

# A Vibrational Analysis of Rhodopsin and Bacteriorhodopsin Chromophore Analogues: Resonance Raman and Infrared Spectroscopy of Chemically Modified Retinals and Schiff Bases<sup>†</sup>

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**ABSTRACT:** Resonance Raman spectroscopy has been used to study chemically modified retinal analogues involving chain substitutions, ring substitutions, or Schiff-base linkages. In addition, retinal fragments and fully deuterated retinals were investigated, and infrared spectra of the four isomers of retinal were obtained. Low-frequency resonance Raman spectra are also reported for all of the isomers of retinal, for the protonated

and unprotonated Schiff bases of *trans*-retinal, for  $\beta$ -ionone, and for *trans*-3-dehydroretinal. Band assignments were made to specific vibrational motions, and these assignments have led to a detailed understanding of the spectral features observed in the resonance Raman spectra of the retinylidene chromophore in rhodopsin and bacteriorhodopsin.

In order to understand in detail the resonance Raman spectra (RRS) of rhodopsin (Lewis et al., 1973; Lewis and Spoonhower, 1974; Oseroff and Callender, 1974; Sulkes et al., 1976; Callender et al., 1976; Mathies et al., 1976, 1977) and bacteriorhodopsin (Mendelsohn, 1973, 1976; Lewis et al., 1974; Marcus and Lewis, 1977; Lewis, 1977; Aton et al., 1977; Campion et al., 1977), we have obtained and compared the resonance Raman spectra of model compounds for the retinylidene chromophore. Specifically, we have studied a series of chemically modified retinal analogues and have combined this data with infrared spectra and the results of normal coordinate calculations reported by Cookingham (1978). In this manner, we have been able to localize the possible origin of many of the vibrational features observed in the RRS of retinal and its Schiff bases.

Robeson et al. (1955) were the first to investigate the vibrational properties of retinal. Subsequently, Rimai and co-workers (Rimai et al., 1971a,b, 1973; Gill et al., 1971; Heyde et al., 1971) studied the RRS of retinal isomers and the all-*trans* Schiff base. Rimai et al. (1973) attempted to explain their observations by comparing the results they obtained to the calculated frequencies for an infinite polyene chain (Rimai et al., 1973; Tric, 1969) and the observed vibrational spectra of other polyenes. Further work on the isomers of retinal in crystalline form (Cookingham et al., 1976; Callender et al., 1976) and protonated Schiff bases of retinal (Sulkes et al., 1976; Mathies et al., 1977) provided more model compound data but little insight into the origin of the spectral features. In addition to these investigations, Warshel and Karplus (1974) used the potential surfaces obtained from a semiempirical method to calculate the vibrational frequencies and relative RRS intensities for *trans*- and 11-*cis*-retinal. Finally, a resonance Raman investigation using 11-*cis*-retinal analogues with butyl substitutions (Cookingham and Lewis, 1978) showed that the scattering frequencies observed from the

methyl groups could be unequivocally assigned.

In this work, we have extended our previous investigation (Cookingham and Lewis, 1978) to include the entire resonance Raman spectrum of each of the isomers of retinal and its Schiff bases. To analyze these spectra, retinal analogues with two fundamentally different types of modifications have been studied. These modifications can be classified as either altering the ionone ring structure or perturbing the isoprenoid chain of the molecule. Thus, the presentation of results on the retinal analogues has been organized along these lines.

Figure 1 shows the structural formulas of retinal, several chemically modified retinals, a retinal Schiff base, and the two structural fragments of retinal that we have investigated. For comparison, we also present RRS of a methylated Schiff base and infrared spectra of retinal in its four most common isomeric configurations. All of the results obtained on the above molecules, together with an investigation of fully deuterated retinal isomers, were analyzed with the aid of normal coordinate calculations by Cookingham (1978). This has led to significant progress in our understanding of the vibrational spectra of model compounds for the retinylidene chromophore of rhodopsin and bacteriorhodopsin.

## Materials and Methods

The chemically modified retinals and 11-*cis*-retinal used in these experiments were the gifts of several generous donors. In most cases, the analogues were received in the *trans* form or as a mixture of isomers. The methods of isomerization, separation, purification, and identification are described in detail in an earlier work (Cookingham and Lewis, 1978). These methods are based on the separation of the isomers by liquid chromatography (Rotmans and Kropf, 1975; Ebrey et al., 1975). The identity of the conformation of the isomers of a given analogue was initially determined from the absorption spectrum. In the cases where published spectra were available, identification was made by direct comparison (Planta, et al., 1962). When no absorption spectra were available for comparison, the measured ratio of the absorption at  $\lambda_{\max}$  to the absorption at 254 nm was used to identify the conformation (as described in Cookingham and Lewis, 1978). A complete table of the spectral parameters used in identifying the isomers has been given by Cookingham (1978).

To further confirm the identifications made on the basis of

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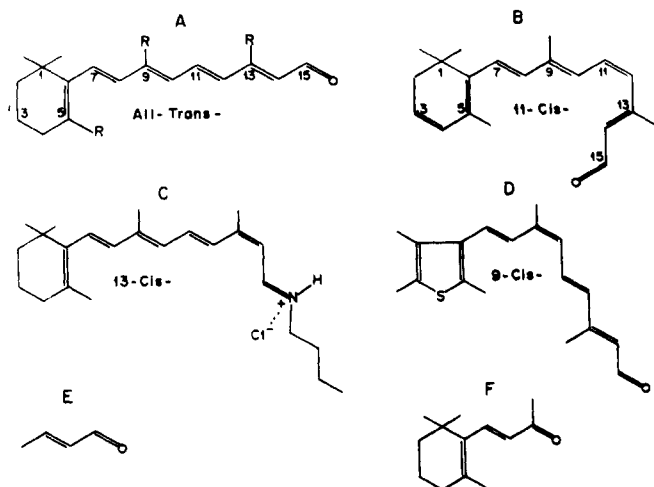


FIGURE 1: (A) *all-trans*-Retinal ( $R = \text{CH}_3$ ) (in analogue compounds,  $R = n\text{-butyl}$  or hydrogen); (B) 11-*cis*-3-dehydroretinal; (C) 13-*cis*-*N*-retinylidene-*n*-butylammonium hydrochloride ( $\text{N}^+\text{RB-HCl}$ ); (D) 9-*cis*-(*E,E,E*)-3,7-dimethyl-9-(2,4,5-trimethyl-3-thienyl)-2,4,6,8-nontetraenal (11-*cis*-sulfur); (E) *trans*-crotonaldehyde; (F)  $\beta$ -ionone.

absorption spectroscopy, we used the fingerprint region of the resonance Raman spectra which has been shown to be characteristic of the retinal isomeric configuration (Rimai et al., 1971a). We found that this was also the case for the analogues, since their fingerprint region had characteristics that were similar to the corresponding retinal conformer. In addition, nuclear magnetic resonance measurements were made on certain critical isomers of the butyl-substituted analogues. Some of the measured chemical shifts are tabulated and discussed in an earlier paper (Cookingham and Lewis, 1978). Our previous assignments were all confirmed by our resonance Raman data and these NMR investigations.

The isomers of retinal and the chemically modified retinals are photolabile, especially under the laser illumination necessary to observe a resonance Raman spectrum. As a result, sample handling and data collection techniques were developed that included liquid chromatographic analysis of the sample before and after all experiments. All measurements of the relative isomeric concentration of a given sample were made by comparing the integrated areas of the absorption vs. elution volume patterns and adjusting for the difference in absorption for the specific isomer at the observation wavelength.

The retinal analogue compounds presented special experimental problems because of the small amounts of material available, generally on the order of 500  $\mu\text{g}$ , from which the four isomers had to be isolated. For some analogues not all isomers could be isolated or even existed. After the separations had been effected, 20 to 40  $\mu\text{g}$  of the pure isomer was concentrated to dryness with a stream of argon and redissolved in 20  $\mu\text{L}$  of acetonitrile for each experiment. The sample was transferred with a syringe to a sample cell consisting of a 1.5-mm capillary tube closed at one end with an optical flat and at the other end with a cork. The sample concentrations were approximately 0.005 M for the mono-*cis* and 0.01 M for the trans isomers. Two samples were prepared of each isomer. A series of three resonance Raman spectra were taken on one sample, and one short spectrum was taken of the other sample. Little or no detectable isomerization or destruction of the retinal was observed upon liquid chromatographic analysis of the sample exposed for a short interval. By studying the time sequence of any impurity band in the series of three Raman spectra taken on the same sample, the development of any isomerization

TABLE I: Summary of Laser Destruction Tests.

sample	exposure time (h)	% destruction
11- <i>cis</i> -retinal ( $10^{-4}$ M)	2	$1.2 \pm 0.8$
	8	$8.7 \pm 1.0$
11- <i>cis</i> -retinal ( $10^{-3}$ M)	2	$1.8 \pm 1.4$
	8	$10.5 \pm 2.7$
9- <i>cis</i> -3-dehydroretinal	1.5	$4.0 \pm 2.9$
	6	$17.0 \pm 2.4$
13- <i>cis</i> -5-desmethylretinal	2.5	$18.0 \pm 2.7$
	10	$39.0 \pm 2.2$
13- <i>cis</i> -13-butylretinal	2	$13.0 \pm 1.1$
	10	$16.0 \pm 1.8$
13- <i>cis</i> -9-butylretinal	2	$0.0 \pm 1.5$
	10	$6.2 \pm 1.4$
<i>all-trans</i> -9-13-dibutylretinal	2.5	$0.0 \pm 2.8$
	10	$4.3 \pm 2.7$

could be followed in the Raman spectra and the impurity bands identified. Liquid chromatography served to identify the isomeric impurities and their relative concentrations after they were observed spectrally.

In the desmethyl samples, long-term laser radiation also caused the conversion of the aldehyde form of the samples to the alcohol form. This could be monitored by measuring the absorption spectrum of the sample after illumination and observing any changes in the absorption spectrum of retinal in the region of the blue-shifted alcohol absorption peaks at 310 and 330 nm. The appearance of a broadening of the main absorption band of the retinal isomers after a long series of three Raman spectral runs was detected, and some small changes were observed in the resonance Raman spectra that could be attributed to the appearance of an alcohol form in the 5- and 9-desmethyl compounds only.

