to tentatively assign the −105.5 and −104.9 ppm signals to the Orn[1] and Gly[2] carbonyls (Figure 3).

Water ^1H, ^2H, and ^{17}O NMR relaxation dispersion studies of aqueous protein solutions have revealed a significant interaction between the solvent and solute.[27] The proposal was formulated that the interaction could impose a slow component of rotational correlation velocity on that fraction of water molecules which presumably is in contact with the randomly tumbling protein.[27] It might well be that the "friction" mechanism transmitting angular momentum from the polypeptide to solvent water is the kind of amide-water pairing effect suggested by the present study.

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(4) Abbreviations: ^{13}C NMR, ^{13}C nuclear magnetic resonance; ^1H NMR, ^1H nuclear magnetic resonance; NMR, nuclear magnetic resonance; Me_2SO, dimethyl sulfoxide or dimethyl-d_6 sulfoxide; TFE, trifluoroethanol or trifluoroethanol-d_2; ppm, parts per million; CW, continuous wave.

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Dielectric Studies on Retinal and Ionone

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Abstract: The dipole moments of *all-trans*-retinal and 11-*cis*-retinal as well as of the analogs α-ionone and β-ionone were determined in the nonpolar solvent methylcyclohexane. No tendency of the molecules to aggregate in this solvent was observed. The calculations of the dipole moments were based on Onsager's theory of dielectric constants of a two-component system. The dipole moment of *all-trans*-retinal was determined to be 4.02 ± 0.05 D. The dipole moment of the sterically hindered 11-*cis*-retinal was found to be 3.89 ± 0.05 D, i.e., only 0.13 D lower than that of *all-trans*-retinal. This is much higher than one would expect if the preferred conformation of 11-*cis*-retinal in solution would be the same as in the crystalline state, viz., a distorted 12-s-cis conformation. This conclusion is based on the finding that dipole moments of conjugated polyene aldehydes strongly depend on the electric polarizability of the π-electrons. In nonpolar solvents, therefore, a polar conformation of 11-*cis*-retinal prevails, probably a distorted 12-s-trans conformation. Assuming the presence of two conformers in thermodynamic equilibrium, an upper limit for the relative amount of the less polar species is estimated. Mixtures containing various relative amounts of 9-*cis*-, 13-*cis*-, 9,13-*dicis*-, and *all-trans*-retinal were produced by photoisomerization of *all-trans*-retinal in nonpolar solution. No formation of 11-*cis*-retinal was detected by high-pressure chromatography. The dielectric constants of all these mixtures were identical with that of pure *all-trans*-retinal. This suggests that the 9-cis, 13-cis, and 9,13-dicis isomers, which are expected to possess planar polyene chains like *all-trans*-retinal, also have dipole moments identical with that of *all-trans*-retinal. In addition, it is found that the dipole moments of analogous aldehydes are strongly correlated with the number of the conjugated π-electrons.

Introduction

Rhodopsin, the visual pigment of rod outer segments, consists of 11-*cis*-retinal covalently bound to the protein, opsin, via a Schiff base linkage with an ϵ-amino group of lysine.[1,2] Light absorption causes photoisomerization to *all-trans*-retinal in the primary photochemical steps of the visual process.[3] There have been extensive conformational studies of the retinal isomers both in crystalline form[4,5] and in solution.[6-9] In crystalline form, 11-*cis*-retinal assumes a distorted 12-s-cis conformation[4] in which the polyene chain largely deviates from a planar structure. In solution, however, a strong temperature effect of the absorption spectra,[8-11] NMR experiments,[12] and theoretical considerations[13] suggest that a distorted 12-s-cis conformation coexists in thermodynamic equilibrium with a distorted 12-s-trans conformation. Little is known regarding the relative amounts of both conformers. The calculated potential energy curve for twisting about the 12-s bond yields the lowest energy minimum for a distorted 12-s-cis conformation.[12,14] However, Warshel and Karplus do not exclude the possibility that in solution the 12-s-trans conformer may prove to be more stable due to solvent interaction.[13]

Different dipole moments of these two conformers have been predicted by several theoretical calculations[15-18] which show the dipole moment of the 12-s-trans conformation to be greater than that of the 12-s-cis conformation. In addition, both conformers of 11-*cis*-retinal would be expected to possess smaller dipole moments than *all-trans*-retinal since steric hindrance leads to a deviation from planarity of the π-electron system. Qualitatively, theoretical work[17] supports this picture. Therefore, if dissolved 11-*cis*-retinal exists predominantly in the 12-s-cis conformation, the dielectric constant should be appreciably smaller than that of an *all-trans*-retinal solution of equal concentration. Little is known, so far, about the actual dipole moments of the retinal isomers in solution although the dipole moments of the first excited state of *all-trans*-retinal and its Schiff base were recently investigated.[19] We report here dielectric studies on *all-trans*-retinal and 11-*cis*-retinal dissolved in the nonpolar solvent methylcyclohexane. In addition, results will be presented for the analogs α-ionone and β-ionone as well as for photoisomerized *all-trans*-retinal.

