Ab Initio Study of ^{13}C Shieldings for Linear π-Conjugated Systems. Theoretical Determination of the C12−C13 Conformation in the Chromophore of Rhodopsin

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Abstract: The *ab initio* shielding calculations are carried out to investigate the conformation dependence of ^{13}C chemical shifts for conjugated compounds like the chromophore of a visual pigment rhodopsin (Rh). First, the calculations are applied systematically to 10 diene derivatives to obtain basic and universal relationships between their conformation and the shieldings of unsaturated carbons. It is indicated that the conjugated carbons are classified into two types according to the profiles of conformation dependence of the shieldings. The shieldings of the carbons forming the rotating bond exhibit complicated angular dependence. It is rigorously evidenced that the behavior of such carbon shieldings can be understood by considering the effect of π-orbital modification, a new concept introduced here. On the other hand, the shieldings of the other carbons essentially follow well-known mechanisms including the steric and charge density effects. One of the most important findings is that the steric effects are reflected predominantly on the σ_{11} component, and the effects originated in electronic perturbation are on the σ_{22} and σ_{33} ones. This classification is hardly disturbed even when both types of effects simultaneously act during a conformational change. It is indicated that these basic data for the dienes are available to interpret the conformation dependence of ^{13}C shieldings for more complicated compounds like retinal. Finally, combining the data for the direct *ab initio* shielding calculations of 11-*cis*-retinal and for those of the dienes, we successfully determine the preferred conformation around the C12−C13 bond of the chromophore in Rh. It is concluded that the chromophore takes *s-trans* conformation around the C12−C13 bond.

Introduction

The photoreceptive protein such as rhodopsin (Rh) or bacteriorhodopsin (bR) possesses a retinal isomer bound to a lysine residue via the protonated Schiff base linkage.[1] Rh exists in the rod cell of the retina of vertebrate and possesses 11-*cis*-retinal (Figure 1), which is isomerized into the *all-trans* form by the absorption of photons, finally leading to signal transduction. On the other hand, bR, which exists in the purple membrane of *Halobacterium halobium*, functions as a light-driven proton pump through a photocycle including the conversion of *all-trans*-retinal into the 13-*cis* isomer. In both pigments, the conformation of retinal closely relates to the appearance of the biological function, especially to the regulation of their absorption maxima. For example, in bR$_{568}$ the C6−C7 bond is likely to be planar *s-trans*,[2] which essentially contributes to the fact that this pigment absorbs yellow-green light.

At present, a diffraction method such as electron microscopy[3,4] seems to be insufficient in resolution to elucidate the conformational state of the chromophore in the pigments. Instead, structural information has been obtained mainly by means of spectroscopic studies. Among them, the observation of ^{13}C NMR chemical shifts for the chromophore provides a good insight not only into its conformation but also into the interaction of the chromophore with the surrounding protein matrix. The solid-state NMR technique has been applied to Rh,[5−7] bR,[8−10] and their photointermediates.[11−13] Consequently, it was revealed that the chemical shifts for the chromophore are significantly different from those for the free protonated retinal Schiff base. As for bR, the chemical shifts of C5 and C8 are displaced significantly to downfield and upfield,

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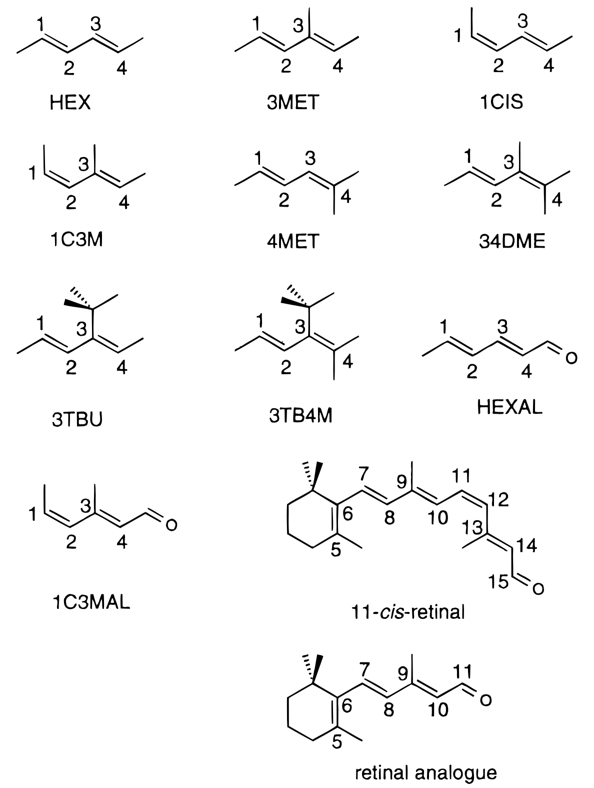

Figure 1. Molecular structures of the linear π-conjugated compounds studied.

respectively, relative to those of model compounds.[9] Recent *ab initio* studies reported by our laboratory[14,15] have successfully provided a rigorous theoretical interpretation for the origin of such chemical shift displacements.

As for the chromophore of Rh, the ^{13}C chemical shifts from C8 to C13 show significant downfield shifts compared with those for the protonated 11-*cis*-retinylidene Schiff base in solution.[6] Recently, Han and Smith[16,17] have attempted to explain this chemical shift difference by assuming that a carboxylate anion exists nearby C12, a model which is based on the so-called external-charge model.[1f,18] Their molecular orbital calculation indicated that both the chemical shift and absorption data for Rh could be consistently explained by this model. However, there seems to be some ambiguities in their analysis of the chemical shift data, because a linear correlation was assumed between chemical shielding and atomic charge density. Although the validity of such a correlation has been widely accepted,[19] this may not necessarily hold when the π-conjugation breaks due to the torsion of a single bond. In fact, our preliminary *ab initio* calculations[20] have demonstrated that the isotropic shielding for C12 of 11-*cis*-retinal changes with rotation of the C12−C13 bond by nearly 6 ppm, which is comparable to the chemical shift difference between the free and protein-bound states. Thus, it is of great necessity to unambiguously determine the conformation around the C12−C13 bond before attempting to build a molecular model of chromophore−protein interaction.

In order to deduce decisive information regarding the conformational state of the chromophore from its chemical shift data, basic data are required to inform us how chemical shieldings are influenced by substituents, *trans*−*cis* isomerization, inductive effects, etc. As for saturated cyclic or acyclic hydrocarbons[21−24] and polypeptides,[25−27] *ab initio* shielding calculations have been extensively carried out to examine the relationship between the chemical shieldings and molecular conformations. The calculations successfully reproduced the empirical rules known as α-, β-, γ-, or δ-substituent effects. A recent study using the solid-state NMR measurements and *ab initio* calculations has elucidated which component of a shielding tensor dominates the steric effects.[28] However, those data accumulated for aliphatic compounds alone may be insufficient to interpret the chemical shift data for conjugated compounds, since the conformation dependence of conjugated carbon shieldings would be influenced with changing π-electronic state, induced by rotation of a single bond. Therefore, a systematic study is newly required for conjugated systems.

The major part of this study is targeted on the search of some empirical rules held between the chemical shifts of conjugated systems and their conformation. For this purpose, *ab initio* shielding calculations are applied to 10 diene derivatives with various conformational states. First, we provide a rigorous theoretical interpretation for shielding changes arising from the modification of π-electronic state. Next, several types of steric effects and an inductive effect are analyzed in conjunction with the data for electronic structures of the conjugated chains. We will find that the additivity is fulfilled among these effects. Through this systematic analysis, we elucidate how a conformational change of conjugated systems influences the principal values of the shielding tensor for each unsaturated carbon. On the basis of this information, we successfully determine the C12−C13 conformation of the chromophore in Rh.

Calculations

Figure 1 shows the 10 diene derivatives examined here: (*E*,*E*)-hexa-2,4-diene (HEX), (*E*,*E*)-3-methylhexa-2,4-diene (3MET), (*E*,*Z*)-hexa-2,4-diene (1CIS), (*E*,*Z*)-3-methylhexa-2,4-diene (1C3M), (*E*)-2-methylhexa-2,4-diene (4MET), (*E*)-2,3-dimethylhexa-2,4-diene (34DME), (*E*,*E*)-3-*tert*-butylhexa-2,4-diene (3TBU), (*E*)-2-methyl-3-*tert*-butylhexa-2,4-diene (3TB4M), (*E*,*E*)-hexa-2,4-dienal (HEXAL), and (*E*,*Z*)-3-methylhexa-2,4-dienal (1C3MAL). These compounds are selected as minimal analogues of partial structures of 11-*cis*-retinal. Here, the numbering of the carbon atoms and the abbreviations (in parenthesis) of these dienes are given to easily compare the chemical shifts of corresponding carbons between different compounds.

