Ab Initio Study of ^{13}C NMR Chemical Shifts for the Chromophores of Rhodopsin and Bacteriorhodopsin. 1. Theoretical Estimation of Their Ring–Chain Conformations

Mitsuhito Wada,† Minoru Sakurai,*,† Yoshio Inoue,† Yusuke Tamura,‡ and Yoichi Watanabe‡

Contribution from the Department of Biomolecular Engineering, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 227, Japan, and Cray Research Japan Limited, 6-4 Ichiban-cho, Chiyoda-ku, Tokyo 102, Japan

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Abstract: The ring–chain conformations of retinals within rhodopsin (Rh) and bacteriorhodopsin (bR) are estimated by means of *ab initio* NMR-shielding calculations, based on the localized orbital/local origin method. First, we calculated the ^{13}C shieldings for the unsaturated carbons of *all-trans*-retinal in double-ζ level and confirmed that there is a good agreement between the calculated and experimental data. Subsequently, the shielding calculations were carried out in the same level for a retinal analogue to investigate the correlation between the ^{13}C chemical shieldings and the C5=C6—C7=C8 dihedral angle (ϕ_{6-7}). Characteristic magnetic properties are found in the C5 and C8 shieldings, which behave in periodic manners depending on the value of ϕ_{6-7}. The major source of the periodicity is attributed to the recovering and breaking of the conjugated system rather than to steric interaction because the σ_{22}- and the σ_{33}-shielding tensor elements in the conjugated plane depend strongly on the ϕ_{6-7} value, whereas the ϕ_{6-7} dependence of the σ_{11} element perpendicular to the plane is less affected. We also propose that the C6–C7 conformation can be estimated through an observation of the chemical shift difference between C5 and C8 ($\Delta\sigma_{C8-C5}$) without requiring the corresponding data for a reference compound. We conclude that only for the in-planar 6-*s-trans* form the calculated value of $\Delta\sigma_{C8-C5}$ has a negative sign as observed experimentally and that the chromophore of bR takes the planar 6-*s-trans* form ($\phi_{6-7} = \sim 180°$). However, information only on the C5 and C8 shieldings is insufficient to determine whether the chromophore of Rh takes a skewed 6-*s-cis* or 6-*s-trans* form.

Introduction

The visual pigment rhodopsin present in the rod cell is responsible for vertebrate dim-light vision. Rhodopsin (Rh) possesses a 11-*cis*-retinal chromophore covalently bound by a protonated Schiff base linkage with a lysine side chain of an apoprotein, opsin.[1] In an initial stage of vision, photon absorption induces the 11-*cis* to *all-trans* isomerization of the retinal.[2] This structural change evokes an enzymatic cascade leading to rod membrane hyperpolarization.[2] Similarly, bacteriorhodopsin (bR) found in the purple membrane of *Halobacterium halobium*[3] consists of a retinal prosthetic group and acts as a light-driven proton pump mediated by the *all-trans* to 13-*cis* isomerization of the chromophore.[2] In spite of the difference in the biological function, bR has also attracted much attention in the study of visual photochemistry due to the structural resemblance to rhodopsin.

For each pigment, an unusual optical property is observed. The main absorption band of protonated retinylidene Schiff base (PRSB) is 440 nm in methanol, whereas bovine rhodopsin and bR absorb maximally at 498 and 568 nm, respectively.[2] Its spectral tuning mechanisms, caused by interaction of the protonated chromophore with apoprotein, have been of major interest for many years in the photobiology of the retinal proteins. Several mechanisms have been proposed to elucidate the red shift, so-called the "opsin shift",[4] including these three factors: (1) weaker interaction between the positively charged Schiff base and its counteranion relative to that of model compounds, (2) electrostatic interactions between the chromophore and the external point charge(s) originating in dissociative amino acid residues, and (3) recovering of the conjugation between the C5=C6 double bond and the rest of the chain owing to conformational change about the C6–C7 single bond.

The solid-state NMR technique may be one of the most powerful methods to elucidate the origin of the opsin shift because ^{13}C chemical shifts of the chromophore bound to opsin (or bacterioopsin) involve invaluable information on perturbations from protein environments to the electronic structure of the chromophore and on structural properties of itself, e.g., conformation around the C6–C7 bond. ^{13}C-labeled experiments for the chromophore have been successfully performed for both Rh and bR.[5-14] At present, we thus know the ^{13}C chemical shift values for all the unsaturated carbons of the chromophore in the binding state. However, there may be some ambiguities about

* Author to whom all correspondence should be addressed.
† Tokyo Institute of Technology.
‡ Cray Research Japan Ltd.
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the interpretation of those experimental values in conjunction with the opsin-shift models mentioned above.

For example, in the case of Rh, the C12 resonance exhibits a fairly large downfield shift (3.1 ppm) relative to that of a model compound, such as the chloride salt of *N*-retinylidenepropylamine.[7] According to the external point-charge model, a negative point charge is presumed to be located equally apart from the C12 and C14 carbons.[15] Thus, if this is the case, the same amount of positive charges would be induced on both C12 and C14 carbons, suggesting the occurrence of a downfield shift at the C14 position equally amounting to that of the C12. However, this prediction is inconsistent with the fact that no significant difference is observed between the chemical shift of C14 in rhodopsin and the corresponding value of the model compounds.

The external point-charge model for bR assumes the presence of a negative charge in the vicinity of C5 and of a positive charge near C7.[16,17] An observation of an unusual downfield shift in the C5 shielding provided the support for the existence of a negatively charged protein residue in the vicinity of C5.[12] Evidences for a positive charge near C7 were deduced from the C7 chemical shift[12] as well as from the absorption data of the artificial bR pigments.[17] However, recent ^{13}C NMR studies of model compounds for bR suggested the possibility to closely mimic the absorption maximum of bR and its C5 chemical shift without requiring a nonconjugated negative charge in the vicinity of the ionone ring,[18] inconsistent with the above external point-charge model.

The third factor mentioned above has also been studied on the basis of the measurements of ^{13}C NMR chemical shifts, especially for the C5 and C8 carbons. As a result of comparison with the data for 6-*s-trans*- and 6-*s-cis*-retinoic acids, it was concluded that the C6–C7 bond takes the *s-trans* form in bR.[12,19] However, Rodman Gilson and Honig, who used semiempirical quantum mechanical methods to guide the analysis of ^{13}C NMR and absorption data in bR, indicated that a planer 6-*s-cis* conformation could not be ruled out.[20]

Theoretical calculation of NMR chemical shieldings may be essentially helpful to remove the above ambiguous points about interpretation of ^{13}C chemical shifts because it could serve ^{13}C chemical shift data for various model compounds closely resembling the active site of Rh and bR. A quantum chemical analysis of the ^{13}C chemical shift for the chromophore has been carried out on the assumption of a linear correlation between experimental chemical shift and π-electron density calculated with a semiempirical MO method.[20,21] However, such an approach seems to have some difficulties in arguing the NMR chemical shift data quantitatively, since chemical shieldings are not only the function of electron density but also the whole of density matrix and energy excitation, namely a second-order property in the perturbation theory.