Experimental Section

Dielectric measurements were carried out using a capacitance bridge (Boonton, Model 75C) with continuously adjustable frequency from 5 to 500 kHz. The balancing of the bridge was reproducible within 0.1%. The measuring cell consisted of two cylindrical electrodes of pure platinum sheets which were insulated with Teflon. The distance between the electrodes was 1.5 mm; the mean diameter of the ring-shaped filling volume (1.5 mL) was 41.5 mm. The outer electrode was kept at a fixed temperature within ±0.01 °C as monitored with a thermistor resistance measuring bridge. Unless stated otherwise, all dielectric measurements were carried out at 20 °C. Cyclohexane, *n*-hexane, carbon tetrachloride, and acetone were used to calibrate the measuring cell. The accuracy of the dielectric measurements was limited by the reproducibility of the pipetting. Using Eppendorf pipets, the standard deviation was 0.7%. Rapid temperature changes in the measuring cell were achieved by switching to another thermostated water bath. Temperature equilibrium was reached within 5 min. The accuracy of the dielectric constants determined at different temperatures was ±1.5%. No frequency dependence of the dielectric constants could be detected until 500 kHz. Therefore, all measurements were carried out at a fixed frequency (300 kHz) with maximal sensitivity of the capacitance bridge. The retinal isomers were analyzed using a Spectraphysics Model 3500 B high-pressure liquid chromatograph equipped with a Schoeffel spectrophotometer (SF 770, spectroflow). A 50-cm column with an inside diameter of 3 mm was used. The stationary phase consisted of Spherisorb (Silica 5 μm) and the eluent was a mixture of 10% diethyl ether (Uvasol, Merck) and 90% petroleum ether (Merck, once distilled). All chromatographic runs were performed at 20 °C at a flow rate of 1.2 mL/min. Complete separation was achieved for all isomers with the sole exception of 9,13-*dicis*-retinal which overlapped with the 13-cis isomer (see e.g., Figure 4). The retention times increased in the order 13-*cis*- < 9,13-*dicis*- < 11-*cis*- < 9-*cis*- < *all-trans*-retinal. The bands of 9-*cis*-, 11-*cis*-, 13-*cis*-, and *all-trans*-retinal were identified with the pure isomers. The position of 9,13-*dicis*-retinal is strongly suggested by the pattern obtained with photoisomerized *all-trans*-retinal. In this case the probability of 9,13-*dicis*-retinal formation is on the order of the product of the probabilities of 9-*cis*-retinal and 13-*cis*-retinal formation. The elutions were monitored at 370 and 251 nm. The relative amounts of the isomers were determined by integration of the peak areas after correction for the different extinction coefficients.

All-trans-retinal was purchased from Fluka A.G., Buchs, Switzerland (puriss.) and was used without further purification. No significant amounts of other isomers could be detected by high-pressure chromatography. 9-*cis*-Retinal and 13-*cis*-retinal were purchased from Eastman Kodak, Rochester, N.Y. α-Ionone and β-ionone were purchased from Merck and were twice distilled under vacuum. 11-*cis*-Retinal was prepared in collaboration with W. Sperling and Ch. Rafferty following mainly standard methods[20,21] with some modifications. The resulting 11-*cis*-retinal was 95.2% pure with 3.3% *all-trans*-retinal and 1.5% 13-*cis*-retinal as determined by high-pressure chromatography. The ratio of the absorption coefficients at 251 and 364 nm was about 0.7 in ethanol. Methylcyclohexane was twice distilled and dried with sodium. Photoisomerization of 0.2 M *all-trans*-retinal solutions in methylcyclohexane was carried out by illumination with white light of about 10^7 lux for various time intervals (up to 2 h) during continuous stirring at 20 °C. Refraction indices were determined with dim red light using an Abbé refractometer (Zeiss, Model G).

