The Effect of Temperature and Solvent Environment on the Conformational Stability of 11-*cis*-Retinal

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Abstract: The effect of temperature and solvent environment on the relative stability of the distorted 12-s-cis and distorted 12-s-trans conformers of 11-*cis*-retinal is analyzed using an approximate solvent effect theory. The formalism includes the partitioning of the solvation energy into van der Waals, electrostatic, and cavity formation terms and the use of multiple linear regression techniques to obtain information about the molecular characteristics of the solute conformers. The 12-s-trans conformer is observed to have a cavity radius 0.03 Å larger, and a dipole moment 2.2 D larger, than those of the 12-s-cis conformer. The "free space" energies of the two conformers differ by only 0.6 kcal/mol with the 12-s-cis the more stable. In hydrocarbon solvent at room temperature, the ratio of 12-s-cis to 12-s-trans conformers is roughly two to one, but increasing solvent polarity or decreasing solution temperature increases the population of 12-s-trans conformer by increasing the importance of the electrostatic term usually at the expense of the cavity formation term. The former term favors population of the conformer with the larger dipole moment (12-s-trans), whereas the latter term favors population of the conformer with the smaller cavity size (12-s-cis). The van der Waals term is relatively insensitive to solute conformational changes and for the solvents investigated is of negligible importance in determining solute conformational stability. The possibility that the 12-s-trans geometry may represent the opsin incorporated conformation of the visual chromophore in rhodopsin is discussed.

Although 11-*cis*-retinal's mechanistic importance in vertebrate vision has been established for many years,[3] many of its photophysical properties are not yet completely understood. For example, 11-*cis*-retinal exhibits unusual band intensity fluctuations in its absorption spectrum with changes in temperature and solvent environment.[4,5] As shown in Figure 1, this characteristic is unique to 11-*cis*-retinal; other isomers of retinal show much smaller intensity changes for identical ranges in solvent polarity and temperature.[7]

A number of investigators[8-12a] have suggested that changes in an equilibrium population of distorted 12-s-cis[13] and distorted 12-s-trans[13] conformers (with the latter more populated at lower temperature[8-11] or higher solvent polarity[11]) are responsible for both the observed temperature and solvent polarity effects (see Figure 2). Although recent nuclear magnetic resonance investigations[12] confirmed the presence of both conformers at room temperature, a quantitative picture of how the solution environment affects conformational stability is still lacking.

In the present paper we investigate the nature of this conformational equilibrium by studying the effect of solvent environment on the band intensities in 11-*cis*-retinal's absorption spectrum. The solvent effect theory that is used to explain the observed spectral changes is presented in the Theoretical Section and applied in Results and Discussion. The prominent features of this formalism include the partitioning of the solvation energy into van der Waals, electrostatic, and cavity formation terms and the use of multiple linear regression techniques to obtain information about the molecular characteristics of the participating solute conformers. The results of this analysis not only provide reasonably accurate estimates of the cavity size, dipole moment, and "free space" energy differences between the 12-s-cis and 12-s-trans conformers, but also supply the first quantitative picture of how solvent environment and solution temperature determine conformational stability in 11-*cis*-retinal.

Experimental Section

Chemicals. 11-*cis*-Retinal, in the form of extensively purified single crystals, was generously donated by Paul K. Brown. Cyclohexane, ethyl ether, and acetonitrile were purchased from Eastman Kodak Co. and were all spectroquality. Propionitrile[14] (Eastman Kodak Co.) and isopropyl ether (MC/B Chemical Co.) were purified by multiple distillations on a 3-ft column packed with glass helices. The distillations were monitored by taking absorption spectra of the middle fraction and were discontinued when the absorption "cutoff" (1 o.d., 1-cm cell) was at a wavelength lower than 2200 Å. The above procedure successfully removed all impurities with absorption bands in the 2200–6000 Å region.

Isopentane and 3-methylpentane (Aldrich Chemical Co.) were purified by passing through a silver nitrate alumina column prepared by the following procedure.[15] Alumina (500 g; Merck chromatographic grade) was added to 1 l. of 4 *N* HNO_3 and stirred for 15 min. The acid was decanted off and the acidic alumina neutralized by continuously rinsing it with distilled water followed by vacuum filtration drying. The silver nitrate solution was prepared by dissolving 60 g of $AgNO_3$ into 150 ml of distilled water. (All steps involving the silver nitrate must be performed in total darkness or under a red safety light.) ACS grade methanol (500 ml) was then added to the $AgNO_3$ solution. The resulting mixture was combined with the neutral alumina and the solvent partially removed using a rotary evaporator. The powdered alumina was then completely dried in a vacuum desiccator in the presence of a neutral desiccant for 24 hr at 50°C.

Absorption spectra were recorded on a McPherson 700 series double-beam spectrophotometer at a spectral bandwidth of 2.0 Å. Room temperature spectra were obtained using matched 1-cm pathlength cells with the appropriate solvent as reference. Low temperature spectra were obtained using 1-cm pathlength "lollipop" cells immersed in liquid nitrogen with air as the reference. The absorption spectra were subsequently corrected for solvent, Dewar, and cell absorption using graphical subtraction.

Theoretical Section

The total energy of a solute–solvent system can be conveniently partitioned into the following contributions

$$E_{\text{total}} = E_{\text{solute}} + E_{\text{vdw}} + E_{\text{es}} + E_{\text{cav}} \tag{1}$$

where E_{solute} represents the energy of a given molecular conformation of the solute under "free space" or "vacuum" conditions, and the latter three terms represent the energy of solvation. E_{vdw} is the energy associated with nonbonded, nonelectrostatic (*van der Waals*[16-19]) interactions between the solute and solvent molecules, E_{es} is the energy due to static and induced solvent-solute dipole-dipole interactions, and E_{cav} is the energy required to form a solvent cavity of sufficient size to accommodate the solute molecule. This latter term is always positive and is balanced by the electro-

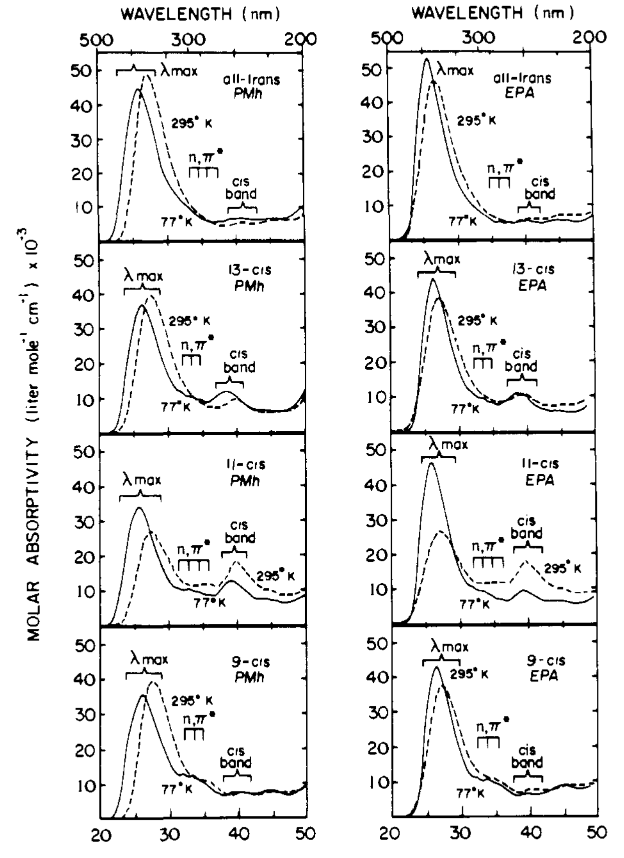


Figure 1. Absorption spectra of *all-trans*, 9-*cis*-, 11-*cis*-, and 13-*cis*-retinal at 295 and 77 K in nonpolar (PMh = isopentane, methylcyclohexane; 5:1, v/v) and polar (EPA = ethyl ether, isopentane, ethanol; 5:2:2, v/v) solvents. The spectra are reproduced with permission from ref 5 and 8, but the cis band, n,π* and $\lambda_{\max}$ labels were added by us. The vertical lines associated with the n,π* labels identify vibronic band maxima in the 77 K spectra.[6]

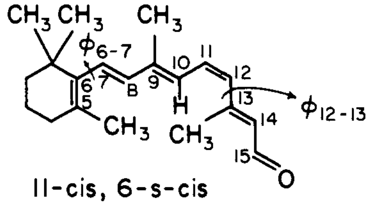
static and van der Waals terms which are always negative. We will observe, as have other investigators,[20] that the van der Waals term is relatively insensitive to solute conformational changes and can be safely neglected provided the solvents are appropriately chosen.

Our formalism is based on a statistical theory which approximates solute-solvent interactions in terms of point dipole interactions. This approach is sufficient as long as there is no appreciable overlap of the electron distributions of the solute and solvent molecules. Consequently, solvents which might form hydrogen bonds (e.g., alcohols) or charge transfer complexes (e.g., halogenated hydrocarbons) with the solute must be excluded from the present analysis.