The geometries of the compounds are fully optimized except for the dihedral angle of C1−C2−C3−C4, which was fixed at every 30° from 0° to 180°. 3TBU and 3TB4M are models of a molecular fragment of retinal including the C1, C5, C6, C7, and C8 carbons. Two methyl groups of the *tert*-butyl moiety of each model correspond to the two methyl groups attached to C1 of retinal. Thus, with reference

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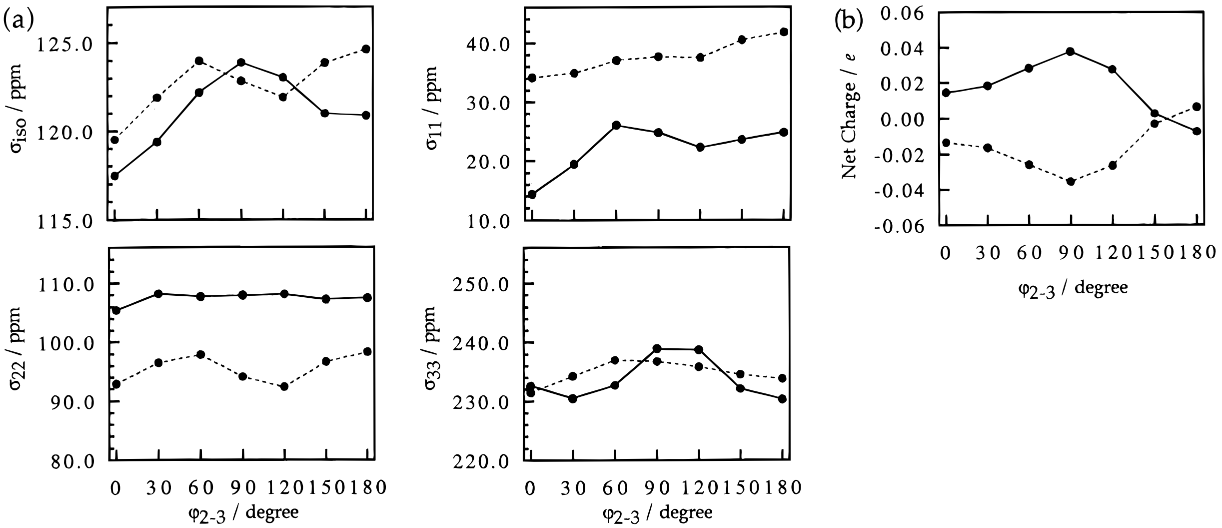

Figure 2. Conformation dependence of the shielding parameters and net charges for the conjugated carbon of HEX. (a) The data for the isotropic shielding and the principal values of the shielding tensor for each unsaturated carbon. (b) The data for net charges which mean the total charge on the corresponding methine (CH) group. In both a and b, the data for C1 (equivalent to C4) and C2 (equivalent to C3) are represented by solid and dashed lines, respectively.

to the optimized structure of retinal,[29] the orientation of the *tert*-butyl group was determined. One of the C—Me bonds of the *tert*-butyl group was rotated by 15° from the eclipsed form against the C3═C4 double bond and fixed at this orientation during geometry optimization. This geometrical constraint is required to reflect the rigidity of the cyclohexene ring of retinal.

The full geometry optimization of 11-*cis*-retinal (Figure 1) was executed by using the X-ray data[30] as an initial structure. Restricted geometry optimization was carried out with the dihedral angle of C11−C12−C13−C14 fixed at every 30° from 0° to 180°.

The geometry optimization and Mulliken population analysis were carried out using the GAUSSIAN92 program.[31b] Due to the CPU limitation of available computer facilities, the shielding calculations were carried out using two different programs, RPAC9.0 and GAUSSIAN94, which were installed on Cray computers at Eagan, MN, and an IBM SP2 cluster system at Institute for Molecular Science, Okazaki, Japan, respectively. The shielding calculation coded in GAUSSIAN94[31c] follows the so-called GIAO (gauge-invariant atomic orbital) theory,[32] while the program RPAC9.0[33] is based on the theory of Hansen and Bouman[34,35] for LORG (localized orbital/local origin) shielding calculations, interfacing to the GAUSSIAN90 program.[31a] All shielding calculations were performed at Hartree−Fock level. The shielding calculation for 11-*cis*-retinal was performed using the RPAC9.0 program, while that for the model compounds mentioned above was done using the GAUSSIAN94 program. PM3 (Parametric Method 3) calculations[36] were performed using the program MOPAC6.01.[37]

We have already reported a detailed study of the basis set dependence of chemical shieldings of conjugated carbons.[38] According to the results of that study, the 4-31G and 6-31G* basis sets were used for 11-*cis*-retinal and the diene derivatives, respectively.[39] In the latter case, we tested the basis sets of 4-31G and 6-31G**, which gave essentially identical results with those from the 6-31G*.

The calculated shieldings were converted to TMS reference, and the positive sign indicates deshielding. The shieldings of TMS are calculated as a 2.1 ppm downfield from methane[19] whose shieldings obtained from the LORG/4-31G and GIAO/6-31G* methods are 222.4 and 201.0 ppm, respectively.

Results

Conformation-Dependent Shifts in HEX. Throughout the former part of this paper, we focus on how the ^{13}C shieldings of the conjugated carbons of the diene derivatives are influenced by rotation of their central single bonds. First, we describe the data for HEX, which provide the most fundamental information on this subject. Figure 2a shows the calculated ^{13}C shieldings for the unsaturated carbons of HEX as a function of φ_{2-3}, defined as the dihedral angle of C1−C2−C3−C4. The angle φ_{2-3} of 180° means an *s-trans* form with respect to the C2−C3 bond. The values of σ_{iso} is the isotropic chemical shielding of each carbon atom, and those of σ_{11}, σ_{22}, and σ_{33} are the principal values of the corresponding shielding tensor. For all of the conjugated carbons, the principal axis for σ_{11} is almost perpendicular to the conjugated plane, whereas both of σ_{22} and σ_{33} are the in-plane elements and the axis of the former is almost parallel to each double bond. During the rotation, the direction of each principal axis was almost kept constant within 5° (data not shown).

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As shown in Figure 2a, the σ_{iso} of C1 (equivalent to C4) exhibits the most deshielded value at $\varphi_{2-3} = 90°$ and the most shielded value at $\varphi_{2-3} = 0°$, resulting in a span of 6.4 ppm in chemical shielding. This profile of the C1 shieldings arises mainly from the behavior of σ_{11} and σ_{33}: σ_{11} shifts steeply to upfield on going from $\varphi_{2-3} = 0°$ to 60° and σ_{33} exhibits a convex-type profile with a maximum around $\varphi_{2-3} = 90°$. Figure 2b shows the net charge of each C−H unit, the value of which was obtained from Mulliken population analysis. The change in σ_{33} appears to be synchronizing with the change in charge density. Thus, the angular dependence of the C1 shielding could be explained by considering the effect of charge distribution and another factor dominating the behavior of σ_{11}, which will be described later in detail.

The σ_{iso} of C2 (equivalent to C3) shows a different profile from that of C1: it has a maximum at $\varphi_{2-3} = 60°$ and a minimum at $\varphi_{2-3} = 120°$. Clearly, there is no apparent correlation between the angular dependencies of the shielding and charge density. In particular, it should be noted that σ_{33} shifts in the direction opposite to that predicted from the change in charge density. Such unusual behavior is thought to be a common phenomenon to the shieldings of carbons forming a rotating bond. Thus, it is of interest to provide a rigorous theoretical interpretation for its origin.

π-Orbital Modification Effect. Any perturbations which modify the π-orbitals of the conjugated system should have a greater affect on the paramagnetic shielding term than the diamagnetic one. According to the formalism of Ramsey,[40] the paramagnetic shielding term depends on two factors: the matrix element of angular momentum and excitation energy. In planar conjugated systems, the $\pi-\pi^*$ transition is not responsible for the shieldings because the matrix element of angular momentum vanishes between π-bonding atomic orbitals.[41] However, rotation of a single bond distorts the π-orbitals, generating some amount of angular momentum. In addition, the $\pi-\pi^*$ transition energy would increase, accompanied by the rotation, because the bond alternation is strengthened due to the breaking of conjugation. The generation of angular momentum contributes to causing a downfield shift for the shielding of the conjugated carbons, while the increase in transition energy contributes to causing an upfield shift. Thus, the fact that the σ_{33} for C2 of HEX shifts downfield with rotation of the C2−C3 bond (Figure 2) cannot be interpreted without explicit theoretical analysis.

In the framework of molecular orbital theory, by setting the gauge origin at the center of atom n, the αα component (α = x, y, or z) of the paramagnetic term is represented as follows

$$\sigma^{\text{para}}_{\alpha\alpha}(n) = \frac{1}{c^2}\sum_{l}^{\text{occ}}\sum_{m}^{\text{vac}}\Delta E_{lm}^{-1}\left\{\langle\psi_l|L_\alpha^{(n)}|\psi_m\rangle\left\langle\psi_m\left|\frac{L_\alpha^{(n)}}{r^{-3}}\right|\psi_l\right\rangle + \text{complex conjugate}\right\} \tag{1}$$

where ψ_l and ψ_m and lth occupied (occ) and mth virtual (vac) molecular orbitals, respectively, and ΔE_{lm} is the difference in orbital energy between them. $L^{(n)}$ is the local angular momentum defined as

$$L^{(n)} = L - R_n \times \text{p} \tag{2}$$

where R_n is a positional vector measured from the atom n.[35]

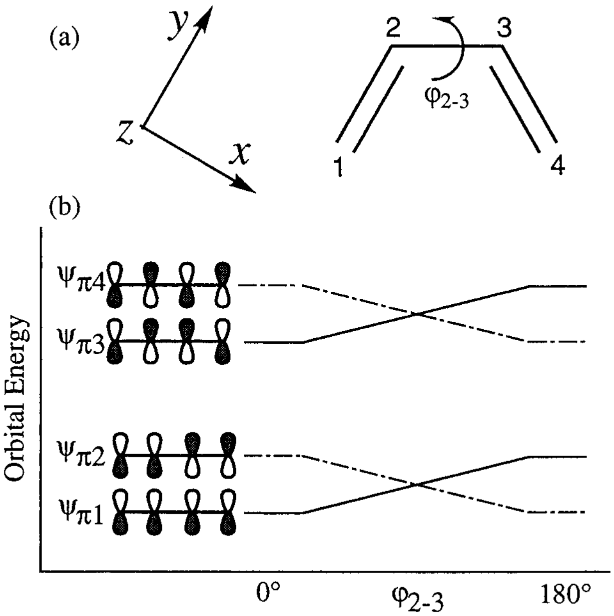

Figure 3. Schematic representations for explaining the π-orbital modification effect in diene. (a) The coordinate system assumed in the formulation (see text). (b) The conformation dependence of π-orbital energy.