In our previous work,[22] *ab initio* NMR shieldings, based on the Hansen and Bouman theory,[23–25] were calculated for some polyenals as models for the chromophore of retinal proteins. It has been shown that the *ab initio* results reproduce well the magnetic properties peculiar to the polyene chemical shifts, suggesting the usefulness of the *ab initio* calculation to build unambiguous models for the active site of Rh and bR.[22] In this report, as the first step of a series of theoretical study for this purpose, the similar method is applied to elucidate the conformation about the C6–C7 bond concerning the above third factor.

Calculations

We have used the program RPAC 9.0[26] which was developed by Hansen and Bouman for *ab initio* shielding calculations, interfacing to the GAUSSIAN90 program.[27] *Ab initio* geometry optimizations and Mulliken population analysis were carried out by GAUSSIAN92.[28] The PM3 (Parametric Method 3)[29] calculations were performed by the program MOPAC 6.01.[30] All the calculations performed here were carried out on Cray YMP8E/8128 supercomputers. The basis sets used are the Pople type, 4-31G,[31] and the Huzinaga–Dunning type, D95V.[32] An outline of the Hansen and Bouman theory is described as follows.

In Ramsey's expression,[33] by use of second-order perturbation theory, the second derivative of the sum of the energy terms, which are linear in the magnetic moment and the applied magnetic field, produces a component of the magnetic shielding parameter

$$\sigma_{ij} = \sigma_{ij}^{d} + \sigma_{ij}^{p} \tag{1}$$

where the diamagnetic part σ_{ij}^{d} is

$$\sigma_{ij}^{d} = (1/2c^2)\langle 0|\sum_{s}[[\epsilon_j \times (\mathbf{r}_s - \mathbf{R})]\cdot[\epsilon_i \times \mathbf{r}_s]]r_s^{-3}|0\rangle \tag{2}$$

and the paramagnetic part σ_{ij}^{p} is

$$\sigma_{ij}^{p} = 1/c^2\sum(E_q - E_0)^{-1}[\epsilon_{i'}\langle 0|\sum \mathbf{L}_s/r_s^3|q\rangle] \times [\epsilon_{j'}\langle q|\sum \mathbf{L}'_s|0\rangle] \tag{3}$$

Here, ϵ_i and ϵ_j are unit vectors along the direction of the nuclear magnetic moment and the applied magnetic field, respectively. The vector $\mathbf{r}_s$ is the position vector of electrons in a coordinate system located on the magnetic

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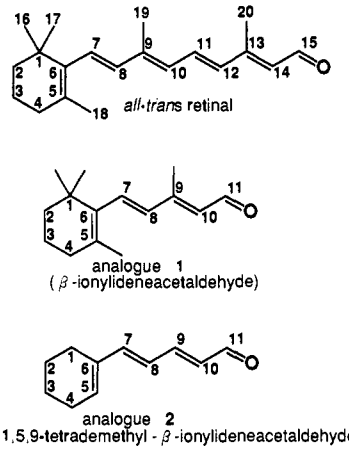

Figure 1. Structures of *all-trans*-retinal and its analogues.

nucleus. Angular momentums, $\mathbf{L}_s = \mathbf{r}_s \times \mathbf{p}_s$ and $\mathbf{L}_s' = (\mathbf{r}_s - \mathbf{R})$, are referred to the nucleus and a gauge origin at **R**.

In the Hansen and Bouman theory,[23-25] identifying the energy differences and matrix elements in the paramagnetic term with the corresponding random-phase approximation (RPA) transition energies and transition moments and using the localized orbital/local origin (LORG) scheme, the next expression is produced.

$$\sigma_{ij} = 1/c^2 \sum_{\alpha} \langle\alpha|[(\mathbf{r}-\mathbf{R}_\alpha)\cdot\mathbf{r}\delta_{ij} - [\epsilon_{f'}(\mathbf{r}-\mathbf{R}_\alpha)] \times [\epsilon_{f'}\mathbf{r}]]r^{-3}|\alpha\rangle +$$
$$i/c^2 \sum_{\alpha\beta} [\epsilon_{f'}\langle\alpha|\mathbf{L}/r^3|\beta\rangle] \times [\epsilon_{f'}[(\mathbf{R}_\alpha - \mathbf{R}_\beta) \times \langle\beta|\mathbf{r}|\alpha\rangle]] -$$
$$2/c^2 \sum_{\alpha_m} \sum_{\beta_n} [\epsilon_{f'}\langle\alpha|\mathbf{L}/r^3|m\rangle](\mathbf{A}-\mathbf{B})^{-1}_{\alpha m,\beta n}[\epsilon_{f'}\langle n|\mathbf{L}^{(\alpha)}|\beta\rangle], \quad (4)$$

where $\mathbf{L}^{(\alpha)} = \mathbf{L} - \mathbf{R}_\alpha \times \mathbf{p}$ is local angular momenta. In eq 4, the reference to an arbitrary gauge origin has been replaced by local origins relative to the magnetic nucleus.

In the LORG theory, occupied orbitals are localized according to the Foster–Boys criterion.[34] In the calculations performed here, we chose the localization scheme that makes "banana" localized molecular orbitals of all multiple bonds and where the centroids of the localized orbitals are chosen as the local origins for all the localized orbitals.

In this study, the above method is first applied to *all-trans*-retinal, including all the constituent atoms (49 atoms, Figure 1), using the 4-31G and D95V basis sets. In order to save CPU time, a series of calculations for studying the ring–chain conformation were carried out for an analogue, **1** (Figure 1), namely β-ionylideneacetaldehyde, whose π-conjugated chain is one isoprene unit shorter than retinal's. The analogue **1** possesses the ionone ring and all the methyl groups except the C20 methyl of retinal, thereby including all steric factors for rotation around the C6–C7 bond. Thus, at least as for structural aspects, this model compound satisfies the necessary conditions for the present purpose.

The geometrical parameters for the unsaturated carbon skeleton of *all-trans*-retinal were taken from the crystal structure.[35] The other parts of geometrical parameters, determining the structure of the ionone ring and locating the hydrogen atoms, were obtained by energy minimization using the same basis sets as in the *ab initio* shielding calculation. Then, the starting structure of the ionone ring was taken from the crystal data of 11-*cis*-retinal which has the 6-*s*-*cis* form[36] about the C6–C7 single bond, and the initial positions of the hydrogens were determined using standard values.[37] Since the crystal structure of analogue **1** is not published, we adopted full optimized geometries from MO calculation. Although it is desirable to perform both the optimization and the shielding calculation on the same level, the PM3 method was used for the geometry optimization to save CPU time. The starting parameters are set up as in *all-trans*-retinal.

All the calculated and experimental shieldings were converted to a methane reference, and the positive sign indicates deshielding. The 4-31G and D95V shieldings for methane shift 222.1 and 193.1 ppm upfield from naked carbon, respectively. The experimental shielding for methane shifts 2.1 ppm upfield from tetramethylsilane.[38]

Results

***Ab Initio* Shielding for *all-trans*-Retinal.** Table 1 summarizes the calculated ^{13}C shieldings for the unsaturated carbons of *all-trans*-retinal together with the corresponding experimental data.[19] Figure 2 shows a correlation between the 4-31G and experimental ^{13}C chemical shifts for the unsaturated carbons. The calculated chemical shieldings are in good agreement with the experimental ones even on the double-ζ levels: the standard deviation except for C15 is fairly small (3.3 ppm for the 4-31G results and 2.7 ppm for the D95V ones). It should be noted that the 4-31G shieldings completely reproduce the relative order of all the carbons studied.