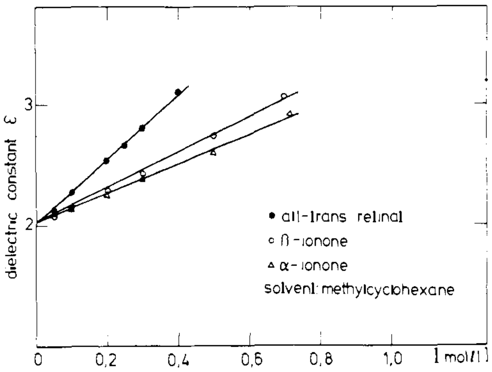

Figure 1. Concentration dependence of the dielectric constants of *all-trans*-retinal, of α-ionone, and of β-ionone dissolved in methylcyclohexane; temperature, 20 °C. The straight lines are obtained by linear regression.

Results

The concentration dependencies of the dielectric constants of α-ionone and β-ionone as well as of *all-trans*-retinal in methylcyclohexane solutions were found to be strictly linear up to concentrations of about 0.5 M (Figure 1). The slopes of the straight lines $\Delta\epsilon/\Delta c$ increase in the order α-ionone < β-ionone < *all-trans*-retinal, indicating that the polarity of the molecules increases in the same order. For α-ionone and β-ionone, which are freely miscible with the solvent, dielectric measurements were carried out in the whole concentration range up to the pure substances (Figure 2). Up to 2.0 M solutions of α-ionone and β-ionone, the dielectric constants fit the theoretical curve (solid line) which was calculated from Onsager's theory (see later). The dielectric constants of pure α-ionone and β-ionone at 20 °C were measured to be 10.5 and 12.7, respectively. These values, however, are about 10% higher than would be expected by extrapolation of the theoretical curve (Figure 2). The dielectric constant of a 0.215 M solution of 11-*cis*-retinal was measured to be 2.52 ± 0.02 at 20 °C which is significantly lower than the dielectric constant of an *all-trans*-retinal solution of the same concentration (2.59 ± 0.02).

The temperature dependence of the dielectric constants was measured for both *all-trans*-retinal and 11-*cis*-retinal solutions in the range from −17 to 40 °C. With decreasing temperature the dielectric constants of both solutions increased linearly with the reciprocal absolute temperature (Figure 3) as is to be expected for noninteracting molecules.

To investigate 9-*cis*-retinal and 13-*cis*-retinal, a 0.2 M solution of *all-trans*-retinal was photoisomerized as described in the Experimental Section. The photoisomerization process was interrupted several times and samples were taken out for analysis of the isomers by high-pressure chromatography and for dielectric measurements. 13-*cis*-Retinal and 9-*cis*-retinal were formed preferentially. The small peak overlapping the 13-cis peak (Figure 4) is due to 9,13-*dicis*-retinal. Virtually no 11-*cis*-retinal was formed under these conditions. No effort was made to achieve photoequilibrium and care was taken to avoid photodecomposition. The dielectric constants were found to be identical within the experimental error of 0.7% for all of

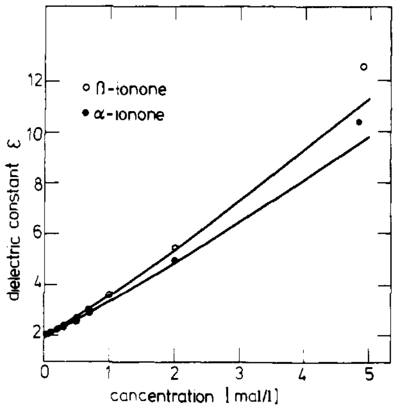

Figure 2. Concentration dependence of the dielectric constant of α-ionone and β-ionone dissolved in methylcyclohexane, measured in the whole concentration range up to the pure substances; temperature, 20 °C. The solid lines represent the calculated dielectric constants based on Onsager's formula (eq 1) when using the dipole moments obtained in the concentration range up to 0.5 M.

