Acknowledgment. This work was supported by Grant GM 24862 from the National Institutes of Health.

References and Notes

(1) Department of Biochemistry, University of Wisconsin, Madison, Wis. 53706.

(2) The following abbreviations are used: CD (circular dichroism), HFIP (hexafluoro-2-propanol), UV (ultraviolet).

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Effect of External Pressure on the Spectroscopic and Conformational Properties of the Visual Chromophores

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Received November 27, 1978

Abstract: The effect of external pressure (3900–23 700 psi) on (1) the spectroscopic properties of *all-trans-* and 13-*cis*-retinal and (2) the conformational equilibrium between the 12-s-cis and 12-s-trans conformers of 11-*cis*-retinal is analyzed in methylcyclohexane solvent. The latter data are interpreted using a pressure and solvent effect formalism which explicitly includes the contributions of dispersive and electrostatic interactions, and the effect of external pressure on those interactions, in the calculation of the total free energy change with applied external pressure. The experimental results indicate that the 12-s-trans conformer is preferentially stabilized at high external pressure despite the fact that this conformer is predicted to have the larger molecular volume in solution. This rather unusual experimental result is explained in terms of a significant preferential electrostatic stabilization of the 12-s-trans species with increased pressure due to the larger dipole moment of this conformer and the increase in the solvent reaction field with external pressure. The 12-s-trans conformer is calculated to have a dipole moment at least 1.6 D larger and a cavity radius at least 0.012 Å larger than the 12-s-cis conformer. The 12-s-cis conformer is calculated to have a vacuum free energy approximately 0.5 kcal/mol lower than that of the 12-s-trans conformer.

I. Introduction

The initial molecular event of vertebrate vision is postulated to involve a photochemical isomerization of the polyene chromophore, 11-*cis*-retinal, which is bound to the opsin protein of rhodopsin via a protonated Schiff base linkage.[1-5] Although the crystal conformation of 11-*cis*-retinal has been demonstrated to have a distorted 12-s-cis geometry,[6] in ambient temperature solution this molecule populates two energetically similar conformers with 12-s-cis and 12-s-trans geometries (see Figure 1).[7-18] Investigators have studied the conformational properties of 11-*cis*-retinal using a variety of experimental[7-13] and theoretical[14-18] techniques. Nevertheless, the conformational properties of this molecule are still a subject of debate (see, for example, ref 7 and 10). Furthermore, the conformation of the chromophore in rhodopsin is still not unequivocally assigned, although spectroscopic data suggest that a 12-s-trans geometry is likely.[19-21]

A recent solvent effect investigation of 11-*cis*-retinal predicted that the 12-s-trans conformer occupies a larger solvent cavity in solution than the 12-s-cis conformer.[7] The difference in cavity radii of the two conformers was calculated to be approximately 0.03 Å,[7] and this relatively large difference encouraged us to investigate the conformational properties of 11-*cis*-retinal using external pressure.[22] We expected to spectroscopically observe an increase in the population of the smaller conformer with increasing external pressure. Our data, however, leads us to the surprising conclusion that applied external pressure stabilizes the larger, 12-s-trans conformer of 11-*cis*-retinal in methylcyclohexane. A detailed analysis of the pressure effect data using the formalism described in the theoretical section provides an explanation for this interesting pressure-induced conformational equilibrium. In particular, our results indicate that pressure-induced electrostatic stabilization is a very important variable in determining the effect of external pressure on the conformational equilibria of polar compounds even in nonpolar solvents. Our calculations indicate that the 12-s-trans conformer has a dipole moment at least 1.6 D larger, a cavity radius at least 0.012 Å larger, and a vacuum free energy approximately 0.5 kcal/mol larger than the 12-s-cis

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conformer. These values are in agreement with the values predicted from our previous solvent effect analysis of 11-*cis*-retinal.[7]

II. Experimental Section

all-trans-Retinal (Eastman), 9-*cis*-retinal (Sigma), and 13-*cis*-retinal (Sigma) were recrystallized from petroleum ether (bp 20–40 °C) and *n*-hexane prior to use. 11-*cis*-Retinal, in the form of extensively purified single crystals, was a generous gift of Paul K. Brown. Methylcyclohexane (Matheson Coleman and Bell) was purchased as "spectroquality" and used without further purification.

The high-pressure optical cell used in the pressure studies is a copy of that used by Zipp and Kauzman.[23] Its attractive feature is an internal sample cell of long path length (3.3 cm) in which the reaction mixture is separated from the high-pressure fluid. Pressure is maintained in the cell by two sapphire to "optically flat" metal seals and two metal to metal seals. The sample vessel, made of flat quartz windows fused to a cylindrical Pyrex body, lies between the thick sapphire windows. The sample vessel is covered with a Teflon sleeve (0.010-in. wall thickness) which transmits pressure to the reaction mixture through an opening in the side of the vessel and prevents contamination of the optical solution by hexane. The pressure is generated using an Enerpac hydraulic hand pump (0–40 000 psi) Model P228 pressure generator attached to a high-pressure valve.[24] This valve (Aminco, three-way, no. 44-13161) is attached to the pressure generator, a direct reading high-pressure gage (American Instrument Co., Inc., Model 47-18350), and the high-presssure optical cell. The three-way valve is designed to be continuously open between the optical cell and the pressure gage, and can be open or closed between the cell and the pressure generator. All o-rings in contact with high-pressure fluid (hexane) in this system are made of Viton. The pressure gage is calibrated using a manganin cell (Harwood Engineering Co., Inc., Model D1902 B).

Master solutions (100 mL) of *all-trans*-, 13-*cis*-, and 11-*cis*-retinal were prepared using spectral grade methylcyclohexane. Concentrations were adjusted to give an absolute absorbance of 0.17–0.20 at λ_{max} for each compound in a 1.0-cm cuvette using the pure solvent as a reference. At ambient pressure these concentrations gave an absolute absorbance reading of approximately 0.65 in the 3.3-cm path length sample vessel inside the high-pressure cell. These solutions were prepared less than 24 h prior to the pressure effect studies, stored under argon in a refrigerator (3 °C), and allowed to warm to room temperature before opening.

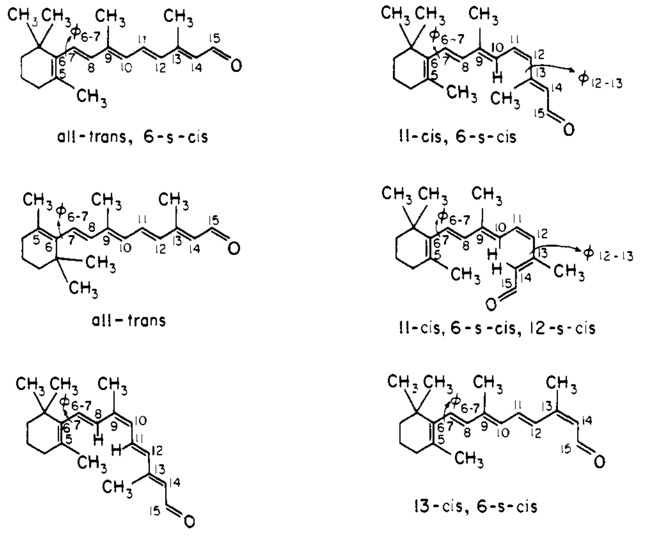
The electronic absorption spectra were obtained on a Cary 14 spectrophotometer at ambient temperature from 300 to 550 nm at pressure intervals of approximately 5000 psi. Pressure cycles were utilized in this study. A sample was pressurized to 3900 psi and allowed to equilibrate for 10 min. A single spectrum was taken. The sample was pressurized to the next pressure interval (8900 psi) and allowed to equilibrate for 10 min, and another spectrum was taken. This sequence continued to the highest pressure. Very slow decompression to 3900 psi, to minimize adiabatic cooling and allowing for a 30-min equilibration time, completed one cycle. The pressure effect data presented in section IV were obtained by averaging data from four cycles for each retinal isomer.