As is well known, it is difficult to quantitatively reproduce shieldings of carbon nuclei bound to polarized groups.[22,23,39] Reproducibility for the C15 shielding may be improved by using more extended basis sets including polarization or diffusion functions.[39] Such calculation was, however, not carried out in the present study because our attention is given particularly to the ^{13}C shieldings of unsaturated carbons from C5 to C8.

The directions of the principal axes were examined for each shielding tensor. For all the unsaturated carbons, the out-of-plane element σ_{11} is aligned perpendicular to the conjugated system, whereas the in-plane elements σ_{22} and σ_{33} are roughly parallel and perpendicular to the relating double bond, respectively. Figure 3 indicates the 4-31G and experimental principal values as a function of carbon position. For all the unsaturated carbons, the calculated σ_{11} and σ_{22} shieldings shift upfield relative to the experimental ones, whereas the calculated σ_{33} shieldings shift downfield relative to the experimental ones. In other words, the calculated results, on the whole, overestimate the anisotropies, σ_{aniso}, of each shielding tensor, usually defined as $\sigma_{33} - (\sigma_{11} + \sigma_{22})/2$. It should be noted that the calculation well reproduces some characteristic features concerning magnetic anisotropy of the unsaturated carbons. First, it is experimentally observed that the σ_{11}'s of the even- and odd-numbered carbons are observed around 50–60 and 30–40 ppm (aside from C15), respectively.[19] This trend, called the odd–even effect,[19] is almost completely reproduced by the calculation irrespective of the basis sets used (4-31G and D95V): the calculated σ_{11}'s of the even- and odd-numbered carbons are around 30–50 and 10–20 ppm, respectively. Second, the calculations apparently reproduce anomalous downfield shifts observed for the σ_{22} elements of C9 and C13. Third, the observed principal values change along the polyene chain in somewhat different ways for each tensor component, even if each plot shows a zig–zag pattern (or the odd–even effect).

The calculation completely reproduces such detailed features of the shielding changes different for each tensor component. Combining this striking result and the good reproducibility for the isotropic shieldings, it seems to be strange that the calculation exhibits poor reproducibility only for the values of anisotropies, as mentioned above. The same trend that the calculated chemical shift anisotropy is greater than that experimentally determined has been also found for *all-trans*-retinylidenebutylimine.[39] The smaller experimental value of the chemical shift anisotropy may be the result of vibrational and librational averaging.[39]

Correlation between the C6–C7 Rotation and the ^{13}C Shieldings. In Figure 4, the ^{13}C isotropic shieldings for analogue **1** are plotted against the rotational angle ϕ_{6-7} around the C6–C7 bond. The shieldings of C5, C6, C7, and C8 change with the value of ϕ_{6-7}, whereas no apparent angular dependence is found in the other carbons distant from the C6–C7 bond. One of the most striking findings is that the shieldings of C5 and C8 exhibit clear periodic dependence on the angle ϕ_{6-7}. For example, the σ–ϕ_{6-7} curve of

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Table 1. Calculated and Experimental ^{13}C Chemical Shifts for *all-trans*-Retinal (ppm from CH_4)

	4-31G				D95V				obsd[a]			
	σ_{iso}	σ_{11}	σ_{22}	σ_{33}	σ_{iso}	σ_{11}	σ_{22}	σ_{33}	σ_{iso}	σ_{11}	σ_{22}	σ_{33}
C5	130.9	13.6	132.3	246.9	131.3	12.9	127.2	252.5	130.6	33.0	143.2	218.0
C6	140.1	37.6	131.6	251.3	139.9	32.3	127.7	259.7	136.2	53.1	143.6	232.5
C7	135.4	20.3	131.3	254.7	129.7	17.8	118.4	253.0	132.3	40.9	136.6	215.9
C8	141.8	48.6	120.3	256.4	138.8	44.2	112.8	259.5	137.3	61.3	146.5	226.3
C9	143.6	18.6	150.8	261.3	145.9	17.7	179.6	270.4	143.5	34.8	170.8	230.1
C10	135.7	46.6	115.9	263.4	132.4	43.8	106.5	246.9	132.3	52.1	131.8	213.3
C11	139.7	17.4	136.9	264.7	139.1	15.7	131.8	269.7	136.0	31.4	144.3	223.0
C12	139.8	48.3	120.6	250.4	137.3	44.5	113.1	254.4	136.1	57.3	133.0	217.7
C13	153.0	14.5	167.7	279.4	156.7	16.1	165.0	289.1	157.6	33.3	189.7	242.0
C14	135.0	46.4	117.9	240.6	134.6	50.3	111.5	241.1	131.9	63.0	133.1	202.8
C15	202.2	62.0	204.6	340.1	197.0	57.1	198.9	335.1	191.5	99.7	215.0	262.0

[a] Taken from ref 34.

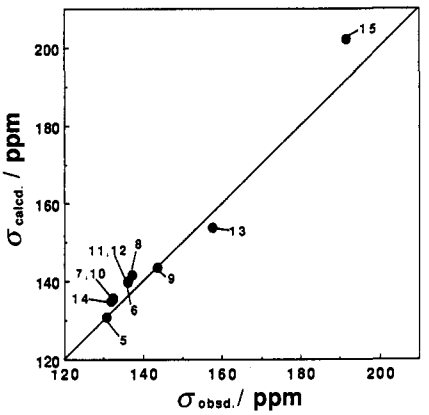

Figure 2. Correlation between the 4-31G and experimental ^{13}C chemical shifts for the unsaturated carbons of *all-trans*-retinal.

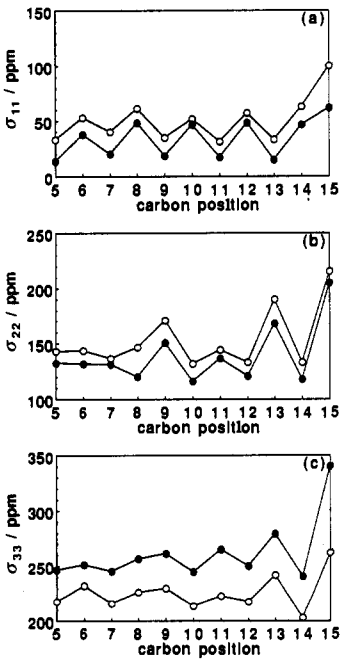

Figure 3. Comparison of the 4-31G (●) and experimental (○) principal values for the unsaturated carbons of *all-trans*-retinal.

C5 appears to be approximated by a sine wave whose period is 180°. On the other hand, the C7 shielding shows less-defined periodicity compared with the C8 shielding, and the C6 shielding shows rather complicated behavior. In a later section, we will discuss the origin of these angular dependencies.

The directions of the principal axes were again examined for C5 and C8 as shown in Table 2. Independent of the angle ϕ_{6-7}, the out-of-plane element σ_{11} is always aligned perpendicular to the conjugated system, whereas the in-plane elements σ_{22} and σ_{33} are parallel and perpendicular to the relating double bond, respectively.