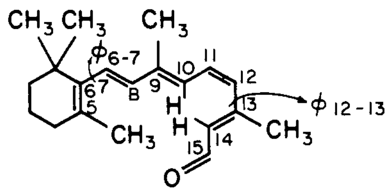
E_{vdw}. The energy due to van der Waals interactions between two molecules includes both attractive and repulsive nonbonded interactions. A simplified formulation based on a combination of the London dispersion equation and Born-type repulsion can be written[21]

$$E_{\mathrm{vdw}} = Br_{ij}^{-12} - (3/2)(\alpha_i \alpha_j r_{ij}^{-6})[I_i I_j/(I_i + I_j)] \qquad (2)$$

where B is a repulsion constant, r_{ij} is the separation between the two molecules,[22] and α_i and I_i are the molecular polarizability and ionization potential for molecule i. This equation can be modified for use in solvent effect calculations by treating the solute and solvent molecules as polarizable spheres with radii, a_u and a_v, respectively. (Throughout this paper, the u subscript will be used to designate so*l*u*te parameters and the v subscript to designate sol*v*ent parameters.) The radii are defined by the volume of the molecule (V) as derived from the bulk density (d)



11-cis, 6-s-cis



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

Figure 2. The 12-s-cis and 12-s-trans conformers of 11-*cis*-retinal. The numbering convention and important dihedral angles are indicated. Since all of the known isomers of retinal have distorted 6-s-cis dihedral angles, the "6-s-cis" label is usually omitted.

$$a = (3V/4\pi)^{1/3} = (3M/4\pi N_{\mathrm{A}} d)^{1/3} \qquad (3)$$

where M is the molecular weight, and N_{A} is Avogadro's number. The intermolecular separation is defined as the sum of the appropriate cavity radii divided by $\sqrt{2}$ [i.e., $r_{\mathrm{uv}} = 0.71(a_u + a_v)$],[22] and the polarizability is defined using the Lorentz-Lorenz expression[23]

$$\alpha = [(n^2 - 1)/(n^2 + 2)]a^3 \qquad (4a)$$

where n is the index of refraction appropriate to the solvent or solute. For the solute, it is convenient to choose a value for n which is reasonable for a broad range of molecules. Although most authors choose a value for n such that $\alpha_u = a_u^3/2$ ($n = 2$),[24a,25] this value considerably overestimates the polarizability of most solute molecules. For large polyenes like 11-*cis*-retinal, the hypothetical solution refractive index is estimated to lie in the range 1.6–1.8. Accordingly, in the present treatment we will use the following relationship to define the solute polarizability

$$\alpha_u = a_u^3/2.5 \qquad (4b)$$

Equation 2 can now be written:

$$E_{\mathrm{vdw}} = -0.6N_n\{a_u^3 a_v^3[0.71(a_u + a_v)]^{-6}\} \times [I_u I_v/(I_u + I_v)][(n^2 - 1)/(n^2 + 2)]G_r \qquad (5a)$$

where N_n is the number of nearest neighbor solvent molecules surrounding a given solute molecule, and the G_r term is defined as

$$G_r = 1 - B'[(a_u + a_v)^{-6}] \qquad (5b)$$

where B' is a semiempirical repulsion constant (different from that used in eq 2) appropriate for the collision radii of the solute and solvent. G_r can range in value from 0.7 to 0.2 based on a comparison of the van der Waals energies calculated using eq 5 with values calculated using Sinanoglu's formalism.[19] For a solute the size of 11-*cis*-retinal in solvents of medium sized molecules, the number of nearest neighbors can be set approximately equal to 15. Choosing a representative value for G_r of 0.5, eq 5a reduces to:

$$E_{\mathrm{vdw}} = -4.5\{a_u^3 a_v^3[0.71(a_u + a_v)]^{-6}\} \times [I_u I_v/(I_u + I_v)][(n^2 - 1)/(n^2 + 2)] \qquad (6)$$

Preliminary calculations indicated that the E_{vdw} term was usually lower in magnitude and always much less sensitive to solute conformational changes (which affect a_u) than the

Table I. Solvent Parameters[a]

Solvent	V, Å^3 [b]	a, Å[c]	I_V, eV	n	ϵ	$k_V(1)$[d]	γ_V[g]	f(cav)[e,g]	$k_V(\psi_{uv})\cdot f$(cav)[f,g]
Cyclohexane	180.6	3.5065	9.8	1.4235	2.016	0.647	24.4	54.3	44.6
Isopentane	194.9	3.5969	10.32	1.3509	1.835	0.674	14.5	41.9	34.6
n-Hexane	218.5	3.7366	10.18	1.3723	1.880	0.672	18.1	43.0	34.9
Ethyl ether	174.0	3.4629	9.6	1.3495	4.249	0.757	16.5	44.2	38.9
Isopropyl ether	236.3	3.8350	9.20	1.3655	3.880	0.738	17.3	45.0	37.8
Acetonitrile	87.8	2.7570	12.2	1.3416	36.50	1.358	27.6	57.5	63.9
Propionitrile	117.7	3.0405	11.7	1.3636	26.51	1.290	26.7	51.5	57.2

[a] All parameters are for 25°C and are extrapolated to this temperature if necessary. Data are from J. A. Riddick and W. B. Bunger, "Organic Solvents", Wiley-Interscience, New York, N.Y., or the CRC Handbook of Chemistry and Physics, 50th ed. [b] $V = 1.66053(M/d)$, where M is molecular weight and d is density in g/ml at 25°C. [c] $a = (3V/4\pi)^{1/3}$, where V is given by footnote b. [d] Calculated using eq 22. [e] Calculated using eq 18. [f] Calculated using eq 18 and 21. [g] In dynes/centimeter.

E_{es} and E_{cav} terms. Since E_{es} and E_{cav} can be calculated at a higher level of approximation than E_{vdw}, and since the vdw term provides no information concerning the solute that is not also available from analysis of the cavity term, eq 6 was used to choose solvents which minimize the importance of E_{vdw}. The solvents that were chosen are listed along with their important parameters in Table I. As we will demonstrate in Results and Discussion, the effect of solute conformational changes on E_{vdw} for the above solvents is roughly one order of magnitude smaller than E_{cav} or E_{es}.

E_{es}. The calculation of the energy due to static and induced solvent-solute dipole-dipole interactions is based on a modification of the Onsager cavity approximation[26] in which the spherical "Onsager" cavity is replaced with an elliptical cavity more appropriate for long chain polyene solutes like 11-*cis*-retinal. In this approximation, the solute is represented as a point dipole at the center of an ellipsoid whose volume equals that of the solute. The electrostatic energy is given by eq 7.[24c,d]

$$E_{es} = -0.5\mathbf{M}_{total} \cdot \mathbf{R}_{00} \tag{7}$$

$\mathbf{R}_{00}$ is the reaction field which physically represents the electrostatic field at the solute molecule created by the polarization of the surrounding medium by the solute's point dipole.[26] $\mathbf{M}_{total}$ is the total dipole moment of the solute expressed as a sum of permanent ($\mathbf{M}_{00}$) and induced ($\alpha_u\mathbf{R}_{00}$) components

$$\mathbf{M}_{total} = \mathbf{M}_{00} + \alpha_u\mathbf{R}_{00} \tag{8}$$

where α_u is the polarizability of the solute. The reaction field for an elliptical cavity is given by eq 9[24b]

$$\mathbf{R}_{00} = (\mathbf{M}_{total}/a_u^3)\{3A(1 - A)(\epsilon - 1)/[\epsilon + (1 - \epsilon)A]\} \tag{9}$$

where ϵ is the static dielectric constant of the solvent, a_u is the mean cavity radius of the solute (see below), and A is the elliptical integral defined in eq 10.

$$A = 0.5(r_1/r_3)(r_2/r_3) \times \int_0^\infty \{[s + (r_1/r_3)^2]^{3/2}[s + (r_2/r_3)^2]^{1/2} \times [s + 1]^{1/2}\}^{-1}\mathrm{d}s \tag{10}$$

The variables r_1, r_2, and r_3 are the lengths of the principal axes of the ellipsoid, where r_1 is defined as parallel to the dipole moment vector. Equation 10 has been constructed in terms of ratios of these axes to emphasize that this integral can be solved by determining only the relative dimensions of the ellipsoid that best encloses the solute molecule. The absolute size of this ellipsoid is, nevertheless, relevant in determining the mean cavity radius, a_u. This latter variable is most easily evaluated, however, using eq 3 and, when necessary (see Results and Discussion), a model compound to determine an appropriate value for the density, d.

The integral A for a given conformation of 11-*cis*-retinal was evaluated using INDO molecular orbitals to predict the direction of the dipole moment vector relative to the Cartesian coordinates of the molecular framework. The geometry of the 12-s-cis conformer was taken from the crystal structure of Gilardi and co-workers.[27] A corresponding structure for the 12-s-trans conformer was generated by rotating the 12-13 torsional angle from 12-s-cis (38.7°) (the crystal dimension) to 12-s-trans (141.3°) to produce equivalent out-of-plane distortion in $\phi_{12\text{-}13}$. Using a six-point Gaussian quadrature numerical integration technique (accurate to ±0.006 for eq 10), the following values for the elliptical integral were obtained: A(12-s-trans) = 0.14, A(12-s-cis) = 0.22. For comparison, A equals ⅓ for a spherical cavity ($r_1 = r_2 = r_3$).