We found that acetonitrile was the solvent of choice because photochemical conversion to the alcohol was approximately an order of magnitude slower in this solvent as compared, for example, to hexane or carbon tetrachloride. The above observation can be understood if polar solvents, such as acetonitrile, perturb the ordering of excited states in a manner which destabilizes the  $^1(n,\pi^*)$  state relative to the  $^1(\pi,\pi^*)$  state and thus decreases intersystem crossing into the triplet manifold. This would reduce the population of molecules in the  $^3(n,\pi^*)$  state from which photochemical conversion to the alcohol probably occurs.

The sample-handling techniques described above required changes in concentration as a part of the analysis procedure after the Raman spectra were obtained. Thus, for a further verification that large-scale destruction of the samples was not taking place, a dilute solution ( $\sim 10^{-4}$  M) of one of the isomers of each analogue sample was prepared. The solution was introduced into two sample cells without further concentrating relative to the remaining stock solution. The two cells were exposed to the same level of laser radiation under conditions identical to those maintained during a Raman experiment. One cell was exposed for the period of time required to rapidly scan the spectrum, and the other cell was exposed for the time required to obtain a sequence of three Raman spectra for that analogue. Liquid chromatography was then used to assay the concentration of the retinal analogue sample remaining in the cells relative to the stock solution. Table I shows the percentage destruction of the sample as a function of exposure time. In all cases, the sample concentration decreases as a function of time. In the case of both 13-*cis*-5-desmethyl- and 13-*cis*-13-butyl-

retinal where a larger percentage of material is destroyed or isomerized, a large change occurs in the shorter time interval, and at longer times a smaller percentage increase occurs as if an equilibrium were being approached. 11-*cis*-Retinal was run at two concentrations to see if any concentration effect could be observed, but the rate of sample decrease did not change significantly. In summary, the worst case estimates of sample loss by either destruction or isomerization are characterized by the 18% and 13% losses during the short experiments in 13-*cis*-5-dmR and 13-*cis*-13-BuR, respectively. Even in these cases, only one small shoulder on the C=C retinal-stretching vibration could be detected. Only bands that could be definitely identified as belonging to the retinal analogues are labeled in the spectra presented in this work.

Crystalline *all-trans*-*N*-retinylidene-*n*-butylamine (NRB) was prepared as previously reported (Sulkes et al., 1976). *N*-Retinylidene-*n*-butylammonium hydrochloride ( $N^+RB^-HCl$ ) was crystallized from a solution of the unprotonated Schiff base in acidified ethanol. The solution of *trans*- $N^+RB^-HCl$  was prepared by bubbling HCl gas through an ethanolic solution of the unprotonated Schiff base until the absorption spectrum showed complete protonation. The protonated Schiff bases of the 13-, 11-, and 9-*cis* isomers were formed in a similar manner. The *trans*- $N^+RBCH_3Br$  was prepared by condensing  $CH_3Br$  with solid *trans*-NRB, forming a solution. This was allowed to sit overnight in the dark at  $-15^\circ C$ , and the excess  $CH_3Br$  was then evaporated. The residue was dissolved in ethanol. The final solutions of the protonated Schiff bases had a 0.2 M concentration, and were allowed to flow at 2 mL/s through a 2-mm capillary tube to obtain the resonance Raman spectrum with minimum sample destruction (Hirsch et al., 1976; Mathies et al., 1976; Callender et al., 1976; Marcus and Lewis, 1977).

A resonance Raman spectrometer of our own design was used in all the Raman experiments described here. A krypton laser at 647.1 nm was employed to illuminate the retinal analogue samples, and a rhodamine 6G dye laser at 637.3 nm was used to irradiate the retinals. In each case, the laser beam power was 45 mW. The protonated Schiff base isomers were excited at 476.2 nm with 7 mW from a krypton ion laser. All of the crystalline samples were excited at 647.1 nm with 2 mW of laser power. The remaining details of our spectrometer are described elsewhere (Sulkes et al., 1976; Cookingham et al., 1976; Collins et al., 1977; Perreault et al., 1976).

Temperature-dependence measurements on the 11-*cis*-retinal Raman spectrum were made by cooling a 0.07 M solution of 11-*cis*-retinal in acetonitrile sealed in a quartz capillary and observing the Raman spectrum as a function of temperature. A Lakeshore Cryotronics Spectrim cryostat was used to maintain the desired temperature. All spectra were excited at 647.1 nm with a krypton laser and observed with  $2\text{-cm}^{-1}$  resolution and a  $1\text{-cm}^{-1}$  step size. The integrated areas were calculated from the digital data collected according to Durant's rule. Two different standards were used to measure the increase in intensity—the  $918\text{-cm}^{-1}$  band of acetonitrile and the  $1576\text{-cm}^{-1}$  ethylenic stretch in 11-*cis*-retinal.

The infrared absorption measurements reported in this work were made on a Perkin-Elmer Model 621 double-beam grating spectrophotometer. The reference beam was attenuated to achieve the maximum pen response. Consequently, the measurements are not quantitative in absolute absorption. The samples were prepared by placing a single drop of 0.8 M retinal in ethanol on an Irtran II plate and allowing it to dry to a thin film. The retinal samples were redissolved in ethanol after the measurements, and LC analysis indicated no isomerization had occurred during the experiment.

## Results and Discussion

The data collected during this study are presented in Figures 2–15 and Figure 16 of the Supplementary Material. The organization is such that Figures 2 to 5 contain all of the retinal analogues with perturbations that occur along the isoprenoid chain, and the figures are arranged by isomer. Figures 6 to 9 compare all of the analogues that have perturbations in the ring structure. The retinal fragments are shown in Figure 10. The deuterated retinal and the protonated and unprotonated Schiff base spectra are arranged with all of the isomers in one figure (Figures 11–13, respectively). Figure 16 (see paragraph concerning Supplementary Material) compares the RRS of each retinal isomer with its corresponding infrared spectrum. The discussion of the results is organized such that a single retinal vibrational Raman band or group of bands will be discussed for all the isomers of retinal with reference to the pertinent data from the analogues, Schiff bases, infrared spectra and previously published normal coordinate calculations (Cookingham, 1978). This format will be used throughout the discussion. All of the solution spectra presented in this paper were studied in acetonitrile with the exception of the 9-*cis*-13-dmR spectrum which is shown in  $CCl_4$ . When reference is made to a Raman vibration of retinal in  $CCl_4$ , the reader should consult the work of Rimai (Rimai et al., 1971a; Gill et al., 1971; Heyde et al., 1971), all of which was verified in our own work. A summary of the suggested assignments for the isomers of retinal is presented in Table II. The subheadings in the discussion section are keyed to the suggested assignment column of Table II for easy cross-reference. The low-frequency crystalline data shown in Figure 15 will be discussed separately.

**The  $1620\text{--}1700\text{-cm}^{-1}$  Region: C=O Stretch.** The vibrations that occur in this region of the Raman spectrum have been shown to be due to the end-group vibration. In retinal this is a C=O vibration and in the Schiff bases it is a  $C=N^+$  or  $C=N$  vibration. The remarkable feature of this Raman vibration is that it is very constant in frequency ( $1656\text{--}1659$ ) for a given solvent, for all isomers of retinal and in all retinal analogues, with only two exceptions. In both 13-dmR analogues (Figures 2F and 3F), the removal of the C(13) methyl group correlates with an increase of approximately  $17\text{ cm}^{-1}$  in the C=O vibration in both  $CCl_4$  and  $CH_3CN$ . In addition, the C=O vibration is solvent dependent ( $CH_3CN$   $1655\text{--}1659$  and  $CCl_4$   $1666\text{--}1668$ ), as one would expect for a polar end group such as the oxygen of an aldehyde.

A small but consistent increase in the C=O stretch of about  $2\text{ cm}^{-1}$  can be noted for all 13-*cis*-isomers compared to the other *cis* and *trans* isomers of a similar compound. No noticeable frequency shift can be seen for a butyl substitution at either C(9) or C(13). This evidence suggests that butyl groups play a role similar to the methyl groups in determining either the electronic distribution in the polyene or the allowed normal mode of vibration at this frequency. Replacing the C(13) methyl with a hydrogen alters either the electronic distribution such that the electron density in the C=O bond (and consequently the frequency of the C=O vibration) increases or the frequency of the allowed normal mode of vibration is altered because of the reduced mass of the hydrogen. In the 13-*cis* isomer, the *cis* bend between the C(13) and the C=O bond as well as possible single-bond rotations about C(14)–C(15) may have a much smaller but similar effect (i.e., increasing the frequency) by partially isolating the C=O bond from C(13) methyl effects and the remainder of the polyene. This could be the cause of the  $2\text{-cm}^{-1}$  increase in frequency observed in this isomer. However, the frequency of the C=O in fully deuterated isomers of retinal occurs between 1637 and 1641

TABLE II: Suggested Vibrational Assignments.

description of vibration	frequencies (cm <sup>-1</sup> )			
	trans	13-cis	11-cis	9-cis
C=O stretch	1656	1659	1658	1656
C=C stretch	1577 1568	1584 1573	1576	1586
C(9) and C(13) asymm Me deformation	1448 (CCl <sub>4</sub> )	1448 (CCl <sub>4</sub> )	1448 (CCl <sub>4</sub> ) 1431 (CCl <sub>4</sub> )	1446 (CCl <sub>4</sub> )
C(9) symm Me deformation	1388 (CCl <sub>4</sub> )	1399 (CCl <sub>4</sub> )	1387 (CCl <sub>4</sub> )	1402 (CCl <sub>4</sub> ) 1374 (CCl <sub>4</sub> )
C(1) <i>gem</i> -Me <sub>2</sub>	1387 (IR) 1362 (IR)	1380 (IR) 1362 (IR)	1375 (IR) 1358 (IR)	1379 (IR) 1360 (IR)
C(13) symm Me deformation	1337	1352 1316	1345	1337 1329
C—C—H bend + C=C stretch or C—C stretch	1282 1272	1282 1274  1222	1271	1295 1280
C—C stretch, C(9) and C(13) Me rock	1198	1193	1219 1206	1216 1201
C—C [C(9) to C(13)] stretch	1163	1163	1143	1147
C(14)—C(15)		1118	1128	1117
C(9)—CH <sub>3</sub> stretch and C(13)—CH <sub>3</sub> stretch	1009	1012	1017 997	1009
C—C—H bend, out of plane [C(5)—CH <sub>3</sub> stretch?]	970	969	970	963

cm<sup>-1</sup>. This effect is less than one would expect on the basis of a simple deuterium substitution and indicates, as one would expect, that the C=O vibration is not totally isolated but is coupled in some manner to the remainder of the polyene.