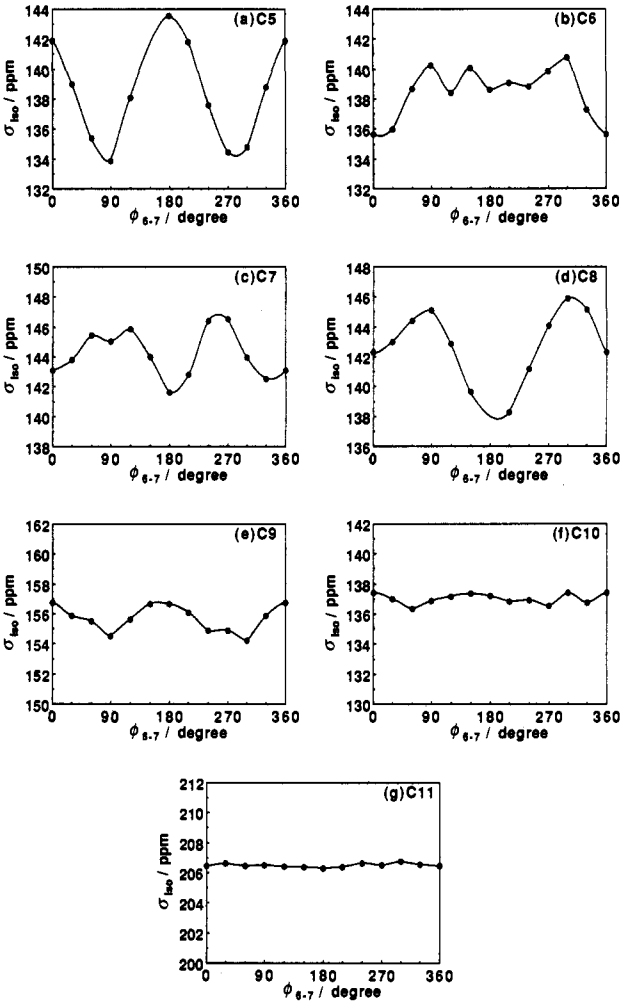

Figure 4. Isotropic chemical shifts for the unsaturated carbons of analogue **1** calculated as a function of the angle ϕ_{6-7}.

In order to analyze the conformation dependence of the C5 and C8 shieldings in more detail, their σ–ϕ_{6-7} curves were approximated by using the Fourier series of expansion as follows:

$$\sigma_{C5} = 138.4 - 1.466 \cos \phi_{6-7} + 4.238 \cos 2\phi_{6-7} \quad (5)$$

$$\sigma_{C8} = 142.4 + 2.729 \cos \phi_{6-7} - 2.368 \cos 2\phi_{6-7} \quad (6)$$

where the terms more than 3-fold ($\cos 3\phi_{6-7}$) were neglected because their coefficients were less than 0.7 ppm. The standard deviations for the C5 and C8 shieldings are 0.6 and 0.8 ppm, respectively. The conformation dependence of the C5 shielding

Table 2. Directions of the Eigenvectors of Principal Values for Retinal Analogue **1** (in deg)[a]

	C5						C8					
	σ_{11}		σ_{22}		σ_{33}		σ_{11}		σ_{22}		σ_{33}	
ϕ 6–7	θ	ξ	θ	ξ	θ	ξ	θ	ξ	θ	ξ	θ	ξ
0	84	10	1	90	91	86	86	5	7	85	85	90
30	88	2	2	92	90	89	84	7	7	85	87	95
60	90	6	0	90	90	84	90	4	5	93	95	92
90	90	2	2	90	88	88	86	5	9	95	98	89
120	89	7	4	92	87	83	95	7	8	96	83	88
150	91	10	3	88	87	80	86	7	7	95	96	95
180	91	3	3	91	92	93	92	3	4	88	93	89
210	91	2	2	91	92	89	91	3	7	90	96	87
240	92	3	3	92	92	87	91	3	9	92	81	92
270	91	2	3	90	88	91	86	6	11	85	80	89
300	90	6	1	90	89	88	86	6	9	95	98	93
330	90	6	0	90	90	84	86	1	10	81	88	88

[a] For C5 (C8), we defined $\mathbf{b}_{//}$ as a vector parallel to the C5=C6 (C7=C8) bond of retinal analogue **1** and defined $\mathbf{b}_{\perp}$ as a vector normal to the plane including C5, C6, and C7 (C6, C7, and C8) of **1**. The values of θ and ξ are defined as the angle made between $\mathbf{b}_{//}$ and the eigen vector of corresponding principal value and that between $\mathbf{b}_{\perp}$ and the eigen vector, respectively.

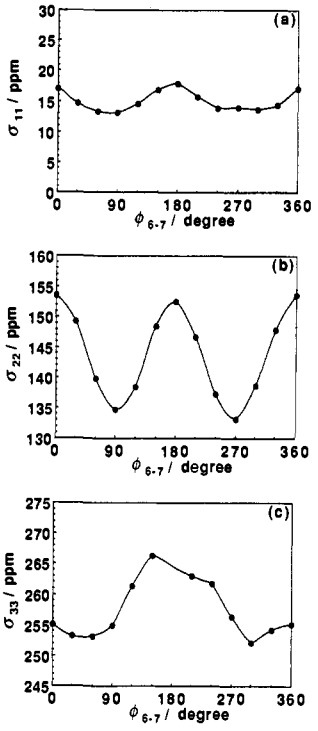

Figure 5. Principal components of the chemical shift tensor for C5 of analogue **5** calculated as a function of the angle ϕ_{6-7}.

arises mainly from the cos $2\phi_{6-7}$ term, whereas for the C8 shielding, both the cos ϕ_{6-7} and cos $2\phi_{6-7}$ terms comparably contribute. The most important difference between the C5 and C8 shieldings is that the signs of the coefficients of the cos ϕ_{6-7} and cos $2\phi_{6-7}$ terms are different between eqs 5 and 6. In other words, the phases of the Fourier series of the terms for C5 gain (or lose) a half period relative to those for C8. From the presence of this phase shift, it is expected that the C6–C7 conformation can be estimated through simultaneous measurements of the C5 and C8 shieldings, which will be discussed in a later section.

In Figure 5, the principal values for the C5-shielding tensor are plotted against the angle ϕ_{6-7}. It is evident that the σ_{22} element dominantly contributes to causing the periodic angular dependence of the C5 shielding. The σ_{11} element is scarcely affected by conformational change about the C6–C7 single bond. The σ_{33} element shows less-clear periodicity but shows some dependence

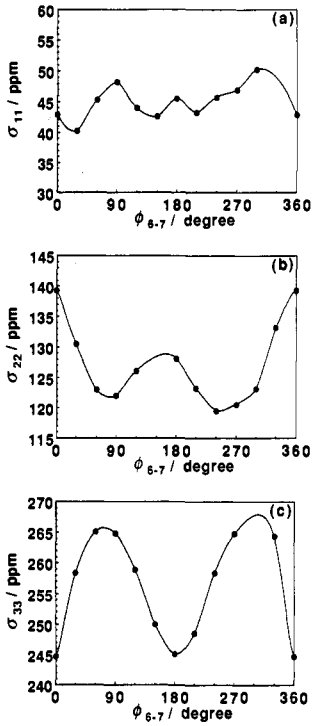

Figure 6. Principal components of the chemical shift tensor for C8 of analogue **1** calculated as a function of the angle ϕ_{6-7}.

on the value of ϕ_{6-7}. The σ_{22} and σ_{33} elements for C5 can be represented by means of the Fourier series of expansion as follows:

$$\sigma_{22} = 143.3 + 9.680 \cos 2\phi_{6-7} \quad (7)$$

$$\sigma_{33} = 258.2 - 6.643 \cos \phi_{6-7} + 2.831 \cos 2\phi_{6-7} \quad (8)$$

where the higher terms whose coefficients are less than 0.5 ppm are neglected. The standard deviations for the σ_{22} and σ_{33} elements are 0.9 and 1.2 ppm, respectively. It is concluded that the σ_{22} and σ_{33} elements are mainly responsible for the cos 2ϕ and cos ϕ_{6-7} terms in eq 5, respectively.