Equations 7, 8, and 9 can now be combined to yield

$$E_{es} = -0.5\{(\mathbf{M}_{00}^2/a_u^3)f_A(\epsilon) \quad (\alpha_u\mathbf{M}_{00}^2/a_u^6)[f_A(\epsilon)]^2\} \tag{11a}$$

where

$$f_A(\epsilon) = 3A(1 - A)(\epsilon - 1)/\{\epsilon + (1 - \epsilon)A - [3\alpha_u A(1 - A)(\epsilon - 1)/a_u^3]\} \tag{11b}$$

Both of these equations can be simplified by treating the α_u/a_u^3 term as a constant appropriate for the solute molecule(s) of interest. For large polyenes, $\alpha_u/a_u^3 = 1/2.5$ (see eq 4b), and eq 11a and 11b reduce to:

$$E_{es} = -0.5(\mathbf{M}_{00}^2/a_u^3)[1 + f_A(\epsilon)/2.5]f_A(\epsilon) \tag{12}$$

where

$$f_A(\epsilon) = 7.5A(1 - A)(\epsilon - 1)/[2.5(\epsilon + A - \epsilon A) - 3A(1 - A)(\epsilon - 1)] \tag{13}$$

In order to use the above electrostatic field equations in a least-squares analysis of solute parameters, it is necessary to separate the solute dependent (A) and solvent dependent (ϵ) variables appearing in $f_A(\epsilon)$. This variable separation is accomplished using eq 14 and 15, which were derived[28] to minimize the error (inevitable in a separation of two functionally interdependent variables) in the parametric regions $[1.8 \le \epsilon \le 40]$ and $[0.1 \le A \le 0.5]$.

$$f_A(\epsilon) = \zeta_A[5(\epsilon - 1)/(3\epsilon + 4.5)] \tag{14}$$

where

$$\zeta_A = 2.7A + 0.95A^2 \tag{15}$$

The cavity ellipticity, ζ_A, diverges from unity in proportion to the extent that the solute cavity deviates from perfectly spherical dimensions. Given the previously defined values for A, the two conformers of 11-*cis*-retinal produce cavity ellipticities of 0.4 and 0.65 for the 12-s-trans and 12-s-cis geometries, respectively. Although errors introduced in calculating the electrostatic field using eq 14 (as an approximation to eq 13) can approach 30% in high dielectric solvents like acetonitrile,[28] uncertainties in other parameters (see below) are such that this source of error is not important for the analysis presented in Results and Discussion. Replacing 11-*cis*-retinal's elliptical cavity with a spherical cavity, however, would produce errors on the order of 300% in the calculated electrostatic field indicating the importance of using elliptical cavities for studying the effect of solvent environment on large polyenes.

Applying the above variable separation to eq 12 we obtain

$$E_{\mathrm{es}} = -0.5[\zeta_{\mathrm{A}}(\mathbf{M}_{00}{}^2/a_{\mathrm{u}}{}^3)] \times [1 + \bar{\zeta}_{\mathrm{A}}2(\epsilon - 1)/(3\epsilon + 4.5)][5(\epsilon - 1)/(3\epsilon + 4.5)] \qquad (16)$$

where the first pair of brackets enclose variables dependent solely upon the characteristics of the solute molecule. The $\bar{\zeta}_{\mathrm{A}}$ term, which is an average cavity ellipticity appropriate for both solute conformers $\{\bar{\zeta}_{\mathrm{A}} = [((\zeta_{\mathrm{A}}(12\text{-s-cis}) + \zeta_{\mathrm{A}}(12\text{-s-trans})]/2 = 0.53\}$, is introduced so that the values of the expressions in the last two brackets of eq 16 can be treated as solely solvent dependent. The advantages of retaining both the ζ_{A} and $\bar{\zeta}_{\mathrm{A}}$ terms in the above equation will be demonstrated in Results and Discussion. In those instances when the solute molecule is nearly spherical, or order of magnitude estimates of the electrostatic stabilization energy are sufficient, ζ_{A} and $\bar{\zeta}_{\mathrm{A}}$ (both of which equal unity for a spherical cavity) can be dropped from eq 16.

E_{cav}. The energy required to form a solvent cavity of sufficient size to accommodate the solute molecule is proportional to the work required to separate the solvent molecules. It is therefore related to the van der Waals and electrostatic forces that attract a solvent molecule to one of its own kind. In the macroscopic approximation, the energy of cavity formation is approximately given by the product of the surface area of the cavity and the surface tension of the solvent, γ. In the highly curved microscopic cavity, however, a correction for curvature must be included. Sinanoglu has described a formalism based on the thermodynamic properties of pure liquids and dilute solutions which includes microscopic curvature corrections,[18,19] and we adopt his procedures with only minor modification.

The cavity formation energy (E_{cav}) is given by eq 17

$$E_{\mathrm{cav}} = 4\pi a^2 k_{\mathrm{v}}(\psi_{\mathrm{uv}})f(\mathrm{cav}) \qquad (17)$$

where $f(\mathrm{cav})$ represents the energy part of the surface tension appropriate to a microscopic cavity

$$f(\mathrm{cav}) = \gamma_{\mathrm{v}}[1 - (\partial \ln \gamma_{\mathrm{v}}/\partial \ln T) - (2\xi_{\mathrm{uv}}T/3)] \qquad (18)$$

γ_{v} is the surface tension of the solvent at absolute temperature, T, and ξ_{uv} is the coefficient of thermal expansion of the cavity defined as

$$\xi_{\mathrm{uv}} = (1/V_{\mathrm{uv}})(\partial V_{\mathrm{uv}}/\partial T)_p \qquad (19\mathrm{a})$$

where V_{uv} is the volume of the cavity. ξ_{uv} is dependent upon the properties of both the solute and solvent, but calculations by Halicioglu and Sinanoglu[29] indicate that ξ_{uv} is closer to the solvent expansivity than to the solute expansivity. Accordingly, we approximate ξ_{uv} as follows

$$\xi_{\mathrm{uv}} = [(d_1/d_2) - 1]/(t_2 - t_1) \qquad (19\mathrm{b})$$

where d_1 and d_2 are solvent densities at temperatures t_1 and t_2, respectively, and $t_1 = T + 5°$, and $t_2 = T - 5°$. Similarly, $\partial \ln \gamma_{\mathrm{v}}/\partial \ln T$ will be approximated as

$$\partial \ln \gamma_{\mathrm{v}}/\partial \ln T = [2T/(\nu_1 + \nu_2)][(\nu_1 - \nu_2)/(t_1 - t_2)] \qquad (20)$$

The $k_{\mathrm{v}}(\psi_{\mathrm{uv}})$ term in eq 17 is a constant dependent upon the volume fraction ψ_{uv} ($= V_{\mathrm{v}}/V_{\mathrm{u}}$) of the solvent (molecular volume $= V_{\mathrm{v}}$) and solute (molecular volume $= V_{\mathrm{u}}$) and is defined in eq 21

$$k_{\mathrm{v}}(\psi_{\mathrm{uv}}) = 1 + \psi_{\mathrm{uv}}{}^{2/3}(k_{\mathrm{v}}(1) - 1) \qquad (21)$$

$k_{\mathrm{v}}(1)$ is a dimensionless microscopic cavity factor appropriate to the pure solvent and is evaluated semiempirically using the following expression

$$k_{\mathrm{v}}(1) = 0.5963 + 75.14f(\epsilon, \mathrm{cav}) - 561.8[f(\epsilon, \mathrm{cav})]^2 \qquad (22\mathrm{a})$$

where

$$f(\epsilon, \mathrm{cav}) = \epsilon[f(\mathrm{cav})]^{-2} \qquad (22\mathrm{b})$$

with $f(\mathrm{cav})$ in dyn/cm (see eq 18).

Results and Discussion

The solvent theory presented above will now be applied to 11-*cis*-retinal by using least-squares regression techniques to determine the molecular characteristics of the 12-s-cis and 12-s-trans conformers needed to account for the solvent effects observed in the absorption spectrum of 11-*cis*-retinal. We will observe that the calculated differences in the conformer cavity radii and dipole moments are close to values predicted using model compounds and approximate molecular orbital theory. Of greatest import, however, is the observation that the "free space" energies of the two conformers differ by only 0.6 kcal/mol with the 12-s-cis conformer the more stable. This result, when evaluated in terms of the predicted conformational dependence of the solvation energy, indicates that the 12-s-cis conformer is populated to roughly twice the extent of the 12-s-trans conformer at room temperature in hydrocarbon solvent. As we will discuss in section B, this observation provides an important insight into the mechanisms operating to produce the observed temperature effects.

(A) Application of Theory to 11-*cis*-Retinal in Solution. Neglecting the effect of the van der Waals term (see above), the difference in energy between an s-cis and s-trans conformer can be written as a combination of eq 1, 16, and 17

$$E_{\mathrm{trans}} - E_{\mathrm{cis}} = E_{\mathrm{solute}}(\mathrm{trans}) - E_{\mathrm{solute}}(\mathrm{cis}) \qquad (23\mathrm{a})$$

$$+0.5[(\zeta_{\mathrm{A}}\mathbf{M}_{00}{}^2/a^3)_{\mathrm{cis}} - (\zeta_{\mathrm{A}}\mathbf{M}_{00}{}^2/a^3)_{\mathrm{trans}}] \times [1 + 1.1(\epsilon - 1)/(3\epsilon + 4.5)][5(\epsilon - 1)/(3\epsilon + 4.5)] \qquad (23\mathrm{b})$$

$$+4\pi[a_{\mathrm{trans}}{}^2 - a_{\mathrm{cis}}{}^2][k_{\mathrm{v}}(\psi_{\mathrm{uv}})f(\mathrm{cav})] \qquad (23\mathrm{c})$$

where the 12-s label is temporarily dropped to conserve space. Applying simple Boltzmann statistics to the relative population of s-cis vs. s-trans conformers, we can write

$$RT \ln (N_{\mathrm{cis}}/N_{\mathrm{trans}}) = E_{\mathrm{trans}} - E_{\mathrm{cis}} \qquad (24)$$

where N_{cis} and N_{trans} are the number of molecules with 12-s-cis and 12-s-trans conformations, respectively. Although the above formula neglects the effect of entropy, calculations indicate that the entropy contribution is relatively small for the present system (see Appendix) and can be safely ignored at the present level of approximation.