III. Theoretical

The method of calculating the effect of external pressure on the solvation energy of the solute is based on the combined use of a modified version of the solvent effect formalism of Birge, Sullivan, and Kohler,[7] and the pressure effect formalisms of ref 25–28. The total energy of solvation at a given external pressure, p, is partitioned into the following contributions:

$$G_{total}(p) = G_{solute} + G_{vdw}(p) + G_{es}(p) + G_{pres}(p) \quad (1)$$

where G_{solute} represents the energy of a given molecular conformation of the solute under "free space" conditions, $G_{vdw}(p)$ is the van der Waals free energy, $G_{es}(p)$ is the electrostatic free energy, and $G_{pres}(p)$ is the free energy associated with internal and external pressure. Implicit in the development of eq 1 is the assumption that G_{solute} is not changed by external pressure. This assumption is not a significant source of error for the pressure ranges investigated in this paper.



all-trans, 6-s-cis

11-cis, 6-s-cis

all-trans

11-cis, 6-s-cis, 12-s-cis

9-cis, 6-s-cis

13-cis, 6-s-cis

Figure 1. Various single-bond and double-bond isomers of retinal (vitamin A aldehyde). The torsional configuration of a given bond is defined as cis if the dihedral angle is less than 90° and trans if the angle is greater than 90°. Consequently, the omission of the "distorted" label in defining, for example, a "12-s-trans" or "6-s-cis" geometry does not imply planarity. The "6-s-cis" label is usually omitted because all of the above isomers are believed to have 6-s-cis linkages in solution due to steric effects (see ref 11 and 14).

$G_{vdw}(p)$. The free energy due to van der Waals interactions between a solute molecule and the first shell of solvent molecules can be approximated using[7]

$$G_{vdw} = -0.338N_n\{a_u^3a_v^3[0.71(a_u + a_v)]^{-6}\} \times [I_uI_v/(I_u + I_v)][(n^2 - 1)/(n^2 + 2)]G_r \quad (2)$$

where N_n is the number of nearest neighbors, a_u is the solute cavity radius, a_v is the solvent cavity radius, I_u is the solute ionization potential, I_v is the solvent ionization potential, n is the refractive index of the solvent, and G_r is a semiempirical constant equal to the fraction of the dispersive stabilization energy *not* lost due to intermolecular "Born-type" repulsion. A derivation of eq 2 has previously been presented (see eq 2-5 and 42 of ref 7 and note that the constant, 0.338, includes the $(G_{vdw}/E_{vdw}$ proportionality given in eq 42 of ref 7).

The number of nearest neighbors, N_n, is calculated using

$$N_n = \pi[\{\sin^{-1}[a_v/(a_u + a_v)]\}^{-2}] \quad (3)$$

where $\sin^{-1}$ returns radians.

The qualitative character of eq 2 should be noted. This equation approximates the complex solute-solvent intermolecular dispersive interactions as simple one-term interactions of isotropic point dipoles. Furthermore, only the first shell of solvent molecules is included in the calculation. A relatively large value for G_r of 0.7 will be used to empirically compensate for the tendency of the above approximations to underestimate the van der Waals stabilization.

The effect of external pressure on G_{vdw} can be analyzed in terms of the compression of the solute and solvent cavity radii as well as the effect of the latter on the solvent refractive index. The density of the solvent at a given external pressure can usually be obtained from literature measurements of compression factors.[29] The cavity radius at a given pressure, $a_v(p)$, can then be calculated using the equation

$$a_v(p) = [3M_v/4\pi N_A d(p)]^{1/3} \quad (4)$$

where M_v is the solvent molecular weight (g/mol), N_A is Avagadro's number, and $d(p)$ is the solvent density (g/cm^3)

calculated as the ratio of the density at atmospheric pressure divided by the compression factor.

The refractive index as a function of solvent density is iteratively calculated using the Lorentz-Lorenz equation[30-32]

$$\left(\frac{[n(p)]^2 - 1}{[n(p)]^2 + 2}\right) = kd(p) \tag{5}$$

where the constant, k, is independent of density and is calculated using literative values for n and $d(1)$ at the appropriate ambient temperature.[33] Rapid convergence is obtained by using $n(1)$ as an initial guess and calculating the next guess, and all subsequent guesses, for n using the equation

$$n_{\lambda+1} = n_\lambda + kd(p) + (n_\lambda{}^2 - 1)/(n_\lambda{}^2 + 2) \tag{6}$$

where λ is an iteration subscript.

Calculating the effect of pressure on the cavity radius of the solute is not a straightforward problem. Compression data are not available for most solute molecules of interest. Furthermore, there is no reason to suspect that such data would be appropriate for the molecule when surrounded by solvent molecules. We will assume that the cavity radius of the solute as a function of external pressure, $a_u(p)$, can be calculated from the solvent compression data using the relationship

$$a_u(p) = a_u(1)[a_v(p)/a_v(1)]^{l_{uv}} \tag{7a}$$

where $a_u(1)$ and $a_v(1)$ are the ambient pressure solute and solvent cavity radii, respectively, $a_v(p)$ is calculated using eq 4, and l_{uv} is an empirical parameter relating the compressibility of the solute relative to the solvent. For example, l_{uv} equals unity if the solute is identical with the solvent with respect to compressibility and $l_{uv} > 1$ if the solute is more compressible than the solvent. A value for l_{uv} can be obtained spectroscopically by comparing the dispersive red shift[34,35] of the solute absorption band $\tilde{\nu}(p)$ as a function of pressure with the red shift observed at ambient pressure as a function of nonpolar solvent refractive index. The latter data can be used to extrapolate to a free-space ($n = 1$) absorption maximum, $\tilde{\nu}_0$, and the cavity size as a function of pressure can be calculated as follows:[36]

$$a_u{}^3(p) = \left\{\frac{a_u{}^3(1)[(n^2(p) - 1)/(n^2(p) + 2)]}{[(n^2(1) - 1)/(n^2(1) + 2)]}\right\} \times \left[\frac{f(p)(\tilde{\nu}(1) - \tilde{\nu}_0)}{f(1)(\tilde{\nu}(p) - \tilde{\nu}_0)}\right] \tag{7b}$$

where $f(1)$ and $f(p)$ are the oscillator strengths of the solute electronic transitions with maxima at $\tilde{\nu}(1)$ and $\tilde{\nu}(p)$, respectively. An analysis of the absorption spectrum of *all-trans*-retinal in a series of hydrocarbon solvents indicates that a value of $l_{uv} = 1.3$ (relative to methylcyclohexane) is necessary to bring eq 7a into agreement with eq 7b.[36b] We will use this value of l_{uv} for all of the retinyl polyenes. Accordingly, the retinyl polyenes are approximately 30% "more compressible" volumetrically than methylcyclohexane owing to the flexibility of the "floppy" polyene chain.

