Quantum Chemical Vibrational Analysis of the Chromophore of Bacteriorhodopsin

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Vibrational spectra of the retinal chromophore in bacteriorhodopsin (BR) are interpreted by a quantum chemical vibrational analysis. The chromophore is represented by a semiempirical MNDO Hamiltonian [modified neglect of diatomic overlap] that describes all valence electrons using Hartree–Fock wave functions. The main sources of errors in a description based on the MNDO force field are identified. We show that the errors are mainly due to the neglect of the effects of electron correlation. A proper understanding of vibrational spectra of the BR chromophore can be obtained only if effects of electron correlation are taken into account.

1. Introduction

In many biological systems the polyene derivative retinal serves as a chromophore for signal detection or energy conversion. For example, upon light absorption retinal initiates the primary processes of vertebrate vision as well as those of phototaxis and photosynthesis in halobacteria. Due to its prominent role as a biological chromophore, the properties of retinal have been investigated in numerous experimental and theoretical studies (for reviews see refs 1 and 2). In retinal proteins the chromophore usually occurs as a protonated Schiff base (RSBH$^+$) and is highly sensitive to the electrostatic properties of its protein environment.[3,4] Depending on local electrostatic fields the spectral position of the main absorption band of RSBH$^+$ shifts between 400 and 650 nm. As a result, RSBH$^+$ can function in a wide spectral range. The function involves photoisomerizations that can be detected by the surrounding protein and, hence, can be used for biological signal processing.

Since retinal's photoisomerization and reisomerization processes are coupled to the protonation state of the Schiff base nitrogen as well as to other electrostatic interactions, retinal can also convert the energy of sunlight into electrogenic membrane potentials. An example is the protein bacteriorhodopsin (BR), which acts as a light-driven proton pump in the cell membrane of *Halobacterium halobium* (for a review see ref 5). In this protein RSBH$^+$ is covalently bound to a lysine residue of the protein (see Figure 1). In the light-adapted state the RSBH$^+$ chromophore undergoes a photoinduced all-trans → 13-cis isomerization and a thermal back-reaction as well as de- and reprotonation reactions. The intermediates can be distinguished spectroscopically and form a reaction cycle that is completed on a millisecond time scale and is kinetically coupled to proton transport.[6]

A key question regarding the mechanism of this photosynthetic process is,[7] which isomerization states does retinal assume? It had been suggested, that retinal works as a proton switch in the photocycle by undergoing an all-trans → 13-cis, 14-s-cis photoisomerization.[8-10] The stereochemical transformations of retinal in the pump cycle of BR occur on time scales between 100 fs and 1 ms. Determination of the exact molecular geometries proved extremely difficult. Currently, the only method that holds the promise to reveal retinal's isomeric states is vibrational spectroscopy. However, this method relies on a theoretical analysis that deduces geometries from vibrational frequencies and intensities. Because of this important role of theory it is of prime interest to investigate to what extent theoretical methods can reliably describe vibrational modes of retinal. Such investigation will be presented in this paper.

We like to illustrate the essential role of vibrational mode analysis of retinal for the case of the proton pump cycle in BR.

On the basis of observations and calculations of vibrational frequencies of BR's chromophore there have been various attempts to prove or disprove the occurrence of 14-s-cis structures in BR's reaction cycle. Smith et al.[11] had argued against an involvement of a 14-s-cis geometry in the early intermediates (K$_{625}$, L$_{550}$) of BR's pump cycle. Gerwert and Siebert[12] as well as two of us[13] subsequently put forward arguments in favor of such involvement. These arguments and counterarguments hinged on observations and interpretations of frequencies of the C$_{14}$–C$_{15}$ stretching vibration in the intermediates K$_{625}$ and L$_{550}$. Recently 14,15-^{2}H rocking vibrations in 14,15-dideuterated retinal were suggested to be indicative for the geometry around the C$_{14}$–C$_{15}$ bond.[14] Resonance Raman (RR) spectra of the L$_{550}$ chromophore were interpreted to rule out 14-s-cis structures in BR's photocycle.[14,15] However, a subsequent quantum chemical investigation of the arguments of Fodor et al.[15] and Mathies et al.[14] has questioned these conclusions.[16]

Interpretations of vibrational spectra are difficult since conclusions strongly depend both on intramolecular force fields used for the vibrational analysis and on assumptions concerning chromophore–protein interactions. In this paper we investigate the MNDO Hartree–Fock (HF) description of the vibrational spectra of the BR chromophore in its equilibrium structure. The absorption maximum of this chromophore lies at 568 nm, and the chromophore, therefore, is referred to as BR$_{568}$. The structure of the chromophore is that of an *all-trans*-RSBH$^+$. We employ the MNDO method to describe the intramolecular force field of RSBH$^+$, since this method has been demonstrated to provide

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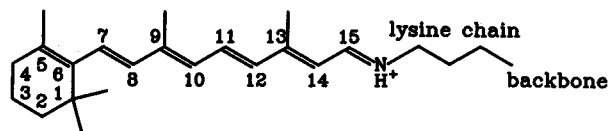
Figure 1. Protonated Schiff base of retinal. The chromophore is attached through the terminal nitrogen to a lysine residue of BR.

qualitatively correct ground-state properties of RSBH$^+$.[17] We have chosen BR$_{568}$ for our investigation because its geometry is well-known and because a wealth of experimental data is available.[18-20]

Our investigation focuses on two issues. The first issue is a description of the normal modes of the BR$_{568}$ chromophore. The second issue concerns the errors introduced by an MNDO-HF description. The paper is structured as follows: In section 2 theoretical methods are characterized and model compounds are introduced. In section 3 we discuss the quality of the MNDO-HF force field. We argue that the neglect of electron correlation effects by the HF method is the main reason for poor descriptions of normal modes, and we characterize these errors. In section 4 we use the MNDO-HF description to analyze vibrational spectra of BR$_{568}$. We investigate to what extent such description, in spite of its shortcomings, allows one to interpret vibrational spectra of RSBH$^+$. A summary (section 5) concludes the paper.

2. Methods

1. Force Field and Molecular Models. For a quantum chemical description of vibrational modes of retinal chromophores we employ the MOPAC program package.[21] This program is based on a semiempirical MNDO Hamiltonian for all valence electrons of a molecule and describes the ground state of this system in the Hartree–Fock (HF) approximation. Details concerning the determination of the molecular force field by MOPAC have been described earlier.[7,13,16] Although during our study the MNDO model became somewhat outdated by the recent development of the improved AM1 model,[22] we continued using MNDO since our considerable experience with that model made it a useful tool in our hands despite of its deficiencies. Furthermore, as we intend to show in this paper, there are essential methodological drawbacks that are common to MNDO, AM1, and all other methods that rely on the HF approximation.

For an investigation of the MNDO force field we consider a very small polyene, namely butadiene, for which vibrational modes are well-known, and all-trans model compounds that represent RSBH$^+$ in BR$_{568}$. The latter models comprise most of the atoms of the complete retinal chromophore and allow one to analyze errors of an MNDO-HF description as well as to investigate the influence of local electric fields on RSBH$^+$ vibrations. The vibrational spectra resulting for these models will be compared with observed vibrational spectra of BR$_{568}$.

The models represent the main structural elements of retinal presented in Figure 1. The structural elements are the following:

(a) The first element comprises retinal except for its β-ionone ring. The ring carbon atoms C_1 and C_4 (the numbering of atoms is defined in Figure 1) are replaced by hydrogen atoms with masses $m_H = 15$. We denote this model which represents the atoms between C_5 and C_{15} by "RET".

(b) The second model, referred to as RETINAL, represents the C_1–C_{15} moiety of retinal. Since the C=C stretch vibrations of the polyene backbone are strongly coupled, we employ RETINAL model compounds for the description of these modes. The
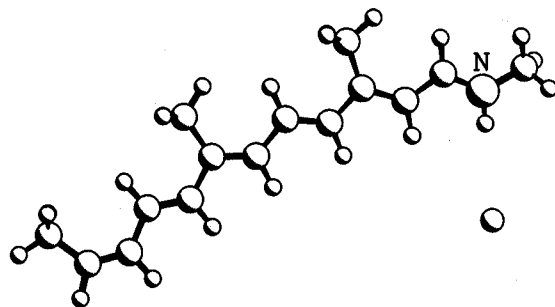
Figure 2. Geometry of the *all-trans*-RET-NH$^+$CH$_3$ model RSBH$^+$ with a Cl$^-$ placed $d = 3$ Å from the proton at the nitrogen.

reason for preferring RETINAL over RET is that the C_5=C_6 double bond of the β-ionone ring is not properly represented by the RET model. As a result, the frequency of the C_5=C_6 stretch vibration in RET models is higher by 86 cm^{-1} compared to that of RETINAL models,[23] the latter providing, of course, the more realistic description.

(c) The retinal models to be considered have to represent the lysine chain to which retinal in bacteriorhodopsin is bound via the Schiff base nitrogen. We denote the representation of the Schiff base nitrogen and lysine moiety by X. We have chosen for X the residue NH$^+$CH$_3$. This choice allows a qualitatively correct description of most normal modes of the chromophore. For a detailed analysis of our choice we refer to refs 16 and 23. Compounds including a representation X of the lysine side chain are referred to as RET-X or RETINAL-X.

(d) The models considered below account for the influence of the protein environment by assuming a single Cl$^-$ counterion at a distance d from the proton of the Schiff base (see Figure 2). The counterion has been placed into the molecular plane on a straight line defined by the N–H bond. A detailed discussion of the validity of such a simple model of the electrostatic environment of the BR chromophore is given in ref 4. Model chromophores with counterion distances of d Å are referred to as d Å chromophores.