Table 1. The Orbital Energies of the Four π-Orbitals of Butadiene as a Function of the Dihedral Angle C1-C2-C3-C4 (in eV)

	0°	30°	60°	90°	120°	150°	180°
$\psi_{\pi 4}$	2.12	1.97	1.59	1.12	1.58	1.98	2.15
$\psi_{\pi 3}$	0.29	0.39	0.68	1.10	0.67	0.37	0.27
$\psi_{\pi 2}$	−9.50	−9.59	−9.86	−10.33	−9.86	−9.56	−9.47
$\psi_{\pi 1}$	−11.97	−11.59	−10.97	−10.35	−10.95	−11.58	−12.00

Here, we consider a four π-electron system such as diene. For the sake of convenience, we let the x, y, and z axes coincide with the direction of the principal axes for σ_{33}, σ_{22}, and σ_{11} of dienes, respectively. The dependence of the orbital energy on φ_{2-3} is schematically illustrated in Figure 3. The scheme indicates that the energy of the orbital with an antibonding character with respect to the central single bond is lowered when rotation of the single bond occurs and that of the orbital with a bonding character is raised. The validity of this scheme is proven by the result from PM3 molecular orbital calculation for butadiene shown in Table 1. It can be assumed that the p_z orbitals of the carbons which form the rotating single bond are perturbed by each other when the bond rotates. This perturbation causes a distortion of the p-orbital, which is represented by the mixing of the p_z orbital with the p_{x-y} orbital defined as

$$\text{p}_{x-y} = {}^1\!/_2(\text{p}_x - \sqrt{3}\text{p}_y) \tag{3}$$

whose direction is perpendicular to both the z-axis and the single bond. Using the mixing parameter λ, the distortion can be described as follows

$$\text{p}_z \rightarrow \frac{1}{\sqrt{1+\lambda^2}}(\text{p}_z \pm \lambda \text{p}_{x-y}) \tag{4}$$

where the double sign takes plus for $\psi_{\pi 1}$ and $\psi_{\pi 3}$ and minus for $\psi_{\pi 2}$ and $\psi_{\pi 4}$, depending on the symmetry of the molecular orbitals. As for the C2 carbon, the matrix elements of angular momentum (for example, $\langle\psi_{\pi 1}|\text{L}_x^{(2)}|\psi_{\pi 3}\rangle$) are calculated as

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$$\langle\psi_{\pi1}|L_x^{(2)}|\psi_{\pi3}\rangle = -\frac{1}{4(1+\lambda^2)}\{\langle \mathrm{p}_z(2)|L_x^{(2)}|\mathrm{p}_z(2)\rangle + \lambda^2\langle \mathrm{p}_{x-y}(2)|L_x^{(2)}|\mathrm{p}_{x-y}(2)\rangle + \lambda\langle \mathrm{p}_z(2)|L_x^{(2)}|\mathrm{p}_{x-y}(2)\rangle + \lambda\langle \mathrm{p}_{x-y}(2)|L_x^{(2)}|\mathrm{p}_z(2)\rangle\} = -\frac{i\lambda}{8(1+\lambda^2)}\int \mathrm{d}r\, z\phi\left\{\left(xy\frac{\partial\phi}{\partial z} - zx\frac{\partial\phi}{\partial y}\right) + \sqrt{3}\left(y^2\frac{\partial\phi}{\partial z} - z\phi - yz\frac{\partial\phi}{\partial y}\right)\right\} + (x-\sqrt{3}y)\phi\left(y\phi + yz\frac{\partial\phi}{\partial z} - z^2\frac{\partial\phi}{\partial y}\right) = \frac{\sqrt{3}i\lambda}{4(1+\lambda^2)} \quad (5)$$

where the function ϕ represents the radial part of p-orbital and the following approximation was used:

$$L_\alpha^{(n)}|\mathrm{p}_\beta(m)\rangle = L_\alpha^{(n)}|\mathrm{p}_\beta(n)\rangle\delta_{mn} \quad (6)$$

The approximation of eq 6 means that the angular momentum of a different-centered p-electron from the atom of interest is neglected. Consequently, we can obtain the following equations:

$$\langle\psi_{\pi1}|L_x^{(2)}|\psi_{\pi4}\rangle = \langle\psi_{\pi2}|L_x^{(2)}|\psi_{\pi3}\rangle = 0$$
$$\langle\psi_{\pi1}|L_x^{(2)}|\psi_{\pi3}\rangle = \langle\psi_{\pi2}|L_x^{(2)}|\psi_{\pi4}\rangle = \frac{\sqrt{3}i\lambda}{4(1+\lambda^2)} \quad (7)$$

It should be noted that the energy differences ΔE_{13} and ΔE_{24} are nearly equal and remain almost unchanged through the bond rotation (see Figure 3). This means that the factor of transition energy does not contribute to the change in shielding. In other words, the conformation-dependent change of the shielding tensor is dominated by the change in the matrix element of angular momentum.

On the basis of these results, we can write the paramagnetic term as follows

$$\sigma_{xx}^{\mathrm{para}}xx(2) = -\frac{1}{c^2}\Delta E^{-1}\langle r^{-3}\rangle\frac{3\lambda^2}{4(1+\lambda^2)} \quad (8)$$

where $\Delta E_{13} = \Delta E_{24} = \Delta E$ and the average of r^{-3} is regarded as a constant. Similarly, the expressions of the other components can be obtained as follows:

$$\sigma_{yy}^{\mathrm{para}}(2) = -\frac{1}{c^2}\Delta E^{-1}\langle r^{-3}\rangle\frac{\lambda^2}{4(1+\lambda^2)}$$
$$\sigma_{zz}^{\mathrm{para}}(2) = 0 \quad (9)$$

According to eqs 8 and 9, the π-orbital modification induced by rotation of the single bond affects mainly the terms of σ_{xx} and σ_{yy}, namely σ_{33} and σ_{22}, in the diene system. Since the value of λ increases with rotation of the bond, an appreciable amount of downfield shift should be induced for these tensor components. This indicates that the shieldings of carbons forming a rotating bond do not necessarily follow the profile of charge density. Therefore, the present formalism is helpful to understand the unusual behavior of the C2 shielding of HEX, especially of its σ_{33} component.

γ-Steric Effect. As is well-known, the ^{13}C shielding of an aliphatic carbon significantly shifts to upfield when a methyl group is introduced at the γ-position relative to the carbon of interest, usually called γ-steric effect. The γ-steric effect appears most remarkably when the carbon atom of interest stays in eclipsed conformation with respect to the γ-methyl group. Accordingly, if a methyl group is introduced at the C3 position of HEX, an upfield shift is expected to be induced for the C1 carbon. Figure 4 shows the calculated results obtained by subtracting the values for HEX from those for 3MET, indicated as Δ3MET. Certainly, an appreciable amount of an upfield shift occurs in the isotropic shielding of C1 when the angle of φ_{2-3} is around 180°, supporting the above conventional picture.

A theoretically more interesting finding is that this upfield shift predominantly originates from a large upfield shift of σ_{11}. This agrees with the experimental result[42] that for the sp^2 carbon, the γ-steric effect is reflected on the principal value whose direction is perpendicular to the conjugated plane. It is also consistent with a recent report indicating that for the sp^3 carbon a significant change occurs in the principal value whose direction is perpendicular to the plane in which the interaction forces operate.[28] Thus, the behavior of σ_{11} can be used to judge whether or not conformation-dependent changes in ^{13}C shielding of a given carbon are due to the γ-steric effect.

Next, along with the above criteria, we examine the data for C1 of 1C3M, the shielding of which is shown as a difference (Δ1C3M) measured from that of HEX in Figure 4. In this case, the isotropic shielding also shifts to upfield when the angle of φ_{2-3} is around 180°, but the σ_{11} component does not. This indicates that the change in the isotropic shielding cannot be ascribed to the γ-steric effect from the C3 methyl group. As can be seen from Figure 4, the conformation dependence of the σ_{11} value for the C1 shielding is very similar to that for the C1 carbon of 1CIS. Thus, the C1 shielding of 1C3M is predominantly influenced by the *trans*−*cis* isomerization of the C1═C2 double bond. On the basis of these results, we could deduce a theorem for the γ-steric effect: the shielding of a C—H carbon of interest receives the γ-steric effect only when the C—H group directs toward the same side of the γ-methyl group. This theorem implicitly states that a quaternary carbon (nonprotonated carbon) is insensitive to the γ-steric effect.