$G_{es}(p)$. The free energy due to static and induced solute-solvent dipole-dipole interactions is calculated using the formalism outlined by Bottcher:[37a]

$$G_{es} = -0.5\mu \cdot \mathbf{R} \tag{8}$$

where μ is the solute dipole moment and $\mathbf{R}$ is the reaction field associated with the polarization of the solvent molecules by the dipole moment of the solute. The spherical Onsager cavity approximation yields

$$\mathbf{R} = \frac{\mu}{a_u{}^3}\left[\frac{5(\epsilon - 1)}{(3\epsilon + 4.5)}\right] \tag{9}$$

where ϵ is the dielectric constant of the solvent. Equation 9 was derived[7] assuming that the mean polarizability of the solute is equal to $(a_u{}^3/2.5)$.[38] Although an elliptical cavity would be more appropriate for the retinyl polyenes,[7] the spherical cavity approximation requires fewer variables and has the advantage of generating dipole moments which can be directly compared with recently measured literature values which were obtained using the spherical cavity approximation.[10,34]

The effect of pressure on G_{es} is calculated by assuming that the static dipole moment of the solute is independent of pressure, evaluating $a_u(p)$ using eq 7a, and calculating $\epsilon(p)$ using Maxwell's relationship (which is appropriate for nonpolar solvents like methylcyclohexane):[37b]

$$\epsilon(p) = n^2(p) \tag{10}$$

where $n(p)$ is obtained from eq 5.

$G_{pres}(p)$. The free energy associated with "pressure" will be calculated as a function of internal and external terms. The internal pressure is associated with the free energy of microscopic cavity formation and is calculated using the following equation (see eq 46 of ref 7):

$$G_{cav} = 4\pi a_u{}^2\gamma_v k_v(\psi_{uv}) \tag{11}$$

where γ_v is the surface tension of the solvent at ambient temperature and pressure and $k_v(\psi_{uv})$ is a constant dependent upon the ratio of the solvent to solute surface areas;[39]

$$k_v(\psi_{uv}) = 1 + (a_v{}^2/a_u{}^2)(k_v(1) - 1) \tag{12}$$

where $k_v(1)$ is a dimensionless microscopic cavity factor appropriate for the pure solvent:[7]

$$k_v(1) = 0.5963 + 75.14\epsilon f(\text{cav})^{-2} - 561.8\epsilon^2 f(\text{cav})^{-4} \tag{13}$$

where ϵ is the solvent dielectric constant and $f(\text{cav})$ is the energy component of the microscopic surface tension in units of dyn/cm as calculated in ref 7 [$f(\text{cav}) = 49.55$ dyn/cm for methylcyclohexane].

The effect of external pressure on the free energy of cavity formation will be determined by substituting $a_v(p)$, $a_u(p)$, and $\epsilon(p)$ for their counterparts in eq 11-13 and calculating these terms using eqs 4, 7a, and 10, respectively. It is important to note that the effect of external pressure on the surface tension is neglected in calculating the internal contribution to G_{pres}. (If the surface tension were corrected for external pressure we would be counting the external term twice, and we can more accurately account for the external pressure effect using the formalism described below.)

The effect of external pressure on G_{pres} can be evaluated by calculating the free energy associated with the PV work required to enlarge the solvent cavity in going from the smaller to the larger solute conformer. If we use a_1 and a_2 to designate the two cavity radii of conformers 1 and 2, the difference in free energy between these two conformers at a given external pressure, $\Delta G_{12}{}^{ext}(p)$, is calculated using the equation

$$\Delta G_{12}{}^{ext}(p) = (4\pi/3)[a_1{}^3(p) - a_2{}^3(p)](P - 1) \tag{14}$$

where P is the external pressure in atmospheres ($\Delta G_{12}{}^{ext}(p) = 0$ at ambient pressure). The corresponding internal term, $\Delta G_{12}{}^{int}(p)$, is calculated as the difference between conformer cavity formation energies and represents a correction term to eq 14 associated with solvent-specific microscopic interactions.

$\Delta G_{12}{}^{total}(p)$. The total difference in free energy of solute conformer 1 vs. conformer 2 at pressure P is calculated as follows:

$$\Delta G_{12}{}^{total}(p) = G_1{}^{total}(p) - G_2{}^{total}(p) \tag{15}$$

$$= G_1{}^{solute} - G_2{}^{solute} \tag{16a}$$

$$+ 0.338\dot{a}_v{}^3\{\dot{N}_{2n}\dot{a}_2{}^3[0.71(\dot{a}_2 + \dot{a}_v)]^{-6} - \dot{N}_{1n}\dot{a}_1{}^3[0.71(\dot{a}_1 + \dot{a}_v)]^{-6}\} \times [I_uI_v/(I_u + I_v)][(\dot{n}^2 - 1)/(\dot{n}^2 + 2)]G_r \tag{16b}$$

Table I. Effect of External Pressure on the Spectroscopic Properties of *all-trans*-, 13-*cis*-, and 11-*cis*-Retinal in Methylcyclohexane

	all-trans-retinal			13-*cis*-retinal			11-*cis*-retinal		
pressure, psi	$\tilde{\nu}_{\max}$, cm^{-1}	fwhm,[a] cm^{-1}	f_{rel}[b]	$\tilde{\nu}_{\max}$, cm^{-1}	fwhm,[a] cm^{-1}	f_{rel}[b]	$\tilde{\nu}_{\max}$, cm^{-1}	fwhm,[a] cm^{-1}	f_{rel}[b]
3 900	26 900	4550	0.990	27 030	4700	0.983	27 260	4640	0.987
8 900	26 780	4550	0.970	26 970	4710	0.981	27 170	4710	0.983
13 850	26 700	4582	0.956	26 850	4740	0.948	27 140	4730	0.968
18 750	26 640	4630	0.947	26 730	4750	0.934	27 030	4790	0.960
23 700	26 580	4635	0.927	26 680	4760	0.922	26 950	4740	0.939
intcp[c]	26 940	4520	1	27 110	4690	1	27 320	4644	1
s(intcp)[d]	(20)	(15)	(0.003)	(20)	(7)	(0.008)	(20)	(36)	(0.005)
slope[e]	−0.016	0.0051	−3.0	−0.018	0.0032	−3.4	−0.015	0.0057	−2.4
s(slope)[f]	(0.001)	(0.0009)	(0.2)	(0.002)	(0.0004)	(0.5)	(0.001)	(0.0024)	(0.3)
(r^2)[g]	(0.98)	(0.91)	(0.98)	(0.98)	(0.95)	(0.94)	(0.98)	(0.66)	(0.95)

[a] Full width at half-maximum calculated by measuring the difference between $\tilde{\nu}_{\max}$ and the long-wavelength half-maximum and multiplying by two. [b] Relative oscillator strength of the ~27 000-cm^{-1} transition corrected for solvent (methylcyclohexane) compression. [c] Linear least-squares intercept of data in column above. [d] Standard error in the intercept. [e] Linear least-squares slope of data in column above. [f] Standard error in the slope. [g] Coefficient of correlation (a perfect fit yields $r^2 = 1$).

$$+ 0.5[(\mu_2^2/\dot{a}_2^3) - (\mu_1^2/\dot{a}_1^3)][5(\dot{\epsilon} - 1)/(3\dot{\epsilon} + 4.5)] \tag{16c}$$

$$+ 4\pi(\dot{a}_1^2 - \dot{a}_2^2)\gamma_{\mathrm{v}}\dot{k}_{\mathrm{v}}(\psi_{\mathrm{uv}}) \tag{16d}$$

$$+ (4\pi/3)(\dot{a}_1^3 - \dot{a}_2^3)(\dot{P} - 1) \tag{16e}$$

where pressure-dependent terms have been indicated by a dot above the symbol. The larger conformer will be preferentially stabilized by the dispersive term (16b) and destabilized by the internal (16d) and external (16e) pressure terms. In general, the conformer with the larger dipole moment will be preferentially stabilized by the electrostatic term (16c).