2. Pariser–Parr–Pople Calculations. Correlation effects in the π-electron system of the chromophores investigated are estimated by comparison of Pariser–Parr–Pople (PPP) HF calculations with PPP-CI calculations. For butadiene the CI results are exact (full-CI); for RSBH$^+$ we use a multireference double-excitation configuration method (MRD-CI)[24] that previously has been applied to describe the mechanisms of wavelength regulation in BR and halorhodopsin (HR).[4] The model geometries required for a calculation of the correlated π-electron ground state of the BR chromophore were chosen to match exactly the geometries of model chromophores employed for the vibrational analysis. For further details on computational methods we refer to ref 4.

3. Quality of the MNDO-HF Force Field

In this section we analyze errors of the MNDO-HF force field. We begin with a compilation of known deficiencies[25] of the MNDO-HF method relevant to the description of normal modes.

Almost all force constants are overestimated. Exceptions are force constants for single-bond torsions, CH$_2$ twists, and CH$_3$ deformations.[25] To approximately correct these errors by rescaling the force matrix, we introduce for each atom type a "spectroscopic" mass. We have chosen the masses $m_H = 1.094$, $m_C = 14.75$, and $m_N = 16.92$. For butadiene this choice results in a reasonable description of most normal modes. Details and consequences of such rescaling are discussed further below.

MNDO-HF calculations systematically underestimate torsional barriers of C–C single bonds and frequencies of torsional modes around such bonds.[13,17] This error is mainly due to the fact that the size of p orbitals of carbon atoms is underestimated in the

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parametrization[26] of the MNDO Hamiltonian. Consequently, the overlap of p orbitals is too small, and this is decisive for the lack of torsional stiffness of C–C bonds. For example, the torsional mode around the central single bond of butadiene is predicted at 40 cm^{-1} and observed[27] at 163 cm^{-1}. A choice of larger p orbitals, as realized in the recently developed AM1 Hamiltonian,[22] leads to an improved description of that vibrational mode, i.e., its frequency is predicted at 87 cm^{-1}. Because C–C torsional modes couple only weakly to the higher frequency hydrogen out-of-plane (hoop), C–C single-bond stretching, C=C double-bond stretching, and hydrogen in-plane rocking vibrations, the error in describing torsional modes around C–C single bonds hardly influences the prediction of these higher frequency modes.[13,23,27]

The response of the nitrogen atom to protonation is improperly described. This error shows up in a comparison between calculated (MNDO-HF) and observed frequencies of the C=N stretching mode in unprotonated and protonated molecules. Upon protonation a 30-cm^{-1} upshift of the C=N stretching vibration is observed, whereas an MNDO-HF description predicts a downshift. Ab initio investigations of López-Garriga et al. have provided evidence that such error should be a common feature of methods that, like MNDO-HF, neglect the effects of electron correlation beyond the HF level.[28–30] That conjecture gains plausibility also from the following qualitative consideration: in an unprotonated Schiff base the Coulomb repulsion of the lone-pair electrons at the nitrogen will lead to a partial occupation of the antibonding π C=N orbital and, therefore, weaken the C=N bond; this is an effect that cannot be described by a HF wave function; in the protonated Schiff base the electron pair is more tightly localized at the C–H bond, and therefore the C=N bond is stronger.

To further examine this issue, we have carried out AM1-HF and MNDO-CI calculations for unprotonated and protonated Schiff bases of hexatriene. Like MNDO-HF, the improved AM1-HF method also fails to qualitatively reproduce the observed blue-shift of the C=N stretching mode upon protonation. The MNDO-CI calculations, in contrast, correctly predict a blue-shift for this mode; the very limited configuration mixing in these calculations involved only 100 configurations. These results demonstrate that there are erroneous descriptions of molecular force fields common to all HF methods. For semiempirical methods such as MNDO or AM1 that type of error is neither caused nor can it be repaired by parametrization. We call errors caused by neglect of specific effects of electron correlation, i.e., effects that depend significantly on the location of bonds in the molecule, "HF errors".

We now present an analysis of MNDO-HF normal mode predictions for polyene derivatives that shows that HF errors affect the stretching force constants of all bonds in the conjugated system. HF errors in σ and π bonds of conjugated hydrocarbons are discussed separately.

The HF approximation generally overestimates electron densities of bonds and, therefore, overestimates stretching force constants. Because of the local character of σ bonds, the corresponding HF error is approximately of the same size in all bonds of the same type. Therefore, corresponding force constants can be corrected to some degree by mass rescaling, i.e., by introducing "spectroscopic" masses for each atom type.[13] Such approach does not discriminate different types of force constants corresponding to stretching, rocking, and deformation modes, respectively. Furthermore, the approach implies that only one kind of σ bond exists for each atom pair X, Y. That assumption holds reasonably well in pure, unsubstituted polyenes if σ–π separation is assumed. It is invalid in polyene derivatives such as retinal, in which hydrogen atoms are substituted by methyl groups. As is well-
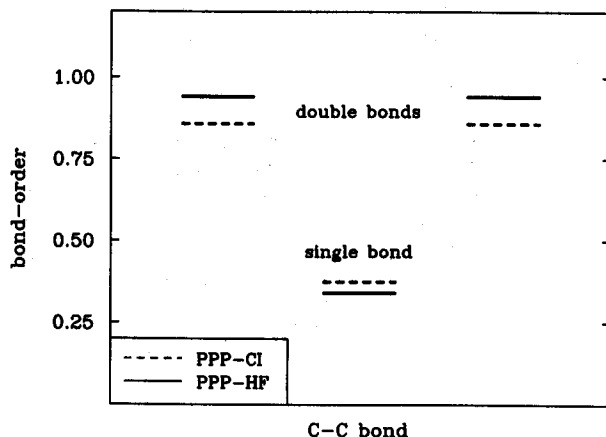
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Figure 3. π-bond orders of butadiene, resulting from PPP-HF and PPP full-CI calculations.

known,[31] methyl groups shift σ electron density into neighboring C–C σ bonds and, thereby, increase the corresponding force constants. We call this effect the σ-CH$_3$ effect. Because the correlation corrections are roughly proportional to the electron density in a bond, this effect is overestimated by HF calculations. We call the corresponding HF error the σ-CH$_3$ overestimate. There are two types of C–C bonds in retinal's polyene chain, bonds with and bonds without an adjacent methyl group. Due to the σ-CH$_3$ overestimate, force constants of the former are more strongly overestimated than those of the latter. Therefore, the global scaling of force constants employed by us causes too large a splitting of the frequencies of polyene backbone stretching modes.

Since π electrons are strongly delocalized in conjugated π bonds, an estimate of HF errors for these bonds is considerably more difficult than for the localized σ bonds. Like for the σ bonds, *large* π electron densities in C=C double bonds are *overestimated* in HF calculations. However, *small* π electron densities in neighboring C–C single bonds are *underestimated*. A proper inclusion of correlation effects can repair both deficiencies leading for double bonds to *smaller* and for single bonds to *larger* π contributions to force constants. Consequently, for C–C single bonds which exhibit large σ and small π electron densities HF errors partially compensate each other (σ–π compensation), whereas in C=C double bonds that show large σ and π electron densities these errors add (σ–π additivity). Therefore, the HF approximation can be expected to be more suitable for the description of polyene-like single bonds than for the description of C=C double bonds. Furthermore, our global mass scaling procedure cannot be expected to properly correct those errors that result from correlation-induced charge density redistributions among single and double π bonds.

1. MNDO-HF Description of the Polyene Butadiene. To demonstrate the existence of the HF errors outlined above, we first discuss π-bond orders of the butadiene ground state resulting from PPP-HF and PPP full-CI calculations. Figure 3 shows that correlation effects increase the π-bond order of the central single bond and decrease the π-bond orders of the double bonds both by approximately 10%. The corresponding σ–π compensation of the HF errors of force constants in the single bond and the σ–π additivity in the double bonds can be elucidated by a simple calculation. For that purpose we have calculated for butadiene with unscaled masses ($m_H = 1$ and $m_C = 12$) the MNDO-HF frequencies of C–C and C=C stretching vibrations. Compared with observations, the calculated overestimate of C=C stretch frequencies (180 cm^{-1}) is indeed considerably larger than the corresponding overestimate of the C–C stretch frequency (98 cm^{-1}). As force constants are proportional to the square of the frequency, the HF approximation overestimates the C=C stretching force constants more than the C–C stretching force constants, the overestimate being more than twice as large. Our

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choice of scaled masses of C-atoms yields a C–C stretch frequency that due to σ–π compensation is slightly red-shifted (13 cm⁻¹) with respect to its observed spectral position and yields C=C stretch frequencies that due to σ–π additivity are blue-shifted by the same amount.

In view of the considerable difference between HF overestimates of C=C and C–C stretch force constants, at a first glance it may surprise that our simple scaling scheme reproduces both observed C=C and C–C stretch frequencies quite well. But if one considers the pronounced nonlinearity of the scaling scheme, the good agreement of observations and theoretical predictions becomes apparent. To explain this, assume all force constants k to be overestimated by the same amount by the HF method. Increase of the reduced mass m by Δm leads to a scaled normal-mode frequency ν' given by

$$\nu'^2 \approx \nu^2\left(1 + \frac{\Delta k}{k} - \frac{\Delta m}{m}\right)$$

Here, the true vibrational frequency ν is given by $(k/m)^{1/2}$. To fit a particular scaled frequency ν' to a corresponding observed value ν, the condition $\Delta k/k \approx \Delta m/m$ has to be satisfied. If this condition holds for the force constant k of a C–C bond, i.e., if

$$\nu'_s \approx \nu_s$$

then, assuming a *constant* HF overestimate Δk, the frequency ν'_d of a C=C bond will be *underestimated*, since C=C force constants are almost twice as large as C–C force constants. Thus, we obtain

$$\nu'_d \approx \nu_d\left(1 - \frac{1}{4}\frac{\Delta m}{m}\right)$$

Since the "spectroscopic" masses employed in the scaling scheme lead to a $\Delta m/m$ value of about 0.2, C=C frequencies would be underestimated by about 80 cm⁻¹. According to the considerations outlined above, a scaling scheme for the correction of HF errors using rescaled masses rests on the assumption that force constants of high-frequency modes are overestimated more strongly than those of low-frequency modes. We call this property of the correction scheme employed by us *high-frequency overcompensation*. Since the assumption above holds for the C–C and C=C bonds of butadiene, the scaled frequencies of the corresponding stretching vibrations are both in good agreement with observations. The scaled masses of H atoms have been chosen to render optimal agreement of observations and calculations for C–H vibrations of butadiene. Therefore, one may expect that couplings of H rock with C–C stretching modes also will be satisfactorily described. Due to larger errors of C=C force constants, a description of corresponding coupling effects of H rock with C=C normal modes will probably yield larger errors.