The σ_{11} shielding of C3 of 1CIS shows an upfield shift when the φ_{2-3} is around 180°, indicating the γ-steric effect from the 1-methyl group. On the other hand, the behavior of σ_{11} or C3 of 1C3M nearly coincides with that for 3MET, supporting the above theorem that the quaternary C3 of 1C3M does not receive the γ-steric effect from the C1 methyl group.

In summary, the σ_{11} component of the shielding tensor can be used as a measure of the γ-steric effect. The maximal shift by the effect occurs at the rotation angle of 180°, that is, *s-trans* conformation. The σ_{11} of C1 of 3MET shifts upfield by 8.0 ppm, whereas that of C3 of 1CIS shifts upfield by 14.6 ppm. This difference may arise from the difference in the direction of steric force. In the former, the γ-methyl group exists nearly in the direction of σ_{33} axis which is almost perpendicular to the C1═C2 double bond. In the latter, the γ-methyl group exists nearly in the direction of σ_{22} which is almost parallel to the C3═C4 double bond.

Other Steric Effects. As described above, the most apparent effect due to the methyl substitution is the γ-effect, but the shieldings of carbon atoms having no γ-substituents also show a characteristic conformation dependence. It was confirmed that the σ_{11} component again reflects the steric effect due to the presence of β- and δ-substituents. Table 2 summarizes the change in the σ_{11} shielding induced by various substituents, as indicated in Figure 5. For example, the difference between the σ_{11} for C2 of 3MET in *s-trans* form and that of HEX is denoted

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(42) Harbison, G. S.; Mulder, P. J. J.; Perdon, H.; Lugtenberg, J.; Herzfeld, J.; Griffin, R. G. *J. Am. Chem. Soc.* **1985**, *107*, 4809.

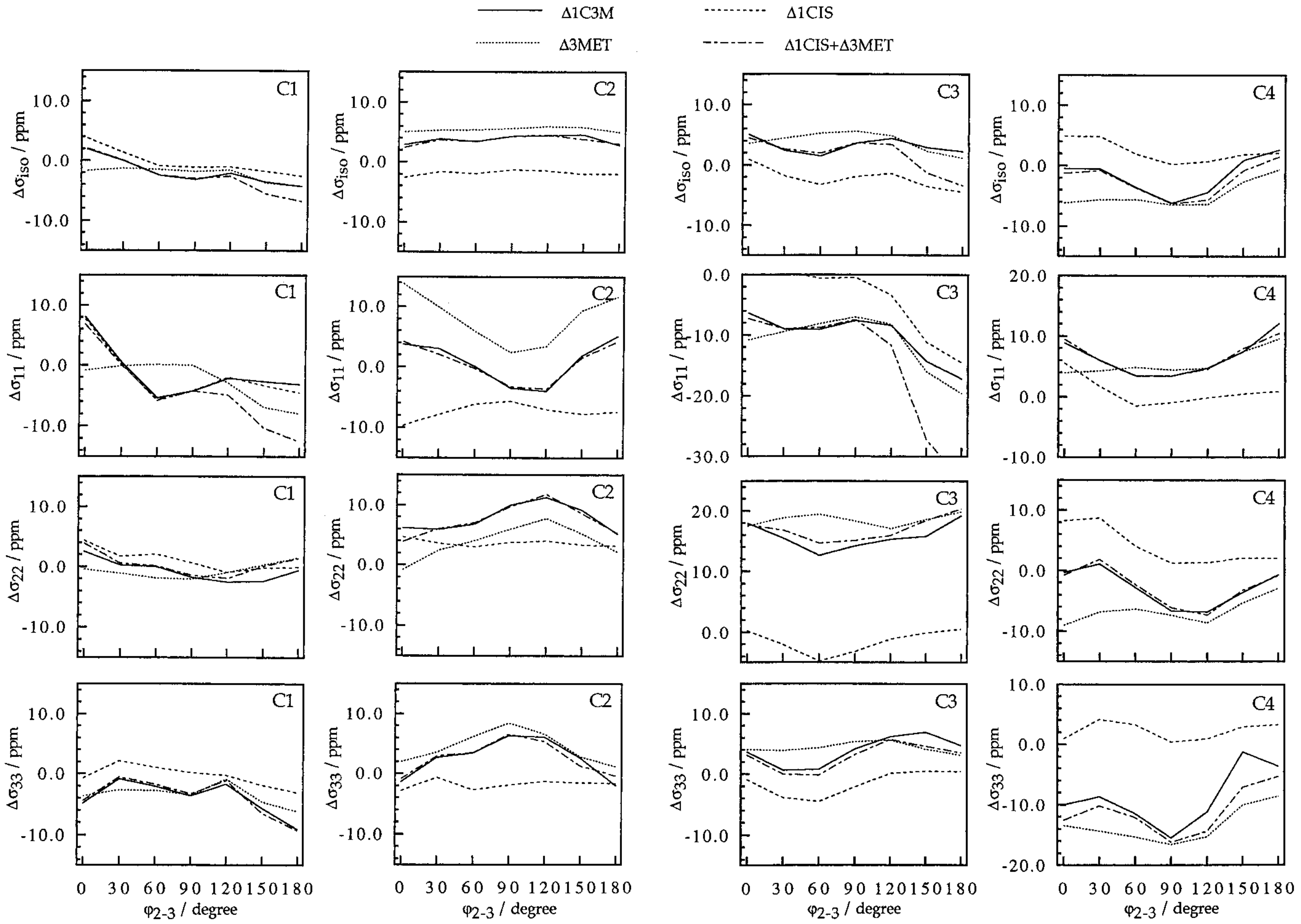

Figure 4. Conformation dependence of the shielding parameters of the conjugated carbons in 3MET, 1CIS, and 1C3M. The value of each shielding parameter is given as the difference from that for the corresponding carbon of HEX with the same angle of φ_{2-3}.

Table 2. Effects of Substituents on the σ_{11} Component of the Shielding Tensor of a Given Carbon

position[a]	$\Delta\sigma_{11}$ (ppm)[b]
β_1	13.8
β_2	12.8 ± 0.6
β_3	11.7
β_4	5.5 ± 2.7
γ_1	−12.8 ± 1.9
γ_2	−7.4 ± 0.6
γ_3	2.9 ± 0.7
δ_2	4.5 ± 1.0

[a] Position where a substituent is introduced (see Figure 5). [b] Net shielding change induced by a substituent. Plus sign indicates a downfield shift. The data, except for β_1 and β_3, indicate the average value with standard deviation over several kinds of diene derivative.

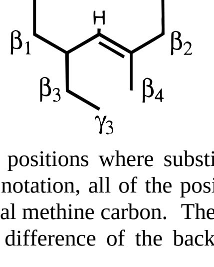

Figure 5. Notation of positions where substituents are introduced. According to the usual notation, all of the positions are first denoted with respect to the central methine carbon. The subscript (1, 2, ...) are added to represent the difference of the backbone conformation or configuration.

as "β_3-steric" effect. Interestingly, an upfield shift is caused only by the γ-substituent, consistent with the results for aliphatic compounds.

Inductive Effect. HEXAL was used to examine the effect of an electron inductive group on the conformation-dependent changes of the conjugated carbon shieldings. The value of ΔHEXAL was obtained by subtracting the chemical shifts of HEX from those of HEXAL. As shown in Figure 6, the data for ΔHEXAL have characteristic features. The shieldings of C1 and C4 exhibit concave and convex curves, respectively. On the other hand, the shieldings of C2 and C3 have less apparent profiles. Thus, as similar to the case of HEX, the carbons relevant to the rotating bond have intrinsically different properties from the other ones in chemical shielding.

It should be noted that the shielding changes of C1 and C4 are dominated by the behavior of σ_{33}. As shown in Figure 7, the σ_{33} values for C1 and C4 of ΔHEXAL change in the opposite sense to each other; that is, as the molecule distorts from the planar conformation, the shielding of C1 shifts to upfield, while that of C4 shifts to downfield. The net charge of each C−H unit of HEXAL, measured from that for HEX, is superposed upon Figure 7. In both carbons, the charge density and chemical shielding are synchronizing with each other. From this correlation, a shielding change of ~600 ppm is expected to occur per change of unit charge. These data clearly indicate that the changes in the C1 and C4 shieldings are dominated by that in electronic distribution of the conjugated system. The behavior of the net charge is explained as follows. When the molecular structure is planar, positive charge should be induced on C1 and C3 due to the so-called resonance effect. When the conjugation is broken as a result of the rotation of the C2−C3 bond, the contribution of the resonance structure is reduced. Consequently, the positive charge density on C1 decreases,