The C=O stretching frequencies in the fragments  $\beta$ -ionone and crotonaldehyde are interesting (Figures 10A,B). In  $\beta$ -ionone this frequency occurs at 1668 cm<sup>-1</sup> and is lower than an isolated C=O vibration which is observed at 1695 cm<sup>-1</sup> in crotonaldehyde where relatively little delocalization of  $\pi$  electron density can occur. The 1668-cm<sup>-1</sup> band in  $\beta$ -ionone and 1695-cm<sup>-1</sup> shoulder in the Raman spectrum of crotonaldehyde are both the most intense bands in the infrared spectra (unpublished observations), which supports the suggestion that these two vibrations are the C=O stretches.

The protonated Schiff bases have a C=N<sup>+</sup>H end group and a vibrational frequency at 1655 cm<sup>-1</sup>. When the Schiff base is formed from a methyl halide instead of a halogen acid, the methyl group attached to the nitrogen causes a frequency shift to 1630 cm<sup>-1</sup>. Unlike the retinals, the 13-cis isomer does not have a consistently higher end-group frequency. The unprotonated Schiff bases are similar to the protonated Schiff bases in this respect.

The infrared vibrational measurements all exhibit a band at about 1660 cm<sup>-1</sup> as the most intense band in the spectrum for each isomer. This vibration should be attributed to the C=O stretch, because the nature of infrared absorption causes a highly polar bond such as a C=O to have a very strong IR absorption.

**The 1500–1620-cm<sup>-1</sup> Region: C=C Stretch.** The C=C stretching mode between 1500 and 1600 cm<sup>-1</sup> is the most intense band in the *resonance Raman spectrum* of retinal, unlike the weak C=O stretching mode previously discussed. It has the greatest amount of resonance enhancement, since it is the vibrational mode with the largest Franck–Condon overlap

between the electronic ground and excited states (Warshel and Karplus, 1974). In contrast, the *nonresonance Raman spectrum* of the fragment crotonaldehyde (Figure 10B) shows a C=O stretching mode (1695 cm<sup>-1</sup>) of similar intensity to the C=C ethylenic mode at 1642 cm<sup>-1</sup> in this molecule (see Figure 10B). This intensity profile is also observed in the *infrared* spectra of the retinal isomers (Figures 16A–16E; see paragraph at the end of this paper concerning Supplementary Material). The frequency of the C=C stretching mode does appear to be sensitive to solvent (Heyde et al., 1971). Our data show that it also varies in position and shape with geometry of the isomers and with substitutions along the chain and in the ring. In general, trans isomers (Figures 2, 6, and 11–13) have C=C stretching modes which are low in frequency compared to 9-cis and 13-cis isomers. The 9-cis isomers (Figures 3, 7, and 11–13) almost always have the highest frequency C=C stretching bands for a given analogue. The 13-cis isomers (Figures 4, 8, and 11–13) have the next highest frequency C=C bands, and they show considerable structure. The observation can be made here that the highest frequency C=C bands occur in the isomers which have a cis bend at a point where there is a methyl group attached to the chain. This highlights once again the fact that the methyl groups are tied into the  $\pi$  electron distribution in the chain. The C=C stretching mode in the 11-cis isomers (Figures 5, 9, 11–13) is equal to or lower in frequency than in the trans isomers. This is especially noticeable in the sulfur analogues (Figure 9).

**Trans Isomer.** An examination of the trans isomers where chain substitutions are predominant (Figure 2) shows very little change in the C=C stretching mode when butyl groups are substituted for methyl groups. However, when the C(9) methyl group is replaced with a hydrogen, there is a splitting in this mode, with the new peaks higher and lower than the 1577-cm<sup>-1</sup> band in *trans*-retinal. In the *trans*-13-dmR analogue,

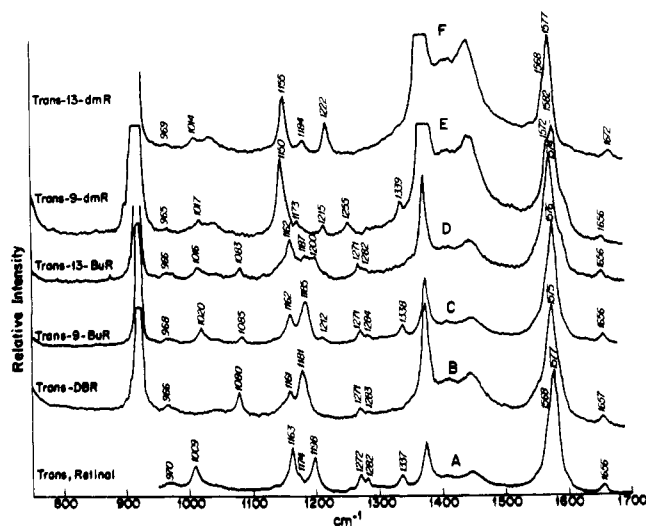


FIGURE 2: Resonance Raman spectra of (A) *trans*-retinal, (B) *trans*-9,13-di-*n*-butylretinal (*trans*-DBR), (C) *trans*-9-*n*-butyl-13-methylretinal (*trans*-9-BuR), (D) *trans*-13-*n*-butyl-9-methylretinal (*trans*-13-BuR), (E) *trans*-9-desmethylretinal (*trans*-9-dmR), and (F) *trans*-13-desmethylretinal (*trans*-13-dmR) in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ ; all positions are accurate to  $\pm 2\text{ cm}^{-1}$ . The solvent bands of  $\text{CH}_3\text{CN}$  have not been removed from the spectra; these bands occur at 918, 1039, 1375, 1412, and  $1447\text{ cm}^{-1}$  and are unlabeled. The apparent intensities of these bands relative to the intensities of the retinal bands vary from spectrum to spectrum, depending on the concentration of the retinal.

this region of the vibrational spectrum is very similar to *trans*-retinal, except for a more predominant shoulder at  $1568\text{ cm}^{-1}$ . Structure in this vibrational mode is also observed in fully deuterated *trans*-retinal along with a lowering of the  $\text{C}=\text{C}$  vibrational frequency, as would be expected with the increase in mass (Figure 11A).

The retinal analogues which are modified in the ring also exhibit some changes in the  $\text{C}=\text{C}$  stretching mode. The downshift of the  $\text{C}=\text{C}$  band in *trans*-5-dmR (Figure 6B) is typical of all the isomers of this analogue. When no C(5) methyl group is present, a steric hindrance between this methyl group and the hydrogen at C(8) is removed, and the ring can move into the same plane as the chain. The  $\text{C}(5)=\text{C}(6)$  bond is now further conjugated with the isoprenoid chain, providing more delocalization and a decrease in the  $\text{C}=\text{C}$  stretching mode. (A concomitant 3–5-nm increase in  $\lambda_{\text{max}}$  of all the 5-dmR analogues was observed, as would be expected from the correlation of  $\lambda_{\text{max}}$  and the  $\nu_{\text{C}=\text{C}}$  stretching frequency (Heyde et al., 1971).)

The major effect of adding another double bond to the ring in *trans*-3-dehydroretinal (Figure 6C) was the addition of a strong shoulder on the low-frequency side. The other isomers of this analogue also show considerable structure in this region. It is possible that the observation of two bands in *trans*-3-dehydroretinal is due to the presence of a second delocalized  $\pi$ -electron system within the ionone ring. In support of this argument, X-ray crystallography has shown that there is a  $59^\circ$  out-of-plane rotation of the ring about the  $\text{C}(6)-\text{C}(7)$  single bond (Hamanaka et al., 1972) which partially isolates the ring and chain double bonds from each other. This is also supported by evidence from model compounds for the ionone ring. The addition of a second double bond in these model compounds, for example, compare cyclohexene and cyclohexadiene, creates a delocalized  $\pi$ -electron system in the cyclohexadiene ring. The  $\text{C}=\text{C}$  stretching frequency in these molecules is lowered from  $1653\text{ cm}^{-1}$  in cyclohexene (Cleveland, 1943) to  $1575\text{ cm}^{-1}$  in cyclohexadiene (DiLauro et al., 1969). However, the spectrum of  $\beta$ -ionone indicates that there is, in addition,  $\pi$ -electron de-

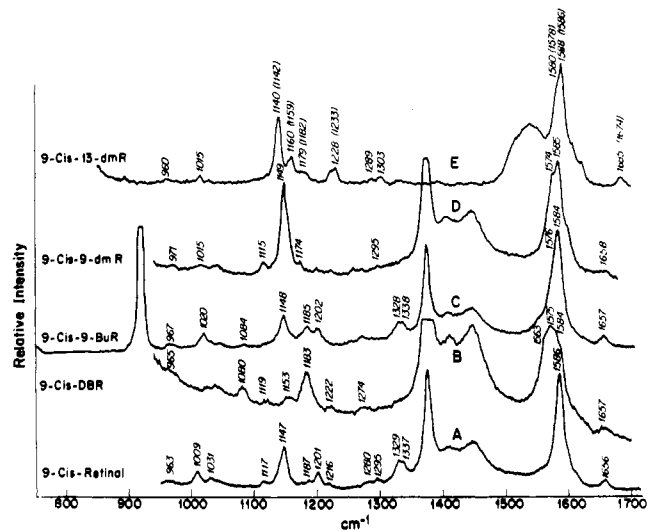


FIGURE 3: Resonance Raman spectra of (A) 9-*cis*-retinal, (B) 9-*cis*-DBR, (C) 9-*cis*-9-BuR, (D) 9-*cis*-9-dmR, and (E) 9-*cis*-13-dmR in  $\text{CH}_3\text{CN}$ . Spectrum E was measured in  $\text{CCl}_4$ . A  $\text{CCl}_4$  band is visible at  $1534\text{ cm}^{-1}$  and is not labeled. Values in parentheses for intense peaks are those measured in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ , and band positions are accurate to  $\pm 2\text{ cm}^{-1}$ .

localization between the ring and the chain. This molecule exhibits two  $\text{C}=\text{C}$  stretching bands at  $1587$  and  $1606\text{ cm}^{-1}$ , one from the ring and one from the chain. Both these frequencies are lower than one would expect for an isolated ring double bond (cyclohexene) or an isolated chain double bond (ethylene). This illustrates that there is partial delocalization of the  $\pi$  electrons between the ring and the chain even in the presence of a  $\text{C}(6)-\text{C}(7)$  twist similar to *trans*-retinal. Thus, it is conceivable that the shoulder in *trans*-3-dehydroretinal at  $1564\text{ cm}^{-1}$  is due to a cyclohexadiene-like  $\text{C}=\text{C}$  stretch with an additional lowering of the frequency by  $11\text{ cm}^{-1}$  due to partial conjugation with the isoprenoid chain.