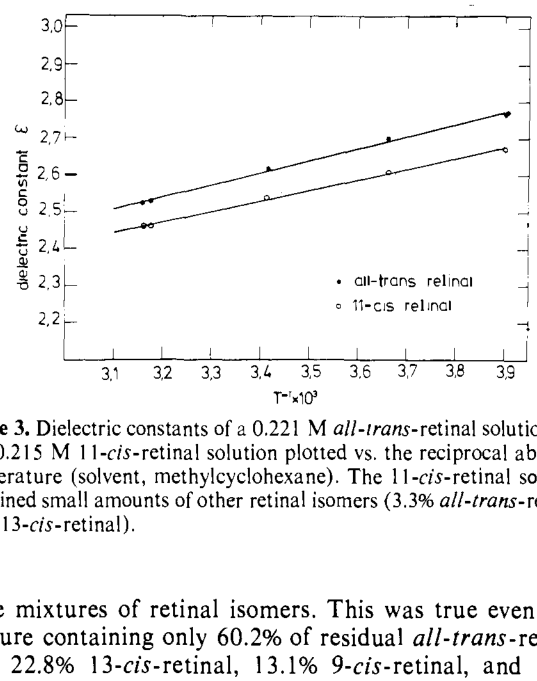

Figure 3. Dielectric constants of a 0.221 M *all-trans*-retinal solution and of a 0.215 M 11-*cis*-retinal solution plotted vs. the reciprocal absolute temperature (solvent, methylcyclohexane). The 11-*cis*-retinal solution contained small amounts of other retinal isomers (3.3% *all-trans*-retinal, 1.5% 13-*cis*-retinal).

these mixtures of retinal isomers. This was true even in a mixture containing only 60.2% of residual *all-trans*-retinal with 22.8% 13-*cis*-retinal, 13.1% 9-*cis*-retinal, and 3.9% 9,13-*dicis*-retinal formed by photoisomerization. This result strongly suggests that the dielectric constants of pure 13-*cis*-retinal and 9-*cis*-retinal are identical with the dielectric constant of *all-trans*-retinal within the measuring accuracy. Since the mixture contained only low amounts of 9,13-*dicis*-retinal, a small difference of the dielectric constant of this isomer to that of *all-trans*-retinal would not have been detected.

Dipole moments were calculated based on Onsager's theory for the two-component system of a polar solute dissolved in a nonpolar solvent[22]

$$\mu = \frac{2\epsilon + n_1^2}{2 + n_1^2} \cdot \sqrt{\frac{9000kT}{4\pi N\epsilon c} \sum_{i=1}^{2} \frac{\epsilon - n_i^2}{2\epsilon + n_i^2}\theta_i} \qquad (1)$$

(μ = dipole moment of the solute; ϵ = dielectric constant; c = concentration of the solute in mol/L; θ_i = volume fraction of component i; T = absolute temperature; N = Avogadro's constant; k = Boltzmann's constant; formula given in electrostatic units). In the above equation the subscript 1 refers to the solute and subscript 2 to the solvent. The "internal refraction indices" n_1 and n_2 are correlated with the electric polarizabilities α_1 and α_2 of the molecules by the following equation which is formally identical with the Clausius-Mosotti equation

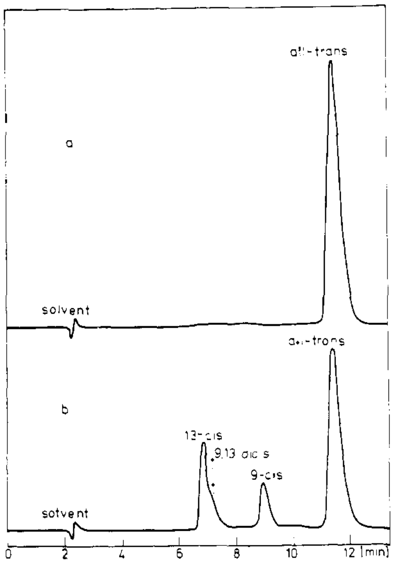

Figure 4. High-pressure chromatography of retinal in a diethyl ether-petroleum ether mixture (1:9) at 20 °C; a, *all-trans*-retinal; b, after photoisomerization of a 0.2 M solution of *all-trans*-retinal for 3 h, as described in the Experimental Section. Ordinate, relative concentration units; monitoring wavelength, 370 nm. The peaks were corrected for the different extinction coefficients. 11-*cis*-Retinal would be eluted 7.8 min after injection.

Table I[a]

Substance	$(\Delta\epsilon/\Delta c)_{20°C}$	$n_{20°C}$	μ
α-Ionone	1.35 ± 0.03	1.5016	3.00 ± 0.05 D
β-Ionone	1.54 ± 0.03	1.5198	3.23 ± 0.05 D
all-trans-Retinal	2.68 ± 0.04	1.73 ± 0.03	4.02 ± 0.05 D
11-*cis*-Retinal	—	1.69 ± 0.03	3.89 ± 0.05 D

[a] Dielectric increments $(\Delta\epsilon/\Delta c)_{20°C}$, refraction indices $n_{20°C}$, and dipole moments μ. The unit of the dipole moment of 1 D is defined as 1×10^{-18} electrostatic unit.

$$\frac{n_i^2 - 1}{n_i^2 + 2} = \frac{4\pi}{3} N_i \alpha_i \qquad (i = 1,2) \qquad (2)$$

(N_i = number of molecules of component i per mL).