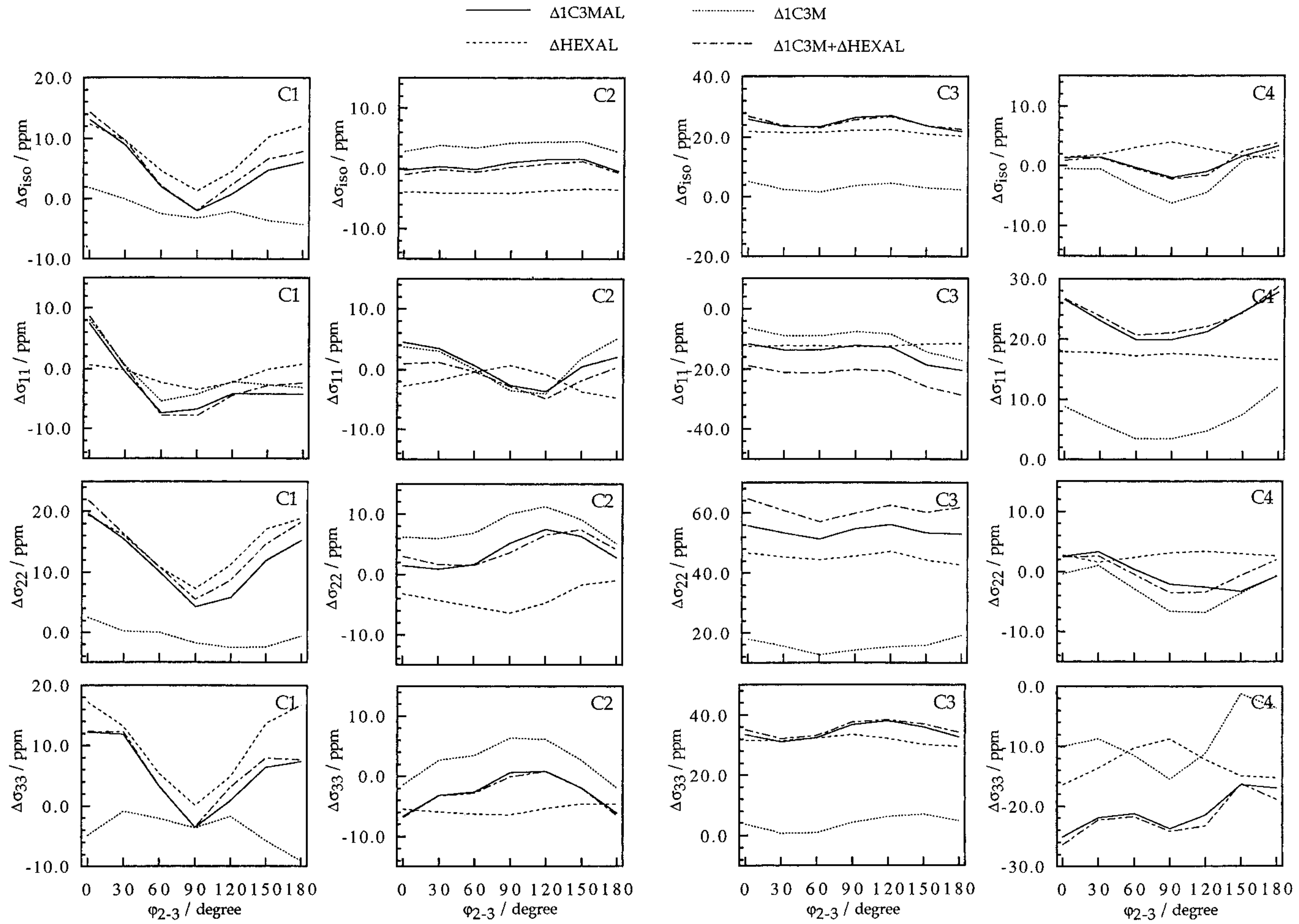

Figure 6. Conformation dependence of the shielding parameters of the conjugated carbons in 1C3M, HEXAL, and 1C3MAL. The value of each shielding parameter is given as the difference from that for the corresponding carbon of HEX with the same angle of φ_{2-3}.

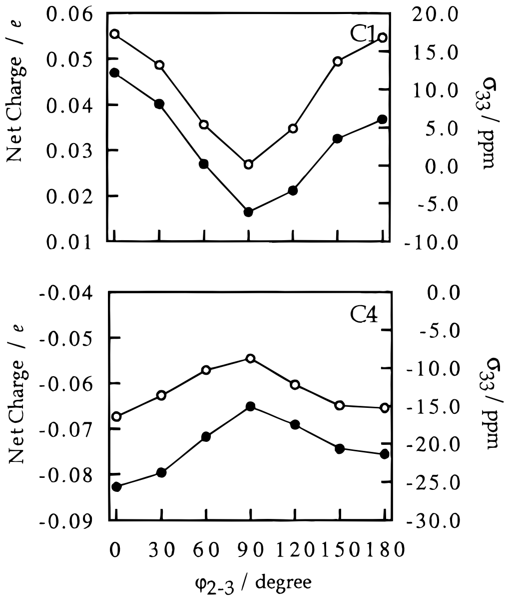

Figure 7. The conformation dependence of the σ_{33} component (○) and the net charge of the CH unit (●) for HEXAL. The value of each parameter is given as the difference from that for the corresponding carbon or CH unit of HEX with the same angle of φ_{2-3}. The upper and lower figures represent the data for C1 and C4, respectively.

whereas that on C4 increases. This successfully explains the fact that C1 exhibits concave-type curves in conformation dependence of both shielding and charge density, but C4 exhibits convex curves.

As for C2 and C3 of HEXAL, there was no apparent correlation between the charge density and chemical shielding. As described above, the shieldings of these carbons should directly receive the π-orbital modification effect. Thus, it is reasonable that their behavior does not follow the simple correlation with charge density. Similarly, for C2 and C3 of HEX (Figure 2), the shieldings of σ_{22} and σ_{33} would be determined by a simultaneous contribution of both electronic distribution and π-orbital modification effects. We could say that in general the shielding of the carbon on the both sides of the rotating single bond shows some complicated conformation dependence due to these two factors.

For all of the other model compounds studied here, it was confirmed that the conformation dependence of charge density coincides with that of σ_{33} (data not shown). Therefore, in contrast to the steric effect, the effect of charge density is reflected on σ_{33}. Since the behavior of σ_{22} resembles that of σ_{33}, it may be expected that σ_{22} also reflects the change in charge density. However, such a correlation was less apparently observed for σ_{22} than for σ_{33}.

Additivity of Miscellaneous Effects. Figure 4 shows the sum of Δ1CIS and Δ3MET, which is indicated as Δ1CIS+Δ3MET. As can be seen from this figure, each value of σ_{iso}, σ_{11}, σ_{22}, and σ_{33} of Δ1CIS+3MET coincides with that of Δ1C3M. This indicates that the methyl substituent effect acting in 3MET and the *trans*−*cis* isomerization effect in 1CIS are almost additive throughout the rotation. Exceptionally, a sizable deviation is observed between Δ1CIS+Δ3MET and Δ1C3M in σ_{11} of both C1 and C3 carbons in the range of φ_{2-3}

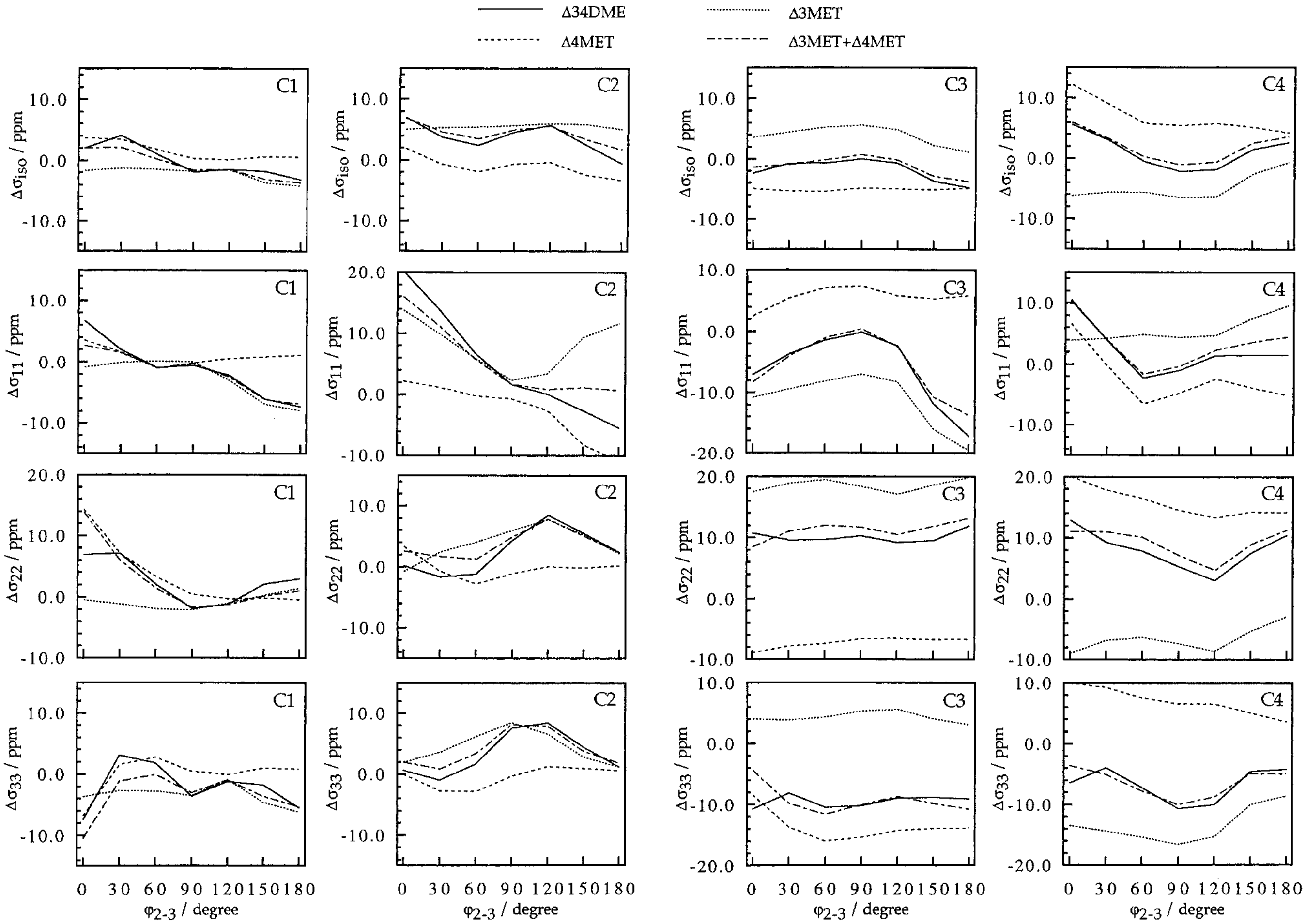

Figure 8. Conformation dependence of the shielding parameters of the conjugated carbons in 3MET, 4MET, and 34DME. The value of each shielding parameter is given as the difference from that for the corresponding carbon of HEX with the same angle of φ_{2-3}.