As similar to the case of C5, the most significant contribution to the angular dependence of the C8 shielding arises from the σ_{22} and σ_{33} elements (Figure 6). The σ_{11} element is again hardly affected by the conformational change. It is of interest that the curve of the σ_{22} element gets out of phase with that of the isotropic shielding. The Fourier series of expansion for the σ_{22} and σ_{33} elements are given as follows:

$$\sigma_{22} = 126.4 + 4.416 \cos \phi_{6-7} + 6.182 \cos 2\phi_{6-7} \quad (9)$$

$$\sigma_{33} = 256.2 + 2.270 \cos \phi_{6-7} - 10.500 \cos 2\phi_{6-7} \quad (10)$$

where the higher terms whose coefficients are less than 0.5 ppm are neglected. The standard deviations for the σ_{22} and σ_{33} elements are 1.7 and 2.5 ppm, respectively. It is concluded that the σ_{22} and σ_{33} elements are responsible for the cos ϕ_{6-7} and cos $2\phi_{6-7}$ terms in eq 6, respectively.

Correlation between Electronic Distribution and the C6–C7 Rotation. Table 3 summarizes the conformation dependence of the atomic net charges obtained from Mulliken population analysis. Figure 7 shows the conformation dependence of the atomic net charge difference $\Delta\rho$ between the carbons adjoined to each other. The value of $\Delta\rho$ can be a measure of polarization of each bond. The $\Delta\rho$'s of C5–C6, C6–C7, C7–C8, and C8–C9 change roughly in a periodic way depending on the value of ϕ_{6-7}. Its periods are roughly estimated to be 180°. The $\Delta\rho$'s of C9–C10 and C10–O hardly show the conformation dependence about the C6–C7 dihedral angle.

Figure 8 shows the conformation dependence of the atomic bond population obtained from Mulliken population analysis.

Table 3. Atomic Net Charge for Retinal Analogue 1

	C6–C7 twist angle (ϕ_{6-7})[a]			
position	0°	90°	180°	270°
C5	0.010967	0.008836	−0.022856	0.010307
C6	0.020157	−0.020167	0.039677	−0.023869
C7	−0.161941	−0.172724	−0.161710	−0.169834
C8	−0.198030	−0.157212	−0.203821	−0.162902
C9	0.039865	0.033152	0.044092	0.032681
C10	−0.302082	−0.294808	−0.302733	−0.294391
C11	0.335405	0.334461	0.335121	0.334486
O	−0.571487	−0.567087	−0.571286	−0.567383

[a] In degrees.

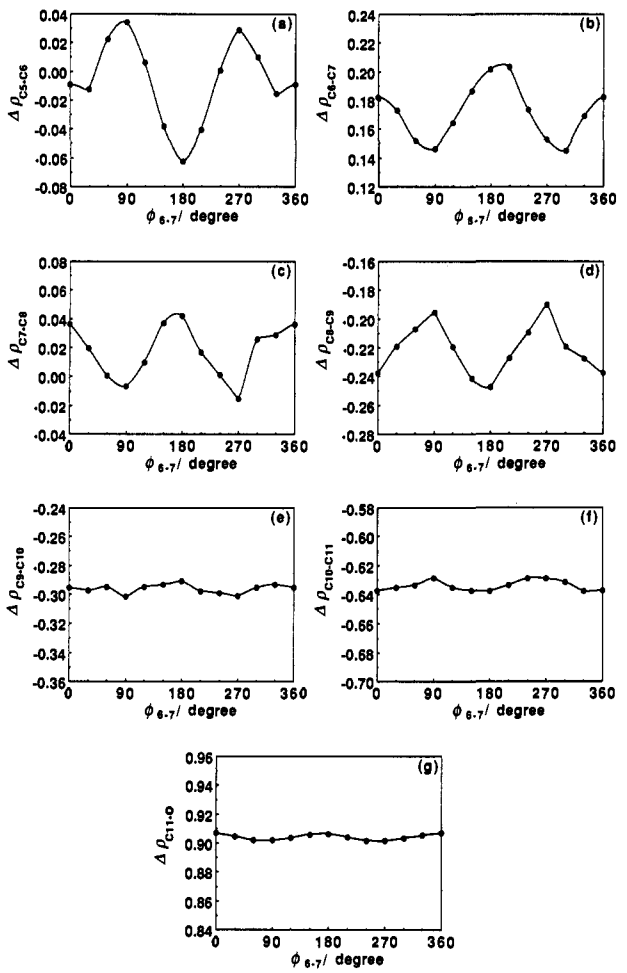

Figure 7. Atomic net charge difference between the carbons adjoined to each other plotted against the angle ϕ_{6-7}.

The atomic bond populations associated with C5, C6, C7, and C8 appreciably change depending on the value of ϕ_{6-7}. The atomic bond populations of C9═C10, C10—C11, and C11═O scarcely change depending on the value of ϕ_{6-7}. The values of atomic populations for C5═C6 and C7═C8 in the planar forms (ϕ_{6-7} = 0°, 180°) are smaller than those in skewed forms: namely, the double-bond strength becomes weak while the π-conjugation is recovering and large while the conjugation is breaking. The values of atomic bond populations for C6–C7 and C8–C9 show the opposite trends to the cases of the double bonds.

These findings directly provide evidence that the recovering and breaking of the conjugated system occur depending on the value of the angle ϕ_{6-7}.

Origin of the Conformation-Dependent Chemical Shift Changes. On the basis of observation of ^{13}C chemical shifts for 6-*s*-*cis*- and 6-*s*-*trans*-retinoic acids, Harbison et al. suggested that a downfield shift of the C5 resonance, on going from the skewed 6-*s*-*cis* to the planar 6-*s*-*trans* forms, is due to the recovering of conjugation