1.1. Intramolecular Couplings in Butadiene. First we investigate the scaled MNDO-HF description of intramolecular couplings of H rock normal modes in butadiene. Because of the high spectral density of such normal modes in polyenes and polyene derivatives, these couplings should be large, and their correct description is an important prerequisite for a reliable normal mode analysis.

For butadiene we have calculated the frequency of the rock vibration with B_u symmetry (B_u rock) at 1290 cm⁻¹ and the frequency of the A_g rock vibration at 1286 cm⁻¹. Due to our optimization of scaled masses these frequencies are very close to observed[27] frequencies of corresponding bands at 1296 (B_u) and 1291 cm⁻¹ (A_g).

To check to what extent couplings between *deuterated* rocking modes are also correctly described, we have calculated A_g and B_u rocking vibrations in fully deuterated butadiene. Computed frequencies of 924 (A_g) and 973 cm⁻¹ (B_u) agree reasonably well with the corresponding observed spectral features at 919 (A_g) and 1009 cm⁻¹ (B_u). In butadiene deuterated at the central positions (butadiene-2,3-d_2) the observed[32] frequency of the A_g rock (942

cm⁻¹) is almost exactly reproduced by the MNDO calculation (931 cm⁻¹). According to the MNDO description, the B_u D rock combination in butadiene-2,3-d_2 is accidentally degenerate with a B_u scissoring mode of the terminal hydrogen atoms. That degeneracy leads to a large splitting resulting in two B_u combination modes calculated at 871 and 1059 cm⁻¹. Unfortunately, we found no experimental data for comparison with these B_u modes. The average frequency of the two modes of B_u symmetry and D-rock character is predicted 30 cm⁻¹ above the frequency of the A_g vibration. In unlabeled butadiene, the B_u scissoring mode is observed[27] at 991 cm⁻¹ and calculated at 979 cm⁻¹.

In the examples mentioned above, the frequency of the A_g rock vibration of the central hydrogen atoms has been calculated and observed *below* the frequency of the B_u rock. Butadiene-1,4,4-d_4 couplings of rocking modes are altered, in that D-rock modes of the terminal hydrogen atoms are decoupled from H-rock modes of the central hydrogen atoms. This leads to a reversal of the spectral ordering of A_g and B_u modes, which is confirmed by the MNDO calculations. The A_g mode (1288 cm⁻¹) in butadiene-1,1,4,4-d_4 is predicted above the B_u mode (1281 cm⁻¹). The corresponding spectroscopic data are[33] 1298 (A_g) and 1274 cm⁻¹ (B_u), respectively.

The good agreement between observations of rocking bands in spectra of deuterated butadiene molecules and MNDO calculations demonstrates that intramolecular couplings of rocking modes are reliably described by the employed method. As shown recently, such reliable description of rock frequencies and rock–rock couplings is not provided by Wilson FG calculations with modified Urey–Bradley force fields.[16]

Though rock–rock couplings in butadiene are well described by MNDO, effective couplings of rocking modes with C–C stretching modes are slightly underestimated. For instance, MNDO calculations for butadiene-2,3-d_2 predict a red-shift of the spectral position of the C–C stretching vibration (13 cm⁻¹), whereas a blue-shift of 13 cm⁻¹ is observed for the corresponding band. This discrepancy is not surprising, since our mass scaling scheme for correction of HF errors renders the force constant for the C–C stretching vibration too small. Consequently, the spectral distance of this vibration to the higher frequency 2,3-H rocks becomes too large and, thus, the corresponding coupling between the C–C stretch and these rocking modes becomes too small. Therefore, a decoupling of these modes through 2,3-d isotope labeling yields a too small blue-shift of the C–C frequency. In contrast to observations, this blue-shift does not compensate the red-shift that arises from the increased effective mass of the C–C stretch.

As one may expect from the overestimates of C=C force constants analyzed further above, the spectral distances between H rocks and C=C stretches are overestimated, and the corresponding couplings are too small. Therefore, MNDO predictions of these couplings deviate significantly from observations. For instance, complete deuteration of butadiene leads to red-shifts of 78 (A_g C=C stretch) and 76 cm⁻¹ (B_u C=C stretch) in the spectrum of butadiene, whereas MNDO calculations predict only red-shifts of 23 (A_g C=C stretch) and 35 cm⁻¹ (B_u C=C stretch).

2. MNDO-HF Description of RSBH⁺. *2.1. Ground-State Properties and Force Constants.* RSBH⁺ is a polyene derivative. The alternation pattern of π-electron densities in single and double bonds differs from that of unsubstituted polyenes for which butadiene served as an example. Quantum chemical calculations that employed RET-X and RETINAL-X model compounds have shown[17] that the distribution of π electrons strongly depends both on the protonation state and on the electrostatic environment of the chromophore. For instance, protonation of the Schiff base has two effects. First, it leads to a global decrease of π-electron density in the polyene chain. Second, π-electron density is shifted along that chain toward the nitrogen atom. This effect induces local dipoles in the polyene chain,[17] increases bond orders of C–C single bonds, and decreases bond orders of C=C double bonds.

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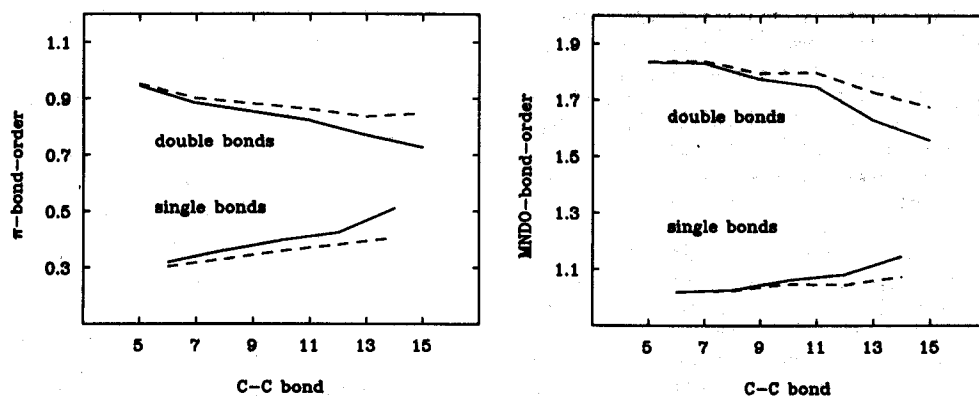
Figure 4. Bond orders of single and double bonds, as predicted for 2- (dashed lines) and 3-Å chromophores (solid lines) from PPP-HF (left) and MNDO-HF calculations (right).

The alteration of bond orders is accompanied by corresponding alterations of bond lengths and torsional barriers of bonds. In case of the latter protonation increases C–C torsional barriers from 5 kcal/mol to up to 20 kcal/mol and decreases C=C torsional barriers from 45 kcal/mol to 10 kcal/mol.[17] The protonation-induced changes of torsional barriers and bond orders decrease with increasing distance of the respective bond from the nitrogen atom.[17]

All protonation effects sketched above, i.e., formation of local dipoles in the polyene chain, changes of torsional barriers of single and double bonds, and adaptation of bond orders, also exist in RSBH$^+$ which is exposed to additional local electric fields. These additional fields can arise, for instance, as assumed in our one-counterion model for the charge environment of the chromophores in BR and HR,[4,17] from an isolated electric charge in the environment of the Schiff base. In that model protonation effects decrease with decreasing distance of the counterion from the Schiff base. Variation of the counterion distance not only controls the chromophores color[4] but also controls ground-state properties.

Figure 4 illustrates for the π-bond orders the control of ground-state properties by variation of the counterion distance d. In this diagram π (PPP) and $\pi + \sigma$ (MNDO) bond orders p_n, as predicted from PPP-HF (left picture) and MNDO-HF calculations (right picture), are shown as a function of the index n of the bond C_n–C_{n+1}. Bond orders are given separately for single and double bonds. According to both types of calculations, bond orders of single bonds increase from the ring to the nitrogen atom and those of the double bonds decrease in this direction. The qualitative agreement of PPP results with MNDO results demonstrates that the variation of bond orders p_n along the polyene chain and their dependence on counterion distance d are caused by the π electrons. We call these variations the $\pi(d,n)$ effects.

To obtain a measure for the $\pi(d,n)$ effects, reference bond orders for double and single bonds are required. As references we have chosen the bond orders p_5 and p_6 of the C_5=C_6 and C_6–C_7 bonds in 2-Å chromophores, respectively. The values of the $\pi(d,n)$ effects for the single bonds are

$$\pi_S(d,n) = p_n(d) - p_6(2\,\text{Å})$$

and those for double bonds are

$$\pi_D(d,n) = p_n(d) - p_5(2\,\text{Å})$$

According to these definitions $\pi_S(d,n)$ values are positive for single bonds and increase with d and n. They indicate a corresponding strengthening of C_n–C_{n+1} bonds and of the respective force constants k_n. For double bonds the $\pi_D(d,n)$ values are negative and decrease with n and d. These values indicate weakenings of C_n = C_{n+1} bonds and of the respective force constants k_n.

