The Bacteriorhodopsin Chromophore Retinal and Derivatives: An Experimental and Theoretical Investigation of the Second-Order Optical Properties

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Abstract: The first hyperpolarizabilities, β, of retinal and related derivatives were measured experimentally by using the hyper-Rayleigh scattering (HRS) technique and compared to the calculated values obtained with the semiempirical intermediate neglect of differential overlap/configuration interaction/sum-over-states (INDO/CI/SOS) method. The experimental and theoretical results are in excellent mutual agreement. By substitution of the *trans*-retinal aldehyde group for stronger or weaker electron-withdrawing groups, the positions of the absorption band maxima could be systematically varied. Theoretical and experimental data confirm the expected linear dependence between β and the inverse of the square of the first transition energy as well as an exponential increase of β with the number of double bonds in the polyene segment. It was found that, upon *trans* to 13-*cis* or 9-*cis* isomerization of a retinal double bond, a constant fraction of the β value is lost, regardless of the nature of the electron-withdrawing group or the solvent. We find an increase in the β value by about 1 order of magnitude in passing from the neutral form of the retinal Schiff base to the protonated form which is present in the bacteriorhodopsin protein. The observed changes in the depolarization ratios are in accordance with the changes in molecular structure.

I. Introduction

Bacteriorhodopsin (bR) is the light-energy transducing protein that is present in the purple membrane of *Halobacterium halobium*. Upon photoexcitation, the protein goes through a photocycle during which a proton is pumped from the cytoplasmic side to the extracellular side of the membrane.[1] It is now established that the key element in the light absorption process and photocycle of bR and related rhodopsins is the chromophore retinal, which is covalently linked to the protein backbone by a protonated Schiff base linkage.[2] There have been extensive efforts directed toward a better understanding of the chromophore−apoprotein interactions that give the protein its characteristic photocycle and a UV−vis absorption which is red-shifted with respect to that of retinal protonated Schiff base (RPSB) in solution. A number of works have focused on thorough studies of the photochemical properties of RPSB and related derivatives in a bulk medium;[3−11] in particular, the pioneering studies of Blatz and co-workers have proved very valuable for understanding the mechanism of the regulation of the protein absorption wavelength.[7,8]

Because of its thermal stability and unique photochemical properties, bR is an excellent candidate for use in opto-electronics and operational devices have already been reported.[12,13] The first hyperpolarizability, β, which is an important parameter for the construction of bR-based three-dimensional optical memories,[13] of the protein is unusually high, and attempts have been made to measure β using two-photon spectroscopy[14] and second-harmonic generation in thin films;[15] these techniques, however, do not give direct access to β, and some critical assumptions have to be made. The more traditional electric-field-induced second-harmonic generation (EFISHG) technique cannot be used due to the presence of charged amino acid residues on the protein. By using the hyper-Rayleigh scattering (HRS) technique,[16−18] we have recently been able to carry out the first direct measurement of β;[19] the HRS β value of 2100×10^{-30} esu at a fundamental wavelength of 1.06 μm is in good agreement with the values measured in the

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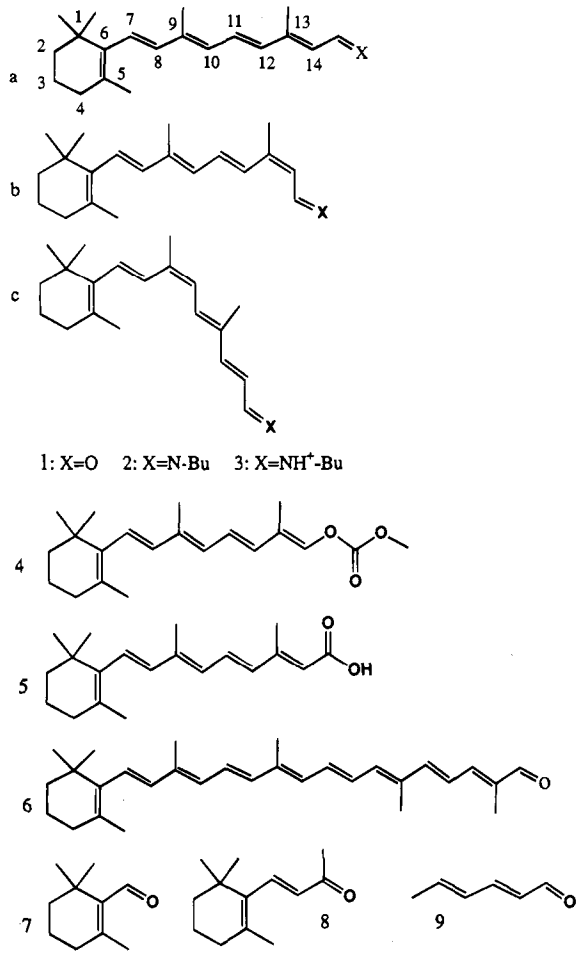

Figure 1. Molecular structure of the compounds studied in this work: **1**, retinal; **2**, retinal Schiff base; **3**, retinal protonated Schiff base (**a**, **b**, and **c** refer to the *trans*, 13-*cis*, and 9-*cis* conformations); **4**, vitamin A acetate; **5**, retinoic acid; **6**, β-apo-8′-carotenal; **7**, β-cyclocitral; **8**, β-ionone; **9**, 2,4-hexadienal.

previous two experiments. We also demonstrated the sensitivity of the β HRS value with regard to the state of aggregation of the protein.

In order to better understand the high hyperpolarizability of the protein, we report in this work a systematic experimental and theoretical study of the first hyperpolarizability of various chromophores based on retinal; these are sketched in Figure 1 and correspond to *trans*-, 13-*cis*-, and 9-*cis*-retinal (**1a**−**c**); *trans*-, 13-*cis*-, and 9-*cis*-retinal Schiff base (**2a**−**c**); *trans*-, 13-*cis*-, and 9-*cis*-retinal protonated Schiff base (**3a**−**c**); vitamin A acetate (**4**), retinoic acid (**5**), β-apo-8′-carotenal (**6**), β-cyclocitral (**7**), β-ionone (**8**), and 2,4-hexadienal (**9**). Note that the retinal derivatives we investigate in this work present 6-*s*-*cis* conformations which are more stable in solution than the 6-*s*-*trans* analog present in the bR protein. The experimental and theoretical methodologies to study the first hyperpolarizability are presented in section II. The theoretical calculations are carried out at the semiempirical level and introduce a series of approximations with respect to *ab initio* methods; therefore, we are interested in only trends rather than in absolute values. Moreover, we do not take into account any solvent effect which is relevant in the case of retinal derivatives.[19] The β results are given in section III; we first examine the relationship between β and the optical absorption in the case of the *trans* derivatives; we then discuss the conjugation length effect on the β values by investigating compounds where the number of double bonds along the conjugated segment increases up to 10; finally, we examine the *cis*−*trans* isomerization effect.

II. Experimental Techniques and Theoretical Methodology

A. Compounds. *trans*-Retinal, 9-*cis*-retinal, 13-*cis*-retinal, β-cyclocitral, vitamin A acetate, and 13-*cis*-retinoic acid were purchased from Sigma-Aldrich, while β-apo-8′-carotenal and 2,4-hexadienal were purchased from Fluka; these compounds were used without further purification. *trans*-Retinoic acid was a gift from BASF.