Another retinal analogue studied was one with two double bonds in a five-membered ring that included a sulfur atom. In this molecule, the  $\text{C}(5)=\text{C}(6)$  double bond and the  $\text{C}(5)$  methyl group are intact compared to unmodified retinals, even though the remainder of the ring is radically different (Figure 1D). The additional double bond in the ring of the sulfur analogue cannot be a part of the conjugation. Thus, there is little effect on the  $\text{C}=\text{C}$  stretching mode in the *trans* isomer of this analogue (Figure 6D). There is an extra band seen between  $1610$  and  $1620\text{ cm}^{-1}$  in all isomers of the sulfur compound, and this may be due to the altered ring structure.

**9-Cis Isomer.** Spectra of analogues of the 9-*cis* isomer which involve chain effects are shown in Figure 3. The substitution of butyl groups in both the C(9) and C(13) positions causes considerable broadening in the  $\text{C}=\text{C}$  band. There appear to be three bands, with two new ones at lower frequency. It is not immediately obvious what the reason for this is, but perhaps it is a mass effect which shows up particularly in 9- and 13-*cis* isomers. These isomers also show the most splitting in the  $\text{C}=\text{C}$  stretching mode of the fully deuterated analogues (Figure 11B,D). The 9-*cis*-9-dmR shows the same splitting as discussed earlier for the *trans* isomer. The ring effects in 9-*cis* isomer analogues (Figure 7) are similar to those observed in *trans* isomers, with a down shift in the  $\text{C}=\text{C}$  band in 9-*cis*-5-dmR, structure in 3-dehydroretinal, and a narrow, symmetric band in the sulfur analogue.

**13-Cis Isomer.** As mentioned before, all 13-*cis* isomers have structure in the  $\text{C}=\text{C}$  band (Figures 4 and 8). When a butyl is substituted in the C(13) position, the low-frequency shoulder at  $1571\text{ cm}^{-1}$  which shows up in the other spectra becomes

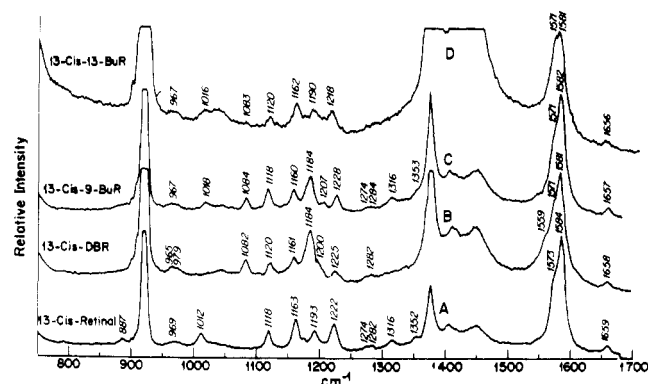


FIGURE 4: Resonance Raman spectra of (A) 13-*cis*-retinal, (B) 13-*cis*-DBR, (C) 13-*cis*-9-BuR, and (D) 13-*cis*-13-BuR in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ , and band positions are accurate to  $\pm 2\text{ cm}^{-1}$ .

almost equal in intensity to the band at  $1581\text{--}1584\text{ cm}^{-1}$ . In the spectrum of 13-*cis*-5-dmR (Figure 8B), the  $\text{C}=\text{C}$  band has shifted down as in the other isomers of 5-dmR. However, in the 13-*cis* isomer there is still a high-frequency shoulder at  $1586\text{ cm}^{-1}$ ; perhaps a bend in the chain at the C(13) position isolates one  $\text{C}=\text{C}$  bond. The 13-*cis*-3-dehydroretinal has at least three bands in the  $\text{C}=\text{C}$  region, one more than in the other 13-*cis* analogues. The normal two-band structure of 13-*cis* (one a shoulder) is seen in the sulfur analogue, and the two bands are easily seen in 13-*cis*-[ $^2\text{H}$ ]retinal (Figure 11B).

**11-*Cis* Isomer.** Unlike the other retinal isomers, the 11-*cis* configuration can attain two distinct conformational states 12-*s-cis* and 12-*s-trans* by rotation around the C(12)–C(13) single bond. However, certain butyl substitutions such as 11-*cis*-13-BuR and 11-*cis*-DBR (Figure 5) force this isomer into the 12-*s-cis* configuration (Cookingham and Lewis, 1978). These two analogues have lower  $\text{C}=\text{C}$  bands than the 11-*cis*-retinal and 11-*cis*-9-BuR, both of which can also be in the 12-*s-trans* configuration. The 11-*cis*-3-dehydroretinal (Figure 9B) has a very broad  $\text{C}=\text{C}$  band with four overlapping peaks, more than we have observed for the addition, in the other isomers, of another double bond in the ring. It may be that the 12-*s-trans*, 12-*s-cis* equilibrium contributes to this effect also. 11-*cis*-[ $^2\text{H}$ ]Retinal is similar to 11-*cis*-retinal except for the lower frequency due to mass effects (Figure 11C).

**Infrared Spectra.** A comparison of the  $\text{C}=\text{C}$  stretching vibrations in the infrared spectra with the corresponding bands in the resonance Raman spectra shows the dramatic decrease in intensity of this band in the infrared (Figures 16A–E; see paragraph at the end of this paper concerning Supplementary Material). The different selection rules in infrared and Raman as well as the effect of resonance enhancement in the Raman should account for this observation. In *trans*-, 9-*cis*-, and 11-*cis*-retinal, the band profiles are very similar in infrared and RRS. Structure is seen in the 13-*cis*-retinal infrared spectrum, just as in the resonance Raman spectrum. There is also considerable structure in the *trans*-3-dehydroretinal infrared spectrum as in the Raman.

**Schiff Bases.** The  $\text{C}=\text{C}$  stretching regions of the protonated Schiff bases are lower in frequency than in retinal, as would be expected with their red-shifted absorption spectra (Heyde et al., 1971). However, as in retinal and the unprotonated Schiff bases, it is the 9-*cis* isomer which has the highest frequency band, with 13-*cis* the next highest and *trans* and 11-*cis* the lowest. The protonated Schiff-base 9-*cis* isomer does have some structure with a low-frequency shoulder. The trans-methylated Schiff base has a  $\text{C}=\text{C}$  band similar in frequency

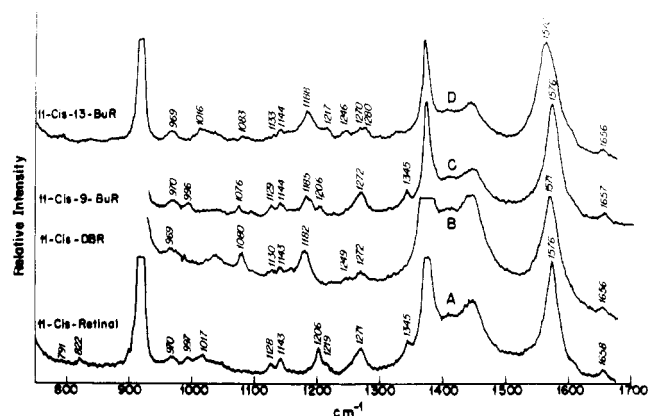


FIGURE 5: Resonance Raman spectra of (A) 11-*cis*-retinal, (B) 11-*cis*-DBR, (C) 11-*cis*-9-BuR, and (D) 11-*cis*-13-BuR in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$  and positions are accurate to  $\pm 2\text{ cm}^{-1}$ . The weak band at  $1039\text{ cm}^{-1}$  is a  $\text{CH}_3\text{CN}$  solvent band visible because of the low concentration of the retinal analogue.

to the protonated Schiff base.

Normal coordinate calculations (Cookingham, 1978) have been able to reproduce most of the above trends. Furthermore, in the 9-*cis* and *trans* isomers of 9- and 13-dmR the calculations suggested that the splittings or shoulders experimentally observed in these isomers may result from a carbon–carbon–hydrogen bend contribution involving the hydrogen at C(9) or C(13) and the nearest double bond. This contribution alters the two lowest energy  $\text{C}=\text{C}$  normal modes.

**The 1300–1500- $\text{cm}^{-1}$  Region: Methyl Deformations.** The frequency region from 1300 to  $1500\text{ cm}^{-1}$  will be considered next because a group of vibrational bands are present which appear to have a similar origin. Unfortunately, the solvent acetonitrile masks some of the weak Raman bands of the analogues between 1380 and  $1500\text{ cm}^{-1}$ ; however, the  $\text{CCl}_4$  spectra of Rimai (Rimai et al., 1971a; Gill et al., 1971) reproduced in this study do show the Raman bands of the retinal isomers at these frequencies. The infrared spectra (Figures 16A–16E; Supplementary Material) show considerable structure in this region and the bands are moderately intense.