Quantitatively, PPP and MNDO calculations predict $\pi(d,n)$ effects for double bonds of similar size. According to the PPP calculations these effects are of the same size for single and double bonds. For single bonds MNDO calculations render markedly different predictions. In MNDO descriptions the $\pi(d,n)$ effect of C–C bonds should be 2 times smaller than those of double bonds. Since in an MNDO description all valence electrons are
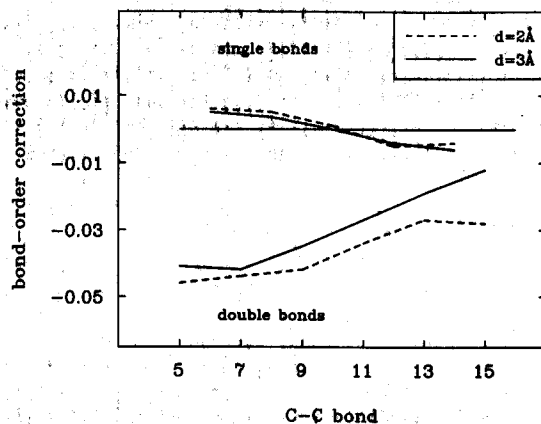
taken into account, MNDO calculations are expected to describe quantitative aspects of $\pi(d,n)$ effects better than PPP calculations. Nevertheless, the qualitative similarity of the results depicted in Figure 4 shows that both methods can be used to judge *qualitative* aspects of the description of protonation effects and of the influence of electrostatic interactions.

2.2. HF Errors for RSBH$^+$. Because the alternation of π-electron densities and π-bond orders of an RSBH$^+$ chromophore strongly depends on protonation state and electrostatic environment, the corresponding HF errors also depend on these parameters. This is illustrated in Figure 5, in which HF errors are presented for two RSBH$^+$ chromophores with counterion distances of $d = 2$ and 3 Å. In this diagram the differences between π-bond orders predicted from PPP-HF and PPP-MRD-CI calculations, are depicted separately for single and double bonds, respectively, as a function of the index n of the bond C_n–C_{n+1}. These differences reflect the correlation corrections to the $\pi(d,n)$ effects discussed above and are denoted as $\Delta_c\pi(d,n)$ corrections.

The $\Delta_c\pi(d,n)$ corrections to the HF bond orders are large and negative for double bonds. Their absolute values decrease from the ring to the nitrogen atom. For single bonds the corrections are small and positive in the neighborhood of the ring, i.e., the π-electron densities in these weak bonds are slightly underestimated by the HF calculations. Near the protonated Schiff base the corrections are negative, i.e., the π-electron densities in these stronger bonds are slightly overestimated. The $\Delta_c\pi(d,n)$ corrections to the HF bond orders of single bonds are also independent of the counterion distance d. In contrast, $\Delta_c\pi(d,n)$ corrections to the double bonds show a pronounced d dependence. The $\Delta_c\pi(d,n)$ corrections to these bonds strongly decrease with increasing counterion distance. As MNDO- and PPP-HF calculations predict similar dependences for bond orders both concerning their variation along the polyene chain and their dependence on

Figure 5. Differences between single bond (upper curves) and double bond (lower curves) bond orders predicted from PPP-HF and PPP-MRD-CI calculations, respectively, for RSBH$^+$ with counterion distances of $d = 3$ and 2 Å. The differences are depicted as a function of the number n of the bond C_n–C_{n+1}.

counterion distance d, we assume for the following discussion of HF errors that the PPP results shown in Figure 5 can be approximately transferred to the MNDO model.

According to Figure 5 HF errors for the description of C–C bond π-electron densities are small and change only slightly along the polyene chain. Therefore, one may expect that the HF errors of corresponding MNDO force constants are mainly due to the σ bonds. Thus, in contrast to the case of the polyene butadiene discussed above, no σ–π compensation of HF errors can be expected for the C–C bonds of RSBH$^+$; in C–C single bonds of a RSBH$^+$ chromophore HF errors resulting from the description of the σ bonds should dominate. In particular, a σ-CH$_3$ HF overestimate should be evident.

For the double bonds of RSBH$^+$ some complications arise. On the one hand, HF errors of π-electron densities are smaller than in butadiene. On the other hand, the strong variation of $\Delta_c\pi(d,n)$ corrections along the polyene backbone suggests that HF force constants of double bonds near the ring are strongly overestimated, whereas the overestimates should be much smaller near the Schiff base. As a result, MNDO force constants of C=C modes are expected to vary more strongly along the chain than the true force constants in RSBH$^+$. This effect should be more pronounced for a counterion distance of $d = 3$ Å than for $d = 2$ Å, because in the latter case the variation of the $\Delta_c\pi(d,n)$ corrections is smaller (cf. Figure 5).

The missing σ–π compensation of HF errors for single bonds in a RSBH$^+$ chromophore has consequences for the couplings of H rock and C–C stretch modes. For butadiene the σ–π compensation together with the global scaling scheme has been shown to underestimate the C–C frequency and, therefore, to underestimate the coupling to the higher frequency hydrogen rocks. For RSBH$^+$ the absence of σ–π compensation will reduce these errors. Due to the σ-CH$_3$ HF overestimate, frequencies of C–C bonds, which are influenced by methyl groups, will probably even be overestimated.

For RSBH$^+$ the HF overestimates of π contributions to C=C force constants are smaller than those for butadiene. Therefore, an improved description of couplings between H rock and C=C stretch modes may be expected. But the variation of $\Delta_c\pi(d,n)$ corrections along the chain will cause a corresponding variation of HF errors for C=C force constants. Since the global mass scaling scheme cannot account for these details considerable errors still have to be expected.

Before we analyze these errors in detail, we like to demonstrate that qualitative aspects of the description of vibrational spectra of RSBH$^+$ are well reproduced by MNDO.

3. Qualitative Effects of Local Electric Fields. We want to investigate now whether the MNDO method is capable of properly describing those electrostatic effects on the intramolecular force field of RSBH$^+$ that are caused by changes in the local charge environment. For this purpose we consider the well-known[2,34] relationship between the color of an RSBH$^+$ chromophore and its intense ethylenic mode which we denote as [C=C]$_+$.

Figure 6 shows calculated [C=C]$_+$ and C=N stretch frequencies of model chromophores with a Cl$^-$ counterion at distances of 2, 3, and 4 Å. The computational results are compared with frequencies observed in BR and in in vitro chromophores like *all-trans*-RSBH$^+$ in solution (PSB$_{sol}$). These chromophores are assigned to model counterion distances according to their respective absorption maximum and according to computational results on optical spectra[23] (see ref 4 for a more detailed justification of that assignment). Besides the effects of a variation of counterion distance d on the [C=C]$_+$ vibration we also include corresponding frequency shifts of the C=N vibration. This serves us to relate the calculated d dependence of the [C=C]$_+$ frequency with frequency shifts for the experimentally well-documented C=N vibration.
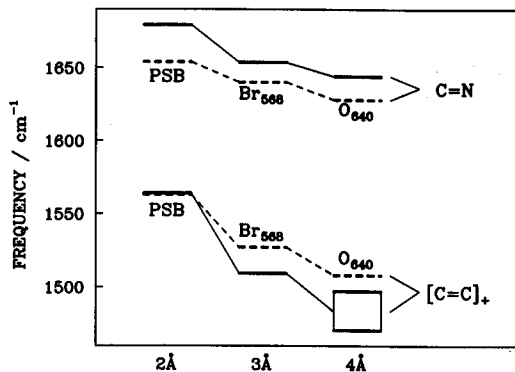
Figure 6. Influence of counterion distance d on C=N and totally symmetric C=C stretching modes of *all-trans*-RSBH$^+$ as calculated according to MNDO; experimental data indicated by dashed lines are taken from refs 35, 19, and 36; the data labeled by "PSB" refer to model compounds in solution with an absorption maximum at about 440 nm.

Figure 6 demonstrates that frequency shifts of ethylenic bands and C=N bands, observed in the sequence O$_{640}$ → BR$_{568}$ → PSB$_{sol}$, are qualitatively reproduced. The relative sizes of the dependencies of the [C=C]$_+$ and C=N frequencies on a change of the charge environment are correctly described. Since MNDO for the 2 Å → 4 Å transition predicts larger red-shifts than observed for PSB$_{sol}$ → O$_{640}$, our assignment of counterion distances to chromophores does not seem to be realistic. The observed red-shifts are close to those calculated for a 2 Å → 3 Å transition. Nevertheless, we still consider our assignment of counterion distances d to chromophores to remain approximately valid because the scaling scheme for the correction of HF errors does not treat compounds with different counterion distances d on an equal footing. For instance, the HF overestimate of the force constant k_{13} of the C$_{13}$=C$_{14}$ bond, which crucially determines the [C=C]$_+$ frequency,[23] significantly decreases with increasing counterion distance. Due to the high-frequency overcompensation of the scaling scheme, this decrease of k_{13} leads to an underestimate of the [C=C]$_+$ frequency with increasing d.

We conclude that an MNDO vibrational analysis provides a qualitatively correct description of effects of local electric fields on the sensitive ethylenic and C=N bands of RSBH$^+$ chromophores if the HF errors outlined above are taken properly into consideration.

4. Normal Modes of BR$_{568}$

*Stretch Vibrations in the Polyene Chain.* In the preceding section bond orders, force constants, and HF errors of stretch vibrations in the polyene chain were discussed. We have shown that all quantities discussed depend strongly on local electrostatic fields in the vicinity of an RSBH$^+$ chromophore. According to our one-counterion model we consider a 3-Å model compound to constitute the best "ad hoc" model for BR$_{568}$.[4,17] But in the following comparisons of observations with theoretical descriptions we will employ also results for 2- and 4-Å model compounds. These additional data will indicate for the individual normal modes the respective sensitivity on the model assumptions concerning the electrostatic environment. Furthermore, they will enable our descriptions to also cover a variety of other all-trans chromophores with absorption maxima in the range between 400 and 600 nm.