= 90° to 180°. In other words, a breakdown of the additivity occurs while the γ-steric effect acts predominantly.

The results for 4MET, 3MET, and 34DME are shown in Figure 8. As for all carbons, the sum of Δ3MET and Δ4MET agrees with Δ34DME. Thus, the effects of the methyl substituent on C3 and C4 are also additive.

As can be seen from Figure 9, Δ3TB4M agrees with the values of the sum of Δ3TBU and 4MET. This indicates that the additivity shown in Figure 8 is also maintained for the *tert*-butyl group. Through comparison of Figure 8 and 9, it is shown that the profile of Δ3TBU is similar to that of Δ3MET. Thus, in the *tert*-butyl group, the carbon which plays a role in exerting steric effect is not that of the outer methyl groups, but the central quaternary carbon. Exceptionally, the outer methyl groups exert a γ-steric effect on the C2 shielding in the range of $\varphi_{2-3} = 0-30°$.

Figure 6 shows that the values of Δ1C3M+ΔHEXAL agree with those of Δ1C3MAL. This indicates that the effect of the aldehyde group is additive to the other effects included in the data of Δ1C3MAL, such as methyl substitution or isomerization. This finding is quite natural because the charge density effect caused by the carbonyl group mainly affects the σ_{22} and σ_{33}, while the steric effect exerts on σ_{11}.

In summary, for most cases, the effects of alkyl substitution, isomerization, and carbonyl substitution are additive. The only situation in which the additivity is disturbed is in the conformational range where the γ-effect is acting predominantly.

Discussion

Features of the ^{13}C Shielding of Conjugated Carbons. In the previous section, we obtained some basic data for conformation dependence of the ^{13}C shieldings of conjugated carbons. Clearly, the conjugated carbons are classified into two types according to the behavior of shielding changes. As found in the data for HEX and HEXAL, the shieldings of the carbons forming the rotating bond exhibit complicated angular dependence. To understand the behavior, one must take into account the effect of π-orbital modifications other than the steric and inductive (charge density) effects. Thus, the direct *ab initio* calculations would be required to predict the shielding of this type of carbon. On the other hand, the shieldings of the other carbons essentially follow the well-known mechanisms, including the steric and charge density effects. Thus, their angular dependence can be understood by using the additivity rule among them and the data for charge density obtained from Mulliken population analysis.

One of the most important findings is that the steric effects are reflected predominantly on the σ_{11} component, and the effects originated in electronic perturbation are on the σ_{22} and σ_{33}. This classification is hardly disturbed even when both types of effects simultaneously act during a conformational change. For example, the σ_{11} and σ_{33} components of C1 (and C4) of HEX are independent of each other. As previously described, the latter changes according to the charge density profile. The

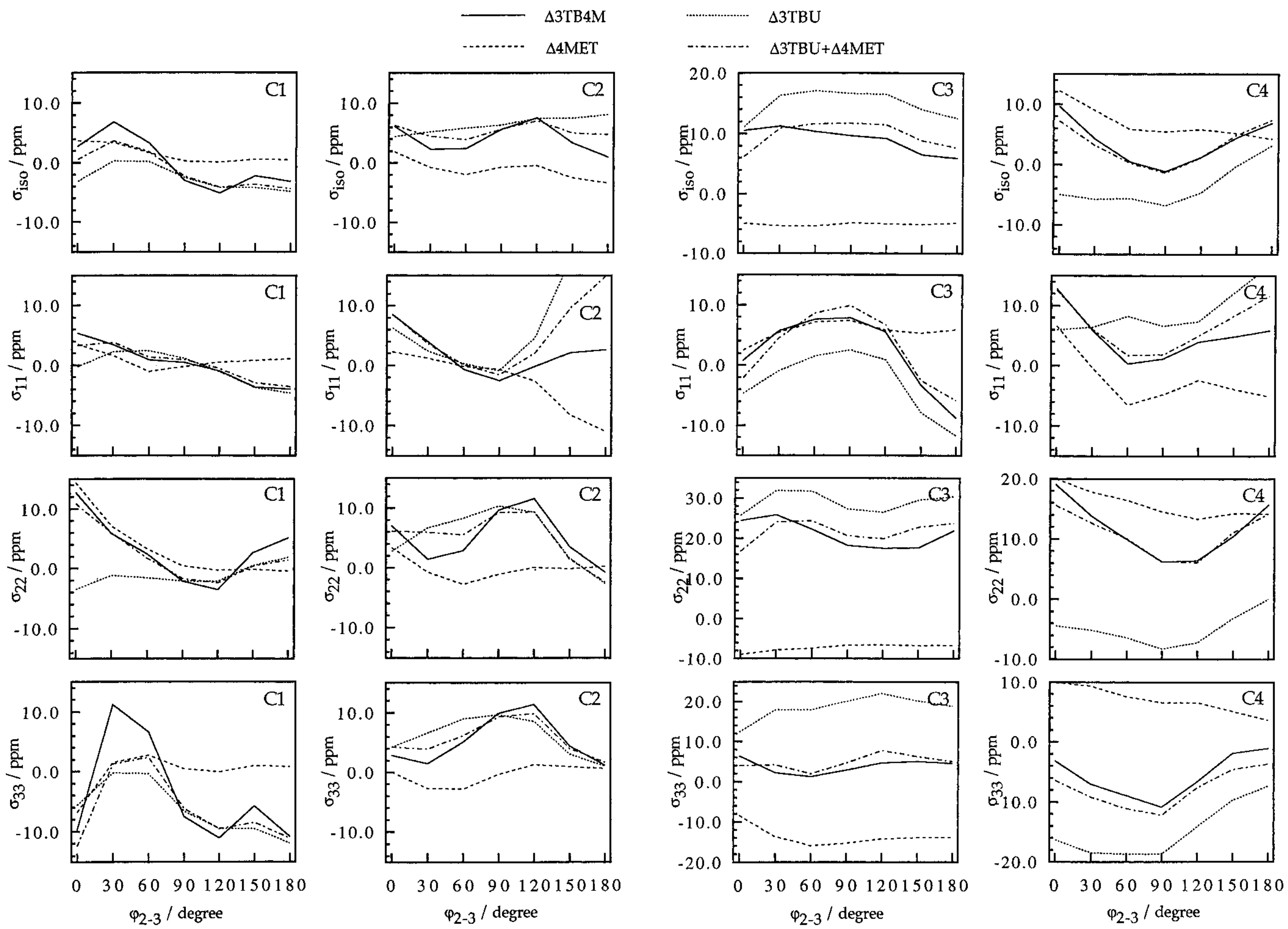

Figure 9. Conformation dependence of the shielding parameters of the conjugated carbons in 3TBU, 4MET, and 3TB4M. The value of each shielding parameter is given as the difference from that for the corresponding carbon of HEX with the same angle of φ_{2-3}.

former exhibits a steep upfield shift of about 10 ppm on going from $\varphi_{2-3} = 0°$ to 30° (Figure 2). Clearly, this shift can be explained by the γ-steric effect between the C1 and C4 carbons.

These results suggest the possibility that by analysis of the shielding tensor one may identify the origin of shielding change caused by unknown factors. In the final section, we attempt to analyze the unusual shifts of retinal in Rh.

Availability of the Diene Data to Retinal. To check the availability of the diene data to retinal and its derivatives, we first selected 11-*cis*-retinal as a target molecule. The shielding calculation for 11-*cis*-retinal was executed for each conformer with a given rotation angle of C12−C13, which is an angle crucial to interpretation of the chemical shift data for rhodopsin.[20] The angle of φ_{12-13} was defined as the dihedral angle of C11−C12−C13−C14, the value of which is taken to be 180° when the C12−C13 conformation is *s-trans*. In Figure 10 the isotropic shieldings of C5 to C14 are plotted as a function of φ_{12-13}.

The C11 carbon is similar to the C1 carbon of HEXAL in the chemical environment. As can be seen from Figures 7 and 10, both carbon shieldings exhibit similar conformation dependence, characterized as a concave-type curve with minima located around $\varphi_{12-13} = 90°$. Similar patterns are also observed for the C9 and C7 shieldings of retinal, although the amplitude in shielding change becomes smaller with increasing distance from the rotating bond: C11 > C9 > C7 > C5. These results imply that the conformation dependence of the C11, C9, C7, and C5 shieldings follows the same mechanism as the C1 shieldings of HEXAL. Namely, the origin of occurrence of the concave-type curve is attributable to breaking of the π-conjugation, leading to a reduction of the contribution of the resonance structure in distorted form. The C13 shielding exhibits a different pattern from those for the other odd-numbered carbons, since the carbon involved in the rotating bond is similar to the C3 carbon of HEXAL.