The Schiff bases of retinal are prepared by adding an 8-fold excess of *N*-butylamine to a 10^{-3} M solution of retinal in methanol at room temperature. *N*-Butylamine is chosen because of its high reactivity toward retinal and its similarity to the ε-amino group of the lysine residue that links the chromophore to the bacteriorhodopsin backbone. The reaction proceeds rapidly and with high yield (>95%), as can be verified with UV−vis spectroscopy. Since the excess *N*-butylamine does not change the HRS signal, no attempt is made to remove it. The Schiff bases are protonated by passing anhydrous HCl through the solution. The concentration of the protonated Schiff bases is determined using UV−vis spectroscopy, and again the yield is found to be larger than 95%. All solutions are prepared directly prior to the measurements under a dim light. The measurements only last for *ca.* 1 h, and during this time, no appreciable degradation of the samples is found to take place. The samples are always kept in the dark during the measurements.

B. Hyper-Rayleigh Scattering. 1. Theory. The first hyperpolarizability of the visual chromophores is measured using the hyper-Rayleigh scattering technique (HRS). Since this technique is not widely used yet, we find it useful to summarize here its main principles and advantages. An HRS measurement is conducted by measuring the intensity of the frequency-doubled light that is generated by focusing an intense laser beam on an isotropic solution.[16−18] The fact that second-order optical processes can occur in an isotropic solution is due to the fluctuations in molecular orientations that instantaneously break the centrosymmetry of the solution.[20,21] These orientational fluctuations play the same role in second-order light scattering as the translational fluctuations (density fluctuations) do in linear light scattering. For a liquid composed of noncentrosymmetric molecules and an incident laser beam of frequency ω, the X component of the macroscopic polarization at frequency 2ω, $P_X(2\omega)$, is proportional to the product of the macroscopic second-order susceptibility tensor and the electric fields:

$$P_X(2\omega) = B_{XYZ}\, E_Y(\omega)\, E_Z(\omega) \tag{1}$$

B_{XYZ} is the XYZ component of the nonlinear susceptibility tensor in laboratory coordinates, and E_Y and E_Z are the electric fields in the Y and Z directions. Due to the orientational fluctuations, it is only the average of B_{XYZ} that is equal to 0. If the incident light travels in the X direction and is polarized in the Z direction while the scattered light is observed in the Y direction, the intensity of the HRS signal will be equal to

$$I_Z(2\omega) = G\,\langle B_{ZZZ}^2\rangle\, I^2(\omega) \tag{2}$$

$I_Z(2\omega)$ is the intensity of the light at frequency 2ω, traveling in the Y direction and polarized in the Z direction; the bracket symbols, $\langle\,\rangle$, indicate orientational averaging. Equation 2 is valid only if orientational correlations between molecules exist over distances that are small compared to the wavelength. G is an instrumental factor that takes into account the scattering geometry, photomultiplier gain, and collection efficiency; it remains unchanged during a series of measurements.

For a solution composed of noninteracting solvent (S) and solute (s) molecules, the macroscopic nonlinear susceptibility can be written as a function of the corresponding microscopic hyperpolarizabilities and number densities:

$$\langle B_{ZZZ}^2\rangle = F_\omega^4 F_{2\omega}^2 (N_S\,\langle\beta_{ZZZ}^2\rangle_S + N_s\,\langle\beta_{ZZZ}^2\rangle_s) \tag{3}$$

where $F_{2\omega}$ and F_ω are the local field factors at frequencies ω and 2ω. N_S and N_s denote the molecular number density of the solvent and solute, respectively. Inserting eq 3 in eq 2 finally yields

$$I_Z(2\omega) = GF_\omega{}^4F_{2\omega}{}^2 (N_S \langle\beta_{ZZZ}{}^2\rangle_S + N_S \langle\beta_{ZZZ}{}^2\rangle_S) I^2(\omega) \quad (4)$$

In a typical hyper-Rayleigh scattering experiment, the dependence of the ratio $I_Z(2\omega)/I^2(\omega)$ is recorded for a delution series of the solute molecule. At low solute concentrations, N_S is approximately constant and $I_Z(2\omega)/I^2(\omega)$ will show a linear dependence on the number density of the solute molecules. This linear dependence observed for *trans*-retinal is shown in Figure 2. From the intercept of this plot, or $I_Z(2\omega)/I^2(\omega)$ for the pure solvent, the product of the instrumental G factor and the local field factors can be determined as

$$\frac{\left(\frac{I_Z(2\omega)}{I^2(\omega)}\right)_S}{N_S\langle\beta_{ZZZ}{}^2\rangle_S} = GF_\omega{}^4F_{2\omega}{}^2 \quad (5)$$

The slope of this plot is equal to $GF_\omega{}^4F_{2\omega}{}^2\langle\beta_{ZZZ}{}^2\rangle_S$. Thus, the value of the slope can be used to calculate the product $GF_\omega{}^4F_{2\omega}{}^2$ if the hyperpolarizability of the solute molecule is known. This factor can then be used to calculate the hyperpolarizability of the solvent molecules.

By using these values for the hyperpolarizabilities of the solvents, the number densities of the solvents, and the ratio $I_Z(2\omega)/I^2(\omega)$ for the solvent, $GF_\omega{}^4F_{2\omega}{}^2$ can be calculated. Knowledge of this product and the obtained value of the slope then allows the hyperpolarizability of the solute molecule to be calculated. By using this approach, called the internal reference method, we can effectively eliminate the local field factors by virtue of always measuring in the same local field. The absence of analytical expressions for the local field factors thus is a major advantage of HRS over EFISHG. Both reference values have been found to reproduce closely the reported EFISHG β values for standard NLO molecules such as *p*-nitroanisol, methoxynitrostilbene, and hydroxynitrostilbene.[16] The HRS β value of 2100×10^{-30} esu for the protein bacteriorhodopsin[19] was also in good agreement with other values obtained via two-photon spectroscopy[14] and second-harmonic generation in thin films.[15]

A linear dependence of $I_Z(2\omega)/I^2(\omega)$ versus N_s is observed, except if the light at 2ω is absorbed by the solution; in this case, a Lambert–Beer correction term has to be taken into account:[22]

$$I_Z(2\omega) = GF_\omega{}^4F_{2\omega}{}^2(N_S \langle\beta_{ZZZ}{}^2\rangle_S + N_s \langle\beta_{ZZZ}{}^2\rangle_S) I^2(\omega)\, 10^{(-\sigma N_s l)} \quad (6)$$

Here, σ is the absorption cross section at frequency 2ω; l denotes an effective optical path length that is approximately equal to the average distance the harmonic light has to travel through the absorbing solution. Notice that, if $\sigma N_s l < 0.1$, the exponential term is close to one and a linear dependence of $I_Z(2\omega)/I^2(\omega)$ versus N_s is found; at higher number densities ($\sigma N_s l > 0.7$), absorption dominates and an exponential decay of $I_Z(2\omega)/I^2(\omega)$ is observed.

