Putidaredoxin and Its Selenium Analog†

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ABSTRACT: Mössbauer spectra of ^{57}Fe-enriched putidaredoxin, a 2Fe–2S protein from *Pseudomonas putida*, and of its selenium analog were measured. The data were analyzed with the help of computer programs that calculate Mössbauer spectra for given sets of magnetic and electric hyperfine interaction tensors. With the *g* tensor and some values of the magnetic hyperfine tensors known from electron spin resonance and electron nuclear double resonance data we were able to find parameters that reproduce the data quite well. The parameters found for the two irons in the reduced, paramagnetic protein are typical of high-spin ferric, $S_a = 5/2$, and ferrous iron, $S_b = 2$, respectively. Spectra measured in strong magnetic fields show conclusively that the spins residing on each individual iron couple antiferromagnetically to a total spin of $S = 1/2$. The magnetic hyperfine tensor of the ferric iron is relatively small and highly anisotropic which implies strong covalency and relatively low symmetry in the arrangement of the ligands. The parameters of the ferrous iron are compatible with distorted tetrahedral symmetry of the ligands. The two iron atoms in the oxidized, diamagnetic protein are interpreted as high-spin ferric, $S = 5/2$, coupled to a total spin of zero.

Putidaredoxin is an iron–sulfur protein of molecular weight 12500 isolated from *Pseudomonas putida* (Cushman *et al.*, 1967). It acts as a specific one-electron transfer agent in a multienzyme system that hydroxylates camphor (Gunsalus, 1968). Each molecule contains two iron and two acid-labile sulfur atoms that can be replaced by isotopically enriched iron and sulfur or selenium, respectively (Tsibris *et al.*,

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