IV. Results and Discussion

The effect of external pressure on the spectroscopic properties of *all-trans*-, 13-*cis*-, and 11-*cis*-retinal was determined for the pressure range 3900–23 700 psi. The raw data, and a linear least-squares analysis of the data, are collected in Table I. The results for *all-trans*- and 13-*cis*-retinal will be discussed first because the effect of external pressure on these two polyenes can be analyzed in terms of a single conformer. Our analysis of these two isomers will then be used to help interpret the pressure-effect data on 11-*cis*-retinal. This latter isomer is known to have two conformers with 12-s-cis and 12-s-trans geometries.[7–18]

Effect of External Pressure on $\tilde{\nu}_{\max}$. The absorption maximum of the strongly allowed electronic transition at ~27 000 cm^{-1} is observed to red shift with increasing external pressure. (Linear least-squares analyses of these data extrapolated to zero pressure intercepts that correspond within the calculated standard error to the absorption maxima observed at ambient pressure in methylcyclohexane.) The observed red shift can be interpreted primarily in terms of increased (pressure-induced) dispersive interactions of the solute with the solvent. The dispersive red shift is approximately proportional to the product of solute oscillator strength, solvent polarizability $[\propto (n^2 - 1)/(n^2 + 2)]$, and the reciprocal of solute volume $[\propto a_{\mathrm{u}}^{-3}]$.[36b] Although the oscillator strength decreases with increasing pressure, the latter two terms increase with pressure and yield a net red shift of the absorption band. A smaller competitive effect is postulated to be present due to the spectroscopic consequences of the pressure-induced compression of the polyene chain. It is likely that out-of-plane torsional distortion of the single bonds will be more significant than distortion of the double bonds at elevated pressure. Accordingly, the excited state will be more destabilized than the ground state upon compression leading to an increase in the transition energy of the solute in the absence of solvent effects. The observed dispersive red shift may therefore be slightly reduced in magnitude by the compression of the polyene chain.

Effect of Pressure on Bandwidth. The lack of vibronic structure in the principal absorption band of the visual chromophores has previously been discussed by Christensen and Kohler.[40] These authors have demonstrated that the spectral broadness is due to the rotational "freedom" of the 6–7 single bond connecting the β-ionylidene ring with the polyene chain. Theoretical calculations support this conclusion by predicting that the ground-state potential surface for 6–7 rotation is very broad while the excited ("$^1B_u^{*+}$") state potential surface is quite narrow with a shifted potential minimum.[14] Accordingly, a range of conformers with varying 6–7 torsional angles will produce an inhomogeneously broadened absorption band (see ref 14 and 40 for a more detailed discussion). The effect of external pressure on the above mechanism can be interpreted in terms of a change in the potential surface of 6–7 rotation in the ground state. As previously discussed, the compression of the polyene chain at increased external pressure is predicted to produce increased out-of-plane distortion in the single bonds. By shifting the potential surface of the 6–7 single bond toward increased torsional distortion the effects of the inhomogeneous broadening mechanism will be enhanced producing an increase in the full width at half-maximum.

Effect of External Pressure on Oscillator Strength. The oscillator strength of the principal absorption band is observed to decrease by approximately 8% in *all-trans*- and 13-*cis*-retinal in going from ambient pressure to 23 700 psi. Both of these isomers exhibit an identical decrease in f within experimental error. (Note that the measured oscillator strengths have been corrected for solvent compression.)

There are numerous theories available to explain the effect of solvent polarizability (refractive index) on the oscillator strength of a solute molecule,[41–45] and the general inability of any single model to explain all of the available data indicates the complexity of the problem. One approach, initially proposed by Chako[41] and recently reviewed by Abe,[42] introduces the effect of solvent polarizability in the form of an effective electric field strength in the solute cavity based on a Lorentz field:

$$E_{\mathrm{cav}} = \frac{(n_\nu^2 + 2)}{3} E_0 \tag{17}$$

where E_0 is the "vacuum" field strength and n_ν is the refractive index of the solvent measured at the frequency of the radiation. Chako's classical derivation leads to the correction[41–43]

$$f_{\mathrm{soln}} = f_{\mathrm{vap}} \frac{(n_\nu^2 + 2)^2}{9n_\nu} \tag{18}$$

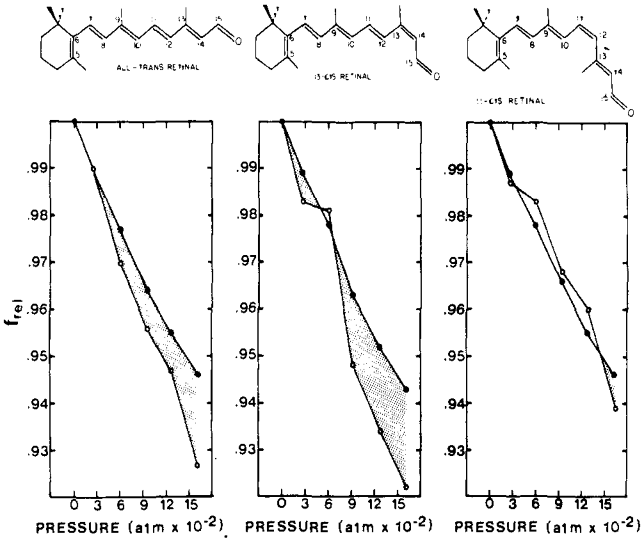


Figure 2. The effect of external pressure on the relative oscillator strengths [$f(p)/f(1)$] of the principal (~27 000 cm^{-1}) absorption bands of *all-trans*-retinal (left), 13-*cis*-retinal (middle), and 11-*cis*-retinal (right). The observed values (Table I) are indicated with open circles; the calculated values based on eq 23 are indicated with solid circles.