The relation between $\langle\beta_{ZZZ}{}^2\rangle$ in laboratory coordinates and $\langle\beta^2{}_{zzz}\rangle$ in the molecular reference frame can be found by performing the orientational average over the direction cosines of the transformation from laboratory to molecular coordinates.[23,24] This relation is given by

$$\langle\beta_{ZZZ}{}^2\rangle = \frac{1}{7}\sum_i \beta_{iii}{}^2 + \frac{6}{35}\sum_{i\neq j}\beta_{iii}\beta_{ijj} + \frac{9}{35}\sum_{i\neq j}\beta_{iij}{}^2 + \frac{6}{35}\sum_{ijk,cycl}\beta_{iij}\beta_{jkk} + \frac{12}{35}\beta_{ijk}{}^2 \quad (7)$$

For the light scattered in the Y direction and polarized in the Z direction,

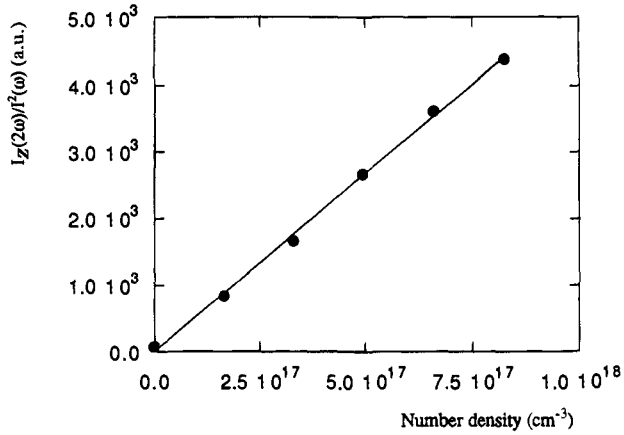

Figure 2. Linear dependence of $I_Z(2\omega)/I^2(\omega)$ on the number density of *trans*-retinal molecules in methanol. From the intercept, slope, hyperpolarizability, and number density of the solvent, the hyperpolarizability of *trans*-retinal can be calculated.

this expression is equal to

$$\langle\beta_{XZZ}{}^2\rangle = \frac{1}{35}\sum_i \beta_{iii}{}^2 - \frac{2}{105}\sum_{i\neq j}\beta_{iii}\beta_{ijj} + \frac{11}{105}\sum_{i\neq j}\beta_{iij}{}^2 - \frac{2}{105}\sum_{ijk,cycl}\beta_{iij}\beta_{jkk} + \frac{8}{35}\beta_{ijk}{}^2 \quad (8)$$

For circularly and elliptically polarized light, it is possible to determine similar expressions. In general, five independent functions of the molecular tensor components can be measured. The quantum-chemical calculations indicate that the main tensor component for these derivatives is β_{zzz}, so the term $\beta_{zzz}{}^2$ will dominate these expressions. If we explicitly evaluate these expressions on one hand using the calculated β tensor for *trans*-retinal and on the other hand for a typical HRS measurement where the sum of the two polarizations is detected, an error of only 2% is introduced if the other tensor components are neglected.

The depolarization ratio, on the other hand, is very sensitive to small, off-axis β tensor components. In the case of a molecular scatterer with $C_{\infty v}$ symmetry and possessing only two significant molecular tensor components, this relation is

$$\frac{\langle\beta_{ZZZ}{}^2\rangle}{\langle\beta_{XZZ}{}^2\rangle} = \frac{15 + 18k + 27k^2}{3 - 2k + 11k^2} \quad (9)$$

with $k = \beta_{xxx}/\beta_{zzz}$. As a result, from the depolarization ratio of the light traveling in the y direction, $\langle\beta_{ZZZ}{}^2\rangle/\langle\beta_{XZZ}{}^2\rangle$, we can obtain information on the ratio of the two main tensor components. For a hypothetical molecule with only a β_{zzz} component, the depolarization ratio is equal to 5.

We stress that in the expressions for HRS, an isotropic average is made of all the molecular β tensor components. This clearly indicates that HRS is sensitive to all the molecular β tensor components. If the number of independent components is limited to five due to symmetry considerations, it is possible to resolve them using linearly, circularly, and elliptically polarized incident light and the different depolarization ratios. The EFISHG technique, on the other hand, is sensitive to molecular tensor components that are nonperpendicular to the molecular dipole moment. For dipolar molecules, where the molecular tensor component in the direction of the charge transfer axis is the largest, HRS and EFISHG give similar results. For ionic species, however, the strong dc field applied with EFISHG will induce migration rather than orientation.

2. Experimental Aspects. The HRS measurements are performed in methanol or chloroform by measuring the quadratic dependence of $I(2\omega)$ on $I(\omega)$ for various number densities in the retinal derivative. The quadratic factor, obtained from the fit, is then plotted as a function of the number density of the solute. To deduce the molecular hyperpolarizability from the plot, the number densities and hyperpo-

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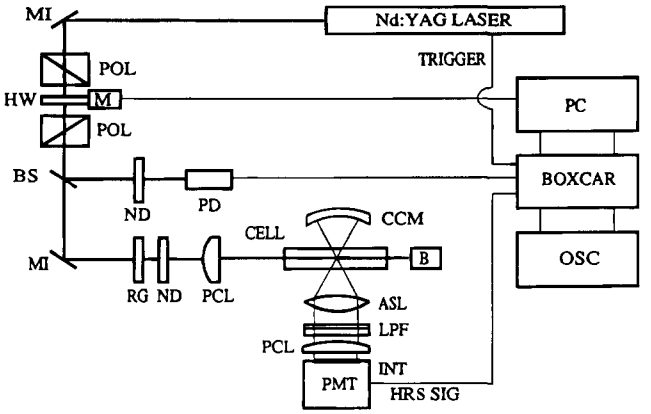

Figure 3. Experimental HRS setup: ASL, aspheric lens condenser; B, beamstop; BS, beam sampler; CCM, concave mirror; HW, half-wave plate; INT, 532 nm interference filter; LPF, low-pass filter; M, stepping motor; MI, mirror; ND, neutral density filter; OSC, oscilloscope; PC, personal computer; PCL, plano convex lens; PD, photodiode; PMT, photomultiplier tube; POL, polarizer; RG, high-pass filter; SIG, signal.

larizabilities of methanol and chloroform from ref 16 are used ($\beta_{methanol} = 0.69 \times 10^{-30}$ esu, $\beta_{chloroform} = -0.49 \times 10^{-30}$ esu). All the hyperpolarizabilities are reported in electrostatic units (esu). They can be converted into SI units ($C^3\ m^3/J^2$) by dividing by a factor of 2.693×10^{20}.

When measurements were repeated several times for a single molecule, the spread on the hyperpolarizabilities was approximately 15%. This was the case for retinal in chloroform and in methanol. A similar error margin was used in previous studies.[16] The spread of 15% is also consistent with a more detailed error analysis, taking into account the errors on the intercept and the slope. Therefore, we estimate a relative error of 15% for the hyperpolarizabilities listed here.

3. HRS Setup. A fundamental wavelength of 1064 nm is obtained from an injection seeded, Q-switched Nd:YAG laser (Quanta-Ray DCR3, 10 ns, 220 mJ, 10 Hz). Fifteen percent of the energy of the fundamental beam is focused into a small cell (volume of 3.5 mL). The intensity of the fundamental beam is varied by rotating a half-wave plate between crossed polarizers and is monitored with a photodiode. The harmonic light that is generated at the focal point is collected by an efficient condenser system, consisting of a concave mirror and two lenses, and is measured by a photomultiplier. By using a 532 nm interference filter and a low-pass filter, we make sure that only second-harmonic light is measured. Gated integrators are used to retrieve actual values for the intensities of fundamental and harmonic light. Data collection and rotation of the half-wave plate are computer controlled. A general layout of the setup is shown in Figure 3. A more detailed description of the setup can be found in ref 17.

The depolarization ratios are measured after removing the lenses from the condensor system and covering the concave mirror. One extra polarizer is placed directly in front of the focusing lens and another in front of the photomultiplier. The numerical aperture is approximately 0.10 to avoid signal averaging over directions significantly deviating from the direction perpendicular to the incident beam.