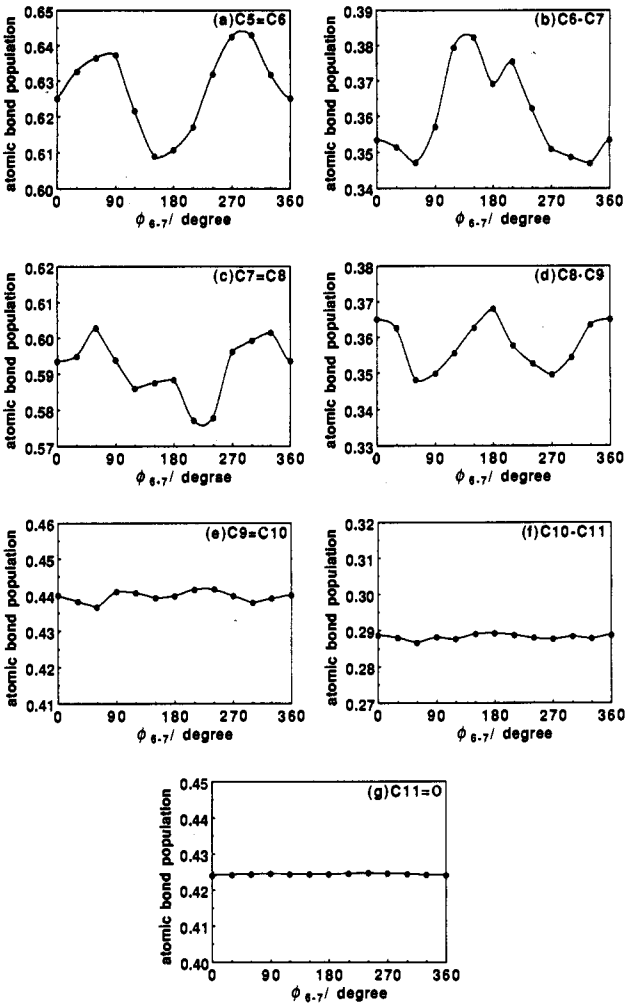

Figure 8. Atomic bond population plotted against the angle ϕ_{6-7}.

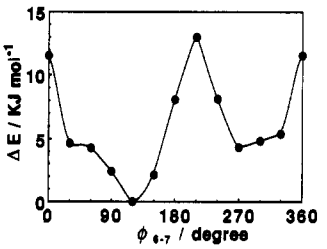

Figure 9. PM3 adiabatic potential energy curve about the C6–C7 bond of analogue 1.

between the C5–C6 double bond and the rest of the chain and that an upfield shift of the C8 resonance in the same conformational change is due to the steric interaction between the H8 hydrogen and the C17 and C18 methyl groups bound to C1.[19] Here, we attempt to check whether such an interpretation is reasonable or not in the light of the present calculated results.

First, we examine the correlation between the ^{13}C shieldings and the steric interaction induced by the C6–C7 rotation. Figure 9 shows the PM3 adiabatic potential curve for the C6–C7 rotation of analogue 1. Two broad minima are found near the ϕ_{6-7} values of 120° and 270°, while two narrow maxima are near 0° and 210°, indicating that skewed forms are energetically favorable relative to planar forms. This is consistent with the experimental results indicating that most of the retinal derivatives, including *all-trans*- and 11-*cis*-retinals,[35,36] have skewed 6-*s*-*cis* conformations about the C6–C7 single bond (ϕ_{6-7} = 40°–62°). In addition, Figure 9 indicates that the planar 6-*s*-*trans* conformation corresponds to a saddle point on the potential surface of interest, consistent with the result from the STO-3G calculation by Poirier

and Yadav[40] and the CNDO/2 calculation by Beppu and Kakitani.[41] We thus believe that the PM3 potential curve well reproduces the steric interaction occurring in retinal and its derivatives. If we compare the angular dependence of the C8 shielding σ_{iso} (Figure 4d) and the potential curve (Figure 9), it is understood that the quantities are well correlated with each other. Namely, the C8 shielding shifts upfield at the angle ϕ_{6-7} giving higher steric energy, which seems to be consistent with Harbison's interpretation mentioned above. However, this may be doubtful in the light of the angular dependence of the principal values. The following points should be noted. First, the conformation-dependent change of σ_{11} is fairly small relative to those of σ_{22} and σ_{33}, although it is not negligible. Second, the angular dependence of σ_{11} does not correlate with the trends of the adiabatic potential curve. On the other hand, according to a conventional picture for the principal values of conjugated carbons,[42] steric effects should be mainly reflected in σ_{11}. Thus, the present findings clearly indicate that the conformation-dependent change in the C8 shielding is governed mainly by the change in the electronic structure of the conjugated system, a change induced by the breaking and recovering of conjugation.

The physical origin of the shielding changes in C5 and C8 can be clearly explained in conjunction with the reason why their shielding changes show periodic behavior. For simplicity, the conjugated system is assumed to be placed on the x–y plane. Then, the π-orbital directs to the z axis. On the other hand, it is shown by the present calculation that the σ_{11} element is aligned parallel to the z axis. The values of σ_{11} are thus determined by the angular momentum matrix element of $\langle P_x|\mathbf{L}_z|P_y\rangle$ and $\langle P_y|\mathbf{L}_z|P_x\rangle$,[43] where P and $\mathbf{L}$ indicate an atomic p-orbital and the angular momentum operator found in eq 3. This clearly indicates that the perturbation to π-orbitals which should be aligned parallel to the z axis would never affect the behavior of σ_{11}. Similarly, since the σ_{22} and σ_{33} elements are placed on the x–y plane, their values are determined by the angular momentum matrix elements of $\langle P_y|\mathbf{L}_x|P_z\rangle$, $\langle P_z|\mathbf{L}_x|P_y\rangle$, $\langle P_x|\mathbf{L}_y|P_z\rangle$, and $\langle P_z|\mathbf{L}_y|P_x\rangle$, which are affected by a perturbation to the π-orbitals.[42] As shown in Figures 7 and 8, the electronic distribution on C5 or C8 and their vicinity shows periodic change, causing a similar periodic change in σ_{22} and σ_{33} of C5 and C8. Therefore, it can be concluded that the angular dependence of the C5 and C8 shieldings mainly arises from the breaking and recovering of the conjugated system.

The above argument may be insufficient to account for less periodicity of the C6 and C7 shieldings. According to eq 3, the paramagnetic contribution to the ^{13}C shielding depends not only on the angular momentum matrix element but also on the excitation energies. Here, if we follow the picture of the localized orbital used in the shielding calculation instead of the canonical orbital, the paramagnetic term is shown to be decomposed into each bond contribution.[23] Although we did not perform such an analysis in this study, it can be easily predicted that the C6 and C7 paramagnetic shieldings have the largest contribution from the rotating bond.[44] The energy of the rotating bond should largely change depending on the angle ϕ_{6-7}. Thus, the conformation dependencies of the C6 and C7 shieldings do not necessarily correlate with these of the electronic distribution and thereby exhibit less apparent periodicity.

The relationship between the σ_{11} component and steric interaction may not be directly deduced from a simple theoretical consideration as described above for the σ_{22} and σ_{33} components. However, the relationship is exemplified by comparing the calculated shieldings for analogue **1** with those for analogue **2**

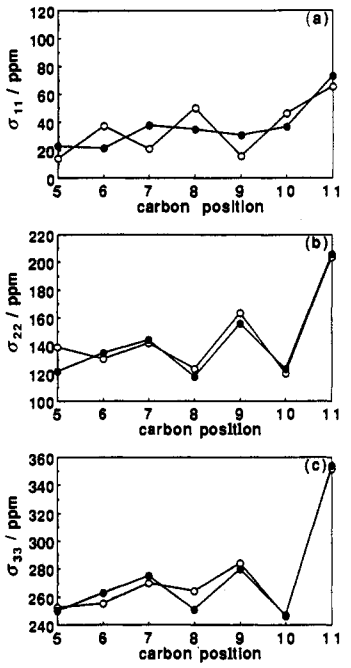

Figure 10. Principal components of the chemical shift tensor for the unsaturated carbons of analogues **1** (○) and **2** (●). The calculations were performed for both analogues by holding the 6-*s*-*cis* linkage at ϕ_{6-7} = 60°.

which is the all-demethylated derivative of analogue **1**. As can be seen from Figure 10, the demethylation, namely reduction of the steric interaction, largely influences the σ_{11} shielding alone, resulting in the disappearance of the odd–even effect.