**C(9) and C(13) Asymmetric Methyl Deformation.** A band between  $1445$  and  $1448\text{ cm}^{-1}$  was observed by Rimai in the Raman spectrum of each of the retinal isomers. In the 11-*cis* isomer an additional Raman band was observed at  $1431\text{ cm}^{-1}$  which was less intense than the other  $1448\text{-cm}^{-1}$  Raman vibration. The original suggestion of Rimai and co-workers (Rimai et al., 1971a; Gill et al., 1971) that this is an antisymmetric deformation within the C(9) and C(13) methyl groups is probably correct, and the observations can be explained as follows. Both methyl group deformations occur at the same frequency and are degenerate in 9-*cis*, 13-*cis*, and *trans* isomers. On the other hand, in 11-*cis*-retinal one deformation frequency may be lowered, since the environments of the C(9) and C(13) methyl groups are different because of the out-of-plane rotation about the C(12)–C(13) single bond (Gilardi et al., 1972) and steric interference from the hydrogen on C(10) in 12-*s-trans* or C(12) in 12-*s-cis*. A similar effect has already been shown to lower the frequency of the carbon methyl stretch for the C(13) methyl by  $20\text{ cm}^{-1}$  in 11-*cis*-retinal (Cookingham and Lewis, 1978). Further support for the above assignments can be found in compounds like acetonitrile and methanethiol, which have been shown to have antisymmetric methyl deformations at  $1440$  and  $1444\text{ cm}^{-1}$ , respectively (Herzberg, 1945; May and Pace, 1968). In addition, the Raman scattering from these vibrations in  $\text{CH}_3\text{CN}$

and  $\text{CH}_3\text{SH}$  is generally weak, while the infrared absorption is moderately intense (Sadler Standard Spectra, 1972; May and Pace, 1968), as is observed for retinal (see Figure 16).

**C(13) Symmetric Methyl Deformation.** For each of the four retinal isomers a band or pair of bands is present in the RRS only when a methyl group is attached to the C(13) position. These bands are located at the following frequencies: trans, 1337; 13-cis, 1316 and 1352; 11-cis, 1345; and 9-cis, 1329 and 1337  $\text{cm}^{-1}$ . In all of the butyl-substituted analogues where the C(13) methyl has been replaced by a butyl group, the above band or bands do not appear. In the desmethyl analogues these bands are absent when the methyl group is removed from the C(13) position with the exception of the 9-cis-9-dmR analogue where neither a 1329- or 1337- $\text{cm}^{-1}$  band appears. These bands are also present in the infrared spectra for the respective isomers at: trans, 1333  $\text{cm}^{-1}$ ; 13-cis, 1309 and 1351  $\text{cm}^{-1}$ ; 11-cis, 1338  $\text{cm}^{-1}$ ; and 9-cis, 1334  $\text{cm}^{-1}$  (see Figure 16).

The 1345- $\text{cm}^{-1}$  band in the 11-cis isomer can be seen in Figure 14 to be temperature dependent. It increases in intensity by approximately 25% on cooling from 25 to  $-45^\circ\text{C}$ . This increase is consistent with the alteration in the 11-cis, 12-s-cis/12-s-trans equilibrium from 40% 12-s-trans at  $25^\circ\text{C}$  to 50% at  $-45^\circ\text{C}$ . These percentages are based on the temperature dependence of this equilibrium which was discovered experimentally with NMR measurements (Rowan et al., 1974) and later verified by calculations (Birge et al., 1976). The resonance Raman temperature measurements indicate that the 1345- $\text{cm}^{-1}$  band is probably present only in the 11-cis-12-s-trans conformer. The observation that the 1345- $\text{cm}^{-1}$  band is missing in all cases where a butyl group occupies the C(13) position (Figure 5B,D) also supports this assignment because these molecules cannot attain the 12-s-trans conformation with a butyl group at the C(13) position. It should be noted, however, that this retinal band cannot be used to monitor the conformation (12-s-cis or 12-s-trans) of the retinylidene chromophore in rhodopsin, since it is sensitive to environmental factors (Cookingham, 1978) and is altered by the nature of the end group.

In summary, based on the above temperature dependence and the effect of chemical modification of these bands, we can assign the vibrational modes in this region to a symmetric deformation of the methyl group at C(13). This assignment is also supported by data on other molecules containing methyl groups, since previous workers (Herzberg, 1968; May and Pace, 1968) have assigned bands at 1376  $\text{cm}^{-1}$  in  $\text{CH}_3\text{CN}$  and 1332  $\text{cm}^{-1}$  in  $\text{CH}_3\text{SH}$  to symmetric methyl deformations.

**gem-Dimethyl.** The infrared spectra of all the retinal isomers contain two moderately strong bands (trans, 1362 and 1387; 13-cis, 1362 and 1380; 11-cis, 1358 and 1375; 9-cis, 1360 and 1379  $\text{cm}^{-1}$ ) which are known to be characteristic of a gem-dimethyl conformation where two methyl groups are attached to the same carbon atom (Silverstein et al., 1974). The doublet structure is due to an in-phase and out of phase interaction between the two methyl groups. Since the ionone ring in retinal contains a gem-dimethyl conformation on C(1), moderate infrared bands would be observed. These bands are not observed in the RRS because the vibrations are localized near the C(1) position, which is well away from the conjugated portion of the molecule that gives rise to the resonance enhancement of the Raman bands.

**C(9) Symmetric Methyl Deformation.** One other weak band is consistently observed in all retinal isomers in this region in both the RRS in  $\text{CCl}_4$  and the infrared. This vibration is a single band in all isomers except 9-cis. In the 9-cis isomer, the Raman frequencies are at 1402 and 1374  $\text{cm}^{-1}$  and the infrared bands occur at 1401  $\text{cm}^{-1}$  and a broad but unresolved

band at 1379  $\text{cm}^{-1}$ , which also contains a contribution from the gem-dimethyl doublet. The Raman and infrared frequencies for the other isomers are: trans, 1388 (R) and 1402 (IR); 13-cis, 1399 (R) and 1400 (IR); and 11-cis, 1387 (R) and 1382 (IR). The occurrence of two bands in the 9-cis isomer lends some support to the assignment of this vibrational mode to a C(9) symmetric methyl deformation, since the cis bend in this isomer may be causing the observed splitting by altering the coupling of normal modes.

**The 1300–1240- $\text{cm}^{-1}$  Region: C–C–H Bend + C=C or C–C Stretch.** An examination of Figures 5 and 9 shows that there is a single, intense band at  $\sim 1271 \text{ cm}^{-1}$  in all 11-cis analogues of retinal which are capable of being in the 12-s-trans conformation. When the equilibrium between 12-s-trans and 12-s-cis is perturbed chemically by forcing 11-cis analogues into the 12-s-cis conformation (as in 11-cis-DBR and 11-cis-13-BuR), this band becomes weaker in intensity and appears to be split. This is also the only band besides the 1345- $\text{cm}^{-1}$  peak in the spectrum of 11-cis-retinal which is temperature dependent (Figure 14). Warshel and Karplus (1974) suggested that bands in this region have contributions from in-plane C–C–H bends and C–C single-bond stretches. Normal coordinate calculations (Cookingham, 1978) indicate that normal modes which mix C–C–H deformations with C=C stretching vibrations would be observed at these frequencies. The admixture of double-bond stretching would probably account for the fact that these bands are observed in the resonance Raman spectrum. Changes made at the aldehyde end of 11-cis-retinal do not affect this band, as is evidenced by its presence in the 11-cis Schiff bases (Figures 12D and 13C). Changes in the ring end of the molecule in 11-cis-3-dehydroretinal and the 11-cis sulfur derivative (Figure 9) do not change the position or intensity of the band either. However, changes in the middle of the molecule effected by a change in conformation to trans, 9-cis, or 13-cis cause the band to become weaker or split. The infrared spectra of the four retinal isomers (Figure 16; Supplementary Material) confirm these observations. Raman bands in this region are not very intense in the 9- and 13-cis-retinals and their analogues, although some distinct features can be seen in analogues of the trans isomer (Figure 2). There is a consistent doublet in trans-retinal and butyl-substituted trans analogues, but trans-3-dehydroretinal (Figure 6C) does not show this doublet. The additional double bond in trans-3-dehydroretinal affects the C=C stretching region and thus could also alter the above normal mode which is an admixture of the in-plane hydrogen bending and C=C stretching vibrations.

The above experimental observations locate the origin of this vibrational mode in the central portion of the isoprenoid chain. This may suggest an explanation for the single intense band in 11-cis-retinal and its analogues that can exist in a 12-s-trans conformation. Retinals in this conformation approximate  $C_{2v}$  symmetry from C(5) through C(13), and this symmetry could contribute to the intensity of the single band observed in this region of the 11-cis-retinal spectrum.

It is interesting to note that the work done on rhodopsin (Sulkes et al., 1976; Callender et al., 1976; Mathies et al., 1976) shows a single, high-intensity band in the resonance Raman spectrum of rhodopsin at 1271  $\text{cm}^{-1}$  as is observed in our spectra of 11-cis-retinals and Schiff bases. In addition, two bands are observed at 1275 and 1296  $\text{cm}^{-1}$  in the spectrum of isorhodopsin (Mathies et al., 1976) as our data on 9-cis isomers indicate. Furthermore, modelling of squid acid metarhodopsin (Sulkes et al., 1976) shows the trans doublet at 1273 and 1287  $\text{cm}^{-1}$  as discussed above. Thus, this region of the retinal spectrum appears to be unperturbed by the nature of the end



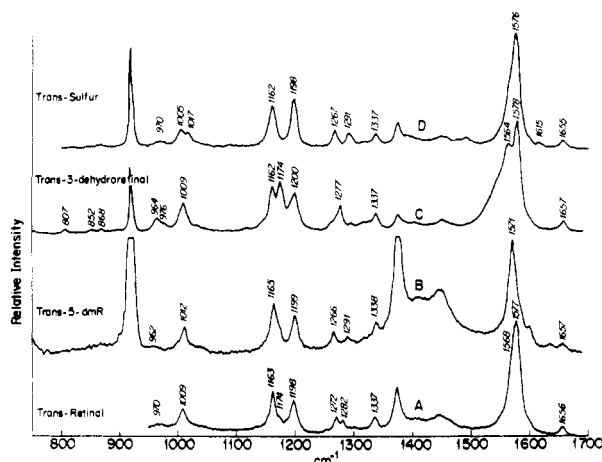


FIGURE 6: Resonance Raman spectra of (A) *trans*-retinal, (B) *trans*-5-desmethylretinal (*trans*-5-dmR), (C) *trans*-3-dehydroretinal, and (D) *trans*-sulfur-retinal (*trans*-sulfur) in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ , and positions are accurate to  $\pm 2\text{ cm}^{-1}$ .