which predicts that increasing solvent polarizability should increase the oscillator strength of the electronic transition. Abe has noted that the use of the Lorentz field correction and quantum mechanical perturbation theory, however, leads to the following equation for Einstein's coefficient of absorption:[42]

$$B_{i0} = \left(\frac{8\pi^2}{3h^2}\right)\left(\frac{1}{n_\nu^2}\right)\left(\frac{n_\nu^2+2}{3}\right)^2 |\mathbf{M}_{i0}|^2 \tag{19}$$

where $\mathbf{M}_{i0}$ is the transition moment for the $i \leftarrow 0$ solute transition occurring at frequency ν. Introduction of the Einstein coefficient into the oscillator strength equation leads to the relationship

$$f_{\text{soln}} = f_{\text{vap}} \left[\frac{(n_\nu^2+2)^2}{9n_\nu^2}\right] \frac{\tilde{\nu}_{i0}}{\tilde{\nu}_{i0}^0} \tag{20}$$

where $\tilde{\nu}_{i0}^0$ is the wavenumber of the solute transition in the vapor phase while $\tilde{\nu}_{i0}$ is the corresponding value in the solution phase. The discrepancies between eq 18 and 20 have been discussed in detail by Abe.[42]

Abe has suggested, however, that a better choice for the cavity correction term is to use the Onsager cavity model in which the reaction field component is neglected:

$$E_{\text{cav}} = \frac{3n_\nu^2}{(2n_\nu^2+1)} E_0 \tag{21}$$

Introduction of this correction factor into B_{i0} (eq 19, third parentheses on right-hand side), and substitution of B_{i0} into the oscillator strength expression, yields

$$f_{\text{soln}} = f_{\text{vap}} \left(\frac{9n_\nu^2}{(2n_\nu^2+1)^2}\right) \frac{\tilde{\nu}_{i0}}{\tilde{\nu}_{i0}^0} \tag{22}$$

Abe demonstrated that eq 22 produced better agreement with experiment than the other formalisms he investigated, and this observation is perhaps the best justification for using the Onsager cavity correction given in eq 21. (It should be noted that eq 22 also includes the perturbation associated with the dispersive shift of the transition energy due to solvent. Although this effect is not related to cavity field effects, we will, for convenience, refer to eq 22 as a microscopic cavity correction equation.)

One rationale for neglecting the reaction field correction in eq 21 can be offered by noting that the correction necessarily contains variables which are solute dependent. Therefore, if one is interested in ratios of oscillator strengths for a given solute under different solvent conditions, one might argue that it is better to neglect the solute-dependent reaction field than to introduce additional parameters into the equation. The fact that Abe found eq 22 to be better than eq 20 suggests that the reaction field factor may approximately cancel out, but the success of eq 22 should be attributed for the most part to fortuitous cancellation of error. The more sophisticated quantum mechanical analysis of Weigang,[43] and the alternative formalisms of Tinoco[44] and DeVoe,[45] indicate that simple field correction equations like those mentioned above are likely to underestimate the effect of solvent polarizability on oscillator strength for strongly allowed transitions because they neglect specific solute-solvent hypochromism effects. In the present instance, these dispersive interactions will also yield a net decrease in the oscillator strength of the solute with applied external pressure. We will return to this consideration shortly.

A quantitative picture of the magnitude of the observed pressure-induced change in oscillator strength in *all-trans*- and 13-*cis*-retinal is shown in Figure 2. For the purpose of comparison, a theoretical prediction of relative oscillator strength based on Abe's eq 22 is also plotted where f_{rel} is calculated using the equation

$$f_{\text{rel}} = \frac{f(p)}{f(1)} = \left[\frac{\tilde{\nu}(p)}{\tilde{\nu}(1)}\right]\left[\frac{n^2(p)\{2n^2(1)+1\}^2}{n^2(1)\{2n^2(p)+1\}^2}\right] \tag{23}$$

where $\tilde{\nu}(1)$ and $\tilde{\nu}(p)$ are the wavenumbers of the principal absorption bands at ambient pressure and externally applied pressure, p, respectively. The effect of external pressure on refractive index was calculated using eq 5. The sodium D line refractive index for methylcyclohexane at ambient pressure and 25 °C was used for $n(1)$ (1.4206). As shown in Figure 1, eq 23 underestimates the pressure-induced decrease in f_{rel}, although it accounts for approximately 80% of the observed effect.

Equation 23 is expected to underestimate the observed effect because it neglects solvent-induced "hypochromism" and pressure-induced conformational changes of the solute. The "hypochromism" is associated with the interaction of the strongly allowed principal absorption band of the solute with higher energy electronic transitions in the solvent. As the external pressure is increased, the solute cavity size decreases thereby enhancing intensity borrowing by the electronic manifolds of the solvent from the strongly allowed solute transition. As noted by one reviewer of this manuscript, this mechanism should be of importance for the molecules investigated here because we observe a pressure-induced dispersive red shift in the 27 000-cm^{-1} absorption bands of the retinal isomers. The dispersive red shift is associated with the same type of solvent-solute transition dipole-transition dipole interactions responsible for hypochromism. Sample calculations using DeVoe's formalism,[45] however, indicate that hypochromism can account for at most 2% of the 8% observed pressure-induced decrease in oscillator strength. An additional source of pressure-induced perturbation in f may be associated with pressure-induced distortions of the polyene chain. These conformational distortions will induce mixing of higher energy forbidden states into the low-lying, strongly allowed "$^1B_u{}^{*+}$" state reducing the transition moment, $\mathbf{M}_{i0}$, for excitation into this state. Accordingly, pressure-induced conformational distortion will also reduce the oscillator strength of the ~27 000-cm^{-1} principal absorption band.

In summary, therefore, we can partition the observed decrease in f_{rel} at 23 700 psi into three competing effects all of which reduce f_{rel} by the approximate percentages given in parentheses: microscopic cavity field effects (4-6%), hypochromism (1-2%), and conformational distortion (1-2%). These combined effects adequately account for the observed

8% decrease in f_{rel} in *all-trans*-retinal and 13-*cis*-retinal in going from ambient pressure to an applied external pressure of 23 700 psi (see Table I).

Effect of External Pressure on the Conformational Stability of 11-*cis*-Retinal. The preceding observations can, in general, also be applied to an analysis of the gross features of the 11-*cis*-retinal pressure effects. A more detailed analysis of the 11-*cis*-retinal data (Table I), however, indicates some important differences between this isomer and *all-trans*- and 13-*cis*-retinal. Our analysis leads to the surprising conclusion that external pressure alters the equilibrium of 12-s-cis and 12-s-trans conformers of 11-*cis*-retinal in favor of the latter despite the fact that the 12-s-trans conformer has a larger molecular volume. This result is unusual because most conformational equilibria favor the smaller conformer at high external pressure due to the dominance of the external pressure effect term (eq 16e).

The most important difference between 11-*cis*-retinal and *all-trans*- and 13-*cis*-retinal is that the oscillator strength decreases less rapidly for the former isomer with applied external pressure. The difference is statistically significant as can be seen by comparing the slopes and standard errors for the relative oscillator strength (f_{rel}) columns in Table I and the data displayed in Figure 2. One might postulate that the difference is solely due to the fact that 11-*cis*-retinal has a lower ambient pressure oscillator strength and therefore will exhibit a smaller relative pressure effect. This argument, however, can be ruled out by noting that 13-*cis*-retinal, which has a lower f value than *all-trans*-retinal (see below), displays a slightly larger pressure effect with respect to f_{rel} than *all-trans*-retinal (although the difference is not statistically significant).