C. Theoretical Methods. The geometries of all the molecules are optimized using the semiempirical Hartree–Fock Austin Model One (AM1) method;[25] this method is known to reproduce accurately the molecular structures of organic molecules. On the basis of these geometries, the first hyperpolarizability, β, is calculated by means of the sum-over-states (SOS) approach based on the perturbation expansion of the Stark energy of the molecule;[26] such an approach has already been successfully exploited in recent years.[27-31] The states are described *via* the semiempirical intermediate neglect of differential overlap/single configuration interaction (INDO/SCI) method using a modified version of the ZINDO quantum-chemical package.[27] We have shown previously the suitability of such a combination of techniques (INDO/SCI/SOS calculations of the states on the basis of AM1 geometries) to investigate the hyperpolarizability, β, of organic molecules.[28] All the details of the present calculations are similar to those explicitly given in ref 28a. Normally, the SOS expressions have to be carried over an infinite set of excited states. Here, we actually use 40 states to calculate the different β components; in our previous work, we have shown that this choice provides fully converged β values in the case of diphenylacetylene donor–acceptor compounds.[28a] We have checked that the same holds true here.

We calculate the norm of the vector part of the β tensor according to

$$\beta_v = (\beta_x^2 + \beta_y^2 + \beta_z^2)^{1/2} \tag{10}$$

where for instance the β_x component is given by

$$\beta_x = \beta_{xxx} + \frac{\beta_{xyy} + 2\beta_{yyx} + \beta_{xzz} + 2\beta_{zzx}}{3} \tag{11}$$

Since we also evaluate the frequency dependence of β, we do not adopt Kleinman symmetry.[33]

By following Oudar,[34] and assuming that the second-order optical response of push–pull organic molecules is governed by the ground state $|0\rangle$ and a low-lying intramolecular charge-transfer excited state $|e\rangle$, a simple expression can be derived for the real part of β_{zzz}:

$$\beta_{zzz}(-2\omega;\omega,\omega) = \underbrace{\frac{3\Delta\mu_{eg,z}M_{eg,z}^2}{(\hbar\omega_{eg})^2}}_{\text{static factor}} \underbrace{\frac{\omega_{eg}^2}{\left(1-\frac{4\omega^2}{\omega_{eg}^2}\right)(\omega_{eg}^2-\omega^2)}}_{\text{dispersion factor}} \tag{12}$$

where $\Delta\mu_{eg,z}$, $M_{eg,z}$, and $\hbar\omega_{eg}$ are the dipole moment difference along the z-axis, the electronic transition moment along the z-axis, and the energy of transition, respectively, between the ground state and the first excited state. This corresponds to the two-state model, which has been shown to give reasonable estimates of β in a number of organic molecules,[34,35,27] Note that, in relation 12, the static factor reflects the intrinsic value of the two-state model (i.e., when $\omega = 0$) and we also introduce the dispersion factor which is usually not included. The two-state model is a simple tool to describe the β response in terms of the electronic and spectroscopic properties of organic molecules; we will also test its validity in the series of retinal compounds by comparing the β values to the converged (40-state) β values.

III. Results and Discussion

A. Dependence of β on the First Transition Energy. We first focus our investigation on the *trans* derivatives. The first hyperpolarizabilities of vitamin A acetate, retinoic acid, retinal Schiff base, retinal, and retinal protonated Schiff base have been measured in methanol and are reported in Table 1, along with the positions of the absorption maxima in methanol as well as the static INDO/SOS results. In solution, these derivatives are

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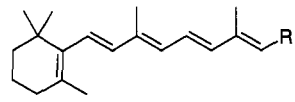
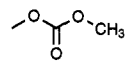
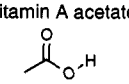
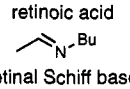
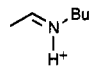
Table 1. Experimental (EXP) and Theoretical (TH) First Hyperpolarizability (in 10^{-30} esu) of Various Retinal Derivatives[a]



R	$\hbar\omega_{eg}$ (eV) MeOH (EXP)	$\hbar\omega_{eg}$ (eV) INDO (TH)	β 1064 nm MeOH (EXP)	β_v^0 INDO/SOS (TH)	β_v^0 two-state INDO/SOS (TH)	β^0 (EXP)
 vitamin A acetate	3.80	3.78	140	2.4	22.9	80
 retinoic acid	3.53	3.52	310	38.0	114.4	160
 retinal Schiff base	3.40	3.60	470	17.9	70.0	220
 retinal	3.26	3.50	730	41.5	120.5	300
 RPSB	2.79	2.42	3600	214.6	388.6	900

[a] The measurements are performed in methanol. The columns refer to the first optical transition energy $\hbar\omega_{eg}$, the experimental $\beta(2\omega; \omega = 1064$ nm) values, the calculated static β_v^0 values (obtained over 40 states and using the two-state model), the experimental extrapolated static β^0 values.

not completely planar due to steric interactions between the 1,1′-methyl groups and the hydrogen atom on carbon 7, which cause the 6−7 single bond to assume a 6-*s*-*cis* conformation.[2] The AM1-optimized geometries of all the molecules indicate a torsion angle between the ring and the polyene chain in the range of 48.7° (corresponding to the protonated form) to 53.3°. This is in agreement with those found in previous studies.[36]

The fact that substituting the aldehyde end group of the retinal polyene system for a stronger electron-withdrawing group induces a bathochromic shift in the position of the absorption band maximum was already thoroughly studied in 1967.[37] Since then, a vast number of retinal derivatives have been prepared, especially in order to achieve a better understanding of the chromophore−apoprotein interactions that give the retinal protonated Schiff base its characteristic absorption spectrum when it is incorporated into the bacteriorhodopsin binding pocket.[3-11] The derivatives we consider in this work have been chosen on the basis of their absorption band maxima, which span the range from 326−445 nm, and their commercial availability.

Typical organic NLO chromophores contain a polarizable π-electron system end-capped with an electron donor and an electron acceptor to create an asymmetric molecular environment.[38] The first hyperpolarizability, β (2ω; $\omega = 1064\ \mu$m) = 730×10^{-30} esu, for retinal in methanol is much higher than that one would generally expect for a molecule without a strong electron donor moiety. Our experimental value for retinal is also higher than the previously reported EFISHG value for retinal in DMSO[39] and the value for retinal in monolayers.[40] Quantum-chemical calculations and experimental data have been used to explain the large solvent dependence of the hyperpolarizability of polyenes in terms of bond-length alternation evolution.[19,41-44] It was found that the bond-length alternation (BLA) of simple donor−acceptor polyenes is reduced in more polar solvents such as methanol. In apolar solvents, the bond-length alternation is large and consequently the hyperpolarizability drops; it comes down to a value of 270×10^{-30} esu for retinal in chloroform.[19] A similar ratio between the hyperpolarizabilities measured in chloroform and in methanol is found for retinoic acid and the *cis* derivatives of retinal and retinoic acid (see Table 4). This indicates that the main effect on the hyperpolarizability is due to the bulk influence of the solvent polarity on the molecule and not to specific hydrogen bonding interactions. Note that the aldehyde group has been found to be an efficient electron acceptor for polyenes.[42-45] The importance of the aldehyde group can be estimated by comparing the β value of *trans*-retinal to that of vitamin A acetate. We stress that possible applications of these molecules in devices will be limited by their very broad absorption band and their instability when exposed to air and light for prolonged periods of time.