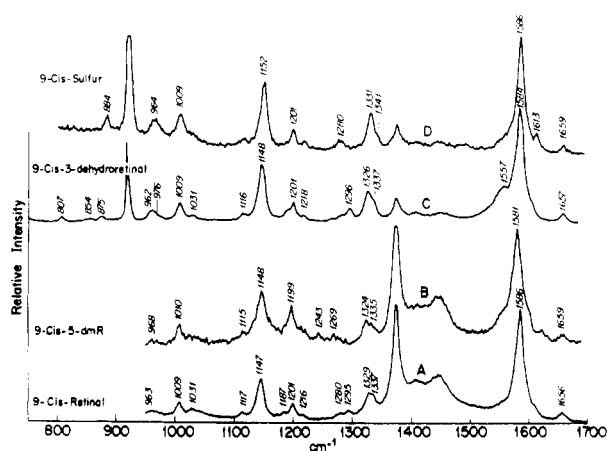


FIGURE 7: Resonance Raman spectra of (A) 9-*cis*-retinal, (B) 9-*cis*-5-dmR, (C) 9-*cis*-3-dehydroretinal, and (D) 9-*cis*-sulfur in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ , and band positions are accurate to  $\pm 2\text{ cm}^{-1}$ .

group and can be used to model visual pigments and possibly even bacteriorhodopsin. Therefore, on the basis of these observations we suggest that the strong  $1271\text{-cm}^{-1}$  band in rhodopsins indicates that the conformation of the 11-*cis*-retinylidene chromophore in opsin is 12-*s-trans*.

**The  $1240\text{--}1100\text{-cm}^{-1}$  Region: Fingerprint.** The region of the resonance Raman spectrum between  $1100$  and  $1240\text{ cm}^{-1}$  has been called the fingerprint region because the distinctive spectral features observed are characteristic for each isomer (Rimai et al., 1971a). Within this region of the retinal spectrum four different general types of vibrations appear to occur, depending on the geometry and structure of the molecule. In this section, the discussion will center on explaining how the retinal analogues were used to isolate the origin of these vibrations.

**C-C Stretch,  $\text{CH}_3$  Rock.** The first group of bands that appears to be affected by similar structural substitutions occurs between  $1180$  and  $1240\text{ cm}^{-1}$ . The analogues with ring substitutions (Figures 6–9) leave this region of the spectrum virtually unchanged in all retinal isomers, except for minor intensity changes. However, the isoprenoid chain substitutions at C(9) and C(13) affect this region dramatically, and these effects can be interpreted with consistency in the *trans*, 11-*cis*, and 9-*cis* analogues. On the other hand, in the 13-*cis* analogue,

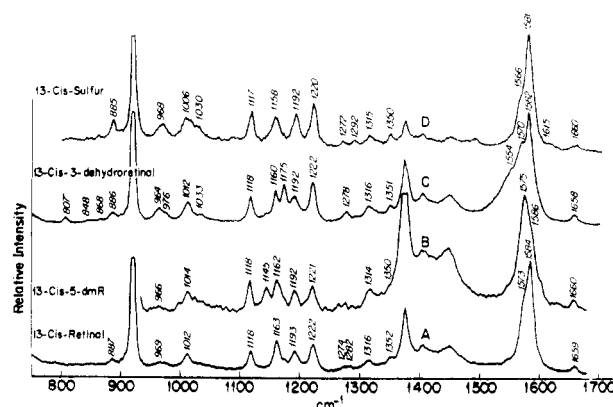


FIGURE 8: Resonance Raman spectra of (A) 13-*cis*-retinal, (B) 13-*cis*-5-dmR, (C) 13-*cis*-3-dehydroretinal, and (D) 13-*cis*-sulfur in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ , and band positions are accurate to  $\pm 2\text{ cm}^{-1}$ .

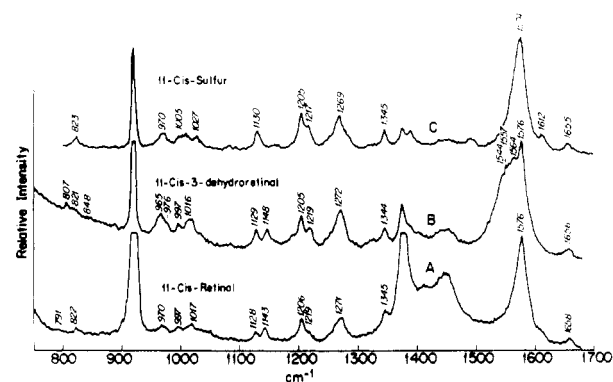


FIGURE 9: Resonance Raman spectra of (A) 11-*cis*-retinal, (B) 11-*cis*-3-dehydroretinal, and (C) 11-*cis*-sulfur in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ , and band positions are accurate to  $\pm 2\text{ cm}^{-1}$ .

some alterations in the normal modes are introduced which cannot be completely resolved by the analogue results we have obtained. With these generalizations as a background, this region will now be discussed in the following order: *trans*, 11-*cis*, 9-*cis*, and 13-*cis*.

In *trans*-retinal the  $1198\text{-cm}^{-1}$  band shifts to lower frequency and increases in intensity (Figures 2B and 2C) when a butyl group is substituted at the C(9) position. A butyl substitution at the C(13) position appears to have an identical effect in the DBR case, but in 13-BuR bands are observed at both the above frequencies. Desmethyl substitutions appear to have the opposite effect. They reduce the intensity of the bands in this region and cause an increase in the frequency of the observed band to  $1222$  and  $1215\text{ cm}^{-1}$  for 13- and 9-dmR, respectively (Figures 2F and 2E). A vibration that consists of different mixtures of C-C single bond stretching and in-plane methyl rocking of one or both methyl groups would explain this series of changes. Such motions are known to occur at these frequencies in alkanes that contain methyl groups (Dollish et al., 1974). The reduction in frequency on substitution of butyl groups is consistent with this suggestion. Additional evidence can be found in work on carotenoids (Rimai et al., 1973), where the compound amphotericin B, which has no methyl groups, has only a very weak  $1195\text{-cm}^{-1}$  band. In order to fully account for our experimental observations, future calculations must be able to explain not only the above frequency shifts but also the origin of the intensity increase in the butyl analogue and decrease in the desmethyl analogues.

The 11-*cis* isomer of retinal has a *cis* bend between C(9) and



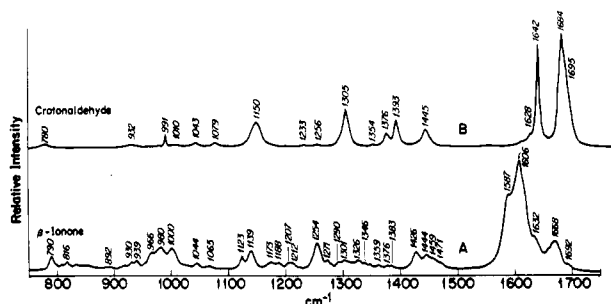


FIGURE 10: Resonance Raman spectra of (A) *trans*-crotonaldehyde and (B)  $\beta$ -ionone. The spectral resolution is  $2\text{ cm}^{-1}$ , and band positions are accurate to  $\pm 2\text{ cm}^{-1}$ .

C(13) which appears to affect the character of this methyl rocking, C-C vibration. Unlike the *trans* isomer which has a single band at  $1198\text{ cm}^{-1}$ , two bands are observed in *11-cis*-retinal, one at  $1206\text{ cm}^{-1}$  and the other at  $1219\text{ cm}^{-1}$  (Figure 5A). In Figure 5B where both methyls are substituted by butyl groups, a single intense band occurs at  $1182\text{ cm}^{-1}$ , as though both the  $1219\text{-cm}^{-1}$  and  $1206\text{-cm}^{-1}$  vibrations had shifted in frequency. When only one methyl is replaced by a butyl group, a strong band between  $1185$  and  $1188\text{ cm}^{-1}$  is observed in addition to a  $1206\text{-cm}^{-1}$  vibration in *11-cis*-9-BuR (Figure 5C) or a  $1217\text{-cm}^{-1}$  vibration in *11-cis*-13-BuR (Figure 5D). The manner in which these bands shift suggests that in *11-cis*-retinal the C(13) methyl rock, C-C vibration occurs at  $1206\text{ cm}^{-1}$ , whereas the C(9) methyl rock, C-C vibration occurs at  $1219\text{ cm}^{-1}$ . This is unlike the *trans* isomer in which these two vibrations apparently occur at the same frequency ( $1198\text{ cm}^{-1}$ ). However, in a manner analogous to *trans*-retinal, the rocking motion involving the butyl groups appears most intense.

The bands in *9-cis*-retinal (Figure 3A) appear to follow a pattern similar to the *11-cis* isomer. The similarity between these two isomers probably reflects the presence of a *cis* bend between C(9) and C(13). In both these molecules, two bands occur above  $1200\text{ cm}^{-1}$  ( $1201$  and  $1216\text{ cm}^{-1}$  in *9-cis* and  $1206$  and  $1219\text{ cm}^{-1}$  in *11-cis*). In addition, in the DBR analogues (Figures 3B and 5B) an intense band appears at  $\sim 1183\text{ cm}^{-1}$ . Furthermore, in the 9-BuR analogue two bands are observed, one at  $1185\text{ cm}^{-1}$  and the other at  $1202\text{ cm}^{-1}$  in *9-cis* (Figure 3C) and  $1206\text{ cm}^{-1}$  in *11-cis* (Figure 5C). Unfortunately, a *9-cis*-13-BuR analogue could not be isolated. In view of these results, we can assign the  $1201\text{-cm}^{-1}$  band in *9-cis*-retinal to the C(13) methyl rock, C-C vibration and the  $1216\text{-cm}^{-1}$  band to the C(9) methyl rock, C-C vibration. In the *9-cis*-dmR analogues (Figures 3D and 3E), the intensity is again reduced as in the *trans* analogues, and the frequency is raised to  $1228\text{ cm}^{-1}$  in *9-cis*-13-dmR. In *9-cis*-9-dmR, the combination of the reduced intensity and the *cis* bend may be sufficient to make the band unobservable or to completely change the character of this vibration.