Furthermore, there is no reason to believe that any of the three mechanisms postulated to account for the pressure-induced diminution of oscillator strength should behave quantitatively differently for 11-*cis*-retinal. The microscopic cavity correction effect (eq 23) is calculated to be essentially identical for all of the retinal isomers (see Figure 2). (The slight differences observed in the calculated pressure effects among the isomers are due to small differences in their dispersive red shifts. As noted in the previous section, this red shift is not associated with cavity field effects but for convenience is nevertheless included in eq 23). If hypochromism were more active in diminishing f_{rel} in *all-trans*- and 13-*cis*-retinal than in 11-*cis*-retinal, we would observe a significant difference in dispersive red shifts in the latter isomer relative to the two former isomers. In fact, dispersive red shifts are identical within experimental error for all three isomers. Finally, there is no evidence to suggest that pressure-induced compression of the polyene chain is significantly different in the three isomers. The statistically identical behavior of the all-trans and 13-cis isomers rules out the possibility that cis isomers are less susceptible to this, or any of the other mechanisms, one might propose to explain the pressure-induced decrease in f_{rel}. Nevertheless, a careful analysis of Table I and Figure 2 leaves little room for doubt that 11-*cis*-retinal is behaving differently than *all-trans*- and 13-*cis*-retinal. We now investigate this observation in more detail.

If we use *all-trans*- and 13-*cis*-retinal as standards and calculate the change in oscillator strength of 11-*cis*-retinal using the equation $2f_{\mathrm{rel}}^{(11)}/(f_{\mathrm{rel}}^{(\mathrm{trans})} + f_{\mathrm{rel}}^{(13)})$ we obtain the results shown in Table II. These data may be viewed as corrections of the relative oscillator strengths to vacuum (or free space) conditions. The observed changes are statistically valid, an observation which is supported by the smoothness of the adjusted values as a function of external pressure.

We interpret the data of Table II as evidence for a pressure-induced change in the relative conformational stability of the 12-s-trans and 12-s-cis conformers. The 12-s-trans conformer is predicted to have a larger ($\lambda_{\max}$) oscillator strength than the 12-s-cis conformer[7,15,21] (see below) and consequently the data indicate that the former is being preferentially stabilized relative to the latter at high external pressures. Additional support for the concept of a pressure-induced change in conformational stability may be found in the full width at half-maxima data for 11-*cis*-retinal which exhibit a maximum at 18 750 psi (with a subsequent decrease at 23 700 psi) suggesting a maximum formation of the conformer favored at higher pressure at 18 750 psi. This observation is in accord with the oscillator strength data of Table II.

Table II. Effect of External Pressure on the Relative Oscillator Strength of 11-*cis*-Retinal Adjusted to Vacuum Conditions Using *all-trans*- and 13-*cis*-Retinal as Standards

psi	$2f_{\mathrm{rel}}^{(11)}/[f_{\mathrm{rel}}^{(\mathrm{trans})} + f_{\mathrm{rel}}^{(13)}]$ [a]
3 900	1.001
8 900	1.008
13 850	1.017
18 750	1.021
23 700	1.016

[a] $f_{\mathrm{rel}}^{(11)}$ = relative oscillator strength of the 27 000-cm^{-1} transition of 11-*cis*-retinal (rightmost column of Table I); $f_{\mathrm{rel}}^{(\mathrm{trans})}$, $f_{\mathrm{rel}}^{(13)}$ similarly defined for *all-trans*- and 13-*cis*-retinal, respectively.

As previously mentioned, our conclusion that external pressure preferentially stabilizes the 12-s-trans conformer is somewhat surprising given the fact that this conformer is believed to occupy a larger solvent cavity than the 12-s-cis conformer. The following calculations indicate, however, that the relatively large difference in the conformer dipole moments produces a significant electrostatic contribution to the pressure-induced differential solvation energy. Furthermore, the magnitude of the electrostatic stabilization increases with applied external pressure owing to the compression of the solvent. The net result is a preferential stabilization of the 12-s-trans conformer at high pressure.

A quantitative prediction of certain conformer properties can be made by applying the theoretical treatment outlined in section II to an analysis of the experimental data (Tables I and II). However, this analysis must depend on a priori assumptions concerning certain average (12-s-cis, 12-s-trans) solute parameters such as ionization potential (I_{u} in eq 2), cavity size, and dipole moment. We will use a value of 8.0 eV for the ionization potential of both conformers based on a comparison with model compounds.[7] A value for the average solute cavity radius can also be obtained by using model compounds to assign a pseudo-solution density and calculating the cavity radius using eq 4. This approach (see footnote 35 of ref 7) yields a value of 4.924 Å for the average cavity radius of the two conformers of 11-*cis*-retinal. A prediction of the average dipole moment of 11-*cis*-retinal can be estimated using theoretical calculations. A recent study of the dipole moments of the isomers of retinal using CNDO/2 molecular orbital theory yielded the following values: 6.42 D (*all-trans*-retinal), 6.02 D (11-*cis*-, 12-*s*-*trans*-retinal), and 4.11 D (11-*cis*-, 12-*s*-*cis*-retinal).[18] We can use the experimental value for *all-trans*-retinal to scale the theoretical data and obtain an estimate for the average dipole moment of the 11-cis conformers:

$$\mu_{\mathrm{av}}(11\text{-cis}) = \frac{\mu_{\mathrm{obsd}}(\text{all-trans})}{\mu_{\mathrm{calcd}}(\text{all-trans})} \times \left[\frac{\mu_{\mathrm{calcd}}(12\text{-s-trans}) + \mu_{\mathrm{calcd}}(12\text{-s-cis})}{2}\right] \tag{24a}$$

$$= \left(\frac{4.6}{6.42}\right)\left(\frac{6.02 + 4.11}{2}\right) = 3.6\ \mathrm{D} \tag{24b}$$

where the experimental dipole moment of *all-trans*-retinal (4.6

Table III. Theoretical Analysis of the Effect of External Pressure on the Conformational Stability of the 12-s-Cis and 12-s-Trans Conformers of 11-*cis*-Retinal in Methylcyclohexane[a]

quantity[b]	eq[b]	external pressure, psi						units
		14.7	3900	8900	13 850	18 750	23 700	
d		0.7651	0.7811	0.7969	0.8163	0.8304	0.8430	g/cm^3
a_v	(4)	3.7055	3.6800	3.6554	3.6262	3.6056	3.5875	Å
n	(5)	1.4206	1.4307	1.4407	1.4531	1.4622	1.4704	
a_u(trans)	(7a)	4.9300	4.8860	4.8436	4.7934	4.7580	4.7270	Å
N_n(trans)	(3)	15.9727	15.9323	15.8932	15.8466	15.8136	15.7845	
G_{vdw}(trans)	(2)	−11.1751	−11.3902	−11.6028	−11.8622	−12.0503	−12.2186	kcal/mol
a_u(cis)	(7a)	4.9180	4.8740	4.8317	4.7817	4.7463	4.7154	Å
N_n(cis)	(3)	15.9248	15.8845	15.8456	15.7992	15.7663	15.7373	
G_{vdw}(cis)	(2)	−11.1532	−11.3678	−11.5799	−11.8387	−12.0264	−12.1943	kcal/mol
ΔG_{vdw}		−0.0219	−0.0224	−0.0229	−0.0235	−0.0239	−0.0243	kcal/mol
G_{es}(trans)	(8)	−0.5582	−0.5848	−0.6119	−0.6459	−0.6712	−0.6944	kcal/mol
G_{es}(cis)	(8)	−0.2305	−0.2415	−0.2527	−0.2667	−0.2772	−0.2868	kcal/mol
ΔG_{es}		−0.3277	−0.3433	−0.3592	−0.3791	−0.3940	−0.4076	kcal/mol
ΔV	(14)	3.6861	3.5882	3.4957	3.3879	3.3135	3.2492	Å^3
ΔG^{ext}	(14)	0.0000	0.0138	0.0308	0.0465	0.0616	0.0763	kcal/mol
G_{cav}(trans)	(11)	8.2166	8.0674	7.9252	7.7588	7.6426	7.5417	kcal/mol
G_{cav}(cis)	(11)	8.1667	8.0183	7.8770	7.7116	7.5961	7.4958	kcal/mol
ΔG_{cav}		0.0499	0.0490	0.0482	0.0472	0.0465	0.0459	kcal/mol
ΔG^{total}	(17)	−0.2996	−0.3028	−0.3031	−0.3089	−0.3098	−0.3096	kcal/mol
$\Delta G_{rel}{}^{total}$		0.0000	−0.0032	−0.0035	−0.0093	−0.0102	−0.0100	kcal/mol