Discussion

The most important findings in this study are that the C5 and C8 shieldings of analogue **1** behave in periodic manners depending on the angle ϕ_{6-7} and, further, that there is the apparent phase shift between their periodic functions (eqs 5 and 6). The latter implies that the difference between the C5 and C8 shieldings at a given angle, ϕ_{6-7}, does not vanish except for a few points and shows an appreciable angular dependence. Figure 11 clearly indicates that the shielding difference also behaves in a periodic manner against the angle ϕ_{6-7}. This fortunate result makes us expect that the C6–C7 conformation can be estimated through an observation of the chemical shift difference between C5 and C8. Although Figure 11b was obtained for analogue **1**, it must be applied to other retinal analogues, including protonated Schiff base. If so, we can estimate the C6–C7 conformation of the chromophores in Rh and bR using Figure 11b because the C5 and C8 shieldings in the proteins have been already reported.[5-8,12-14] In order to examine the applicability of Figure 11a,b, available experimental data for some fixed values of the angle ϕ_{6-7} were compared with the corresponding predicted values from eqs 5 and 6. *all-trans*-Retinoic acid and 13-*cis*-retinal both crystallize in the skewed 6-*s*-*cis* form and the planar 6-*s*-*trans* form.[45,46] In the former, the C5 and C8 resonances shift to downfield by 7.1 ppm and upfield by 8.0 ppm, respectively, in going from the skewed 6-*s*-*cis* form (ϕ_{6-7} = 42°) to the planar 6-*s*-*trans* form (ϕ_{6-7} = 165°).[19] In the latter, the C5 resonance shifts to downfield by 10.1 ppm in going from the skewed 6-*s*-*cis* form (ϕ_{6-7} = 65°) to the planar 6-*s*-*trans* form (ϕ_{6-7} = 175°).[19] The corresponding calculated values are summarized in Table 4 together with these experimental data. The calculated values tend to underestimate the magnitudes of shielding changes but

(40) Poirier, R. A.; Yadav, A. *Chem. Phys. Lett.* **1989**, *156*, 122–124.
(41) Beppu, Y.; Kakitani, T. *Chem. Phys.* **1990**, *148*, 333–346.
(42) Salem, L. In *The Molecular Orbital Theory of Conjugated Systems*; Benjamin, W. A., Ed.; 1967; pp 202–204.
(43) Ebraheem, K. A. K.; Webb, G. A. *Prog. NMR Spectr.* **1977**, *11*, 149–181.
(44) Because the π–π overlap of the rotating bond (C6–C7) should strongly depend on the angle ϕ_{6-7}.
(45) Stam, C. H. *Acta Crystallogr.* **1972**, *B28*, 2936–2945.
(46) Simmons, C. J.; Liu, R. S. H.; Denny, M.; Seff, K. *Acta Crystallogr.* **1981**, *B37*, 2197–2205.

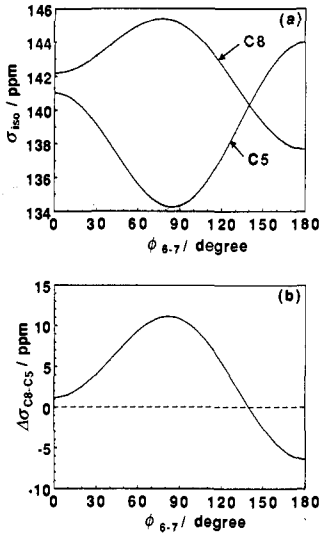

Figure 11. (a) ϕ–σ_{iso} curves obtained from the Fourier series of terms of eqs 5 and 6. (b) ϕ–σ_{C8-C5} curve obtained from the subtraction of eq 5 from eq 6. For the sake of convenience, the C6–C7 conformation is classified into the following four types: planar 6-*s*-*cis* (0°–30°), skewed 6-*s*-*cis* (30°–90°), skewed 6-*s*-*trans* (90°–150°), and planar 6-*s*-*trans* (150°–180°).

Table 4. Chemical Shift Difference of Retinal Derivatives in going from a 6-*s*-*cis* to 6-*s*-*trans* Form

	ϕ_{6-7}[a]		$\Delta\sigma$(C5)[b]		$\Delta\sigma$(C8)[b]	
	cis	*trans*	calcd	obsd	calcd	obsd
all-trans-retinoic acid	41.2[c]	165.8[c]	5.6	7.1[e]	–6.4	–8.0[e]
13-*cis*-retinal	65.4[d]	174.8[d]	8.9	10.1[e]	–7.8	unknown

[a] In degrees. [b] Defined as $\Delta\sigma = \sigma_{trans} - \sigma_{cis}$ (in ppm). A positive sign indicates deshielding. [c] Taken from ref 43. [d] Taken from ref 44. [e] Taken from ref 19.

Table 5. Chemical Shift Difference between the C5 and C8 Carbons in Retinal Derivartives, Rhodopsin, and Bacteriorhodopsin

		$\Delta\sigma_{C8-C5}$[b]	
	ϕ_{6-7}[a]	calcd	obsd
6-*s*-*cis*-retinoic acid	41.2[c]	6.1	10.1[f]
retinol acetate	57.9[d]	9.0	10.2[f]
6-*s*-*cis*-13-*cis*-retinal	65.4[d]	9.9	12.2[f]
all-trans-retinal	62.0[e]	9.6	6.6[f]
6-*s*-*trans*-retinoic acid	165.8[c]	–6.0	–5.0[f]
rhodopsin			8.9[g]
bacteriorhodopsin			–12.1[h]

[a] In degrees. [b] Defined as $\Delta\sigma_{C8-C5} = \sigma_{C8} - \sigma_{C5}$ (in ppm). [c] Taken from ref 45. [d] Taken from ref 46. [e] Taken from ref 35. [f] Taken from ref 19. [g] Taken from ref 7. [h] Taken from ref 12.

properly reproduce the relative order. Table 5 summarizes the calculated and observed shielding differences $\Delta\sigma_{C8-C5}$, obtained by subtracting the value of the C5 shielding from that of the C8 one. As can be seen from this table, the skewed *s*-*cis* and *s*-*trans* forms can be unambiguously distinguished from each other by examining the value of $\Delta\sigma_{C8-C5}$, which is also reproduced in the present calculation. Therefore, as far as judged from the comparisons with the available observed data, it is concluded that at least in qualitative ways Figure 11a,b is applicable to a wide variety of retinal derivatives. In other words, such a successful consequence supports the above argument that the physical origin of the angular dependence involved in eqs 5 and 6 is essentially ascribed to the common nature of conjugated systems, namely the breaking and recovering of conjugation induced by a single bond rotation.