In order to calculate ($N_{\mathrm{cis}}/N_{\mathrm{trans}}$), we will take advantage of a general feature of polyene electronic absorption spectra. In the 2000–6000 Å region, linear polyenes exhibit one intense absorption band which corresponds to the canonically allowed $\mathrm{B_u} \leftarrow \mathrm{A_g}$, $\pi^* \leftarrow \pi$ transition. When cis linkages are present, however, a second, less intense, absorption band appears roughly 1400 Å to the blue of the longest wavelength band.[30] This dependence on conformation of the intensity of this higher energy transition leads to the desig-

Table II. Observed and Calculated Solvent Effect on the Absorption Spectrum of 11-*cis*-Retinal

	$RT \ln (A_{\text{cis band}}/A_{\lambda_{\max}})$		
		Calcd	
Solvent	Obsd.	Neglects S^a	Max S^b
Cyclohexane	-0.18_6	-0.198	-0.202
Isopentane	-0.21_1	-0.209	-0.211
n-Hexane	-0.22_8	-0.210	-0.207
Ethyl ether	-0.24_6	-0.259	-0.259
Isopropyl ether	-0.25_5	-0.255	-0.253
Acetonitrile	-0.30_3	-0.297	-0.302
Propionitrile	-0.30_3	-0.303	-0.299
Regression coefficients		Value^{a,c}	Value^{a,c}
a_0, b_0, kcal/mol		$-0.229(a_0)$	$-0.198(b_0)$
a_1, b_1, $D^2/Å^3$		$-0.00593(a_1)$	$-0.00543(b_1)$
a_2, b_2, $Å^2$		$1.187(a_2)$	$1.299(b_2)$
Coefficient of determination (r^2)		0.943	0.924

^{a} Calculation neglects the contribution of surface entropy to the energy of cavity formation (see Results and Discussion). ^{b} Calculation based on "maximum entropy" approximation (see Appendix). ^{c} The symbol which is used in the text to label a given regression coefficient is given in parentheses.

nation "cis band", and the intensity of this transition serves as a sensitive diagnostic tool for detecting cis configurations in polyenes.[31] The intensity of the "cis band" is considerably enhanced by the presence of cis linkages near the center of long chain polyenes.[32] In particular, recent calculations on 11-*cis*-retinal predict that the oscillator strength for excitation into the 2500 Å cis band increases from 0.2 to 0.6 as the geometry changes from a distorted 12-s-trans (150°) to a distorted 12-s-cis (30°) conformation.[11,33] Conversely, these calculations predict that the total oscillator strength in the $\lambda_{\max}$ region at 3620 Å decreases from 1.0 to 0.5 for the same geometry change. Accordingly, the intensities of these two transitions are predicted to be sensitive measures of 12-s-cis vs. 12-s-trans conformational populations.

Applying Beer's law to the determination of $(N_{\text{cis}}/N_{\text{trans}})$, we can write

$$\frac{N_{\text{cis}}}{N_{\text{trans}}} = \frac{A_{\lambda_{\max}}(\text{trans}) \cdot R - A_{\text{cis band}}(\text{trans})}{A_{\text{cis band}}(\text{cis}) - A_{\lambda_{\max}}(\text{cis}) \cdot R} \tag{25a}$$

where

$$R = (A_{\text{cis band}}/A_{\lambda_{\max}})_{\text{obsd}} \tag{25b}$$

A is the molar absorptivity at the band maximum of the transition designated in the subscript. The labels "(trans)" and "(cis)" in eq 25 refer to absorption bands for the bands for the pure 12-s-trans and pure 12-s-cis conformers, respectively, and N_{cis} and N_{trans} are mole fractions (i.e., $N_{\text{cis}} + N_{\text{trans}} = 1.0$). Although experimental molar absorptivities are not available for the pure 12-s conformers, ranges for the absorptivities can be postulated by reference to the other isomers of retinal.[34]

$$35000 < A_{\lambda_{\max}}(\text{trans}) < 45000 \tag{26a}$$

$$18000 < A_{\lambda_{\max}}(\text{cis}) < 26000 \tag{26b}$$

$$6000 < A_{\text{cis band}}(\text{trans}) < 11000 \tag{26c}$$

$$18000 < A_{\text{cis band}}(\text{cis}) < 26000 \tag{26d}$$

$$42000 < [A_{\lambda_{\max}} + A_{\text{cis band}}] < 50000 \tag{27}$$

The above ranges apply to room temperature and polar and nonpolar solvent environments and are in units of l. mol^{-1} cm^{-1}.

In order to evaluate $N_{\text{cis}}/N_{\text{trans}}$, it is convenient to express the right hand side of eq 25 as a power function in the observable molar absorptivities

$$N_{\text{cis}}/N_{\text{trans}} = C_{\text{A}}[(A_{\text{cis band}}/A_{\lambda_{\max}})^{C_{\text{B}}}{}_{\text{obsd}}] \tag{28}$$

where the two constants, C_{A} and C_{B}, are empirically defined to fit eq 25 for given values of the parameters in eq 26. To evaluate C_{A} and C_{B}, least-squares regression techniques were applied to match eq 28 to eq 25 for the observed (Table II) and predicted (eq 26) absorptivities. The full range of values in "A" (eq 26) were tested subject to the restrictions imposed by eq 27 and, in each case, the least-squares coefficient of determination, r^2, was greater than 0.95 (mean = 0.992). This behavior indicates that eq 28 represents a satisfactory approximation to eq 25 for the entire range in conformer populations expected. C_{A} was observed to have a large range, whereas C_{B} showed minimal variation:

$$4.2 < C_{\text{A}} < 10.4,\ C_{\text{A}}\ (\text{average}) = 7.4 \tag{29a}$$

$$2.5 < C_{\text{B}} < 3.1,\ C_{\text{B}}\ (\text{average}) = 2.8 \tag{29b}$$

The uncertainties in the calculated solute parameters introduced as a result of the uncertainties in the above constants are dependent upon the property being calculated. Accordingly, a quantitative discussion must await the final analysis of the solvent data.

Equations 23 and 24 can now be written:

$$RT \ln (A_{\text{cis band}}/A_{\lambda_{\max}}) = \mathbf{a}_0 + \mathbf{a}_1[1 + 1.1(\epsilon - 1)/(3\epsilon + 4.5)][5(\epsilon - 1)/(3\epsilon + 4.5)] + \mathbf{a}_2[k_{\text{v}}(\psi_{\text{uv}})f(\text{cav})] \tag{30}$$

where

$$\mathbf{a}_0 = (1/C_{\text{B}})[E_{\text{solute}}(\text{trans}) - E_{\text{solute}}(\text{cis}) - RT \ln (C_{\text{A}})] \tag{31}$$

$$\mathbf{a}_1 = [1/(2C_{\text{B}})][(\zeta_{\text{A}}\mathbf{M}_{00}{}^2/a^3)_{\text{cis}} - (\zeta_{\text{A}}\mathbf{M}_{00}{}^2/a^3)_{\text{trans}}] \tag{32}$$

and

$$\mathbf{a}_2 = (4\pi/C_{\text{B}})[a^2(\text{trans}) - a^2(\text{cis})] \tag{33}$$

$(A_{\text{cis band}}/A_{\lambda_{\max}})$ was measured for the seven solvents listed in Table I, and Figure 3 shows the effect of a representative set of these solvents on 11-*cis*-retinal's absorption spectrum. The values of the constants $\mathbf{a}_0$, $\mathbf{a}_1$, and $\mathbf{a}_2$ were determined by the multiple linear least-squares method. The complete solvent effect data are compiled in Table II and graphically presented in Figure 4.

As Figure 4 demonstrates, the simple theory embodied in eq 30–33 is sufficient for semiquantitative prediction of the observed solvent effects. We note that the displacements of solvent data points from the linear regression line are not proportional to the polarizability of the particular solvent (eq 4), indicating that our neglect of the van der Waals term is not the principal source of error.

If we substitute the $\mathbf{a}_2$ regression coefficient from Table II into eq 33 and include the range of values predicted for C_{B} (eq 29b), we obtain

$$a^2(\text{12-s-trans}) = a^2(\text{12-s-cis}) + 0.27\ (\pm\ 0.03)\ Å^2 \tag{34}$$

Assuming an average solution volume for 11-*cis*-retinal of 500 Å^3 (see ref 34), eq 34 predicts a(12-s-trans) = 4.937 Å and a(12-s-cis) = 4.910 Å. The difference between these two values is of greater physical significance

$$a(\text{12-s-trans}) = a(\text{12-s-cis}) + 0.027\ (\pm\ 0.005)\ Å \tag{35}$$

It is interesting to compare the result in eq 35 with the differences in cavity radii calculated for the crystal geometries of all-trans vs. 11-*cis*-retinal.[27,36,37]

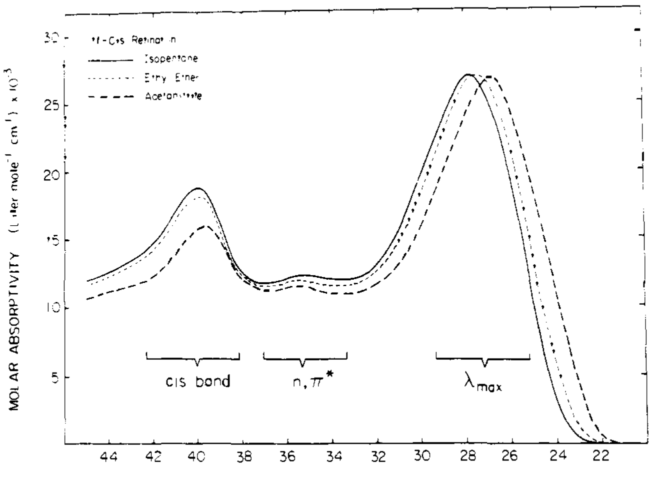


Figure 3. The effect of solvent environment on the absorption spectrum of 11-*cis*-retinal.

$$a(\textit{all-trans}\text{-retinal}) = a(11\text{-}\textit{cis}\text{-retinal}) + 0.047\ \text{Å} \qquad (36)$$

The smaller difference in cis vs. trans cavity radii for rotation about the 12,13 single bond relative to rotation about the 11,12 double bond is believed to involve two factors. First, the equilibrium dihedral angles for the 12-s-cis and 12-s-trans conformers of 11-*cis*-retinal are predicted to be very distorted from planarity,[12,33] minimizing the cavity size differences between these two geometries. Second, eq 35 represents cavity radii differences for a solution environment, whereas eq 36 is for the pure crystal; we might expect a highly ordered crystalline lattice to be more sensitive to conformational differences.