[a] Calculation is based on the ambient pressure solute parameters a_u(trans) = 4.9300 Å, a_u(cis) = 4.9180 Å, μ(trans) = 4.3895 D, μ(cis) = 2.8105 D, I_u(eq 2) = 8 eV, the solvent parameter I_v(eq 2) = 9.8 eV, and the general parameters G_r(eq 2) = 0.7, I_{uv}(eq 7a) = 1.3. [b] All quantities are pressure dependent and are calculated using the equation indicated. The density of solvent at various pressures was calculated using the compression data of Bridgeman.[29] Negative values for Δ quantities indicate preferential stabilization of 12-s-trans conformer.

Table IV. Observed[a] and Calculated[b] Oscillator Strengths of the 27 000-cm^{-1} Absorption Band of the Retinal Polyenes in Methylcyclohexane at Room Temperature

isomer	obsd[a]	calcd[b]	geometry calcd
all-trans	1.15	1.19	all-trans (standard)[c]
		1.21	all-trans (crystal[d])
9-cis	0.95	0.99	9-cis (standard)[c]
11-cis	0.69	0.98	11-cis,12-s-trans (150°) (standard)[c]
		0.48	11-cis,12-s-cis (30°) (standard)[c]
		0.50	11-cis,12-s-cis (crystal[e])
13-cis	1.00	1.13	13-cis (standard)[c]

[a] Observed values were obtained by fitting higher energy bands to Gaussians and subtracting these contributions before integrating under the principal absorption band. [b] Calculated values based on Pariser–Parr–Pople SCF-MO-CI procedures including full single and double excitation CI. Contributions due to the "$^1B_u{}^{*+}$" ← S_0 and "$^1A_g{}^{*-}$" ← S_0 low-lying transitions are added to obtain the value listed (see ref 15). [c] Standard geometries analogous to those defined in ref 15. [d] Reference 48. [e] Reference 6.

D) is from ref 34. The average cavity radius and dipole moment were used as initial guesses for both conformers and iteratively adjusted to fit specified values for the net stabilization of the 12-s-trans conformer at 23 700 psi relative to ambient pressure ($\Delta G_{rel}{}^{total}$ in Table III). The direction of adjustment was chosen so that $a_{trans} > a_{cis}$ and $\mu_{trans} > \mu_{cis}$ and the magnitude of the incremental adjustments was determined by input parameters. Iterations were continued until the specified value of $\Delta G_{rel}{}^{total}$ was obtained, and, if the desired value was "overshot", the incremental values were halved and their signs were changed. It is important to note that our calculations do not provide a least-squares estimate of the solute parameters; our formalism can only provide solute parameters that are consistent with (but not necessarily unique with respect to) the experimental observation. The iterative procedures that we have adopted are expected to underestimate the differences in the 12-s-cis vs. 12-s-trans cavity radii and dipole moments owing to the fact that the initial guesses are the average values.

Net stabilization free energies [$\Delta G_{rel}{}^{total}$ (23 700 psi)] in the range 0.0 to −0.1 kcal/mol were investigated to determine if our formalism could account for a calculated maximum stabilization of the 12-s-trans species at an intermediate pressure of 18 750 psi. We observed the correct behavior for a small range of $\Delta G_{rel}{}^{total}$ (23 700 psi) energies from −0.008 to −0.012 kcal/mol. The calculated results for the intermediate value of −0.01 kcal/mol are shown in Table III and yield the following differences in the ambient pressure conformer cavity radii and dipole moments:

$$a_u(\text{12-s-trans}) = a_u(\text{12-s-cis}) + 0.012\ \text{Å} \qquad (25)$$

$$\mu(\text{12-s-trans}) = \mu(\text{12-s-cis}) + 1.6\ \text{D} \qquad (26)$$

Because of the iterative approach used (see above), the above values are best viewed as lower limits to the conformer differences. In that regard, eq 25 and 26 compare favorably with the previous solvent effect study which predicted a cavity radius difference of 0.03 Å and a dipole moment difference of 2.2 D.[7] The CNDO/2 calculations of Weiman et al. predict a dipole moment difference of 1.9 D between the 12-s conformers (12-s-trans larger).[18]

The calculated conformer differences in dipole moment and cavity radius are not overly sensitive to our choice of $\Delta G_{rel}{}^{total}$ (23 700 psi). For example, if we choose $\Delta G_{rel}{}^{total}$ = −0.04 kcal/mol, a value four times more negative than that given in Table III, the resultant solute conformer differences are $\Delta\mu$ = 1.9 D, Δa_u = 0.010 Å (12-s-trans larger in both cases).

The above results are not consistent with the conclusions of Bauer and Carl that the difference between the 12-s-trans and 12-s-cis conformer dipole moments is in the range 0.5–0.8 D.[10] These authors also concluded that 11-*cis*-retinal preferentially assumes the more polar 12-s-trans conformation in nonpolar solvent at room temperature. We also disagree with this conclusion for the reasons outlined below.

An estimate of the mole fraction of the 12-s-trans conformer of 11-*cis*-retinal present in room temperature methylcyclohexane solution can be obtained by analyzing the oscillator strength of the 27 000-cm^{-1} band. The oscillator strength is

Table V. Comparison of Various Experimentally Observed and Theoretically Calculated Properties of the 12-s-Cis and 12-s-Trans Conformers of 11-*cis*-Retinal

quantity	value	method	ref
μ(12-s-trans) − μ(12-s-cis), D	0.65 ± 0.15	dielectric measurements	10
	>1.6	pressure effects	this work
	2.2 ± 0.3	solvent effects	7
	1.91	SCF-MO-CNDO/2	18
	2.51	SCF-MO-CNDO/S	18
a(12-s-trans) − a(12-s-cis), Å	>0.012	pressure effects	this work
	0.027 (±0.005),	solvent effects	7
G(12-s-trans) − G(12-s-cis), kcal/mol	0.5 ± 0.2	(see text)	this work
	0.6 ± 0.3	solvent effects	7
	0.8	MO theory	16
	1.5	CFF (PPP-SCF-MO)	11

a sensitive experimental indicator of the conformation of the retinal polyene chain and theoretical calculations using the Pariser-Parr-Pople SCF-MO-CID formalism are known to provide relatively accurate estimates of the observed f values.[15,21] A comparison of observed and calculated oscillator strengths of the 27 000-cm^{-1} band of four retinal isomers is shown in Table IV. The calculated values include the contributions due to the low-lying "$^1B_u{}^{*+}$" ← S_0 and "$^1A_g{}^{*-}$" ← S_0 transitions in keeping with recent two-photon investigations which indicate that both transitions lie within the range of the 27 000 cm^{-1} inhomogeneously broadened absorption band.[46,47] As can be seen from Table IV, the observed and calculated values agree fairly well for the all-trans, 9-cis, and 13-cis isomers, but the observed f value for 11-*cis*-retinal lies between the calculated values for the "pure" 12-s-trans and 12-s-cis conformers of this isomer.