Among the diene compounds studied, 1C3MAL is the model that most resembles the molecular fragment of 11-*cis*-retinal including the C11, C12, C13, and C14 carbons. The calculated isotropic shifts for 1C3MAL are superposed upon Figure 10. Clearly, the conformation-dependent profiles of the C1, C2, C3, and C4 shieldings of 1C3MAL coincide with those for C11, C12, C13, and C14 of 11-*cis*-retinal. In addition, the conformation dependence of the principal values are also reproduced well by the calculation for the model as shown in Figure 11, where the data for the C12 shielding are indicated as an example.

In a previous study using an analogue compound (β-ionylideneacetaldehyde, see Figure 1),[14] we investigated the change of carbon shieldings as a function of the C6−C7 torsion. In that paper, it was indicated that the C5 shielding shows a concave-type dependence while the C8 shielding shows a convex-type. At first sight, the behavior of C5 and C8 shielding seems strange since both carbons are located in the equivalent position to C1 of HEX, whose shielding shows a convex-type profile (Figure 2). However, we can show that these profiles represent intrinsic properties of the β-ionone ring moiety. The 3TB4M is an appropriate model in which C4 and C1 are in similar chemical environments to C5 and C8 of retinal, respectively. As shown in Figure 12, the shieldings for C5 and

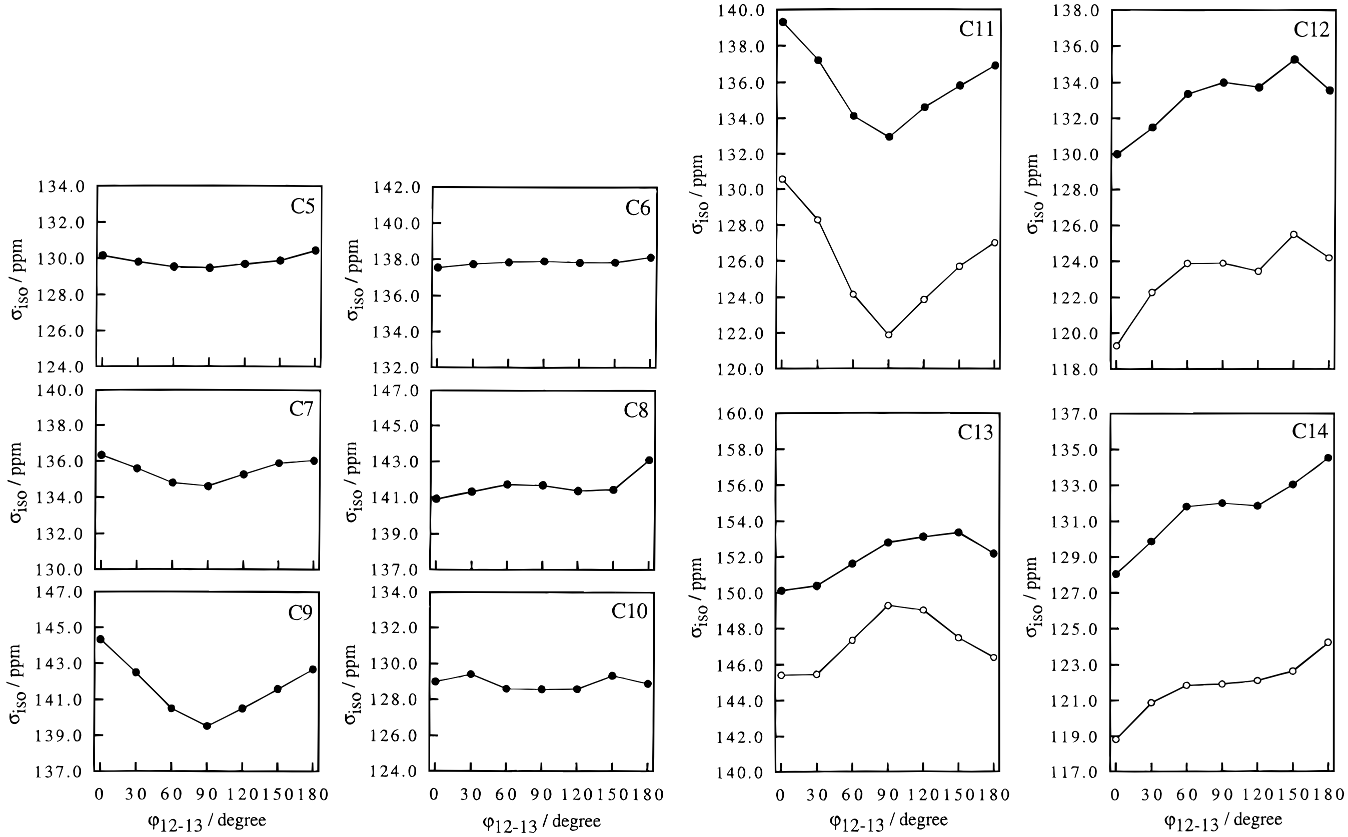

Figure 10. Conformation dependence of the isotropic shieldings of conjugated carbons of 11-*cis*-retinal. The data for C1, C2, C3, and C4 of 1C3MAL are superposed on the figures for C11, C12, C13, and C14, respectively.

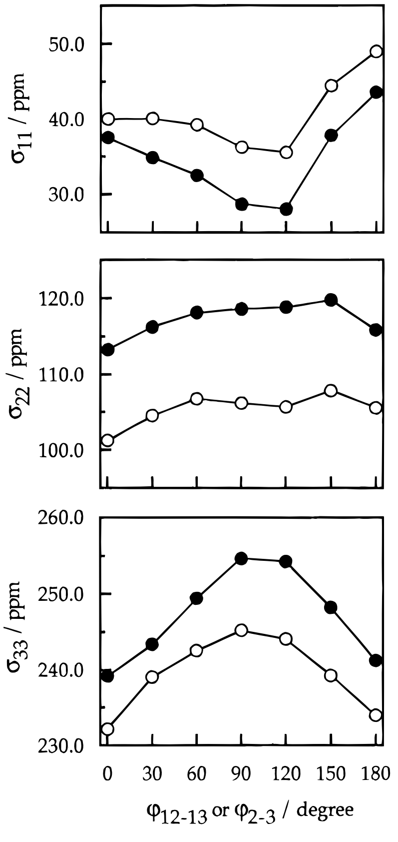

Figure 11. Comparison of the principal values for the C12 shielding of 11-*cis*-retinal (●) and those for the C2 shielding of 1C3M (○). The former and latter data are plotted against φ_{12-13} and φ_{2-3}, respectively.

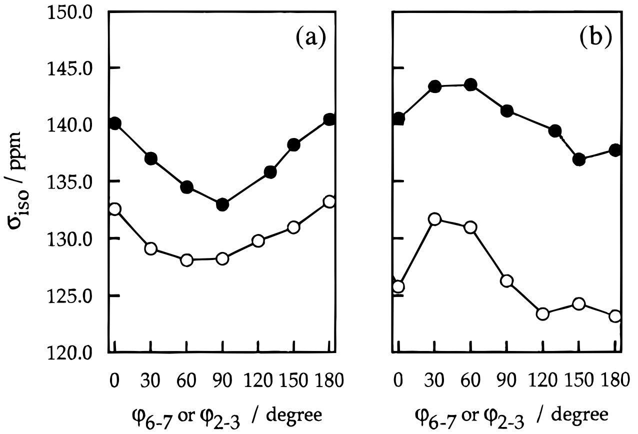

Figure 12. Comparison of the isotropic shieldings of conjugated carbons of the retinal analogue (●) and those for 3TB4M (○). The former data are cited from ref 14. (a) The data for the C5 shielding of the retinal analogue and those for the C4 shielding of 3TB4M. (b) The data for the C8 shielding of the retinal analogue and those for the C1 shielding of 3TB4M. In both a and b, the shielding data for the retinal analogue and 3TB4M are plotted against φ_{6-7} and φ_{2-3}, respectively.

C8 of the retinal analogue coincide with those for C4 and C1 of 3TB4M, respectively. This indicates again that the data for the dienes can be used to interpret the shieldings of carbons apart from the rotating bond.

From these results, we believe that the data for the present model compounds are applicable to the analysis of the conformation dependence of chemical shifts for the conjugated carbons of retinal, even if the length of the conjugated chain is shorter than that of retinal.

The C12−C13 Conformation of the Chromophore of Rh. The isotropic shielding of C12 of the chromophore of Rh shows a ~3 ppm downfield shift relative to that of protonated 11-*cis*-retinylidene Schiff base in solution. It has been hypothesized

Table 3. Experimental Chemical Shift of C12

	σ_{iso}	σ_{11}	σ_{22}	σ_{33}
all-trans PSB	134.3[a]	58	133	212
Rh[b]	132.1	(56)	(131)	(210)
Rh[c]	133.5	41	149	209

[a] $(\sigma_{11}+\sigma_{22}+\sigma_{33})/3$. [b] Taken from ref 6. Each principal value shown in parentheses was obtained by subtracting 2 from the corresponding value of *all-trans* PSB. [c] Taken from ref 7.