1. C–C Stretch Vibrations. Figure 7 depicts C–C stretch vibration frequencies calculated for 2-, 3-, and 4-Å *all-trans*-RET-NH$^+$CH$_3$ model chromophores as well as frequencies observed for these vibrations in BR$_{568}$. Since the C$_6$ atom is part of the β-ionone ring, the spectral position of the C$_6$–C$_7$ stretch vibration is incorrectly described in RET-X chromophores,[23] and we have omitted that vibration in Figure 7. In spite of this disadvantage of RET-X compounds we have decided not to employ RETINAL-X compounds for a discussion of C–C modes. In RETINAL-X compounds additional couplings as well as accidental degeneracies between ring modes and C–C stretching modes may occur, which sensitively depend on the detailed assumptions concerning the charge environment of the chromophore. Addi-

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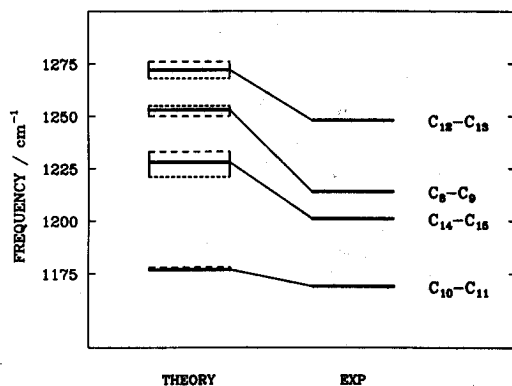
Figure 7. Comparison of C–C stretch frequencies observed for BR_{568} with MNDO calculated C–C frequencies in 2, 3, and 4-Å *all-trans*-RET-NH⁺CH₃ model chromophores. C–C stretch frequencies of 3-Å models are depicted as solid lines; respective frequencies of 2- and 4-Å models are depicted as dashed lines. C–C stretch frequencies in 4-Å model compounds are always larger than respective frequencies in 2-Å compounds, except for the frequencies of the C_8–C_9 vibration, which show the opposite behavior.

tional charges, which in BR are assumed to exist near the ring moiety,[3,37,38] can easily change the interaction between ring modes and C–C stretches. As we did not investigate the influence of such charges in detail, we prefer to quote RET–X results in the following discussion.

It is evident from Figure 7 that the MNDO calculations reproduce the observed spectral order of C–C stretch vibrations in BR_{568}. This spectral order has two causes: (1) As a result of the methylation of carbon atoms C_9 and C_{13} and of the corresponding σ-CH₃ effects, the force constants k_8 and k_{12} of the C_8–C_9 and C_{12}–C_{13} bonds are larger than those of the C_{10}–C_{11} and C_{14}–C_{15} bonds. (2) Due to the protonation of the Schiff base, the π contributions to single bonds increase along the polyene chain from the ring to the nitrogen atom [$\pi(d,n)$ effects, cf. section 3.2.1].

The σ-CH₃ effect, both for the chromophore of BR_{568} and for the model chromophores, is larger than the $\pi(d,n)$ effects and leads to a blue-shift of the C_{12}–C_{13} and C_8–C_9 stretch frequencies relative to C_{14}–C_{15} and C_{10}–C_{11} frequencies. The $\pi(d,n)$ effects modulate the σ-CH₃ effect. Thus, the C_{12}–C_{13} vibration frequency lies above that of the C_8–C_9 vibration. Similarly, the C_{14}–C_{15} stretch has a larger frequency than the C_{10}–C_{11} stretch. As the $\pi(2\text{ Å},n)$ effects are smaller than the $\pi(3\text{ Å},n)$ and $\pi(4\text{ Å},n)$ effects, a decrease of the counterion distance generally causes small red-shifts. For the C_{10}–C_{11} mode such a red-shift is absent since that vibration couples to a lower frequency N–Lys mode. The spectral position of the N–Lys mode close to the C_{10}–C_{11} mode probably is an artifact of MNDO.[23] Correspondingly, the missing red-shift of the C_{10}–C_{11} frequency presumably is a MNDO artifact as well. For the C_8–C_9 vibration the inverse relation between counterion distance and frequency is due to an alteration (upon variation of d) of its coupling with the C_{14}–C_{15} stretch mode.

Figure 7 shows furthermore that all calculated C–C frequencies are blue-shifted with respect to observations, independent of counterion distance. In contrast to butadiene, for a RSBH⁺ the absence of σ–π compensation of HF errors actually causes a slight overestimate of C–C force constants. In our mass scaling scheme this overestimate on the average amounts to 24 cm⁻¹. Its main contribution derives from the σ-CH₃ overestimate which can be concluded from two features in Figure 7: first, the C_8–C_9 stretch vibration, which is affected by the σ-CH₃ overestimate, is strongly (39 cm⁻¹) blue-shifted with respect to observation; in our description this vibration is strongly localized such that its spectral position is mainly determined by the value of k_8; second, the C_{10}–C_{11} stretch, which is unaffected by the σ-CH₃ overestimate, is only slightly (9 cm⁻¹) blue-shifted.

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TABLE I: ¹³C Effects Observed[19] for the C_{14}–C_{15} Band in Vibrational Spectra of the Chromophore of BR_{568} (Exptl) Compared with Calculated Frequency Shifts of the C_{14}–C_{15} Stretch Vibration (Theor)[a]

isotope label	$\Delta\nu$, cm⁻¹		isotope label	$\Delta\nu$, cm⁻¹	
	exptl	theor		exptl	theor
¹³C₈	0	-1	¹³C₁₃	-2	-7
¹³C₉	0	-1	¹³C₁₄¹³C₁₅	-24	-22
¹³C₁₀	0	-2	N–D	0	0
¹³C₁₁	-2	-2	14-D	-8	+1
¹³C₁₂	-1	-1	15-D	+14	+13

[a] For the unlabeled compound the band is observed at 1201 cm⁻¹ and calculated at 1228 cm⁻¹.
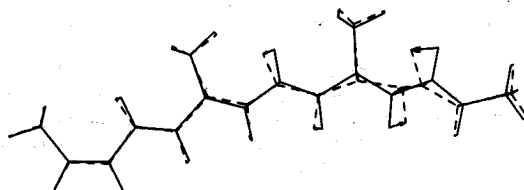
Figure 8. Normal mode pattern of the C_{14}–C_{15} stretch vibration of the 3-Å *all-trans*-RET-NH⁺CH₃ model chromophore predicted by MNDO calculations.

The spectral position of the C_{10}–C_{11} vibration offers the possibility to examine the mass scaling scheme in some more detail. If the PPP results depicted in Figure 5 are transferred to the MNDO model, then the $\Delta_c\pi(d,n)$ correction for the π contribution to k_{10} should vanish. Therefore, k_{10} should reveal the mass-scaled MNDO description of an unsubstituted C–C σ bond. As compared to observations, the slight blue-shift of the calculated C_{10}–C_{11} frequency indicates that our scaling scheme does not correct completely the HF error of such a bond (cf. Figure 7).

Additional information can be obtained from the calculated frequency of the C_{14}–C_{15} normal mode. Figure 5 shows that the HF approximation overestimates the π contribution to k_{14}. Thus, in contrast to the single bond of butadiene, the HF errors of σ and π bonds are additive for the C_{14}–C_{15} bond of RSBH⁺. That σ–π additivity explains the blue-shift of the calculated C_{14}–C_{15} vibration, which is 18 cm⁻¹ larger than that of the C_{10}–C_{11} frequency.

The overestimate of the C_{12}–C_{13} frequency is slightly smaller than those of the C_8–C_9 and C_{14}–C_{15} frequencies. In this case no detailed conclusions can be drawn because (i) this C–C vibration is considerably delocalized, (ii) it is the C–C stretch vibration with the highest frequency, and (iii) it is, therefore, strongly coupled to higher frequency rock modes.

These comparisons of observed and calculated C–C stretch frequencies of unlabeled compounds have shown that MNDO provides a satisfactory description. That conclusion is corroborated by the fact that most observed and calculated frequency shifts of the C–C stretch modes experienced upon ¹²C → ¹³C substitution agree very well. To illustrate the agreement, we compare in Table I observed and calculated ¹³C isotope effects for the C_{14}–C_{15} stretch mode. The agreement demonstrates that the description of calculated C–C normal modes is quite realistic as far as the vibrational amplitudes of the relevant atoms C_{14} and C_{15} are concerned.

Deuteration effects on C–C modes are indicative of couplings with hydrogen rocking modes. In BR_{568}, the C_{14}–C_{15} stretch mode is strongly coupled to the higher frequency 15-rock mode. 15-D isotope labeling decouples these modes from each other. The effect is reproduced by the MNDO description. For a 3-Å *all-trans*-RET-NH⁺CH₃ compound, MNDO predicts 15-D labeling to cause a blue-shift of 13 cm⁻¹ of the C_{14}–C_{15} stretch vibration, which agrees well with the observed blue-shift. In keeping with the size of this blue-shift, the 15-rock mode exhibits large amplitudes in the normal mode pattern of the C_{14}–C_{15} stretch as depicted in Figure 8.

In contrast to the 15-D effect, the effect of 14-D labeling on the C_{14}–C_{15} vibration is not reproduced by our model calculations.
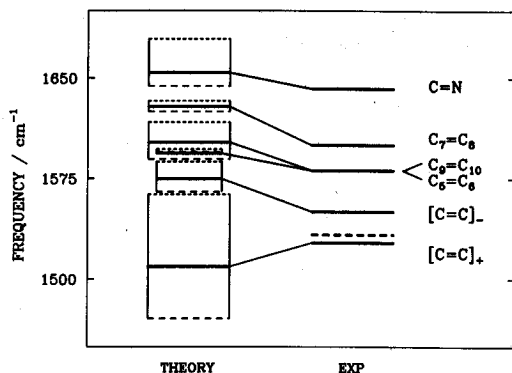
Figure 9. Comparison of C=C stretch frequencies observed in BR$_{568}$ with calculated frequencies of 2-, 3-, and 4-Å *all-trans*-RETINAL-NH$^+$CH$_3$ model chromophores. Vibrational frequencies of 2- and 4-Å chromophores are depicted as dashed lines (4-Å frequencies are always smaller than respective 2-Å frequencies). Experimental data also comprise—as a dashed line—the frequency of a band, which is assigned to the [C=C]$_-$ vibration by Smith et al.[19]

In BR$_{568}$ this vibration is observed to red-shift by 8 cm^{-1} upon 14-D labeling. However, our MNDO-HF calculations yield a vanishing frequency shift. The observation indicates that the effective mass of the C$_{14}$ atom increases upon deuteration and causes a larger red-shift than the blue-shift that arises from the decoupling of the C$_{14}$-C$_{15}$ stretch and 14-rock modes. Consequently, the coupling must be small in BR$_{568}$. Our calculation overestimates this coupling between 14-rock and C$_{14}$-C$_{15}$ stretch modes. Accordingly the 14-rock mode exhibits a large amplitude in the vibrational pattern of the calculated C$_{14}$-C$_{15}$ stretch (cf. Figure 8).