The absorption spectra of *trans*-retinal and its derivatives are shown in Figure 4. The first transition energy of retinal has been found to be sensitive to both solvent polarity and specific (hydrogen bonding) interactions, but the observed evolution is not as strong as that observed in the case of pure donor−acceptor polyenes.[43] These specific interactions also give rise to an increase in the quantum yield of fluorescence due to state order reversal;[5] this fluorescence was, however, too weak to affect the HRS measurements. The *trans*-retinal protonated Schiff base is the only compound that weakly absorbs at 532 nm. As was mentioned before, a more electron-withdrawing end group shifts the position of the absorption maximum to longer wavelengths. The experimental values at 1064 nm have been extrapolated using the dispersion factor of the two-state model to correct for the dispersive enhancement.

Both the experimental extrapolated static results and the theoretical results show a strong correlation between β^0 and $(1/\hbar\omega_{eg})^2$ (see Figure 5), which derives from the two-state model. The larger slope found in the experimental curve most likely originates in solvent effects that act to increase β. Note that there is an inversion in the trends between theory and experiment

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(37) Rosenberg, B.; Krigas, T. M. *Photochem. Photobiol.* **1967**, *6*, 769.
(38) Cheng, L.-T.; Tam, W.; Stevenson, S. H.; Meredith, G. R.; Rikken, G.; Marder, S. R. *J. Phys. Chem.* **1991**, *95*, 10631.
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(40) Huang, J.; Lewis, A.; Rasing, Th. *J. Phys. Chem.* **1988**, *92*, 1756.
(41) Marder, S. R.; Beratan, D. N.; Cheng, L. T. *Science* **1991**, *252*, 103.
(42) Marder, S. R.; Gorman, C. B.; Meyers, F.; Perry, J. W.; Bourhill, G.; Brédas, J. L.; Pierce, B. G. *Science* **1994**, *265*, 632.
(43) Bourhill, G.; Brédas, J. L.; Cheng, L. T.; Marder, S. R.; Meyers, F.; Perry, J. W.; Tiemann, B. G. *J. Am. Chem. Soc.* **1994**, *116*, 2619.
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(45) Meyers, F.; Brédas, J. L.; Zyss, J. *J. Am. Chem. Soc.* **1992**, *114*, 2914.

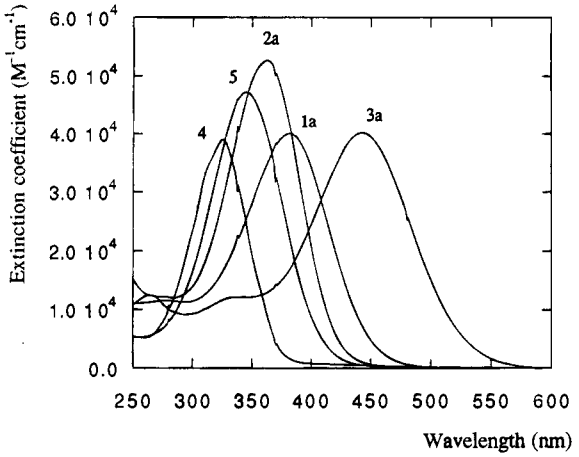

Figure 4. Absorption spectra of vitamin A acetate (**4**), retinoic acid (**5**), retinal Schiff base (**2a**), retinal (**1a**), and retinal protonated Schiff base (**3a**).

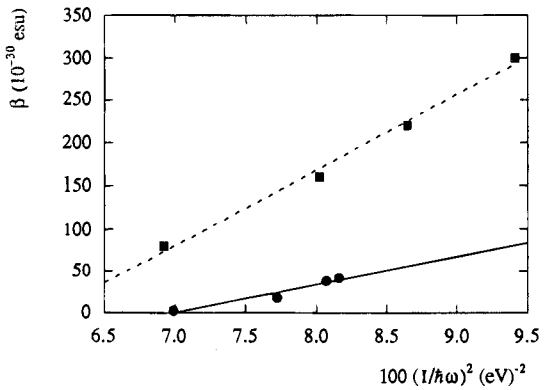

Figure 5. Dependence of the experimental extrapolated static hyperpolarizabilities (dashed line) and the INDO/SCI/SOS theoretical static hyperpolarizabilities (solid line) on the transition energy. (left to right) Experimental values: vitamin A acetate, retinoic acid, retinal Schiff base, retinal protonated Schiff base. Theoretical values: vitamin A acetate, retinal Schiff base, retinoic acid, retinal (*trans*-retinal protonated Schiff base is omitted for reasons of clarity).

with regard to retinoic acid and retinal Schiff base; we have no detailed explanation for this discrepancy.

In Table 1, we make the comparison between the static β_v^0 values obtained, on the one hand, using 40 states in the SOS approach and, on the other hand, only two states (the ground state and the first excited state). The two-state model reproduces the trends followed by the converged β_v^0 values. We stress that this does not mean that the first excited state gives the only significant contribution to the β_v^0 value; it is clear from the theoretical results that other states do contribute to the converged β_v^0 value; however, the contributions from the first excited state provide the trend followed by the first hyperpolarizabilities within the series of investigated molecules. Such two-level pictures have been recently reviewed by Kanis *et al.*[46]

By following the two-state model, the β value can be described as a function of the spectroscopic properties of the molecule. The parameters in the two-state model, *i.e.*, $\Delta\mu_{eg}$, $(M_{eg})^2$, and $(\hbar\omega_{eg})$,[2] can be obtained from the INDO/SCI analysis of the first excited state and are presented in Table 2. We observe that the evolution of the β values is mostly governed by the evolution of the dipole moment difference between the ground and excited states and the evolution of the first transition energy; the evolution of $(M_{eg})^2$ is weak except in the case of the retinal protonated Schiff base. Note that some enhancement

(46) Kanis, D. R.; Ratner, M. A.; Marks, T. J. *Chem. Rev.* **1994**, *94*, 195.

Table 2. INDO/SCI Calculated Electronic Characteristics of the First Excited State in *trans*-Retinal Derivatives[a]

derivative	$\Delta\mu_{eg}$	M_{eg}^2	$\hbar\omega_{eg}$
vitamin A acetate	0.84	121.17	3.78
retinoic acid	8.97	129.24	3.52
retinal Schiff base	5.65	142.67	3.60
retinal	8.92	133.46	3.50
retinal protonated Schiff base	10.11	198.15	2.42

[a] The M_{eg}^2 and $\Delta\mu$ values are in D^2 and D, respectively, and the $\hbar\omega_{eg}$ values, in eV.

Table 3. Theoretical Static and Dynamic (at 1.165 eV) β_v Values (in 10^{-30} esu) Obtained with the INDO/SCI/SOS Method

derivative	β_v^0 INDO/SOS (40 states)	β_v 1064 nm INDO/SOS (40 states)
vitamin A acetate	2.4	7.1
retinoic acid	38.0	100.0
retinal Schiff base	17.9	47.0
retinal	41.5	109.0
retinal protonated Schiff base	214.6	2228

in the β value is expected due to a resonance effect in the case of the retinal protonated Schiff base. Experimentally, the two-photon resonance occurs at $\hbar\omega_{eg}/2 = 1.4$ eV. This resonance is not far from the frequency used in the hyper-Rayleigh measurements ($\hbar\omega = 1.165$ eV). In Table 3, we report the INDO/SOS β values calculated at 1.165 eV for the different compounds. The dynamic β values are about 3 times greater than the static values except for the retinal protonated Schiff base, for which the β value is strongly affected by resonance and is increased by 1 order of magnitude with respect to the static case. The experimentally observed difference between the static and dynamic responses for retinal protonated Schiff base is less pronounced than the corresponding theoretical difference. The theoretical values show a dramatic increase because the $\hbar\omega/2$ calculated value (1.21 eV) nearly coincides with the Nd:YAG frequency (1.165 eV). However, in both the theoretical and experimental cases, this resonance enhancement effect is strongest in the case of RPSB.