All parameters on the right side of eq 1 are known with the exception of θ_i and n_i. The volume fractions θ_i were measured pycnometrically. The internal refraction indices n_i could not be measured directly, but in our case, they are not expected to differ very much from the optical refraction indices (see Discussion). The optical values were used for the calculation of the dipole moments. Refraction indices for α-ionone and β-ionone were measured over the whole concentration range up to the pure substances and were found to increase strictly linearly with the concentration. Refraction indices of *all-trans*-retinal were determined up to 0.4 M solutions. Again, a linear concentration dependence was observed. Assuming that a linear relationship holds for retinal as well over the whole concentration range, a refraction index of 1.73 ± 0.03 for pure *all-trans*-retinal was estimated by linear extrapolation.

The refraction index of 11-*cis*-retinal was determined in the same way to be 1.69 ± 0.03. Using these values, the dipole moments calculated from the dielectric constants of *all-trans*-retinal up to 0.4 M solutions as well as for α-ionone and β-ionone up to 2.0 M solutions were found to be independent of the concentration. The results are summarized in Table I. In addition, dipole moments of *all-trans*-retinal and of 11-*cis*-retinal have been evaluated from the dielectric constants measured at different temperatures from −17 to 40 °C. The temperature dependence of the refraction index of the solvent, which is due to the temperature dependence of the density, was thereby taken into account. The dipole moments of both *all-trans*-retinal and 11-*cis*-retinal were found to be temperature independent within the accuracy of these measurements (1.5%).

Discussion

The linear relationship between the dielectric constants and the concentration, observed for α-ionone, β-ionone, and *all-trans*-retinal up to concentrations of about 0.5 M, strongly suggests that no aggregation occurs. Solute aggregation would result in a deviation of the dielectric constant from a linear concentration dependence. In the simplest case of a dimerization, a quadratic deviation from linearity would be expected. This, however, is clearly not observed up to 0.5 M solutions. Furthermore, the nonlinear increase of the dielectric constant measured for higher concentrations of α-ionone and β-ionone (Figure 2) is up to 2.0 M solutions in excellent quantitative agreement with Onsager's theory which applies only for noninteracting molecules. We therefore conclude that there is virtually no tendency of ionone and retinal to aggregate at moderate concentrations. This conclusion is further supported by the observation that the dielectric constants of *all-trans*- and 11-*cis*-retinal have a strict linear correlation with the reciprocal absolute temperature (Figure 3). This is expected for noninteracting polar molecules since the orienting force in an electric field is opposed by the random temperature motion. If aggregation did occur, the effect of temperature on the aggregation constant would result in a nonlinear correlation between the dielectric constant and the reciprocal absolute temperature.

The dielectric constants for pure α-ionone and β-ionone are about 10% greater than calculated by extrapolation of Onsager's formula using the dielectric data obtained at lower concentrations. This deviation may be due to partial aggregation. It may also indicate that some of the idealizing assumptions which enter Onsager's equation are not completely valid for pure ionone.

These observations are consistent with spectroscopic data which show no deviation from Beer's law for *all-trans*-retinal and 11-*cis*-retinal[23] up to the highest spectroscopically accessible concentrations (ca. 5 mM for the absorption maximum) and for different solvents (*n*-hexane, ethanol). As opposed to this, tail to tail dimerization of *all-trans*-retinal via the aldehyde groups was recently suggested.[24] About 40% dimerization was estimated for a 3.2 mM ethanolic solution. In the proposed model, the dipole moments of the aldehyde groups nearly cancel. Therefore, the dipole moments of such dimer aggregates would be virtually zero and this should result in a dielectric constant with a pronounced negative deviation from linearity with increasing retinal concentration. There is, however, no evidence from our dielectric data for such a dimerization process.

Onsager's theory of dielectric constants which was used to calculate dipole moments treats the polar molecules as polazable dipoles at the center of a spherical cavity. The dielectric constant of a solution of a polar solute in a nonpolar solvent is a function of both the low-frequency polarizabilities of the solvent and the solute and the dipole moment of the solute. Generally, the polarizability at radiofrequencies may be up to 10% greater than in the visible spectral range due to the atomic polarizability.[25] No atomic polarizability is observed for the solvent, methylcyclohexane, since the dielectric constant at low frequencies equals the square of the optical refraction index. The atomic polarizability of retinal is expected to be negligibly small since for polyene molecules the electronic component largely prevails due to the high polarizability of the π-electron system. In addition, the extended conjugated π-electron system tends to stabilize a planar molecular structure so that an appreciable atomic polarizability is unlikely. In the worst case, assuming a 5% higher polarizability, dipole moments for retinal would result which are 2.5% (i.e., ca. 0.1 D) smaller than those shown in Table I.