In the vibrational pattern of the C$_{12}$-C$_{13}$ stretch vibration, which in BR$_{568}$ is observed at 1248 cm^{-1} and for the 3-Å all-trans compound calculated at 1272 cm^{-1}, rock modes also have large amplitudes. Therefore, the C$_{12}$-C$_{13}$ stretch in BR$_{568}$ is significantly blue-shifted by 14-D isotope labeling (69 cm^{-1}). That blue-shift is reproduced qualitatively by the MNDO description, but its size is underestimated (29 cm^{-1}).

2. C=C Stretch Vibrations. The RR and IR spectra of retinal chromophores are dominated by the very intense ethylenic band. Shape and spectral position of this band in the range 1500–1600 cm^{-1} characterize the various chromophores. Therefore, a number of spectroscopic investigations have been devoted to reveal structure and composition of this and of its neighboring C=C bands in BR$_{568}$. Smith et al.,[19] for instance, have presented recently a comprehensive RR study of isotope effects in which each C atom of retinal's polyene backbone had been replaced by a ^{13}C isotope. The resulting patterns of complex band shifts have been analyzed by using band fitting procedures. These data allow a detailed evaluation of the quality of theoretical methods employed for the description of the corresponding C=C normal modes and, in particular, of the results obtained from our calculations on 2-, 3-, and 4-Å RETINAL-NH$^+$CH$_3$ model chromophores. That evaluation will be presented below. Our analysis has led us to a partial revision of some of the assignments of bands to normal coordinates given by Smith et al.[19] on the basis of Wilson-FG calculations. Unfortunately, the proof of that revision requires a rather detailed discussion.

To provide an overview, Figure 9 shows the results of our calculations on the frequencies of C=C normal modes of 2-, 3-, and 4-Å models, corresponding data on observed bands and band shoulders in the RR spectrum of BR$_{568}$, and our assignments.

The spectral order of C=C stretch frequencies and their dependence on counterion distance d can be rationalized qualitatively in terms of the $\pi(d, n)$ effects (cf. section 3.2.1). According to these effects, the force constants k_n of the C$_n$=C$_{n+1}$ bonds of an RSBH$^+$ chromophore strongly decrease from the ring toward the nitrogen atom ($k_5 > k_7 > k_9 > k_{11} > k_{13}$). Therefore, the C$_{13}$=C$_{14}$ mode should contribute mainly to the C=C normal coordinate with the lowest frequency, whereas the C$_5$=C$_6$ mode should contribute to that with the highest frequency. Furthermore, the
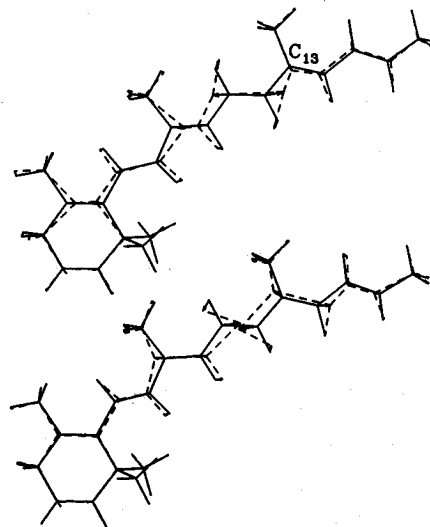
Figure 10. Normal-coordinate pattern of the [C=C]$_-$ stretch vibration in 2- (upper part) and 3-Å (lower part) RETINAL-NH$^+$CH$_3$ chromophores. The hydrogen atoms of the methyl group that models the lysine chain and the proton of the Schiff base have been deuterated to prevent artificial couplings of nitrogen modes and lysine modes with C=C modes.

$\pi(d, n)$ effects increase with d. That increase is more pronounced near the Schiff base nitrogen than near the cyclohexene ring. Therefore, the sensitivity of C=C frequencies with respect to variation of d should decrease with increasing distance of the respective C=C mode from the nitrogen atom or equivalently with increasing frequency of the respective normal mode.

According to the computational results in Figure 9, these expectations, which are derived from the $\pi(d, n)$ effects, generally are confirmed. But these expectations represent slight oversimplifications. First, they neglect the particular molecular environment of the C$_5$=C$_6$ bond. That bond is an integral part of the cyclohexene ring. Therefore, the corresponding stretch mode has a larger effective mass than the other C=C modes that are part of retinal's polyene chain. As a result, the C$_5$-C$_6$ stretch vibration is calculated spectrally below the C$_7$-C$_8$ and for $d = 2$ and 3 Å also below the C$_9$=C$_{10}$ vibration. Second, the two low-frequency C=C normal coordinates cannot be characterized simply in terms of *single* localized C=C modes. These normal coordinates are strongly delocalized. We denote them as [C=C]$_+$ and [C=C]$_-$ normal modes since they are composed of in-phase (+) and out-of-phase (−) combinations of local C=C stretches. In keeping with the order of force constants resulting from the $\pi(d, n)$ effects, the C$_{13}$=C$_{14}$ mode predominantly contributes to the [C=C]$_+$ vibration and the C$_{11}$=C$_{12}$ mode predominantly contributes to the [C=C]$_-$ vibration (cf. Figure 10 for the [C=C]$_-$ vibration).

The general observation that the MNDO-results on the C=C modes are explained by the $\pi(d, n)$ effects leads to the conclusion that σ-CH$_3$ effects, which had been shown to control the spectral order of C-C stretches, are of minor importance for the C=C modes in an RSBH$^+$ chromophore. In contrast, for *unprotonated* retinal Schiff bases the σ-CH$_3$ effects are also decisive for the spectral order of the C=C modes. In these compounds $\pi(d, n)$ effects do not exist. Consequently, the C$_{13}$=C$_{14}$ and C$_9$=C$_{10}$ modes of unprotonated retinal Schiff bases contribute to the two highest frequency C=C normal modes.[39]

Comparison of the MNDO results in Figure 9 with observed C=C band positions shows that the spectrum calculated for the 3-Å chromophore on the average is blue-shifted by 14 cm^{-1} with respect to observations. This average overestimate of C=C force constants is of similar size as that obtained for butadiene (cf. section 3.1). Hence, our scaling scheme does not sufficiently correct HF errors in an RSBH$^+$ chromophore, although in such compound HF errors of the π contributions to the force constants of the C=C bonds are smaller than in butadiene. Note that the

(39) Grossjean, M. F.; Tavan, P. Unpublished results.

TABLE II: ^{13}C Effects Observed[19] for the Ethylenic Band in Vibrational Spectra of the Chromophore of BR$_{568}$ (Exptl) Compared with Calculated Frequency Shifts of the [C=C]$_+$ Stretch Vibration (Theor)[a]

	$\Delta \nu$, cm^{-1}	
isotope label	exptl	theor
$^{13}C_8$	−1	0
$^{13}C_9$	−5	0
$^{13}C_{10}$	−5	−1
$^{13}C_{11}$	−7	−4
$^{13}C_{12}$	−7	−5
$^{13}C_{13}$	−7	−15
$^{13}C_{14}{}^{13}C_{15}$	−10	−12
11,12-D	−36	−10

[a] For the unlabeled compound the band is observed at 1527 cm^{-1} and calculated at 1509 cm^{-1}.

frequency of the [C=C]$_+$ vibration in the 3- and 4-Å compounds is underestimated by the scaling, because for the C_{13}=C_{14} bond the $\Delta_c\pi(d, n)$ correction of the π bond order becomes very small at large d (cf. Figure 5). As a consequence, the high-frequency overcompensation of our scaling scheme entails an underestimate of the force constant of the C_{13}=C_{14} mode.

As for butadiene, for RSBH$^+$ the blue-shift of the C=C stretches should lead to an underestimate of the effective coupling with rock modes (cf. section 3.1).

Figure 9 reveals a further interesting feature of our theoretical description. If we call the difference between the C_7=C_8 and [C=C]$_+$ frequencies the C=C band width of the vibrational spectrum, we note that for the 3-Å model chromophore this C=C band width is larger by 47 cm^{-1} than observed in BR$_{568}$. The cause for this overestimate of the C=C band width is the strong variation of the $\Delta_c\pi(d, n)$ corrections along retinal's polyene chain (cf. Figure 5), which is not taken into account by our global scaling scheme. As a result, for the 3-Å model the differences of calculated C=C force constants are too large. In particular, the differences between k_9, k_{11}, and k_{13} should be smaller than predicted from MNDO calculations, since the $\Delta_c\pi(d, n)$ correction of π contributions to C=C force constants varies most strongly in the region between C_9 and C_{13} (cf. Figure 5). The overestimate of these differences and the underestimate of the effective couplings to rocking modes render calculated C=C normal coordinates that are too strongly localized at the individual C=C bonds. Particularly the C_9=C_{10}, C_{11}=C_{12}, and C_{13}=C_{14} stretch modes in BR$_{568}$ are expected to form linear combinations in which their relative weights are more alike than predicted by MNDO for the corresponding C_9=C_{10}, [C=C]$_-$, and [C=C]$_+$ normal modes.

To check these expectations and to present the arguments for the assignments given in Figure 9, we now want to discuss the individual C=C normal modes and their MNDO description in some more detail.