B. Influence of Conjugation Length. The length dependence of the second-order polarizability in conjugated organic molecules is a recurring theme in nonlinear optics work. A lot of studies have been performed for instance on disubstituted α,ω-diphenylpolyene and diphenylacetylene oligomers,[28,35,47-49] on disubstituted α-phenylpolyene oligomers,[47-51] and on monosubstituted benzodithia polyenals.[45] For these structures, capped at one or both ends with an aromatic group, an exponential increase of β with the number of π electrons has always been observed. The calculated exponential dependence of the first hyperpolarizability on the number of π electrons is weaker than that determined experimentally for a series of α,ω-disubstituted α-phenylpolyenes and 4,4′-disubstituted α,ω-diphenylpolyenes.[52]

In Table 4, we present the measured and calculated values (both at zero frequency and 1064 nm) of the first hyperpolarizability for β-cyclocitral, β-ionone, retinal, and β-apo-8′-carotenal. These are polyene derivatives with 2, 3, 6, and 10 double bonds, respectively (if we include the CO double bond in the polyene system). The spectral properties of these molecules have been studied in detail to determine the nature

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(48) Huijts, R. A.; Hesselink, G. L. J. *Chem. Phys. Lett.* **1989**, *156*, 209.
(49) Stiegman, A. E.; Graham, E.; Perry, K. J.; Khundkar, L. R.; Cheng, L. T.; Perry, J. W. *J. Am. Chem. Soc.* **1991**, *113*, 7568.
(50) Dehu, C.; Brédas, J. L. *Int. J. Quantum Chem.* **1994**, *52*, 89.
(51) Beljonne, D.; Dehu, C.; Brédas, J. L. *Nonlinear Opt.*, in press.
(52) Matsuzawa, N.; Dixon, D. A. *Int. J. Quantum Chem.* **1992**, *44*, 497.

Table 4. Dependence of the Experimental (EXP) and Theoretical (TH) First Hyperpolarizabilities (in 10^{-30} esu) on the Number of Double Bonds in the Polyene Chain

derivative	no. of double bonds	β 1064 nm $CHCl_3$ (EXP)	β^0_{ext} $CHCl_3$ (EXP)	$\hbar\omega_{eg}$ (eV) INDO (TH)	β_v^0 INDO/SOS (TH)	β_v 1064 nm INDO/SOS (TH)	β_v^0 two-state (TH)
β-cyclocitral	2	5	3.2	4.77	2.5	3.8	5.1
β-ionone	3	10	5.7	4.28	9.0	15.8	19.1
retinal	6	270	103	3.50	41.5	108.0	120.5
carotenal	10	1040	220	2.93	115.5	538.5	355.3

of the electronic states of retinal derivatives.[5] The calculated values at 1064 nm follow the same trend as the measured values: $\log(\beta) = 3.02 \log(n) - 0.297$ for the calculated values and $\log(\beta) = 3.54 \log(n) - 0.481$ for the measured values, where n is the number of double bonds.

A strong similarity exists between the absorption spectra of the polyenals, related to retinal, and the corresponding polyenones, where the hydrogen atom of the aldehyde function is substituted by a methyl group. For polyenones, the absorption maxima are in general at slightly higher energies and the intensities are in many cases lower. Futhermore, as is dicussed in section III, these molecules have different conformations. These effects, combined with the reduced electron-withdrawing properties of the ketone group, can explain the observation that the hyperpolarizabilities do not correlate perfectly with the number of double bonds.

The values for retinal and especially carotenal are strongly enhanced by resonance, as can be seen from Table 4. For β-cyclocitral, the presence of a weak, but well-separated, n$-\pi^*$ (a′$-$a″) transition is noted below the onset of the main absorption band that corresponds to the a″$-$a″ (π-π^*) transition. Upon chain elongation, the n$-\pi^*$ band is completely swamped by the $\pi-\pi^*$ transition that shifts to higher wavelengths.[5]

Here also, the two-state model fits the trend followed by the converged β values. The fact that this model remains valid for the extended derivatives can be explained by the presence of a very intense peak dominating the low-wavelength region of the spectra, related to the $\pi-\pi^*$ transition. Note that, in our INDO/SCI analysis and in agreement with the experimental spectra, we also observe the n$-\pi^*$ transition below the $\pi-\pi^*$ transition for β-cyclocitral and β-ionone. However, the associated oscillator strength is extremely weak. As a result, we can expect that there is no significant contribution to β coming from such a transition.

The experimental β^0 values were estimated using the ratio between the calculated INDO/SOS values at zero frequency and 1064 nm:

$$\beta^0_{est} = \beta^{1064}_{exp} \times \frac{\beta^0_{cal}}{\beta^{1064}_{cal}} \qquad (13)$$

The estimated extrapolated β^0_{est} values evolve with the number of double bonds according to $\log(\beta^0_{est}) = 2.87 \log(n) - 0.433$. In Figure 6 we compare this evolution with the theoretical INDO/SOS static relation $\log(\beta^0) = 2.35 \log(n) - 0.248$. In the resonant case as well as in the static case, the theoretical equations agree very well with their experimental counterparts but seem to underestimate the magnitude of the slope. However, we have to keep in mind that we do not take into account the solvent effect in our calculations. We already pointed out that this effect boosts the β value in the case of the *trans*-retinal molecule,[19] and one can suspect this effect to be greater in longer compounds, since the stronger the conjugation, the stronger the effect would be. Recently, simulating the solvent effect by a self-consistent reaction field at the semiempirical level, Yu *et al.* have found that the magnitude of the slope of the log(β)/log(conjugation) relation in the case of push$-$pull molecules can be larger when the solvent is taken into account.[53]

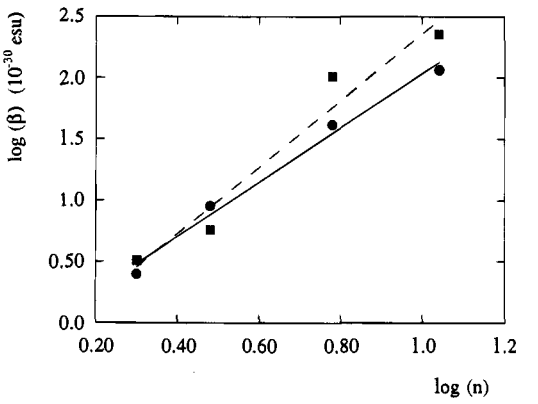

Figure 6. Conjugation length dependence of the theoretical static first hyperpolarizability (solid line, circles) and experimental static first hyperpolarizability calculated from eq 13 for (from left to right) β-cyclocitral, β-ionone, retinal, and β-apo-8′-carotenal.

Table 5. Influence of the 6,7-*s*-*cis* to 6,7-*s*-*trans* Isomerization:Experimental (1064 nm) First Hyperpolarizabilities (in 10^{-30} esu) and Depolarization Ratios of 2,4-Hexadienal and β-Ionone

species	β 1064 nm $CHCl_3$	depolarization dioxane
2,4-hexadienal	13	2.9
$\beta-$ionone	9.9	1.9

The magnitudes of the slopes found here fall in the range of the observed slopes for the α,ω-diphenylpolyene derivatives, where two double bonds are counted for each phenyl unit.[38,44] These derivatives were substituted with a strong electron donor$-$acceptor system, and the magnitude of the slope was shown to be dependent upon the nature of the end groups. Because of the difference in the counting schemes used and the nature of the end groups, there is no simple way of comparing these results to ours.