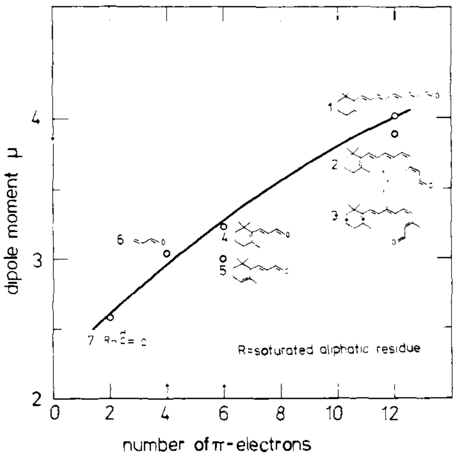

Figure 5. Dipole moments of various aldehydes with conjugated polyene chains. The dipole moments for acrolein (3.04 D; gas) and for aliphatic aldehydes (2.58 D) are taken from literature;[25] the other values are contained in Table I. The structure formulas are: *all-trans*-retinal (1); 11-*cis*-12-*s*-*trans*-retinal (2); 11-*cis*-12-*s*-*cis*-retinal (3); α-ionone (5); β-ionone (4); acrolein (6); aliphatic aldehydes (7). The dipole moments of the molecules 1, 4, 6, and 7 are interpolated with a quadratic regression curve (solid line).

The dipole moments of the retinal isomers are appreciably greater than those of the ionone isomers (Table I) although in both cases the only polar group is the aldehyde group. In addition, the dipole moments of saturated aliphatic aldehydes (e.g., propionaldehyde, butyraldehyde) are within experimental error independent of the length of the hydrocarbon chain and are significantly smaller than the dipole moments of the ionone and retinal isomers. This indicates that the polarizability of polyene aldehydes and their overall dipole moments are correlated. The electric dipole field of the conjugated aldehyde group obviously induces a strong polarization of the highly polarizable delocalized π-electrons which increases with the length of the polyene chain, although the polyene chain as such is essentially nonpolar (e.g., butadiene, hexatriene[25]). The correlation between the length of the polyene chain and the dipole moment of several molecules is depicted in Figure 5 which shows that the dipole moments of *all-trans*-retinal, β-ionone, acrolein, and aliphatic aldehydes closely fit a quadratic interpolating curve (Figure 5, solid line). The dipole moment of α-ionone is appreciably smaller than the dipole moment of β-ionone and is practically identical with the dipole moment of acrolein. This is obviously due to the fact that α-ionone possesses only four conjugated π-electrons and hence a reduced polarizability (i.e., smaller refraction index, see Table I) than β-ionone. It should be noted in this context that the conjugation of the π-electrons of the cyclohexene ring is partially disturbed due to steric hindrance of the 6-s-cis bond.[26] This steric hindrance, however, is common to all ionone and retinal isomers.

According to this picture, the highest polarizability, and hence the highest dipole moment of all retinal isomers, is expected for *all-trans*-retinal due to the straight planar structure of the polyene chain.[12] A distortion of the planarity of the π-electron system due to steric hindrance will lead to a decrease of the polarizability and therefore to a decrease of the dipole moment of the molecule. It is likely, however, that the mobility of the conjugated π-electrons for isomers with sterically unhindered planar side chains will hardly change for different isomers. In fact, we obtain within measuring accuracy identical dielectric constants for *all-trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal all of which possess planar side chains.[27] This indicates that the dipole moments of these retinal isomers are probably identical.

The main results for the sterically hindered 11-*cis*-retinal are: (a) the dipole moment is only 0.13 D smaller than that of *all-trans*-retinal, and (b) no temperature dependence of the dipole moment is observed between −17 and 40 °C. The smaller dipole moment of 11-*cis*-retinal as compared to that of *all-trans*-retinal is in agreement with our expectation that a nonplanar π-electron system reduces the dipole moment of the molecule. As to the actual conformation of 11-*cis*-retinal in solution, it is generally believed that two energetically similar conformations coexist in thermal equilibrium, viz., a distorted 12-s-trans and a distorted 12-s-cis conformation.[11,12,14,27,28] This is suggested by the strong temperature effect of the absorption spectra,[8-10] NMR experiments,[12] and theoretical considerations.[13] An alternative possibility is that the observed temperature effects are due to changes in a single conformation.[9,13] It is impossible from our dielectric measurements to decide which one of these possibilities is actually realized. The following discussion is based on the two-conformation model which is more commonly adopted. However, it will be shown later that both models lead essentially to the same conclusions.