The lowest frequency C=C band in the spectrum of BR$_{568}$, the intense ethylenic band, is observed at 1527 cm^{-1}. It is assigned to the [C=C]$_+$ normal mode, for which the 3-Å model yields a frequency of 1509 cm^{-1}. The [C=C]$_+$ frequency varies with d between 1564 (2 Å) and 1471 cm^{-1} (4 Å; cf. discussion in section 3.3).

As expected, the comparison of observed and calculated ^{13}C effects in Table II shows that the [C=C]$_+$ normal mode predicted by MNDO for the 3-Å model actually is more localized than the ethylenic mode of BR$_{568}$. Whereas calculated $^{13}C_5$, $^{13}C_6$, ..., $^{13}C_{10}$ effects are considerably smaller than observed, the observed strong contributions of the C_{13}=C_{14} and C_{11}=C_{12} stretch modes are reproduced rather closely by the calculations. The latter observations are significant because they provide evidence that the order of force constants predicted by the $\pi(d, n)$ effects actually agrees with that in the real chromophore of BR$_{568}$.

The [C=C]$_-$ normal mode is predicted to be the second lowest C=C normal mode. Its frequency ranges between 1564 ($d = 4$ Å) and 1588 cm^{-1} ($d = 2$ Å). For the 3-Å model strong amplitudes of the C_{11}=C_{12} and C_{13}=C_{14} stretch vibrations and weaker contributions of C_7=C_8 and C=N stretch vibrations are

found (cf. Figure 10). In the 2-Å model, which exhibits a C=C band width similar to that of the chromophore of BR$_{568}$, the [C=C]$_-$ vibration is more strongly delocalized than in the 3-Å model. Due to the small variation of HF errors near the ring (cf. Figure 5) and the correspondingly stronger coupling of C=C modes in that part of the molecule, the vibrational amplitudes of carbon atoms near the cyclohexene ring are slightly larger whereas the amplitudes of carbon atoms near the nitrogen atom are slightly smaller (cf. Figure 10). The normal mode patterns depicted in Figure 10 and calculated ^{13}C effects[23] show that C_9=C_{10}, C_{11}=C_{12}, and, for 3- and 4-Å models, also C_{13}=C_{14} modes are strongly coupled.

The Wilson–FG calculations of Smith et al.[19] predict the spectral distance of the [C=C]$_+$ and [C=C]$_-$ normal modes to be very small. These authors calculate the frequency of the [C=C]$_+$ vibration at 1528 cm^{-1} and that of the [C=C]$_-$ vibration at 1534 cm^{-1}. Since a peak-fit analysis of the RR spectrum of BR$_{568}$ predicts a weak band at 1533 cm^{-1}, i.e., almost exactly at the calculated frequency of the [C=C]$_-$ vibration, Smith et al. assign this weak band to that vibration.

However, as Smith et al.[19] admit, the mode composition calculated by Wilson–FG for the [C=C]$_+$ and [C=C]$_-$ normal modes is incorrect. All ^{13}C effects on the ethylenic band observed by Smith et al. are at variance with the C=C mode character described by the Wilson FG force field; particularly, the red-shifts of the ethylenic band upon ^{13}C labeling of the atoms C_{13} and C_{14} are strongly underestimated, whereas they are strongly overestimated for C_{11} and C_{12}. According to their calculations, the [C=C]$_+$ vibration has mainly a C_9=C_{10} mode character. Furthermore, in the [C=C]$_-$ vibration the C_{11}=C_{12} mode has the largest contribution to the normal-mode pattern. No sizeable C_{13}=C_{14} contributions are found. A normal mode with C_{13}=C_{14} contributions, as observed for the ethylenic mode of BR$_{568}$ and calculated by MNDO for the [C=C]$_+$ and [C=C]$_-$ normal modes, is predicted at a much higher frequency (1580 cm^{-1}). Thus, it seems that the $\pi(d, n)$ effects which render the sequence $k_{13} < k_{11} < k_9$ of C=C force constants have not been accounted for in the empirical construction of the Wilson–FG force field. As a consequence, the assignments of Smith et al. are incorrect.

If we take spectral distances and frequencies of the [C=C]$_+$ and [C=C]$_-$ vibrations calculated for the 3-Å chromophore as a basis and if we further take into account that the $\Delta_c\pi(d, n)$ correction of k_{11} is larger than that of k_{13}, then we expect the frequency of the [C=C]$_-$ vibration of the chromophore of BR$_{568}$ in the spectral range between 1540 and 1560 cm^{-1}. Indeed, a band shoulder at 1550 cm^{-1} is observed in the spectrum of BR$_{568}$. We assign that shoulder to the [C=C]$_-$ vibration. Smith et al. assign this band shoulder to a "combination mode". As we will show further below, our assignment allows a consistent interpretation of observed isotope effects. But before we embark on this interpretation, we will continue with the assignment of the remaining C=C bands.

The third C=C band in the spectrum of BR$_{568}$ is observed at 1581 cm^{-1}. According to the MNDO calculations only the C_9=C_{10} and C_5=C_6 stretch vibrations can give rise to that band. These vibrations are predicted, spectrally close, in the frequency range 1589–1600 cm^{-1} (cf. Figure 9). Figure 11 depicts calculated normal coordinate patterns of the C_9=C_{10} stretch vibration in 2- and 3-Å models. As argued further above, that vibration is too strongly localized in the 3-Å model and should contain larger contributions of the C_{11}=C_{12} and C_{13}=C_{14} stretch modes. In the 2-Å model the smaller C=C band width leads to a larger delocalization of the C_9=C_{10} normal mode (cf. Figure 11). But that effect is not yet large enough to explain all observations of isotope effects.

Our assignment of the band at 1581 cm^{-1} to *two* C=C stretch vibrations, the C_9=C_{10} and C_5=C_6 vibrations, is at variance with the assignment of Smith et al. of this band to only *one* vibration with a predominant C_{13}=C_{14} character. In addition, our assignment implies a lower spectral position of the C_5=C_6 stretch vibration (about 1580 cm^{-1}) than suggested by Smith et al. on the basis of their Wilson–FG normal mode calculations (1598
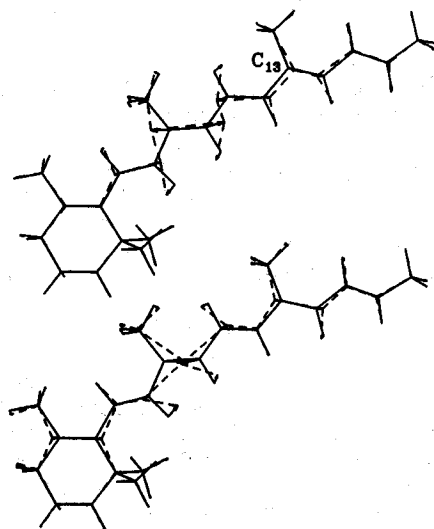
Figure 11. Normal-coordinate pattern of the C_9=C_{10} stretch vibration in 2- (upper part) and 3-Å (lower part) RETINAL-NH$^+$CH$_3$-chromophores. As in Figure 10 the methyl groups that models the lysine chain and the Schiff base have been deuterated (cf. caption to Figure 10).

cm^{-1}). In the spectrum of BR$_{568}$ we found no hint for a band at 1598 cm^{-1}. Our assignment is supported by the band pattern of C=C vibrations in the spectrum of $^{13}C_9$ isotopically labeled BR$_{568}$. In the RR spectrum of that chromophore, a band, lying in the unlabeled chromophore at 1581 cm^{-1}, looses intensity, and a small band at 1580 cm^{-1} remains, which we assign to the C_5=C_6 vibration. The band shoulder, which in the unlabeled chromophore lies at 1550 cm^{-1}, gains intensity, is strongly broadened, and thus indicates the appearance of the red-shifted C_9=C_{10} vibration. The remaining observed ^{13}C effects on the 1581-cm^{-1} band can also be explained by our assignment.

The highest frequency C=C band in the spectrum of BR$_{568}$ is observed at 1600 cm^{-1}. In the spectra of $^{13}C_7$- and $^{13}C_8$-labeled chromophores this band is red-shifted. Therefore, it has been identified as the C_7=C_8 vibration by Smith et al. The MNDO results confirm this assignment.

To substantiate our assignments of the C=C normal modes, we consider the RR spectrum of *11,12-d*-labeled BR$_{568}$. That is the only spectrum published by Smith et al. in which all five C=C bands are clearly separated from each other. Here, the ethylenic band is observed at 1491 cm^{-1}, i.e., *11,12-d* labeling entails a red-shift of 36 cm^{-1} for this band. Further C=C bands are observed at 1514, 1544, 1581, and 1595 cm^{-1}. The MNDO calculations predict for the [C=C]$_+$ and [C=C]$_-$ vibrations red-shifts of 10 and 6 cm^{-1} upon *11,12-d* labeling. Due to the general underestimate of the couplings between rocks and C=C stretches outlined further above, these frequency shifts are too small on an absolute scale. On a relative scale the red-shift of the [C=C]$_+$ vibration is larger than that of the [C=C]$_-$ vibration.

At a first glance, the latter MNDO result may appear to be somewhat puzzling, since the C_{11}=C_{12} character of the [C=C]$_+$ normal mode is weaker than that of the [C=C]$_-$ normal mode. But the size of the *11,12-d* effect on a chosen C=C normal mode depends not only on the size of the contribution of the C_{11}=C_{12} mode but also on the size of the coupling to the corresponding H rocks. According to the MNDO description that coupling is much stronger in the [C=C]$_+$ than in the [C=C]$_-$ normal mode. Consideration of HF errors and scaling scheme explains why MNDO arrives at that prediction: As argued further above, k_{13} is underestimated and k_{11} is overestimated, leading to corresponding errors of the frequencies of the [C=C]$_+$ and [C=C]$_-$ normal modes, respectively. These HF errors for the [C=C]$_+$ mode render a much smaller spectral distance to the H-rock modes than for the [C=C]$_-$ mode and, hence, a much larger effective coupling.