C. Influence of *cis*$-$*trans* Isomerization. At this stage, it should be noted that the derivatives we investigate have different conformations due to the change in the steric ring-chain interactions as the polyene chain is elongated. This causes the 6$-$7 single bond to assume a planar 6-*s*-*cis* conformation in β-cyclocitral and β-ionone and a 6-*s*-*cis* conformation, with an angle of approximately 50° between the ring and the polyene chain, for the retinal derivatives, as was mentioned above. Apart from this bond, all other single and double bonds are in the *trans* conformation. The importance of the 6-*s*-*cis* to 6-*s*-*trans* isomerization can be seen in comparing the experimental data of β-ionone to those of 2,4-hexadienal (see Table 5).

Whereas the 25% decrease in the hyperpolarizability can be attributed to both the single bond conformation and the different end groups, the change in the depolarization ratio is related directly to the *s*-*cis* isomerization. As was discussed in section II, a molecule with a single dominant β tensor component should have a depolarization ratio equal to 5. Since β-ionone is a bent

(53) Yu, J.; Zerner, M. C. *J. Chem. Phys.* **1994**, *100*, 7487.

Table 6. Influence of the *trans*-to-*cis* Isomerization of a Double Bond on the Experimental (1064 nm) Hyperpolarizability (in 10^{-30} esu) of the Various Retinal Derivatives

derivative (solvent)	β *trans*	β 13-*cis*	β 9-*cis*
retinoic acid (chloroform)	110	67	
retinal (chloroform)	270	170	161
retinoic acid (methanol)	310	160	
retinal Schiff base (methanol)	470	260	220
retinal (methanol)	730	480	470
RPSB (methanol)	3600	2440	2450

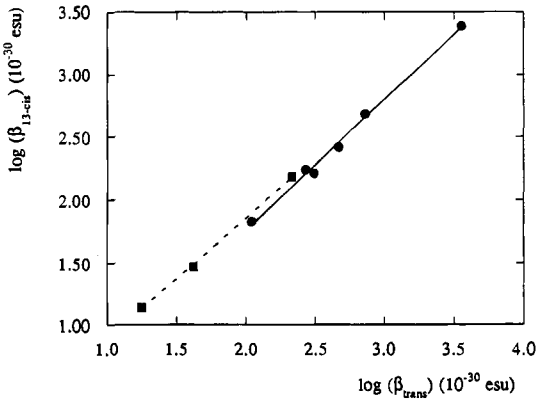

Figure 7. Effect of *trans*-to-13-*cis* isomerization on the experimental (1064 nm, circles, solid line) and INDO/SCI/SOS static theoretical (squares, dashed line) first hyperpolarizability of retinal derivatives. (left to right) Experimental: retinoic acid ($CHCl_3$), retinoic acid (MeOH), retinal ($CHCl_3$), retinal Schiff base (MeOH), retinal (MeOH), retinal protonated Schiff base (MeOH). Theoretical: retinal Schiff base, retinal, retinal protonated Schiff base.

Table 7. Theoretical (static) Results of the *trans*-to-*cis* Isomerization Effect on the First Hyperpolarizability (in 10^{-30} esu

derivative	β_v *trans*	β_v 13-*cis*	β_v 9-*cis*
retinal Schiff base	17.9	13.7	13.0
retinal	41.5	29.8	32.2
RPSB	214.6	151.3	117.3

molecule, we expect the off-axis β component to become more important. This results in a lower depolarization ratio for β-ionone as compared to 2,4-hexadienal (1.9 *vs* 2.9).

The experimental results for the 13-*cis* and 9-*cis* derivatives are shown in Table 6, along with the values for the corresponding *trans* derivatives from Table 1. The absorption maxima of the *cis* derivatives are within 0.06 eV of the absorption maxima of their *trans* counterparts. From the plot of $\log(\beta_{13\text{-}cis})$ to $\log(\beta_{13\text{-}trans})$, shown in Figure 7, it can be seen that, upon *trans*-to-*cis* isomerization of a retinal double bond, a constant fraction of β is lost. This fraction does not seem to be dependent upon the solvent, since it is the same for the derivatives dissolved in methanol and in chloroform.

The theoretical results of the *trans*-to-13-*cis* isomerization effect on retinal, retinal Schiff base, and retinal protonated Schiff base confirm the experimental observations (see Table 7); the β value decreases by 30% for the 13-*cis* derivatives whereas the evolution from the 13-*cis* to 9-*cis* isomers does not result in a significant change of the β value except for the protonated form where the 13-*cis* to 9-*cis* isomerization results in another decrease of 20% in the β value. The *trans*-to-*cis* evolution can be theoretically understood by analyzing the evolution of the different parameters which appear in the two-state model (see Table 8). In agreement with experimental results, passing from the *trans* to the 13-*cis* form hardly affects the first transition energy (the larger shift being 0.02 eV). However, we observe that the dipole moment difference as well as the electronic

Table 8. INDO/SCI Calculated Electronic Properties of the First Excited States of *trans*-, 13-*cis*-, and 9-*cis*-Retinal Derivatives[a]

derivative	$\Delta\mu_{eg}$	M_{eg}^2	$\hbar\omega_{eg}$
trans-retinal	8.92	133.46	3.50
13-*cis*-retinal	8.45	122.40	3.49
9-*cis*-retinal	8.33	119.63	3.49
trans-retinal Schiff base	5.65	142.67	3.60
13-*cis*-retinal Schiff base	4.65	134.08	3.59
9-*cis*-retinal Schiff base	5.09	129.94	3.59
trans-retinal protonated Schiff base	−10.12	198.15	2.42
13-*cis*-retinal protonated Schiff base	−9.25	162.89	2.42
9-*cis*-retinal protonated Schiff base	−7.31	162.50	2.43

[a] The M_{eg}^2 and $\Delta\mu$ values are in D^2 and D, respectively, and the $\hbar\omega_{eg}$ values, in eV.

transition moment between the two states is weaker for the *cis* form with respect to the *trans* form.

The negative sign of $\Delta\mu$ in the case of the protonated derivatives can be explained by analyzing the INDO/SCI total charge distribution in the ground state and in the first excited state, for example in the case of *trans*-retinal protonated Schiff base (Figure 8). In the ground state, we observe that the positive charge is mostly localized on the right part of the molecule containing the protonated amino group (0.7|e|) (the molecule is arbitrarily separated into two parts by the dashed line in the figure), whereas in the first excited state, the charge is delocalized more along the molecule. As a result, and contrary to what usually happens in donor−acceptor systems in the gas phase, the high and negative $\Delta\mu$ value is due to a weaker charge separation in the first excited state than in the ground state.

Garito and co-workers have already pointed out that the effect of *trans*-to-*cis* isomerization decreases the second hyperpolarizability γ value in polyenes.[54] For polyenes with an equal number of double bonds, they calculated the value of γ to be smaller for the *cis* conformation. The shorter distance along the conjugated axis produced by the *cis* geometry is the main reason for the observed decrease. They concluded that γ is more sensitive to the length of the chain than to its conformation. Keeping this in mind, we have also analyzed theoretically the evolution of β with the distance along the conjugated bridge (obtained from the AM1-optimized geometries) in the case of the *trans* derivatives (compounds **7**, **8**, **1a**, and **6**), *i.e.*, the distance (d), in angström, between C1 in the ionone ring and the aldehyde acceptor group (see Figure 1). In Figure 9 we present the linear dependence found between $\log(\beta_{v,trans})$ and $\log(d)$ which is characterized by the following equation:

$$\log(\beta_{v,trans}) = 1.88 \log(d) - 0.442$$

If we consider the $\log(\beta_v)/\log(d)$ values corresponding to the 13-*cis* and 9-*cis* retinal derivatives (compounds **1b** and **1c**, respectively), we find that the two sets of coordinates obey the above equation (see Figure 9). The smaller β values observed for a molecule in the *cis* conformation and with the same number of double bonds can thus be traced back to the shorter distance separating the β-ionone ring from the aldehyde acceptor group. The conclusions drawn by Garito and co-workers concerning the *trans*-to-*cis* isomerization effect thus also hold true for β in our series of compounds.