If we substitute the $\mathbf{a}_1$ regression coefficient from Table II into eq 32, assume $a^3 = 120$ Å^3 (see ref 35), use the previously defined values for ζ_A (see Theoretical Section), and include the full range of values for C_B, we find

$$0.4\mathbf{M}_{00}{}^2(12\text{-s-trans}) = 0.65\mathbf{M}_{00}{}^2(12\text{-s-cis}) + 4.0\ (\pm\ 0.3)\ \text{D}^2 \qquad (37)$$

Assuming the dipole moment for 11-*cis*-retinal lies in the range 3-7 D, the difference in dipole moments for the two conformers is

$$|\mathbf{M}_{00}|(12\text{-s-trans}) = |\mathbf{M}_{00}|(12\text{-s-cis}) + 2.2\ (\pm\ 0.3)\ \text{D} \qquad (38)$$

The above value is in agreement with the dipole moment differences calculated for the 12-s conformers of 11-*cis*-retinal by Weimann and co-workers using CNDO molecular orbital procedures.[38] These authors studied a range of 12-13 torsional angles and calculated dipole moments for the 12-s-trans conformer 1.8-2.3 D (CNDO/2) [2.5-2.9 D (CNDO/S)] larger than those calculated for the 12-s-cis conformer.

Substituting the $\mathbf{a}_0$ regression coefficient into eq 31 and including the full range of values predicted for C_A and C_B (eq 29), the difference in the "free space" energies of the two 12-s conformers is predicted to be

$$E_{\text{solute}}(12\text{-s-trans}) = E_{\text{solute}}(12\text{-s-cis}) + 0.6\ (\pm\ 0.3)\ \text{kcal/mol} \qquad (39)$$

The above result is consistent with the NMR analysis and consistent force field (CFF) calculations of Rowan, Warshel, Sykes, and Karplus.[12a] The CFF calculations predict the "free space" energy of the 12-s-cis conformer to be 1.5 kcal/mol lower in energy than the 12-s-trans conformer, a value slightly larger than that observed. In addition, the NMR analysis indicates that, at room temperature in acetone solvent, the difference in the total conformer energies is less than 1 kcal/mol and that, at a temperature of −47°C in the same solvent, the 12-s-trans conformer is more populated. Using our solvent effect theory, we calculate a differential solvation energy (ΔE_{sol} in footnote *e* of Table III) for 11-*cis*-retinal in acetone of 0.3 kcal/mol at 25°C and ~0.5 kcal/mol at −47°C with both values energetically favoring the 12-s-trans conformer. (The latter value is a rough estimate based on the analysis presented in section B below.) These values suggest that, in acetone at room temperature, the 12-s-cis conformer dominates by ~60% but that, at a reduced temperature of −47°C, the populations of the two conformers are essentially equivalent. Since the uncertainty in our calculation of the low temperature equilibrium is at least ±15% in conformer population, our results are not inconsistent with the NMR analysis, indicating a higher population of the 12-s-trans conformer at −47°C.

The important numerical results of the solvent effect analysis are summarized in Table II for three representative solvents. The following observations can be made:

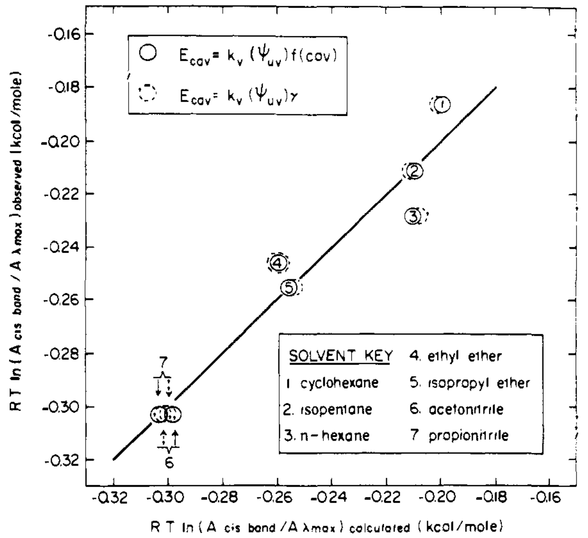


Figure 4. Observed vs. calculated values of $RT \ln (A_{\text{cis band}}/A_{\lambda_{\max}})$ for the solvent effect on 11-*cis*-retinal's absorption spectrum. Values calculated neglecting the contribution of surface entropy to cavity formation are represented by solid circles. Values calculated assuming a maximum contribution of surface entropy to cavity formation (see Appendix) are represented by dashed circles.

Table III. The Effect of Conformation on the Solvation Energy (kcal/mol) of the 12-s-cis and 12-s-trans Conformers of 11-*cis*-Retinal

Parameter[a]	Solvent Isopentane	 Ethyl ether	 Acetonitrile
ΔE_{vdw}[b]	−0.04	−0.03	−0.04
ΔE_{es}[c]	−0.13	−0.34	−0.62
ΔE_{cav}[d]	0.16	0.19	0.31
ΔE_{sol}[e]	−0.01	−0.18	−0.35

[a] Calculated using the solvent parameters listed in Table I and the following solute parameters: $I_u = 8.0$ eV, ζ_A(12-s-trans) = 0.40, ζ_A(12-s-cis) = 0.65, a(12-s-trans) = 4.937 Å, a(12-s-cis) = 4.910 Å, $|M_{00}|$(12-s-trans) = 6.2 D, and $|M_{00}|$(12-s-cis) = 4.0 D. [b] $\Delta E_{\text{vdw}} = E_{\text{vdw}}$(12-s-trans) − E_{vdw}(12-s-cis), calculated using eq 6. [c] $\Delta E_{\text{es}} = E_{\text{es}}$(12-s-trans) − E_{es}(12-s-cis), calculated using eq 16. [d] $\Delta E_{\text{cav}} = E_{\text{cav}}$(12-s-trans) − E_{cav}(12-s-cis), calculated using eq 17. [e] $\Delta E_{\text{sol}} = \Delta E_{\text{vdw}} + \Delta E_{\text{es}} + \Delta E_{\text{cav}}$. A negative value of ΔE_{sol} indicates that the 12-s-trans conformer is preferentially stabilized by the solvent environment.

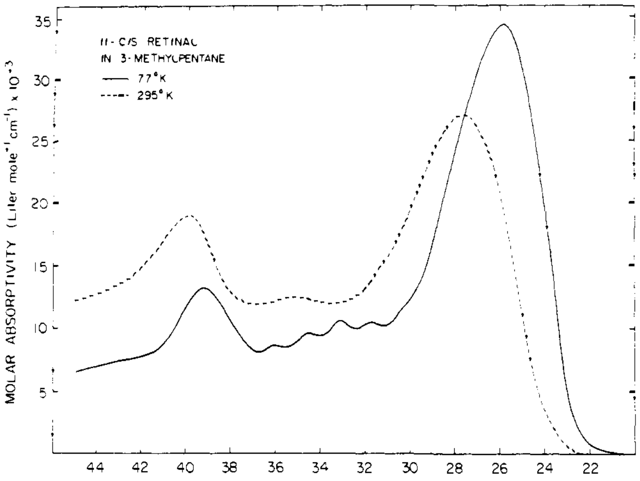


Figure 5. The effect of temperature on the absorption spectrum of 11-*cis*-retinal in 3-methylpentane. The vibronic structure appearing in the 30000-37000 cm^{-1} region of the 77 K spectrum is associated with the $\pi^* \leftarrow n$ transition.[6]

(1) The effect of the van der Waals term on conformational stability is relatively unimportant for the solvents in Table I, and its neglect in the least-squares analysis (eq 30-39) was justified. This term is not inherently negligible, however, and for solvents with high refractive indices this term might become relatively important.

(2) For hydrocarbon solvents, the electrostatic and cavity terms cancel to within 0.1 kcal/mol. This indicates that, in these solvents, the conformer equilibria depend primarily on the relative "free space" energies of the 12-s-cis and 12-s-trans conformers.

(3) As a consequence of observation 2 and the "free space" energies in eq 39, the 12-s-cis conformer predominates by roughly 2 to 1 over the 12-s-trans conformer at room temperature in hydrocarbon solvent. This result has important mechanistic implications for the temperature effect on the absorption spectrum of 11-*cis*-retinal (see below).

(4) As solvent polarity increases, the electrostatic term becomes increasingly dominant resulting in a preferential stabilization of the 12-s-trans conformer because of its larger dipole moment (eq 38).

(B) The Effect of Temperature on the Absorption Spectrum of 11-*cis*-Retinal. The effect of temperature on the absorption spectrum of 11-*cis*-retinal was first described by Wald and co-workers in 1959.[4] They noted that the intensity of the λ_{max} absorption band increased markedly on cooling in contrast to the behavior of the all-*trans*-, 9-*cis*-, and 13-*cis*-retinals which showed little or no band intensity changes in their absorption spectra with temperature. More detailed measurements by Sperling and Rafferty[5,8] and by Becker and coworkers[9] demonstrated that, in 11-*cis*-retinal, the "*cis* band" decreased in intensity in proportion to the increase in the λ_{max} intensity, and that the temperature effect was more pronounced in polar solvents. All of these effects are shown in the spectra of Figures 1 and 5.