On the basis of that analysis and on the published data on ^{13}C effects,[19] we estimate for BR$_{568}$ that the *11,12-d*-induced red-shifts of the [C=C]$_+$ and [C=C]$_-$ vibrations should be approximately

of the same size, i.e., of about 36 cm^{-1}. According to that estimate, the band at 1514 cm^{-1} in the spectrum of *11,12-d*-labeled BR$_{568}$ should arise from the shoulder at 1550 cm^{-1} in the spectrum of the unlabeled chromophore. Because of significant C_{11}=C_{12} contributions, the C_9=C_{10} stretch vibration should also exhibit a large red-shift upon *11,12-d* labeling. Therefore, the band at 1544 cm^{-1} in *11,12-d*-labeled BR should be due to the C_9=C_{10} vibration, which in the unlabeled chromophore was assigned to a band at 1581 cm^{-1} together with the C_5=C_6 vibration. We assign the band at 1581 cm^{-1} to the strongly localized and, consequently, frequency-invariant C_5=C_6 stretch mode and the band at 1595 cm^{-1} to the less localized C_7=C_8 vibration, which exhibits a small red-shift of 5 cm^{-1} upon *11,12-d* labeling of the chromophore.

3. Hoop and Rock Vibrations of Hydrogen Atoms. In the following paragraph we compare observed band positions of Hoop and H-rock vibrations, localized near the nitrogen atom, with MNDO results for the 3-Å RET-NH$^+$CH$_3$ model. As these modes do not couple strongly to ring modes and do not directly depend on the counterion distance d, we need to consider solely that model compound. In the real chromophore BR$_{568}$ a d dependence arises indirectly through couplings to d-dependent. N-hoop and N-rock modes. As these modes are poorly described, possible MNDO-HF artifacts have to be avoided carefully.[23]

3.1. Hoop Vibrations. The 14-hoop mode is coupled with the ill-described N-hoop mode. The latter in BR$_{568}$ is observed[19] at 942 cm^{-1} but by the MNDO description predicted[23] at 723 cm^{-1}. However, in N–D-labeled compounds the coupling between the 14-hoop and H-hoop is negligible, and the deficiency of the MNDO method does not matter. For this reason we compare observed and calculated frequencies in N–D-labeled chromophores. In such a chromophore the observed 14-hoop frequency of 887 cm^{-1} agrees with the calculated frequency of 889 cm^{-1}.

The 15-hoop mode is coupled more strongly with the N-hoop mode than with the 14-hoop mode. In an N–D-labeled 3-Å model chromophore the 15-hoop vibration is predicted at 973 cm^{-1}. This prediction agrees with the frequency of a band in N–D-labeled BR$_{568}$ at 976 cm^{-1}, which has been assigned to the 15-hoop vibration.[40] That assignment, which deviates from the results of Smith et al.,[19] who on the basis of their Wilson–FG calculations assign a band at 996 cm^{-1} to the 15-hoop vibration in N–D-labeled BR$_{568}$, has recently been confirmed by IR dichroism experiments that were combined with a MNDO vibrational analysis.[41]

We like to conclude that differences between observed and calculated 14-hoop and 15-hoop frequencies and differences between observed and calculated N–D isotope effects can be explained as consequences of an underestimate of the frequency of the N-hoop mode.[23]

3.2. Rock Vibrations. To date experimentalists did not find agreement in assigning the 15-rock vibration in unlabeled BR$_{568}$. FTIR studies[20] assign that vibration to a band at 1334 cm^{-1}, whereas RR studies identify a band near 1346 cm^{-1} as the 15-rock mode.[19,18] In our model chromophore the frequency of that vibration is calculated at 1355 cm^{-1}. It is hardly influenced by changes of the counterion distance or N–D labeling of the chromophore. In 15-D labeled BR$_{568}$ the 15-rock the 15-rock vibration is observed at 974 cm^{-1} and calculated in the corresponding model chromophore at 965 cm^{-1}. The good agreement of observed and calculated frequencies demonstrates that the 15-rock force constant is rather well described by MNDO calculations.

The 14-rock vibration has been assigned to a band at 1358 cm^{-1} by Stockburger et al.[18] According to the MNDO calculations of RETINAL-NH$^+$CH$_3$ chromophores the 14-rocking mode is coupled with 8-, 10-, and 12-rock modes as well as with ring modes. Therefore, we employ that model chromophore for analysis of 14-rock vibrations. For the 3-Å RETINAL-NH$^+$CH$_3$ model the MNDO calculations predict four normal vibrations at 1315, 1338,

(40) Alshuth, T. Thesis, Universität Göttingen, 1985.
(41) Fahmy, K.; Grossjean, M. F.; Siebert, F.; Tavan, P. *J. Mol. Struct.* **1989**, *214*, 257.

1422, and 1429 cm^{-1} with 14-rock character, to which the 14-, 12-, 10- and 8-rock modes mentioned above contribute with different phase combinations and slightly different amplitudes. The frequencies of these vibrations are scarcely influenced by changes of the counterion distance. Furthermore, these vibrations are only weakly coupled with the ill-described N-rock mode. For 14-D-labeled BR$_{568}$ observed frequencies of the split 14-D rocking mode (988/974 cm^{-1}) agree well with calculated spectral positions (990/1027 cm^{-1}).

4. C=N Stretch Vibration. In BR$_{568}$ the C=N stretch vibration is observed at 1642 cm^{-1} and for the 3-Å chromophore predicted at 1654 cm^{-1}. The theoretical overestimate indicates an underestimate of the "spectroscopic" mass of the nitrogen atom by our scaling scheme. The overestimate entails a difference between the calculated force constants of the C=C and C=N bonds which is much larger than in BR$_{568}$. Therefore, couplings between the C=N stretch mode and the other C=C modes are underestimated. For instance, in N–D labeled BR a red-shift of 7 cm^{-1} of the C$_7$=C$_8$ band is observed,[19] whereas the C$_7$=C$_8$ vibration remains frequency-invariant in our calculations.

5. Summary

We have investigated in this paper to what extent a quantum chemical MNDO vibrational analysis provides a correct description of vibrational spectra of polyenes and polyene Schiff bases. We have focussed our investigated in this respect on the influence of protonation and of environmental electrostatic interactions on C$_n$–C$_{n+1}$ bond orders and normal modes. If systematic errors of the MNDO-HF method are being taken into consideration, vibrational modes of polyenes and of protonated Schiff bases of retinal (RSBH$^+$) are described well. The errors of the MNDO-HF method are mainly due to its neglect of electron correlation.

We have shown that some of the deficiencies of the MNDO-HF method can be corrected by a global scaling of the force matrix, e.g., by introducing "spectroscopic" masses for each atom type. However, this correction scheme cannot properly account for the details of the correlation effects in polyenes and polyene derivatives. To elaborate on this point we have compared for butadiene and RSBH$^+$ bond orders from MNDO-HF, PPP-HF, and PPP-CI calculations. We have shown that a global scaling of the force matrix yields reasonably accurate vibrational frequencies only for unsubstituted polyenes. We have also demonstrated that such global rescaling leads to considerable errors in the quantitative description of the normal mode frequencies of an RSBH$^+$ chromophore. The errors could be rationalized to arise from the variation of σ-electron densities introduced by methylation of the polyene backbone (σ-CH$_3$ effects) and from the variation of the π-electron densities in RSBH$^+$ [$\pi(d, n)$ effects, see section 3]. It has been demonstrated, that the $\pi(d, n)$ effects vary with the distance between the respective C$_n$–C$_{n+1}$ bond from the protonated Schiff base nitrogen atom and with the counterion distance d. Corresponding to the variation of electron densities, caused by

the σ-CH$_3$ and $\pi(d, n)$ effects, correlation effects and thus HF overestimates of force constants vary along the polyene chain. This variation cannot be corrected by a global scaling scheme of the force matrix. Due to this deficiency, the spectral distances between lower and higher frequency C–C and C=C modes, between lower frequency C–C modes and H-rock modes, between H-rock modes and higher frequency C=C modes, and between the lowest frequency C=C mode and the C=N mode are overestimated. Therefore, corresponding couplings are underestimated.

In spite of the flaws of the mass-scaled MNDO-HF vibrational analysis, a comparison of calculated spectra with observations on BR$_{568}$ and other *all-trans*-RSBH$^+$ chromophores, such as O$_{640}$ and *all-trans*-RSBH$^+$ in solution, has demonstrated that several essential features in the spectra of these compounds are reproduced: the spectral order of C–C and C=C stretching modes is correctly predicted; isotope effects observed for these modes are satisfactorily calculated; correlations between the frequencies of the RSBH$^+$'s ethylenic and C=N modes and the absorption maximum of the chromophore are also fairly well described. If HF errors in the description of $\pi(d, n)$ and σ-CH$_3$ effects are taken into account, the remaining deviations of theoretical results from observations can be properly understood.

In comparison with empirical methods for the description of vibrational modes, such as Wilson–FG calculations employing Urey–Bradley type force fields, our mass-scaled MNDO vibrational analysis yields a more reliable description of vibrational spectra of polyenes and RSBH$^+$. In particular, the influences of local electric fields, protonation effects [$p(d, n)$] and substituent effects [σ-CH$_3$] are directly and systematically included in our quantum chemical description. Furthermore, our description provides a systematic approach toward an understanding of the remaining errors. All these aspects are lacking in an empirical method such as Wilson–FG. Such method does not comprise the clues for an explanation of the considerable deviations between its predictions and the observations.

Therefore, we suggest that a quantum chemical vibrational analysis using a semiempirical valence-shell model can furnish reliable interpretations of vibrational spectra of polyenes, polyene derivatives, and other conjugated molecules. Since most of the errors arise from the HF approximation and are not due to the restriction to a semiempirical Hamiltonian (like MNDO), we suggest that an inclusion of correlation effects in a quantum chemical (MNDO, AM1) vibrational analysis will render much improved theoretical descriptions of normal modes. Efficient algorithms for the calculation of correlation effects using large CI expansions become available today, and their use for the vibrational analysis of biomolecules appears to be inevitable.