Since the π-electron system of the 12-s-cis conformer is much more twisted than that of the 12-s-trans conformer an appreciable difference in the electric polarizability would be expected which should result in significantly different dipole moments for the two different conformations. It is obvious from Figure 5 that the maximal difference of the dipole moments of retinal and of an aliphatic aldehyde is 1.4 D. The dipole moment of the s-cis conformer is, however, estimated to be somewhat higher than that of acrolein[25] (3.04 D) due to partial conjugation of all π-electrons. Based on this, we estimate that the actual difference between the dipole moments of the two conformers is in the range of 0.5 to 0.8 D. It is remarkable that the dipole moment of 11-*cis*-retinal $\bar{\mu}$ calculated from our dielectric measurements is only 0.13 D smaller than that of *all-trans*-retinal. We therefore conclude that a polar conformer, most probably a distorted 12-s-trans conformation, of 11-*cis*-retinal largely predominates in solution at room temperature. The relative amount of both conformers is more quantitatively estimated in the following way. From Debye's formula[29] it follows that the actual dipole moment of 11-*cis*-retinal $\bar{\mu}$ is

$$\bar{\mu}^2 = x \cdot \mu_{12\text{-t}}^2 + (1 - x) \cdot \mu_{12\text{-c}}^2 \qquad (3)$$

(x = mole fraction of the 12-s-trans form, $\mu_{12\text{-t}}$ = dipole moment of the 12-s-trans conformer, $\mu_{12\text{-c}}$ = dipole moment of the 12-s-cis conformer).

If we take $\bar{\mu}$ equal to 3.89 D and assume for $\mu_{12\text{-t}}$ dipole moments of 3.9 to 4.0 D and for $\mu_{12\text{-c}}$ dipole moments of 3.2 to 3.5 D, the mole fraction of x ranges from 0.77 to 0.97. These values correspond to an energy difference between the conformers of 1 to 2 kcal/mol. Assuming these energy values, the value of x varies in the temperature range of −17 to 40 °C from 6% (for 1 kcal/mol) to 2% (for 2 kcal/mol). This results in a temperature dependence of 1% to 3% for the mean dipole moment $\bar{\mu}$ of 11-*cis*-retinal according to eq 3. Since such small changes of $\bar{\mu}$ could not be reliably measured, it was impossible to gather further support for the two-conformation model from these experiments. Therefore, our results are equally consistent with 11-*cis*-retinal in solution existing as essentially one conformation (at least a mole fraction of 0.77; see above). The main conclusion for both models is that in a nonpolar solvent 11-*cis*-retinal assumes preferentially a polar conformation which is most probably identical with a distorted 12-s-trans conformation.

NMR experiments[12] suggest that the same conformation is the more stable one in the polar solvent deuterioacetone. Furthermore, a thermodynamic equilibrium between the two conformations, with the 12-s-trans conformer being the more stable one, can explain the well established temperature effect of the absorption spectrum.[11,12] Theoretical calculations predict that the 12-s-cis conformer should be 1.5 kcal/mol lower in energy than the 12-s-trans conformer.[12] However, such a small energy difference may be overcompensated by secondary interactions with the solvent.[13,18] In contrast to these results, it was concluded from spectroscopic studies on 14-methyl-11-*cis*-retinal that the 12-s-cis form of 11-*cis*-retinal is more stable in solution.[27,30] This conclusion is based on the assumption that the 12-s-cis conformation of 14-methyl-11-*cis*-retinal is sterically hindered. However, the theoretical potential energy functions for twisting about the 12-s bond yield qualitatively similar pictures for both 11-*cis*-retinal and its 14-methyl analog: two energetically stable conformations with preference of a twisted 12-s-cis conformation.[27]

Dipole moments of both *all-trans*-retinal and 11-*cis*-retinal were theoretically calculated by several authors.[15-18] There is general agreement that the 12-s-trans conformer of 11-*cis*-retinal is more polar than the 12-s-cis conformer. This conclusion was also reached by us from a different point of view (see above). Theoretically calculated dipole moments, however, largely depend on the method of approximation. For 11-*cis*-12-s-*trans*-retinal, dipole moments ranging from 2.65 D[15] to 7.86 D[17] were obtained; dipole moment differences between both conformers of 11-*cis*-retinal range from 0.35 D[15] to 2.2 D.[18] The calculated dipole moment of *all-trans*-retinal[17] is twice that found in our measurements.