Next, we attempt to estimate the C6–C7 conformation of the chromophores of bR and Rh on the basis of Figure 11b and the available observed data (Table 5). Hereafter, for the sake of convenience, the C6–C7 conformation is classified into the following four types: planar 6-*s*-*cis* (0°–30°), skewed 6-*s*-*cis* (30°–90°), skewed 6-*s*-*trans* (90°–150°), and planar 6-*s*-*trans* (150°–180°).

As shown in Table 5, in bR the observed value of $\Delta\sigma_{C8-C5}$ is –12.1 ppm.[12,13] Figure 11b shows that the value of $\Delta\sigma_{C8-C5}$ has a minus sign only in the planar *trans* form defined above. It is thus reasonable to conclude that the chromophore of bR takes the planar 6-*s*-*trans* form around the C6–C7 bond, although the value of the angle ϕ_{6-7} is uncertain within an allowed range of 140°–180°. However, further discussion may be needed to judge whether this conclusion is acceptable or not because the observed value of –12.1 ppm is too large in magnitude compared with the calculated values, a minimum of which is –6.7 ppm at the angle ϕ_{6-7} of 180°. A similar anomalous shift has been found in the σ_{22} component of the C5 shielding: the observed value for bR shifts to downfield by –27.0 ppm compared to that for 6-*s*-*trans*-retinoic acid.[12] Such a large shift can not be explained from eq 7.

In order to examine the origin of the above discrepancy for the isotropic shielding, the C5 and C8 shieldings are separately compared with the corresponding observed values. As reported by Harbison et al., the C8 shielding of the chromophore of bR shifts to upfield by 8.1 ppm compared with that of a protonated retinal Schiff base (PRSB), such as the chloride salt of *all-trans*-*N*-retinylidenebutylamine.[12] The value of 8.1 ppm is quite reasonable in comparison with the calculated value (7.7 ppm) obtained when the ϕ_{6-7} angles of the chromophore of bR and PRSB are assumed to be 180° and 62°, respectively, where the latter value was taken from the X-ray structure of *all-trans*-retinal.[47] It is thus safely said that the shielding change in C8, in going from PRSB to bR, mainly originates from the conformational change around the C6–C7 bond. On the contrary, the C5 shieldings of bR shift to downfield by 16.1 ppm relative to that of PRSB.[12,13] Clearly, this anomalous downfield shift can not be explained only from the above conformation change, the contribution of which is expected to be no more than 8.8 ppm on the basis of Figure 11a. As pointed out by Harbison et al., the residual 7.3 (= 16.1–8.8) ppm shift may be induced by an electrostatic perturbation from a negative point charge near C5.[12] However, a recent model study using various analogues has suggested that most of the above anomalous shift of the C5 resonance can be explained without locating such a negative charge.[18] In order to solve this problem, a more rigorous calculation for PRSB with all atomic representations would be required, which is under investigation now in our laboratory.

In Rh, the observed value of σ_{C8-C5} is given to be 8.9 ppm (Table 5).[7] On the basis of Figure 11b, there are two solutions for the ϕ_{6-7} angle, namely 58° and 135°, which can explain the observed value. This indicates that information only on the C5 and C8 shieldings is insufficient to determine whether the chromophore of Rh takes a skewed *s*-*cis* or *s*-*trans* form. This conclusion is also derived from the following separate consideration about the calculated C5 and C8 shieldings. In going to Rh from PRSB, the C5 resonance shifts to downfield by 1.6 ppm and the C8 one to upfield by 1.6 ppm.[7,19] Combining these data with that of Figure 11b, possible ϕ_{6-7} angles are estimated to be 49° and 119° from the C5 shielding and to be 26° and 109° from the C8 one. Thus, the angle ϕ_{6-7} of the chromophore is estimated to be in a range of 26°–58° or 109°–135°.

Smith et al. and Mollevanger et al. have suggested that the chromophore of Rh has a skewed 6-*s*-*cis* conformation on the basis of the fact that the C5 and C8 shieldings of Rh resemble the corresponding values of 6-*s*-*cis*-retinoic acid, respectively.[5-7] However, such a definitive statement is dangerous in light of the

(47) Tallent *et al.* carried out a molecular dynamics simulation of PRSB by holding the 6-*s*-*cis* linkage at ϕ_{6-7} = 50°. (Tallent, J. R.; Hyde, E. W.; Findsen, L. A.; Fox, G. C.; Birge, R. R. *J. Am. Chem. Soc.* **1992**, *114*, 1581–1592.)

fundamental nature of the angular dependence of the C5 and C8 shieldings. As can be seen from eq 5, the angular dependence of the C5 shielding is predominantly governed by the cos 2ϕ term, which makes it difficult to distinguish between the planar 6-*s-trans* and planar 6-*s-cis* forms and between the skewed 6-*s-cis* and skewed 6-*s-trans* forms. The C5 shieldings are thus useful only to distinguish between the skewed forms and the planar forms as defined above. On the other hand, the angular dependence of the C8 shielding has almost equivalent contributions from the cos ϕ_{6-7} and cos $2\phi_{6-7}$ terms, which makes it possible to distinguish the planar 6-*s-cis* forms from the planar 6-*s-trans* forms. However, as similar to the case of C5, the presence of the minima around 90° again makes it difficult to distinguish between the skewed 6-*s-cis* and skewed 6-*s-trans* forms. Consequently, the distinction between the skewed 6-*s-cis* and 6-*s-trans* forms is impossible only through the observation of the C5 and C8 shieldings.

The structural study of the bR chromophore has been carried out by other NMR techniques including the measurement of rotationally resonant magnetization exchange between the C8 and C18 carbons[48] and the solid-state deuterium NMR measurement in which the specific orientations of three labeled methyl groups were calculated from the deuterium quadrupole splittings.[49] Those studies unexceptionally concluded that the chromophore of bR takes the planar 6-*s-trans* form, consistent with the present result.

(48) Ulrich, A. S.; Heyn, M. P.; Watts, A. *Biochemistry* **1992**, *31*, 10390–10399.

(49) Creuzet, F.; McDermott, A.; Gebhard, R.; van der Hoef, K.; Spijker-Assink, M. B.; Herzfeld, J.; Lugtenburg, J.; Levitt, M. H.; Griffin, R. G. *Science* **1991**, *251*, 783–786.

Concluding Remarks

The present *ab initio* shielding calculation revealed that the parameter of $\Delta\sigma_{C8-C5}$ involves significant information on the ring–chain conformation of retinal and its derivatives. We thus estimated the conformations of the chromophore within Rh and bR by examining the value of $\Delta\sigma_{C8-C5}$. Such a method of conformational analysis is unique in the point that it does not require the ^{13}C-shielding data for reference compounds to determine the conformation of interest. A conclusion to be drawn is that the chromophore of bR takes the planar 6-*s-trans* conformation, probably with the presence of an electrostatic perturbation near the C5 carbon. On the other hand, it is concluded that in Rh the C6–C7 bond is considerably twisted, but there remains two possibilities of the skewed 6-*s-cis* and skewed 6-*s-trans* forms. In principle, this ambiguity can not be excluded only by the observation of ^{13}C shieldings of the unsaturated carbons.

The present study is the first to describe the *ab initio* shielding data for *all-trans*-retinal with full atomic representations. The results showed that the calculation is very promising for full understanding of the ^{13}C-shielding data of the chromophores of Rh and bR. We are now investigating the ^{13}C shielding of PRSB, including the point-charge model.

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