A number of investigators[8-12a] have recently suggested that the equilibrium between the 12-s-cis and 12-s-trans conformers, with the latter conformer more populated at lower temperatures, provides the most reasonable explanation for the temperature effect on the absorption spectrum of 11-*cis*-retinal. Our solvent effect analysis, however, indicates that, in hydrocarbon solvents at room temperature, the 12-s-cis conformer is more stable by approximately 0.6 kcal/mol (see observation 3 and eq 39). Consequently, Boltzmann statistical effects would work against the population of 12-s-trans conformers with decreasing temperature and, in order to explain the observed results, it is necessary to show that, as temperature decreases, the 12-s-trans conformer is increasingly solvated (stabilized) relative to the 12-s-cis conformer by the solvent environment.

A quantitative description of low temperature solvent effects is hindered by the lack of appropriate solvent data at reduced temperatures. However, a qualitative picture can be presented based on the following considerations.

(i) As the temperature is decreased, the solvent contracts, resulting in an increase in its refractive index and dielectric constant. These parameter changes increase the magnitude of the van der Waals and electrostatic terms, both of which preferentially stabilize the 12-s-trans conformer (see Table III).

(ii) As the temperature is decreased, the cavity radius of the solute decreases by an amount proportional to the expansivity of both solvent and solute.[29] A decrease in cavity radius increases the magnitude of the electrostatic term at the expense of both the van der Waals and cavity terms. As above, the net effect is an increased stabilization of the 12-s-trans conformer with decreasing temperature.

The above two effects will be slightly counteracted by an increase in the f(cav) term (eq 18) with decreasing temperature. However, preliminary calculations indicate that the increase is relatively small and is of secondary importance in determining the effect of temperature on E_{cav}. Consequently, the 12-s-trans conformer is predicted to be preferentially stabilized as the temperature is decreased, even in hydrocarbon solvents. In polar solvents, the temperature effect is magnified because the electrostatic term increases in importance relative to the cavity term in the same fashion as observed for room temperature solvent effects (see observation 4).

Implications for Rhodopsin Stereospecificity

The stereospecificity of the opsin matrix in rhodopsin has been the subject of numerous investigations.[39-42] Although it has long been known that the visual chromophore in rhodopsin has an 11-cis double-bond linkage,[39] the assignment of the torsional conformation of the 12-13 single bond remains uncertain.[41,42] The semiempirical calculations of Honig and Karplus predict the 12-s-cis conformer to be 0.83 kcal/mol more stable than the 12-s-trans conformer.[42] On the basis of these calculations, these investigators suggested that the 12-s-cis geometry may represent the opsin incorporated conformation of the visual chromophore in rhodopsin.

Assuming that the conclusions drawn for retinal also apply to the protonated Schiff's base, our observation of a free space energy difference between the two 11-*cis*-retinal 12-s conformers of 0.6 kcal/mol suggests that the relative stability of the 12-s-cis conformer is not sufficiently large to preclude 12-s-trans from being preferred in rhodopsin. Stereospecific binding in opsin could easily "override" such a small energy difference. Furthermore, extrapolation of our solvent effect data to the aqueous solvent environment of the chromophore prior to incorporation suggests that the 12-s-trans conformer dominates by approximately 2:1. Thus, the thermodynamic implications of the solvent effect analysis support neither conformer over its 12-s counterpart.

Evidence in favor of a particular chromophore geometry in rhodopsin, however, may be found by applying our interpretation of the effect of temperature on the conformational stability of 11-*cis*-retinal in solution (see Results and Discussion, section B) to an analysis of the retinal photoisomerization data of Kropf and Hubbard.[43] These authors demonstrated that 11-*cis*-retinal displayed an anomalous temperature dependence in its quantum efficiency for photoisomerization. For all-*trans*-, 9-*cis*-, and 13-*cis*-retinal, the quantum efficiency decreases with decreasing temperature as would be expected for a process with a barrier to isomerization. In contrast, the value for 11-*cis*-retinal *increases* as the temperature is lowered.[43] Assuming that the equilibrium shifts toward the 12-s-trans conformer as temperature decreases (Results and Discussion, section B), the increase in quantum efficiency is consistent with the conformationally variant level ordering scheme proposed by Birge, Schulten, and Karplus.[11] It is interesting to compare the above effects with those observed when 9-*cis*- and 11-*cis*-retinal are combined with opsin. For example, the cis → trans photoisomerization quantum efficiency for 9-*cis*-retinal decreases from 0.5 to 0.2 upon incorporation into isorhodopsin.[43] This decrease is to be expected since the pigment matrix will increase the barrier to isomerization due to stereospecific binding which must be destroyed to accommodate cis–trans interconversion. The quantum efficiency for 11-*cis*-retinal, however, increases from 0.2 to 0.6 upon incorporation into rhodopsin.[43] This increase suggests that the 12-s-trans geometry represents the opsin incorporated conformation of the visual chromophore in rhodopsin since this conformer is expected to have a lower barrier to photoisomerization.[11,44] Consequently, the increase in 11-*cis*-retinal's quantum efficiency upon either lowering solution temperature or incorporation into the pigment system is associated with similar conformational effects.

These arguments are consistent with recent investigations of retinal analogues which found that 11-*cis*-14-methylretinal,[41] 11-*cis*-13-desmethyl-14-methylretinal,[41] and 11-*cis*-13-desmethylretinal[45] combined with opsin to form stable pigment systems. For stereochemical reasons, these analogues are expected to highly favor the 12-s-trans conformation.

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Appendix

Analysis of the Effect of Entropy on the Calculation of Conformer Parameters. In deriving the solvent effect equations used in Results and Discussion, the effect of entropy of solvation was neglected. By applying a "maximum entropy" approximation to the calculation of the free energy of solvation, we now demonstrate that the neglect of entropy was appropriate. The differences in the calculated solute parameters based on the "maximum entropy" approximation, relative to those calculated neglecting entropy (Results and Discussion), are well within the experimental errors associated with the uncertainties in the C_A and C_B constants.

Equation 24 can be written in the thermodynamically correct form as follows:

$$RT \ln (N_{cis}/N_{trans}) = G_{trans} - G_{cis} \tag{40}$$

where G_{trans} and G_{cis} are the total free energies of the 12-s-trans and 12-s-cis conformers, respectively, and the remaining terms retain their previous meanings (see eq 24). The total free energy is partitioned in the same fashion as for the total energy

$$G_{total} = G^\circ_{solute} + G_{vdw} + G_{es} + G_{cav} \tag{41}$$

where G°_{solute} represents the standard free energy of a given molecular conformation of the solute under "free space" conditions. The latter three terms represent the free energy of solvation and are defined as follows.

G_{vdw}. The van der Waal's free energy can be expressed in terms of E_{vdw} (eq 6) using the relationship

$$G_{vdw} = (1 - x)E_{vdw} \tag{42}$$

where x is a proportionality constant that relates the vdw entropy to the vdw energy. Halicioglu and Sinanoglu[19] have found that this proportionality is fairly constant for both polar and nonpolar solvents and assign x a value of 0.436. G_{vdw} is therefore smaller in magnitude than E_{vdw} and can be disregarded in an analysis of conformational stability for reasons identical with those given for neglecting E_{vdw} in the solvent effect analysis of Results and Discussion.

G_{es}. Within the confines of Onsager reaction field theory, no differentiation exists between electrostatic energy and electrostatic free energy. This result can be understood by evaluating the thermodynamic probability, Ω, associated with a point dipole at the center of a sphere. This parameter measures the number of ways that a given macrostate can be achieved and is equal to unity for a point uniquely defined in space.[46] Hence, the entropy ($S = k \ln \Omega$) is zero, and G_{es} is equivalent to E_{es}:

$$G_{es} = E_{es} - kT \ln \Omega = E_{es} \tag{43}$$

In deriving eq 43, energy and enthalpy are considered indistinguishable. This approximation is appropriate for both the electrostatic[18] and cavity formation[47] terms.

G_{cav}. The free energy of cavity formation is given by eq 44

$$G_{cav} = E_{cav} - TS_{cav} \tag{44}$$

where

$$S_{cav} = -4\pi a^2 k_v^{(s)}(\psi_{uv})\gamma_v(\partial \ln \gamma_v/\partial T + 2\xi_{uv}/3) \tag{45}$$

S_{cav} is the surface entropy of the cavity, $k_v^{(s)}(\psi_{uv})$ is the microscopic cavity entropy factor defined below, and the remaining terms have the same meaning as in eq 18. $k_v^{(s)}(\psi_{uv})$ can be calculated using eq 21 except that the $k_v(1)$ term appearing in this expression must be replaced by $k_v^{(s)}(1)$, the microscopic cavity entropy factor appropriate to the pure solvent. This latter parameter is more difficult to evaluate than $k_v(1)$ but, for the considerable majority of solvents for which this term has been determined, $k_v^{(s)}(1)$ is smaller than $k_v(1)$.[48] For the purposes of testing the effect of entropy on our solvent effect analysis, we can assume a maximum value for S_{cav} by setting $k_v^{(s)}(1) = k_v(1)$. Within this "maximum entropy" approximation, G_{cav} takes the simple form:

$$G_{cav}(\max S) = 4\pi a^2 k_v(\psi_{uv})\gamma_v \tag{46}$$

Equation 40 can now be written (see derivation of eq 30):

$$RT \ln (A_{cis\ band}/A_{\lambda_{max}}) = \mathbf{b}_0 + \mathbf{b}_1[1 + 1.1(\epsilon - 1)/(3\epsilon + 4.5)][5(\epsilon - 1)/(3\epsilon + 4.5)] + \mathbf{b}_2[k_v(\psi_{uv})\gamma_v] \tag{47}$$

where $\mathbf{b}_0$, $\mathbf{b}_1$, and $\mathbf{b}_2$ are constants dependent upon the solute and are defined in an identical fashion as the $\mathbf{a}_0$, $\mathbf{a}_1$, and $\mathbf{a}_2$ coefficients in eq 31–33, respectively. Least-squares values for these constants were determined using the same data and regression techniques outlined for the analysis of eq 30. The results are tabulated in the right hand column of Table II and yield the following calculated differences in