In *13-cis*-retinal the substitution of a butyl group at the C(9) position is the only one that significantly alters the appearance of the spectrum in this region. In the case of this substitution in both 9-BuR and DBR, the vibrational mode at  $1193\text{ cm}^{-1}$  is reduced in frequency to  $1184\text{ cm}^{-1}$  and the intensity is increased (Figure 4B,C), as was previously observed for butyl substitutions. On the other hand, the substitution of a butyl group at the C(13) position does not significantly alter vibrational modes in this region (Figure 4D). It may be possible to explain these results if the *13-cis* isomer has a C(9) methyl rock, C-C vibration at  $1193\text{ cm}^{-1}$  (similar to the frequency of this mode in *trans*-retinal) and a much weaker contribution

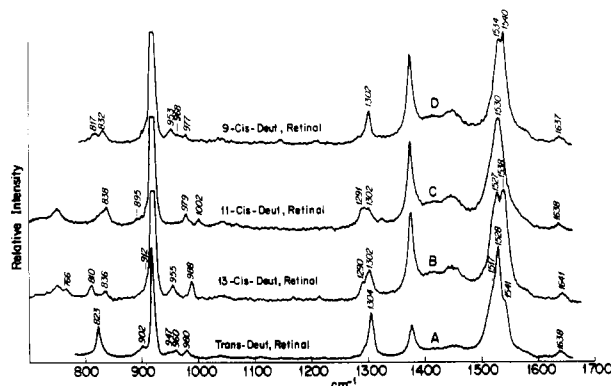


FIGURE 11: Resonance Raman spectra of (A) *trans*-[ $^2\text{H}$ ]retinal, (B) *13-cis*-[ $^2\text{H}$ ]retinal, (C) *11-cis*-[ $^2\text{H}$ ]retinal, and (D) *9-cis*-[ $^2\text{H}$ ]retinal in  $\text{CH}_3\text{CN}$ . The spectral resolution is  $2\text{ cm}^{-1}$ , and band positions are accurate to  $\pm 2\text{ cm}^{-1}$ . The existence of bands between  $900$  and  $950\text{ cm}^{-1}$  was verified by taking spectra in methanol which has no bands in this region.

from the C(13) methyl rock C-C vibrational mode. However, this explanation cannot account for the strong  $1222\text{-cm}^{-1}$  band in *13-cis*-retinal which is insensitive to butyl substitution. It appears that the structure of the *13-cis* isomer alters the normal modes in this region, giving rise to bands which are unique to this isomer.

All of the vibrations discussed above are visible in the infrared spectra. They are not especially intense and therefore probably do not involve significant changes in dipole. Thus, the infrared data do not contradict any of the suggested assignments of coupled methyl rocking and C-C stretches made above.

The normal coordinate calculations of Cookingham (1978) suggest that modes involving methyl rocking motions, methyl stretching, and single-bond stretching could occur in this frequency range. In the calculations, the C(9) methyl group makes a much larger contribution than the C(13) methyl group, in accord with our experimental observations.

**C-C Stretch.** A consistently intense band occurs in both the resonance Raman and infrared spectra of *9-cis*-, *13-cis*- and *trans*-retinal between  $1145$  and  $1165\text{ cm}^{-1}$ . The conspicuous absence of this band in *11-cis*-retinal (Figure 5A) suggests that the planar portion of the retinal from C(7) to C(13) is involved in the normal mode giving rise to this vibration. The planar arrangement of the isoprenoid chain is significantly distorted in *11-cis*-retinal. A symmetric stretch involving several single bonds in the isoprenoid chain, particularly those between C(7) and C(13), seems the most likely assignment for this band. Both strong infrared and strong Raman bands would be expected for such a C-C single bond stretch. The nonplanar nature of the isoprenoid chain in *11-cis*-retinal, which would disrupt any in-phase stretching motion of single bonds, explains the absence of this band in the *11-cis* spectra. The resonance Raman intensity varies somewhat depending on the analogue and seems to be less intense in the dibutylretinal isomers. This band becomes more intense in *trans*-13- and *trans*-9-dmR. It is most intense in the *trans*-9-dmR isomer, where an extended chain of C-C single bonds exists without an additional perturbation from the C(9) methyl group. Further support for our assignment can be found in a very intense band at a similar frequency ( $1158\text{ cm}^{-1}$ ) in carotenoids which has been assigned (Rimai et al., 1970) to a C-C stretching vibration.

In all analogues studied, the *9-cis* isomer has a significantly lower C-C stretching frequency at about  $1147\text{ cm}^{-1}$  compared to *trans* and *13-cis* (Figures 2-4). This observation is interesting in view of the previous observation that the C=C

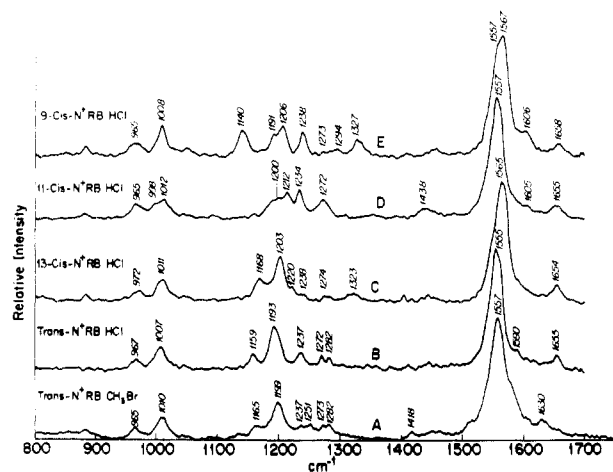


FIGURE 12: Resonance Raman spectra of (A) *trans*-N<sup>+</sup>RBCH<sub>3</sub>Br, (B) *trans*-N<sup>+</sup>RB-HCl, (C) 13-*cis*-N<sup>+</sup>RB-HCl, (D) 11-*cis*-N<sup>+</sup>RB-HCl, and (E) 9-*cis*-N<sup>+</sup>RB-HCl in ethanol. The spectral resolution is 4 cm<sup>-1</sup>, and band positions are accurate to  $\pm 2$  cm<sup>-1</sup>.

stretching frequency for 9-*cis*-retinal was the highest of all the retinal isomers. This effect, which results from less electron delocalization, would be expected to have an opposite effect on single- and double-bond stretches. Thus, the above result can be taken as additional evidence supporting this assignment.

**C-C Stretch, CH<sub>3</sub> Rock; C-C Stretch: Schiff-Base Comparisons.** In many instances, the resonance Raman scattering from visual pigments and bacteriorhodopsin can be better modeled with a Schiff-base spectrum than by the spectrum of the corresponding retinal isomer. The suggested assignments for retinal may be useful in interpreting the origin of some of the bands in the fingerprint region of the Schiff bases. In the Schiff bases, the 1140- to 1170-cm<sup>-1</sup> band may be due to a single-bond stretching motion of the Schiff base isoprenoid chain between C(9) and C(13) similar to that observed in retinal. In an analogous manner the structure between 1180 and 1215 cm<sup>-1</sup> in the Schiff bases may be due to vibrations involving methyl-rocking motions and C-C single-bond stretching motions as in retinal.

The Schiff bases do exhibit one vibration that is not present in any of the retinals or model compounds. This vibration occurs at approximately 1236 cm<sup>-1</sup> in all isomers of the protonated Schiff bases (Figure 12) and at approximately 1224 cm<sup>-1</sup> in the isomers of the unprotonated Schiff bases (Figure 13). The Schiff bases differ from retinal in that they contain a butyl group attached through a protonated Schiff base to the C(15) carbon of the polyene (Figure 1C). The 1236- and 1224-cm<sup>-1</sup> bands are probably due to motions involving the Schiff-base nitrogen. This is supported by the fact that upon deprotonation of the Schiff base this band shifts from 1236 to 1224 cm<sup>-1</sup>. This shift is similar to that observed for the C=N stretch. Further evidence to support this suggestion is found in the spectrum of a *trans*-methylated Schiff base (Figure 12A) where the methyl group is attached to the nitrogen and the 1237-cm<sup>-1</sup> vibration is split into two vibrations at 1237 and 1251 cm<sup>-1</sup>. A vibration at  $\sim 1236$  cm<sup>-1</sup> is very prominent in the spectra of visual pigments (Sulkes et al., 1976; Mathies et al., 1976; Callender et al., 1976), and thus this assignment should be useful in interpreting the visual pigment spectra.

**C(14)-C(15) Stretch.** All of the 9-, 11-, and 13-*cis* isomers of retinal and the analogues (except 9-*cis*-13-dmR) have a band that occurs between 1114 and 1133 cm<sup>-1</sup>. This band is also present in the infrared spectrum with increased intensity

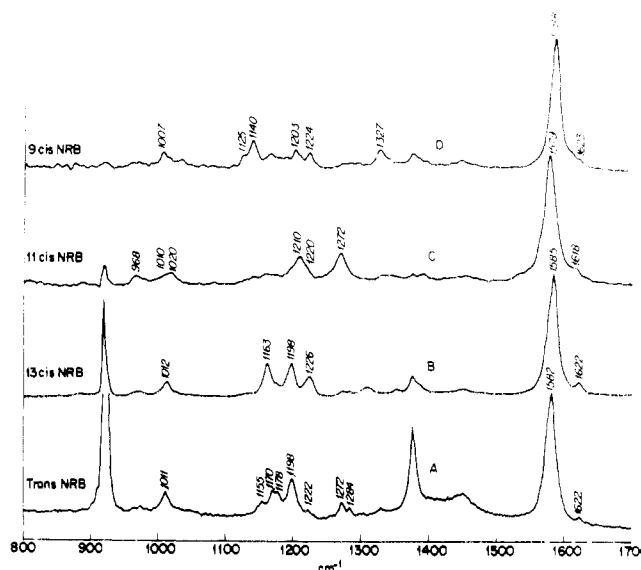


FIGURE 13: Resonance Raman spectra of (A) *trans*-NRB, (B) 13-*cis*-NRB, (C) 11-*cis*-NRB, and (D) 9-*cis*-NRB in CH<sub>3</sub>CN. Samples were cooled to 15 °C and excitation was with  $\sim 50$  mW of 647.1-nm radiation. The spectral resolution is 2 cm<sup>-1</sup>, and band positions are accurate to  $\pm 2$  cm<sup>-1</sup>.

and, as in the Raman, it is the strongest in 13-*cis*-retinal. Aliphatic aldehyde groups are known to have C-C stretching vibrations associated with the carbon-carbon single bond adjacent to the carbonyl group. The frequency of this stretching vibration occurs between 1120 and 1090 cm<sup>-1</sup> (Dollish et al., 1974). Since electron delocalization causes the C=C stretch to be somewhat lower in retinal (1650 cm<sup>-1</sup>) than in aliphatic aldehydes (1920 cm<sup>-1</sup>), the C(14)-C(15) single-bond stretch of the aldehyde group would be expected to occur at a somewhat higher frequency than in aliphatic aldehydes. An additional observation is that the *cis* bend appears to be important in determining the intensity. The closer it is to the aldehyde group, the stronger the C(14)-C(15) stretch at about 1120 cm<sup>-1</sup>. In *trans*-retinal, no Raman vibration is observed; however, a strong infrared band is observed at 1110 cm<sup>-1</sup>. The intensity of this band in the infrared spectra of all the retinal isomers can be taken as further support for the above assignment.