Table 4. Comparison between the Experimental and Calculated Data for the Shielding Parameters of C12

		σ_{iso}	σ_{11}	σ_{22}	σ_{33}
exptl[a]		−0.8	−17	+16	−3
calcd[b]	$\varphi_{12-13} = 180°$	−4.2	−2.7	−2.7	−7.1
(full atom)	$\varphi_{12-13} = 120°$	−4.0	−18.1	+0.3	+5.9
calcd[c]	$\varphi_{2-3} = 180°$	−2.2	−6.5	−3.0	−3.1
(model)	$\varphi_{2-3} = 120°$	−3.2	−19.9	+3.1	+7.0

[a] Obtained by subtracting the data for protonated *all-trans*-retinylidene Schiff base (ref 6) from those for rhodopsin (ref 7) (positive sign denotes downfield shift). [b] Obtained by subtracting the data for *all-trans*-retinal (ref 14) from those for 11-*cis*-retinal (positive sign denotes downfield shift). [c] Obtained by subtracting the data for 3MET from those for 1C3M (positive sign denotes downfield shift).

that this shielding difference arises from the electrostatic interaction between the chromophore and charged amino acid(s) of the protein, a model which has been originally proposed by Nakanishi's group.[15] However, there is still no direct evidence for the hypothesis from either theoretical or experimental viewpoint. According to accumulated data for ^{13}C shieldings of conjugated compounds,[19] only a few ppm of the shielding change could also be explained by other factors including nonspecific interactions like solvent effects[43,44] and structural changes of molecules as studied here. Thus, the information on isotropic shielding alone is insufficient to identify the real origin of the above shielding difference. Here, we abandon the attempt to interpret the isotropic shielding. Instead, we focus on the analysis of the principal values of the shielding tensor.

Unfortunately, the solid-state NMR data for 11-*cis*-retinal Schiff base have not been published. Thus, we used protonated *all-trans*-retinylidene Schiff base as a reference compound. There have been two reports refering to the C12 shielding in the chromophore of Rh (see Table 3).[6,7] First, we consider Mollevanger's data. Table 4 summarizes the differences in the principal values between the C12 shielding of the Rh chromophore and that for the reference. A notable feature is that the σ_{11} and σ_{22} components are largely shifted (−17 ppm and +16 ppm, respectively) as compared with those for the reference. On the basis of the present results for the dienes, we determined that σ_{11} reflects steric effects like γ-effect, while σ_{22} and σ_{33} reflect electronic perturbations to the conjugated systems. Thus, it may be concluded that the C12 shielding of the Rh chromophore receives both types of effects. Furthermore, the detailed analysis of steric effects responsible for the change in σ_{11} may enable us to speculate the conformation about the C12−C13 bond.

Table 4 summarizes the calculated values for shielding changes that would be induced on C12 as a result of *trans*−*cis* isomerization of the C11═C12 double bond and the rotation of the C12−C13 bond. The effect of these factors was estimated first by subtracting the C12 shielding of *all-trans*-retinal from that of 11-*cis*-retinal. When the angle φ_{2-3} is kept at 180°, the σ_{11} component shifts upfield by 2.7 ppm upon going from *all-trans* to 11-*cis*-retinal. 3MET and 1C3M are the minimal models available for examining the effect of the *trans*−*cis* isomerization: the C1, C2, C3, and C4 of the models correspond to C11, C12, C13, and C14 of retinal, respectively. As shown in Table 4, the σ_{11} component of C2 shifts upfield by 6.5 ppm upon going from 3MET to 1C3M. These results indicate that the net effect of the *trans*−*cis* isomerization on the σ_{11} shielding is not more than several ppm, which is insufficient to explain the above experimental data for Rh.

As the C12−C13 bond of 11-*cis*-retinal is rotated from the *s-trans* form, the σ_{11} shielding gradually shifts upfield. As shown in Figure 11, the σ_{11} of C12 exhibits a concave-type profile with a minimum at $\varphi_{2-3} = 90-120°$. When $\varphi_{2-3} = 120°$, the σ_{11} shifts upfield by 18.1 ppm, as measured from that of the *all-trans* isomer with $\varphi_{2-3} = 180°$. A comparable amount of upfield shift (19.9 ppm) was also obtained from the calculation using the model compounds (Table 4). When the C12−C13 takes the *s-trans* conformation, the C12 shielding should receive the β_3-steric effect from the C13 methyl. Similarly, the C2 shielding of 1C3M receives the effect from the C3 methyl. As previously described in Table 2, the β_3-steric effect causes a large downfield shift to the σ_{11} shielding of a carbon of interest. The rotation of the C12−C13 or C2−C3 bond should decrease the β_3-steric effect on the C12 or C2 shielding, resulting in a large upfield shift. Thus, the occurrence of the concave-type profile as shown in Figure 11 can be regarded as an intrinsic property of molecules possessing the local structure like 1C3M.

For retinal and the models, the σ_{11} shielding changes upon going from φ_{12-13} (φ_{2-3}) = 180° to φ_{12-13} (φ_{2-3}) = 120° are quite close to the experimental value for Rh. The uniqueness of such an agreement is assured from Figure 11. Therefore, on the basis of the Mollevanger results, we can conclude that the chromophore of Rh takes the skewed *s-trans* form around the C12−C13 bond.

Recently, Smith et al. have reexamined the C12 shielding for Rh and found that the principal values of the C12 shielding tensor are largely unperturbed in comparison with that of *all-trans* protonated retinylidene Schiff base chloride salt (*all-trans* PSB), which is in contrast to Mollevanger's results.[6] According to this new finding, it may be reasonable to be concluded that the conformation about the C12−C13 bond is nearly planar *trans*. However, as can be seen from Table 3, an appreciable discrepancy arises in the isotropic shielding on the assumption that all of the principal values of the C12 shielding are equal between the Rh and *all-trans* PSB cases. Namely, the isotropic shielding (134.3 ppm) calculated from the principal values shift downfield about 2 ppm relative to the actually observed value (132.1 ppm) for Rh. This discrepancy may arise from the fact that *all-trans* isomer is used as a reference compound. To consistently explain both isotropic shielding and principal values for Rh, it is sufficient to assume that each of the principal values for Rh is actually about 2 ppm smaller than those for *all-trans* PSB: namely, σ_{11}, σ_{22}, and σ_{33} are 56, 131, and 210 ppm, respectively. Such a modification does not bring about any change on the value of magnetic anisotropy and the NMR spectral pattern (shown in Figure 4B of reference 6).

The present calculation provides information about the extent of shielding change induced for the isotropic shielding of C12 purely as a result of *cis*−*trans* isomerization of the C11−C12 bond. As shown in Table 4, a 2−4 ppm downfield shift is expected upon going from *cis* to *trans* when the rotational angle about the C12−C13 bond is kept 180°. In addition, each of the principal values exhibits a similar amount of downfield shift,

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although the shielding change of σ_{33} for retinal and that of σ_{11} for the model are somewhat larger. Consequently, the major part of the above discrepnacy between Rh and *all-trans* PSB could be ascribed to the difference in the configuration of the C11−C12 bond. Therefore, on the basis of the later NMR results given by Smith et al., it could be accepted that the C12−C13 conformation of the Rh chromophore takes nearly planar *trans* one, in agreement with that of *all-trans* PSB.

One of the main purposes in this study is to present a theoretical procedure by which a conformational structure of linear polyene is deduced from given experimental data for isotropic shieldings and the corresponding principal values. As described above, this was successfully achieved by using the chromophore of Rh as an example of linear polyene. A biologically significant finding is that the occurrence of 12-*s-trans* conformer alone is deduced from both of the two different sets of experimental data, although there is a difference in the rotational angle about the C12−C13 bond.

Throughout this section, we focused mainly on the C12 shielding of the chromophore, since our attention was given to the conformational property about the C12−C13 bond. However, for a thorough understanding of interactions of the retinal chromophore with opsin, it is necessary to consistently explain the occurrence of the shielding differences extending over several carbon atoms from C5 to C14. A recent theoretical study[16,17] has successfully indicated that such shielding differences can be interpreted in terms of charge polarization effects on the conjugated system by the putative Glu side chain. In that study, the conformation of the C12−C13 bond was assumed to be *s-trans*, the validity of which is supported by the present calculations.

Concluding Remarks

The results for the dienes can be summarized as follows: (1) the presence of the effect uniquely occurring in the conjugated systems, called π-orbital modification effect, was evidenced with rigorous theoretical analysis; (2) some characteristics of the steric and substituent effects in acting for the conjugated system were revealed with tensor analysis; (3) the additivity of all the effects studied was confirmed. In addition to these results, the direct *ab initio* shielding calculation of 11-*cis*-retinal enabled us to determine the preferred conformation around the C12−C13 bond of the chromophore in Rh.

As earlier theoretical study[45] has indicated that there are two energy minima at the skewed *s-trans* and skewed *s-cis* conformations around the C12−C13 bond of 11-*cis*-retinal. According to NMR studies of protonated 11-*cis*-retinal Schiff base, in solution the thermal equilibrium holds between the two conformers.[46] The C12−C13 conformation is a key factor to determine the orientation of the chromophore in the protein pocket and thereby the chromophore−protein interaction. In spite of this, the C12−C13 conformation of the chromophore in Rh has not been determined yet, probably because there is no direct method available to determine the conformational population in the protein. This is the first study to answer this problem.

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