12-s-cis vs. 12-s-trans conformer parameters. (For a detailed discussion of the approximations involved in generating the following relationships, see the derivations of eq 34-39.)

$$a(\text{12-s-trans}) = a(\text{12-s-cis}) + 0.029\ (\pm 0.005)\ \text{Å} \quad (48)$$

$$|\mathbf{M}_{0\sigma}|(\text{12-s-trans}) = |\mathbf{M}_{0\sigma}|(\text{12-s-cis}) + 2.1\ (\pm 0.3)\ \mathbf{D} \quad (49)$$

$$G^{\circ}_{\text{solute}}(\text{12-s-trans}) = G^{\circ}_{\text{solute}}(\text{12-s-cis}) + 0.7\ (\pm 0.3)\ \text{kcal/mol} \quad (50)$$

The above values, when compared with those given in eq 35, 38, and 39, demonstrate that the uncertainties in the C_A and C_B constants are a far more significant source of error than our neglect of entropy in the analysis presented in Results and Discussion. Since the values in eq 48-50 are derived using the "maximum entropy" approximation, they may (or may not) be more accurate than the solute parameter relationships given in Results and Discussion. It is worth noting that the coefficient of determination for the least-squares fit for the "maximum entropy" approximation is 0.924, a value slightly smaller than that observed for the analysis in neglecting surface entropy entirely (0.943, see Table II).

References and Notes

(1) National Institutes of Health Postdoctoral Fellow, 1974-75. Address after September 1975: Department of Chemistry, University of California, Riverside, Calif. 92502.

(2) Alfred P. Sloan Research Fellow. Address after September 1975: Department of Chemistry, Wesleyan University, Middletown, Conn. 06457.

(3) G. Wald, *Science*, **162**, 230 (1968); R. Hubbard and G. Wald in "Structural Chemistry and Molecular Biology", A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968; W. E. Abrahamson and S. E. Ostroy, *Prog. Biophys. Mol. Biol.*, **17**, 170 (1967).

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(5) W. S. Sperling and C. N. Rafferty, *Nature* (*London*), **224**, 591 (1969).

(6) (a) The vibrationally structured absorption bands in the 270-330 nm region of the 77 K absorption spectra of the retinal isomers can be spectroscopically identified with the $\pi^* \leftarrow n$ transition.[6b] This assignment is based primarily on the following observations: (1) a blue shift in the transition energy upon increasing solvent polarity (cf. Figure 3); (2) a localized character in the dispersive red shift upon changing solvent refractive index; and (3) carbonyl group localized effects in the vibrational electronic development. Implications and further discussion of the above assignment will be presented in ref 6b. (b) R. R. Birge, M. J. Sullivan, and B. E. Kohler, to be published.

(7) Although *all-trans*-, 9-*cis*-, and 13-*cis*-retinal exhibit small intensity changes with change in temperature and solvent polarity, careful measurements[5,8] indicate that the observed oscillator strengths for the absorption bands remain constant. In contrast, the absorption band oscillator strengths in 11-*cis*-retinal exhibit considerable temperature and solvent dependence.[5,8]

(8) W. S. Sperling in "Biochemistry and Physiology of Visual Pigments", H. Langer, Ed., Springer-Verlag, New York, N.Y., 1973, p 19.

(9) A. M. Schaffer, W. H. Waddell, and R. S. Becker, *J. Am. Chem. Soc.*, **96**, 2063 (1974); see also R. S. Becker, K. Inuzuka, and D. E. Balke, *ibid.*, **93**, 38 (1971).

(10) A. Warshel and M. Karplus, *J. Am. Chem. Soc.*, **96**, 5677 (1974).

(11) R. R. Birge, K. Schulten, and M. Karplus, *Chem. Phys. Lett.*, **31**, 451 (1975).

(12) (a) R. Rowan, A. Warshel, B. D. Sykes, and M. Karplus, *Biochemistry*, **13**, 970 (1974); (b) R. S. Becker, S. Berger, D. K. Dalling, D. M. Grant, and R. J. Pugmire, *J. Am. Chem. Soc.*, **96**, 7008 (1974).

(13) For convenience, we will define a single bond as s-cis if the dihedral angle, ϕ, is in the range $0° \leq \phi < 90°$ and s-trans if the angle is $90° < \phi \leq 180°$. Consequently, the subsequent omission of the "distorted" label in the remainder of this paper does not imply planarity.

(14) (a) Propionitrile is extremely toxic.[14b] Sample cells should be prepared in a well ventilated hood and sealed prior to transport to the spectrophotometer. (b) J. A. Riddick and W. B. Bunger, "Organic Solvents", Wiley-Interscience, New York, N.Y., p 806, and references therein.

(15) J. van Egmond, private communication. van Egmond's method is based on the following literature procedures: B. de Vries, *Chem. Ind.* (*London*), 1049 (1962); L. R. Chapman and D. F. Kuemmel, *Anal. Chem.*, **37**, 1598 (1965); E. C. Murray and R. N. Keller, *J. Org. Chem.*, **34**, 7, 2234 (1969).

(16) Many authors include electrostatic interactions within the context of van der Waal's forces.[17] In the present paper, however, we will adopt the more restricted definition used by Sinanoglu.[18,19]

(17) See, for example, B. Linder, *Adv. Chem. Phys.*, **12**, 225 (1967); L. Pauling and E. B. Wilson, "Introduction to Quantum Mechanics", McGraw-Hill, New York, N.Y., 1935, pp 383-388.

(18) O. Sinanoglu in "Molecular Associations in Biology", B. Pullman, Ed., Academic Press, New York, N.Y., 1968, p 427.

(19) T. Halicioglu and O. Sinanoglu, *Ann. N.Y. Acad. Sci.*, **158**, 308 (1969).

(20) D. L. Beveridge, M. M. Kelly, and R. J. Radna, *J. Am. Chem. Soc.*, **96**, 3769 (1974).

(21) F. London, *Z. Phys.*, **63**, 245 (1930); M. Born and J. E. Mayer, *ibid.*, **75**, 1 (1932); L. Pauling, "The Nature of the Chemical Bond", 3rd ed, Cornell University Press, Ithaca, N.Y., 1960, pp 505-509.

(22) The attractive component of the van der Waal's interaction involves the polarization of one molecule in the rapidly changing field associated with the instantaneous electron configuration of the second molecule. The mean interaction distance term (r_{ij} in eq 2) associated with the above effect is well defined only for interactions between one-atom molecules (in which case r_{ij} = internuclear separation). For dispersive interactions between polyatomic molecules, a first approximation to r_{ij} could be the distance between the molecular centers as given by the sum of the appropriate cavity radii. This sum, however, underestimates the mean interaction distance because localized polarizability effects are neglected. In the present analysis, we take these local effects into account in an approximate way by treating the interacting cavities as an ensemble of smaller polarizable spheres within the larger cavity and calculating the mean interaction distance in terms of a force weighted average of all of the small sphere dispersive interactions. Sample calculations indicate that, within this approximation, the mean interaction distance for the two spherical cavities is given as the sum of the appropriate large sphere cavity radii divided by the square root of two.

(23) H. A. Lorentz, *Ann. Phys.*, **9**, 641 (1880); L. Lorenz, *Ann. Phys.*, **11**, 70 (1880).

(24) (a) C. J. F. Bottcher, "Theory of Electric Polarization", Elsevier, Amsterdam, 1952, p 206; (b) *ibid.*, pp 72-74; (c) *ibid.*, pp 133-135, 138-139. (d) Equation 7 neglects the attenuating contribution to the electrostatic energy associated with charge separation. This term arises from the work required to reorganize the solute's electronic structure to an extent necessary to account for the induced component of the total dipole moment.[24c] Although the inclusion of this term would simplify the final expression for E_{es}, its introduction is not appropriate within the theoretical constraints imposed by our use of a space fixed point dipole and would result in a less physically realistic description of the electrostatic stabilization energy.

(25) E. G. McRae, *J. Phys. Chem.*, **61**, 562 (1959); S. Basu, *Adv. Quantum Chem.*, **1**, 145 (1964).

(26) L. Onsager, *J. Am. Chem. Soc.*, **58**, 1486 (1936). See also J. N. Wilson, *Chem. Rev.*, **25**, 377 (1939); C. P. Smyth, "Dielectric Behavior and Structure", McGraw-Hill, New York, N.Y., 1955.

(27) R. D. Gilardi, I. L. Karle, and J. Karle, *Acta Crystallogr., Sect. B*, **28**, 2605 (1972).

(28) A more detailed discussion of the error associated with the use of the variable separation defined in eq 14 and 15 will be presented in a subsequent paper: R. R. Birge and B. E. Kohler, to be published.

(29) See footnote on page 311 of ref 19.

(30) L. Zechmeister, A. L. LeRosen, W. A. Schroeder, A. Polgar, and L. Pauling, *J. Am. Chem. Soc.*, **65**, 1940 (1943); L. Zechmeister and A. Polgar, *ibid.*, **65**, 1522 (1943).

(31) L. Zechmeister, "Cis-trans Isomeric Carotenoids", Academic Press, New York, N.Y., 1962.

(32) For example, *all-trans*-retinal has a 6-s-cis linkage in its polyene chain, but as can be seen in Figure 1, the intensity of its "cis band" is relatively small.