It is relevant to examine the hyperpolarizability of the 13-*cis* derivatives because, upon light absorption, the *trans*-retinal protonated Schiff base in bacteriorhodopsin undergoes in the first part of the photocycle,[1] a *trans*-to-13-*cis* photoisomerization to the so-called L state. In the following step, the Schiff base

(54) Garito, A. F.; Heflin, J. R.; Wong, K. Y.; Zamani-Khamiri, O. *Nonlinear Optical Properties of Polymers*; Heeger, A. J., Orenstein, J., Ulrich, P. R., Eds.; Materials Research Society: Pittsburgh, PA, 1988; Vol. 109, p 91.

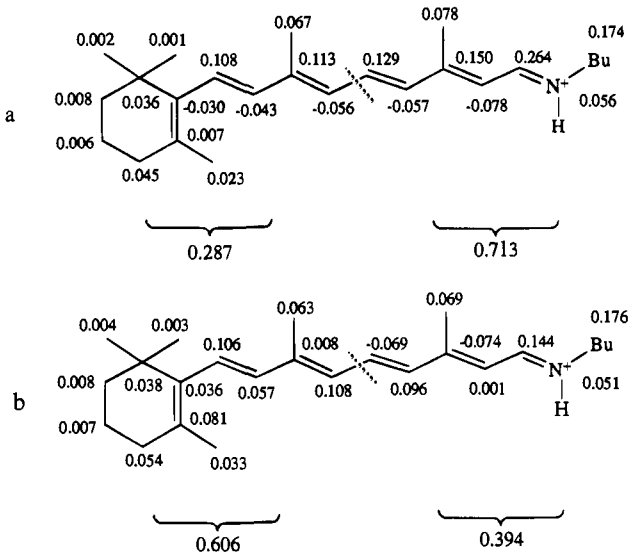

Figure 8. INDO total charge distribution per atom (in |e|) in the ground state (a) and in the first excited state (b) for the *trans*-retinal protonated Schiff base.

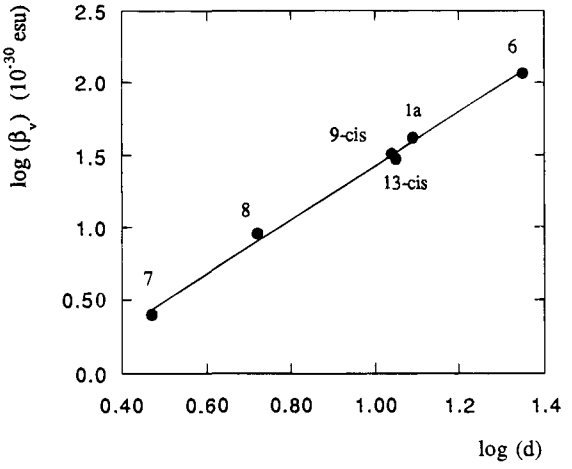

Figure 9. Theoretical relation between $\log(\beta_{v,trans})$ and $\log(d)$, where d is the molecular length in angstroms.

is deprotonated, which causes a strong blue shift of the chromophore absorption maximum from 570 to 412 nm in the intermediate M state.[1] Since the opto-electronic devices that are constructed with bR very frequently use the M state as well as the ground state, the hyperpolarizability of this state is important[12] (it was measured experimentally by using second-harmonic generation in purple membrane poly(vinyl alcohol) films[15]). It is most interesting to compare the β values for the *trans*-retinal protonated Schiff base to those of bacteriorhodopsin in the ground state and in the M state. It can be seen that the *trans*-to-*cis* isomerization and deprotonation cause a drastic reduction of the hyperpolarizability of the free chromophore (experimentally, at 1064 nm, from $\beta = 3600 \times 10^{-30}$ esu to $\beta = 480 \times 10^{-30}$ esu and theoretically, in the static case, from $\beta = 214.6 \times 10^{-30}$ esu to $\beta = 29.8 \times 10^{-30}$ esu).

In Table 9, the depolarization ratios of the *trans*-, 13-*cis*-, and 9-*cis*-retinal derivatives are shown. For the more bent 13-*cis* and 9-*cis* derivatives, we expect a larger off-axis β component than in the more linear *trans* derivatives. Just as was the case for 2,4-hexadienal and β-ionone, this results in a lower depolarization ratio for the 13-*cis* and 9-*cis* derivatives. The depolarization ratio among the *trans* derivatives is largest for the retinal protonated Schiff base, followed by that of retinal and retinal Schiff base. As the position of the absorption band maximum comes closer to 532 nm, the dispersive enhancement of β becomes more important. Calculations on β show that the dispersive enhancement is the largest for the tensor component that is parallel to the direction of the charge transfer,[55] which is directed along the polyene chain for retinal derivatives. The off-axis β tensor component thus becomes less important compared to the component parallel to the polyene chain upon going from *trans*-retinal Schiff base to *trans*-retinal and the *trans*-retinal protonated Schiff base, which results in an increasing depolarization ratio. The depolarization ratio for *trans*-retinal protonated Schiff base is very close to the theoretically predicted value of 5 for a molecule with a single dominant β tensor component.

Table 9. Influence of the *trans*-to-*cis* Isomerization of a Retinal Double Bond on the Depolarization Ratios

derivative	9-*cis* depolarization	13-*cis* depolarization	*trans* depolarization
retinal Schiff base	2.1	2.3	3.8
retinal	2.6	2.5	4.3
RPSB	3.0	2.7	4.8

Conclusions

We have investigated, experimentally and theoretically, the first hyperpolarizability of retinal and related chromophores. We have analyzed a variety of effects on the β values: the relationship between β and the first transition energy, the influence of the conjugation length, and the *cis*-to-*trans* isomerization. In all cases, the experimental and theoretical results follow the same trends, and we can draw the following conclusions:

(i) We find the expected linear dependence between β and $1/(\hbar\omega_{eg})$,[2] which derives from the two-state model.

(ii) We observe a linear relationship between $\log(\beta)$ and $\log(n)$ in the case of the *trans* derivatives (where n is the number of double bonds in the conjugated segment).

(iii) Upon the isomerization from *trans* to 13-*cis*, a constant fraction of the β value is lost, as is pointed out by a linear plot of $\log(\beta_{13\text{-}cis})$ versus $\log(\beta_{trans})$. For molecules with an equal number of double bonds, the lower β values obtained for the *cis* isomers can be explained by the shorter distance between the ionone ring and the aldehyde group induced by the *cis* conformation.

Finally, we find the two-state model to provide reliable trends, which confirm that the hyperpolarizability is highly dependent on the position and the nature of the first excited state. In our case, this state is characterized by a strong dipole moment change with respect to the ground state. Our results demonstrate that the measurements of the hyperpolarizability in retinal and related visual chromophores are a powerful tool for investigating the electronic structure of the retinal polyene systems.

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