The mole fraction of 12-s-trans species (χ_{12t}) present in ambient temperature methylcyclohexane solvent can be estimated using the equation

$$\chi_{12t} = (f_{\mathrm{obsd}} - f_{12c})/(f_{12t} - f_{12c}) \qquad (27)$$

where f_{obsd} is the observed oscillator strength of 11-*cis*-retinal and f_{12t} and f_{12c} are the calculated f values for the pure 12-s-trans and 12-s-cis conformers, respectively. Application of eq 27 to the data of Table IV (using the standard 11-cis geometries) yields a value of 0.42 for the mole fraction of 12-s-trans conformer present in ambient temperature methylcyclohexane solvent.

The above result is subject to error in both the experimental and theoretical f values used in evaluating eq 27. For example, the calculations can be seen to overestimate the oscillator strengths of the other isomers by approximately 5% (see Table IV). However, the experimental values for 9-*cis*- and 11-*cis*-retinal are also believed to be overestimated because interferences due to the moderate intensity band system at ~36 000 cm^{-1} were corrected for in an approximate fashion which would tend to underestimate their net contribution (see footnote *a*, Table IV). This tendency is reflected in the closer agreement between the observed and calculated oscillator strengths for 9-*cis*-retinal. A more quantitative estimate of our experimental error can be made by reference to Sperling's careful measurements of relative oscillator strengths of 11-*cis*-retinal vs. *all-trans*-retinal which yielded values for $f_{11\text{-cis}}/f_{\text{all-trans}}$ of 0.566 (isopentane, methylcyclohexane) and 0.567 (EPA).[9] These values are to be compared with our relative value of 0.60 (methylcylcohexane) and suggest that we are overestimating the experimental oscillator strength of 11-*cis*-retinal by approximately 5%. We conclude, therefore, that the experimental and theoretical f values of 11-*cis*-retinal are both comparably too large. Assuming a (symmetrical) error of ±15% in the previously calculated value of $\chi_{12t} = 0.42$, the predicted mole fraction of 12-s-trans conformer in methylcyclohexane falls in the range of 0.35–0.48.

Accordingly, if the solvent provides no differential stabilization of the two conformers, the above analysis indicates that the 12-s-cis conformer has a vacuum free energy 0.05–0.36 kcal/mol lower than the 12-s-trans conformer. The calculations in Table III, however, indicate that methylcyclohexane solvent preferentially stabilizes the 12-s-trans conformer by approximately 0.30 kcal/mol at ambient temperature and pressure. Accordingly, the 12-s-cis conformer must have a vacuum free energy 0.35–0.66 kcal/mol lower than the 12-s-trans conformer leading to the relationship

$$G(\text{12-s-trans}) = G(\text{12-s-cis}) + 0.5\ (\pm 0.2)\ \text{kcal/mol} \qquad (28)$$

This free energy relationship is in good agreement with the difference in internal energy obtained from an ambient temperature solvent effect analysis of 11-*cis*-retinal which predicted a vacuum internal energy difference of 0.6 (±0.3) kcal/mol.

A summary of experimental and calculated parameters for 11-*cis*-retinal is presented in Table V.

Acknowledgments. The authors would like to thank Paul Brown for generous gifts of 11-*cis*-retinal. R.R.B. gratefully acknowledges many helpful conversations with Professor W. H. Orttung. This work was supported by grants from the National Institutes of Health (EY -02202), the National Science Foundation (CHE75-19172), and the Committee on Research, University of California.

References and Notes

(1) Although there is general agreement on the fact that the chromophore in rhodopsin is bound via a protonated Schiff base linkage, the observation[3] that bathorhodopsin is formed in less than 6 ps has prompted some investigators to challenge the conventional belief that the initial photochemical step involves a cis–trans isomerization. Although a number of interesting alternative mechanisms have been proposed,[4] recent picosecond studies on isorhodopsin suggest that isomerization can, in fact, occur within a few picoseconds in rhodopsin.[5]

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Co-oligopeptides Containing Two Aromatic Residues Spaced by Glycyl Residues. 11. A Conformational Study of Tryptophan- and Glycine-Containing Oligopeptides Based on the Temperature Dependence of ^1H NMR Spectra

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Received October 2, 1978

Abstract: Proton magnetic resonance spectra were recorded in the temperature interval from +51 to −64 °C at 360 MHz with alkaline CD_3OD solutions of the following compounds: H-Trp-OH, H-Trp-Gly-OH, H-Gly-Trp-OH and H-Gly-Trp-Gly-OH (which contain one tryptophyl residue); H-Gly-Trp-Gly-Trp-Gly-OH and H-Gly-Trp-$(Gly)_2$-Trp-Gly-OH (which contain two tryptophyl residues spaced by glycyl residue(s)); H-Trp-Trp-OH, H-Trp-Trp-Gly-OH, H-Gly-Trp-Trp-OH, and H-Gly-Trp-Trp-Gly-OH (which contain two adjacent tryptophyl residues). The following parameters have been derived from the experimental spectra and their dependence on temperature is discussed: (1) chemical shifts of the aromatic protons; (2) chemical shifts and coupling constants of the $C_\alpha H$–$C_\beta H_2$ fragment of the tryptophyl residue; (3) anisochronism of the glycine C_α protons (also in view of the possible presence of hydrogen-bonded structures of the peptide backbone). On the basis of these parameters, the conformational properties of the aromatic side chains (in terms of rotamer populations) and of the backbone (in terms of possible hydrogen-bonded conformers) are discussed. A thermodynamic analysis of the rotamer populations around the χ_1 torsion angle of the tryptophyl side chain in the investigated compounds has been carried out. The enthalpy differences between the three classical rotamers G^+, G^-, and T are found to be linearly related to the respective entropy differences. For the favored conformer of H-Trp-Trp-O^- at low temperature, the detailed geometry is proposed, which is in agreement with the previously described spectroscopic properties of this molecule.

Introduction

This paper is part of a general study on the conformational properties of peptides containing one or two aromatic amino acid residues and glycyl residues. Previous CD studies[1,2] have shown that sequences containing two adjacent aromatic residues (in particular the pair -Trp-Trp-) may possess some degree of conformational rigidity.[3] A ^1H NMR study at room temperature[4] enabled us to describe the conformational equilibrium of the aromatic side chains. However, only subtle

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0002-7863/79/1501-5170$01.00/0