(33) Consistent force field calculations[12a] predict that the 12-s-cis and 12-s-trans conformers have 12,13 dihedral angles[13] of approximately 30 and 150°, respectively.

(34) At 77 K in a polar solvent glass (EPA, see Figure 1), the λ_{max} and cis band molar absorptivities for 11-*cis*- and 13-*cis*-retinal are very similar in value: $A_{\lambda_{max}}$(11-cis) = 46000, $A_{\lambda_{max}}$(13-cis) = 44000, $A_{cis\ band}$(11-cis) = 10000, and $A_{cis\ band}$(13-cis) = 11000 (all values in l. mol^{-1} cm^{-1}). The 12-s-trans conformer is expected to be highly preferred in a low temperature polar solvent (see ref 11 and section III-B) but, assuming a small amount of 12-s-cis conformer remains, its presence will tend to decrease $A_{\lambda_{maax}}$ and increase $A_{cis\ band}$ relative to the pure 12-s-trans absorptivities. Consequently, the following inequalities are expected to hold: $A_{\lambda_{max}}$(all-trans) $\gg$ $A_{\lambda_{max}}$(11-cis, 12-s-trans) $>$ $A_{\lambda_{max}}$(13-cis), and $A_{cis\ band}$(all-trans) $\ll$ $A_{cis\ band}$(11-cis, 12-s-trans) $<$ $A_{cis\ band}$(13-cis). Using data for nonpolar and polar ambient temperature solvent environments [$A_{\lambda_{max}}$(all-trans) = 49000 (PMh), 46000 (EPA); $A_{cis\ band}$(all-trans) = 5300 (PMh), 6100 (EPA); $A_{\lambda_{max}}$(13-cis) = 40000 (PMh), 38000 (EPA); $A_{cis\ band}$(13-cis) = 9700 (PMh), 11000 (EPA); see Figure 1 and ref 5 and 8], the above inequalities lead to the ranges given in eq 26a and 26c. The absorptivities for the pure 11-cis, 12-s-cis conformer are more difficult to estimate. Theoretical calculations predict that this conformer should exhibit roughly equal λ_{max} and cis band absorptivities.[11] Furthermore, since a mixture of 12-s-cis and 12-s-trans conformers exist in solution at room temperature,[12] the following inequalities are predicted: $A_{\lambda_{max}}$(11-cis, 12-s-cis) $<$ $A_{\lambda_{max}}$(11-cis) and $A_{cis\ band}$(11-cis, 12-s-cis) $>$ $A_{cis\ band}$(11-cis), where the "11-cis" values refer to the following ambient temperature solution measurements: [$A_{\lambda_{max}}$(11-cis) = 27000 (PMh), 26500 (EPA); $A_{cis\ band}$(11-cis) = 17500 (PMh), 18000 (EPA); see Figure 1]. The above considerations yield the ranges given in eq 26b and 26d. Finally, it is observed that all of the cis isomers have total ($A_{\lambda_{max}}$ + $A_{cis\ band}$) absorptivities in the range given in eq 27.

(35) The hypothetical solution volume for a molecule which does not exist as a liquid can be estimated using the relationship $V = M/N_A d$, where M and N_A are defined in eq 3, and d is the density of a similar compound which exists as a liquid at the temperature of interest. β-Ionone is an appropriate model compound for 11-*cis*-retinal and has a density of 0.9462 at 20°C. Accordingly, 11-*cis*-retinal (M = 284.45) has a hypo-

thetical solution volume of 500 Å^3.

(36) The molecular cavity radii for the crystals of *all-trans-* and 11-*cis*-retinal were calculated using the formula, $a = (3W/4\pi n)$,[13] where W is the observed[27,37] unit cell volume, and n is the number of molecules per unit cell (for the above crystals, $n = 4$): a(*all-trans*-retinal)[37] = 4.774 Å, a(11-*cis*-retinal)[27] = 4.727 Å.

(37) T. Hamanaka, T. Mitsui, T. Ashida, and M. Kakudo, *Acta Crystallogr., Sect. B*, **28,** 214 (1972).

(38) L. J. Weimann, G. M. Maggiora, and P. E. Blatz, submitted for publication.

(39) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36,** 269 (1952).

(40) For a recent review, see A. Kropf, B. Whittenberger, S. Goff, and A. Waggoner, *Exp. Eye Res.*, **17,** 591 (1973).

(41) W. T. Chan, K. Nakanishi, T. G. Ebrey, and B. Honig, *J. Am. Chem. Soc.*, **96,** 3642 (1974).

(42) B. Honig and M. Karplus, *Nature*, (*London*), **299,** 558 (1971).

(43) A. Kropf and R. Hubbard, *Photochem. Photobiol.*, **12,** 249 (1970).

(44) The extent to which the conformationally variant level ordering and photochemical reactivity arguments advanced in ref. 11 for the 12-s conformers of 11-*cis*-retinal also apply to the protonated Schiff's base is currently under study: R. R. Birge and M. Karplus, to be published.

(45) R. Nelson, J. K. deRiel, and A. Kropf, *Proc. Natl. Acad. Sci. U.S.A.*, **66,** 531 (1970).

(46) M. W. Zemansky, "Heat and Thermodynamics", McGraw-Hill, New York, N.Y., 1957, p 270.

(47) A. W. Adamson, "Physical Chemistry of Surfaces", Interscience, New York, N.Y., 1960, Chapter II.

(48) Of the 12 solvents investigated by Halicioglu and Sinanoglu,[19] aniline was the only liquid for which $k_v^{(s)}(1) > k_v(1)$. In general, $k_v^{(s)}(1) \sim 0.8k_v(1)$.

Proton Magnetic Resonance Studies of the Effects of Sugar Hydroxyl Dissociation on Nucleoside Conformation. Arabinosyl Nucleosides with an Intramolecular Hydrogen Bond between the Pentose O(5′) and O(2′)

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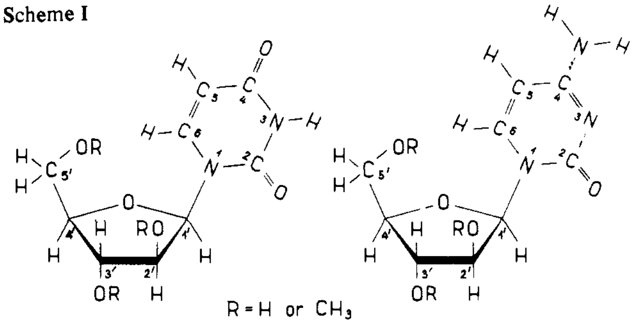
Abstract: Analyses have been made of the proton magnetic resonance spectra, in neutral and alkaline media, of 1-β-D-arabinofuranosylcytosine and some of its O′-methyl derivatives and of some *O*′-methylarabinofuranosyluracils. In particular, changes in chemical shifts of specific protons were correlated with ionization of the sugar 2′ and 3′ hydroxyls. In aqueous medium the neutral forms of the foregoing derivatives possess comparable populations of the forms C(2′)endo and C(3′)endo and of the three classical exocyclic 5′-CH_2OH conformers. For those derivatives with free 2′ and 5′ hydroxyls, dissociation of the "up" 2′-OH leads to an unusual modification in the sugar conformation, hitherto not encountered in solution, viz., C(2′)endo-C(3′)exo and gauche-gauche, accompanied, and probably constrained to this form, by formation of an intramolecular hydrogen bond with the 5′-OH as donor, 5′-OH···O(2′)$^{(-)}$; this conformation is similar to that observed in the solid state for the *neutral* forms of arabinofuranosyluracil and arabinofuranosylcytosine. No marked changes in conformation occur when either the 2′-OH or 5′-OH is etherified, nor with cytidine. Dissociation of the 3′-OH does not lead to detectable changes in conformation of arabinonucleosides, but, in the case of 2′-*O*-methylcytidine leads to a preference for the form C(2′)endo. The marked preference for the conformation anti is maintained on dissociation of the sugar hydroxyls. In the case of 3′-*O*-methylarabinofuranosyluracil, and of arabinosylcytosine, it proved possible to evaluate the p*K* for dissociation of the 2′-OH from the dependence of chemical shifts and coupling constants on the degree of alkalinity of the solutions. It is shown that ^{1}H NMR titration of sugar hydroxyls in general is a feasible procedure. The lower rate of alkali-catalyzed exchange of the pyrimidine H(5) in arabinofuranosylcytosine, relative to its 5′-O-methyl and 3′,5′-di-O-methyl derivatives, is shown to be related to the intramolecular hydrogen bond in the former.

Considerable attention has been devoted to studies on the possible effects on nucleoside and nucleotide conformations of protonation and/or ionization of the heterocyclic base residues. No attempts appear to have been made to analyze the potential influence of dissociation of the sugar hydroxyls; in fact, in a ^{1}H NMR investigation of the influence of base ionization on the conformation of pseudouridine, Deslauriers and Smith[2a] avoided the use of pH values above 11 so as to eliminate possible complications from sugar hydroxyl dissociation. Much earlier the ^{1}H NMR spectra of some nucleosides were recorded at pH values up to 14 by Jardetzky and Jardetzky,[2b] but no attempt was made to interpret the observed effects.

It was long ago shown that dissociation of the sugar hydroxyl(s) of pyrimidine nucleosides is reflected in important modifications of the electronic absorption spectra of the heterocyclic bases,[3] indicative of some type of interaction between the two. More recently, the use of the aglycone as a uv "probe" has made possible in some instances reasonable estimates of the acidities of these hydroxyls.

During the course of some studies on the acidities of the sugar hydroxyls of some nucleosides, it was noted that very marked changes in the coupling constants of the pentose protons of araC[4] and 3′-maraU (Scheme I) occurred under

Scheme I



Shugar et al. / Sugar Hydroxyl Dissociation and Nucleoside Conformation