The fact that the dipole moment of 11-*cis*-retinal is very close to that of *all-trans*-retinal suggests that the deviation of the polyene chain from planarity may be even smaller than theoretically predicted. This may indicate that solvent interactions relieve the steric hindrance. Solvent effects may also explain why the calculated conformation of 11-*cis*-retinal does not prevail in solution. It is remarkable that a polar conformation of 11-*cis*-retinal prevails even in a nonpolar solvent. The stability of a polar conformer relative to a less polar conformer is expected to increase with the polarity of the solvent.[16,17] As to the actual conformation of the chromophore, however, secondary interactions of the retinal residue with the specific, nonisotropic chromophore site of rhodopsin are likely to be of paramount importance.

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The Zero-Point-Average Structure of Bicyclo[3.1.0]hexane as Determined by Electron Diffraction and Microwave Spectroscopy[1a]

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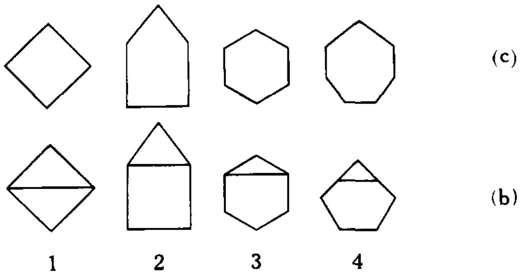
Abstract: The structure of bicyclo[3.1.0]hexane has been determined by a combined analysis of electron diffraction and microwave spectroscopic data. The molecule was observed to prefer the boat conformation with flap angles of 70.6° (1.1) and 25.2° (2.8) for the cyclopropane and cyclopentane moieties, respectively. On the basis of calculated vibrational amplitudes and a combined analysis including microwave rotational constants, the average C-C bond length, 1.524 (3) Å, was resolved into three distinct distances: C_1-C_5 = 1.454 (9) Å, C_1-C_6 = 1.515 (8) Å, and C_1-C_2, C_2-C_3 = 1.543 (4) Å. Although the bridge bond (C_1-C_5) appears to be quite short for a single bond, the bond lengths for the remaining bonds in the three- and five-membered rings are comparable to the corresponding parameters reported for cyclopropane (1.512 Å) and cyclopentane (1.546 Å).

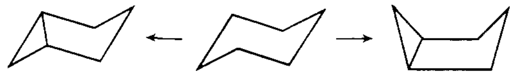
Introduction

It is interesting to speculate on the structural changes which arise from the introduction of a 1-3 intraannular C-C bond in the cyclic systems **1c**–**4c**. Of particular interest are the conformational differences between the resulting bicyclo[*n*.1.0]alkanes, **1b**–**4b**, and their cyclic counterparts.

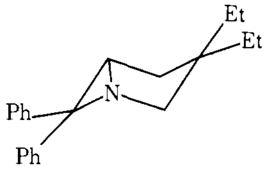


Precise comparisons, based on gas-phase structural data, are possible for the first two pairs: **1c**[2] with **1b**[3], and **2c**[4a] with **2b**.[4b] Structural data on **3c**[5] and **4b**[6] are also available; however, their respective analogs have not yet been investigated. Cycloheptane **4c**[7] is currently under investigation, and a comparison should be forthcoming for the fourth pair in this series. The purpose of this paper is to report on the structural parameters of **3b**, bicyclo[3.1.0]hexane (henceforth referred to as BCH).

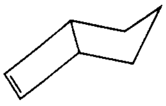
In the case of the six-membered rings **3b** and **3c**, one might propose two possible conformations for the bicyclo compound, namely a chair and and a boat form. In view of the preferred conformation of cyclohexane, one might expect **3b′** to be the more stable form for BCH, and, in fact an early x-ray study of 3,3-diethyl-6,6-diphenyl-1-azabicyclo[3.1.0]hexane[8] (**5**) found this to be the case. More recently, Chiang and Bauer[9] have also found a preference for the chair conformation in the related compound Δ^6-bicyclo[3.2.0]heptane (**6**).



3b′ 3c 3b″



5



6

Subsequent x-ray studies have shown, however, that several BCH derivatives, not substituted in the 3 and 6 positions, definitely prefer the boat conformation **3b″**.[10-13] The same conclusion has been reached for BCH itself on the basis of molecular mechanics calculations[14] and a recent microwave spectroscopic investigation.[15] In this latter study, however, a complete structural analysis was not possible since isotopic substitutions on the parent compound were not undertaken. We therefore felt that a combined microwave spectroscopic-electron diffraction investigation of BCH would be worthwhile in determining a precise geometry for this molecule. Such an

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