11-*cis*-Retinal has one additional band that occurs at 1143 cm<sup>-1</sup> in all 11-*cis* analogues with the exception of the sulfur analogue. The infrared spectrum has a moderately intense band at 1140 cm<sup>-1</sup>. The absence of this vibration in the sulfur analogue with the different ring structure suggests this vibration may be due to a single-bond stretch in or near the ring.

**The 990-1030-cm<sup>-1</sup> Region: C-CH<sub>3</sub> Stretch.** There is one prominent band between 990 and 1030 cm<sup>-1</sup> in all of the retinal isomers, except for 11-*cis*. The band is found at 1009 in *trans*-, 1009 in 9-*cis*-, and 1012 cm<sup>-1</sup> in 13-*cis*-retinal. In 11-*cis*-retinal, two bands are seen at 997 and 1017 cm<sup>-1</sup>. Rimai (Rimai et al., 1971a) suggested that the band or bands in this region are due to chain carbon-methyl stretching modes. Work from this laboratory (Cookingham and Lewis, 1978) established that the two bands in 11-*cis*-retinal can be assigned as follows: 997 cm<sup>-1</sup> to the C(13) methyl group and 1017 cm<sup>-1</sup> to C(9) methyl group. This assignment was made by substituting butyl groups for methyl groups. For example, in 11-*cis*-DBR (Figure 5B) no bands are found in this region; however, a new band appears at 1080 cm<sup>-1</sup>. In 11-*cis*-9-BuR (Figure 5C), the 997-cm<sup>-1</sup> band remains, the 1017-cm<sup>-1</sup> band is absent, and a 1076-cm<sup>-1</sup> band is present at about one-half

the intensity as in the DBR spectrum. These results were used as the basis for assigning the  $997\text{-cm}^{-1}$  band to the C(13) methyl stretch (Cookingham and Lewis, 1978). In the 11-*cis*-13-BuR (Figure 5D) spectrum, the  $997\text{-cm}^{-1}$  band is absent, the  $1017\text{-cm}^{-1}$  band remains, and there is a  $1083\text{-cm}^{-1}$  band. Thus, the  $1017\text{-cm}^{-1}$  band was assigned to the C(9) methyl stretch (Cookingham and Lewis, 1978). The new band at  $\sim 1080\text{ cm}^{-1}$  in all the butyl analogues cannot be considered a carbon-*n*-butyl stretching mode, because a simple butyl mass effect would decrease rather than increase this stretching frequency. Skeletal stretching modes of alkanes (Simananouti and Mizushima, 1949; Brown et al., 1954) occur at about these frequencies, although it is difficult to understand how such a vibrational mode would be resonance enhanced.

An  $\sim 1080\text{-cm}^{-1}$  vibrational mode is also observed in the other butyl analogues studied. In *trans*-DBR (Figure 2B) the band at  $1009\text{ cm}^{-1}$  is absent and a new band appears at  $1080\text{ cm}^{-1}$ . The 9- and 13-BuR show about half the intensity at  $1009\text{ cm}^{-1}$  and half at  $\sim 1084\text{ cm}^{-1}$ . The very same pattern is seen in butyl-substituted 9- and 13-*cis*-retinals (Figures 3 and 4), except the intensity of the  $1080\text{-cm}^{-1}$  band is weak when it is next to a *cis* bend. The removal of methyl groups in the desmethyl retinals shows a decrease in the intensity of the  $1009\text{-cm}^{-1}$  band and no new bands appearing. In the 5-dmR spectra (Figure 6B-8B) there is no apparent decrease in intensity of the  $1009\text{-cm}^{-1}$  band, indicating no contribution from the C(5) methyl to this vibrational mode. This conclusion is also supported by the observation that substitution of the C(9) and C(13) methyl groups by butyl groups leaves no residual intensity at  $1009\text{ cm}^{-1}$  which could be attributed to the C(5) methyl (Figures 2B-5B).

In the two separate discussions above, our data suggested that the C(13) methyl group is directly involved in a specific normal mode. The C(9) and C(13) methyl group assignments made in this section for 11-*cis*-retinal further support these suggestions. As we have demonstrated above, the frequency of the C(13)-CH<sub>3</sub> stretching vibration in 11-*cis*-retinal is  $20\text{ cm}^{-1}$  lower than the corresponding C(9)-CH<sub>3</sub> stretching vibration. It is encouraging to note that in 11-*cis*-retinal this same effect is detected for all the vibrational modes having a C(13)-CH<sub>3</sub> component in their description. These normal modes (for example, the asymmetric methyl stretch and the methyl rocking C-C stretch) were discussed and previously assigned using data on all the retinal isomers. However, in 11-*cis*-retinal, because of the unique characteristic of the C(13) CH<sub>3</sub> group, the asymmetric methyl stretch involving the C(13) methyl occurs at  $1431\text{ cm}^{-1}$ , whereas a similar vibrational mode consisting of the C(9) methyl is detected at  $1448\text{ cm}^{-1}$ . In complete analogy, the C(13) methyl rocking, C-C stretch occurs at  $1206\text{ cm}^{-1}$ , while the C(9) methyl rocking, C-C stretch is observed at  $1219\text{ cm}^{-1}$ .

The Schiff bases (Figure 12) show a carbon-methyl stretching region similar to that seen in retinals. There are single bands in *trans*, 9-*cis*, and 13-*cis* analogues and two bands in 11-*cis*-N<sup>+</sup>RB. The 3-dehydroretinals also show this general trend, but have some slight intensity at  $1030\text{ cm}^{-1}$  in the 13- and 9-*cis* isomers. There is also extra intensity between  $1020$  and  $1030\text{ cm}^{-1}$  in some of the sulfur analogue spectra which may be attributable to changes in the ring in these compounds or ring-methyl stretches.

The infrared spectra show very low-intensity multiple bands in the carbon-methyl stretching region. All of the carbon-methyl stretching modes should show up in the infrared, including ring methyls which may account for the additional infrared bands.

*The 950-980-cm<sup>-1</sup> Region: C-C-H Bend, Out of Plane.*

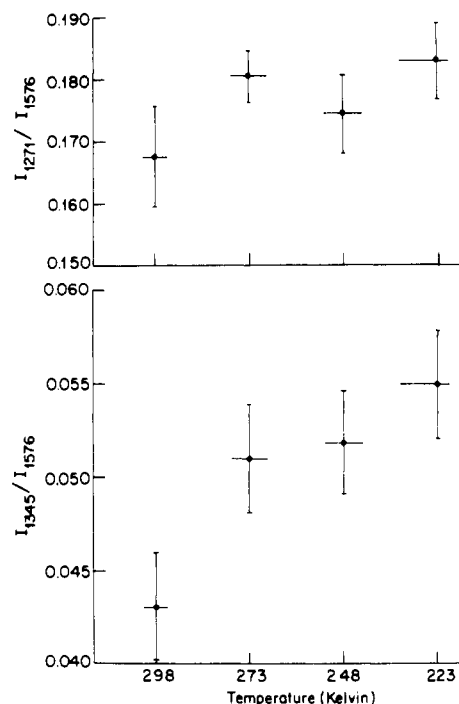


FIGURE 14: The temperature dependence of the vibrational bands at  $1345$  (lower) and  $1271\text{ cm}^{-1}$  (upper) in 11-*cis*-retinal.

There are low-intensity bands between  $950$  and  $980\text{ cm}^{-1}$  in all the Raman spectra of retinals and Schiff bases which are very intense in the infrared spectra shown in Figure 16 (Supplementary Material). The frequency and intensity of these bands appear to be unaltered by changes on the chain or ring. Rimai (1971a) attributed these bands to out of plane hydrogen bends on the isoprenoid chain. Warshel and Karplus (1971) suggested that this band could also have contributions from the C(5)-methyl stretching mode. An examination of the *trans*-5-dmR spectrum (Figure 6B) shows no noticeable changes in intensity in this band when the C(5) methyl is absent; however, with such a small band it would be difficult to notice small-intensity differences. In all 3-dehydroretinals there appears to be a high-frequency shoulder at  $976\text{ cm}^{-1}$ , which could be due to the C(5)-methyl stretch, brought into resonance when the double-bond conjugation is extended further into the ring in this analogue.

*The 790-950-cm<sup>-1</sup> Region.* In the solution spectra of the retinal isomers and the analogues (Figures 2-9) a few resonance Raman bands appear at frequencies between  $750$  and  $950\text{ cm}^{-1}$ . The infrared spectra (Figure 16) also show some structure in this region. Normal coordinate calculations (Cookingham, 1978) suggest that these vibrations may come from C=C-C bending vibrations of the polyene.

*The 790-cm<sup>-1</sup> Region and Below.* Considerable theoretical work has predicted the existence of low-frequency vibrations in retinylidene-like molecules (Warshel and Karplus, 1974; Warshel and Dauber, 1977). The existence of these modes has been verified by our experiments in all of the isomers of retinal and the protonated and unprotonated Schiff bases as well as in the fragment  $\beta$ -ionone. Figure 15 depicts the spectra of all of these compounds from  $10$  to  $1100\text{ cm}^{-1}$ . All of the samples are the crystalline form of these molecules with the exception of  $\beta$ -ionone which is a liquid at room temperature. The spectra of the retinal isomers were also studied in solution, but the Rayleigh scattering masked the low-frequency modes. Vibrations in the higher frequency region ( $150$ - $750\text{ cm}^{-1}$ ) were weak in all cases except for 11-*cis*-retinal.

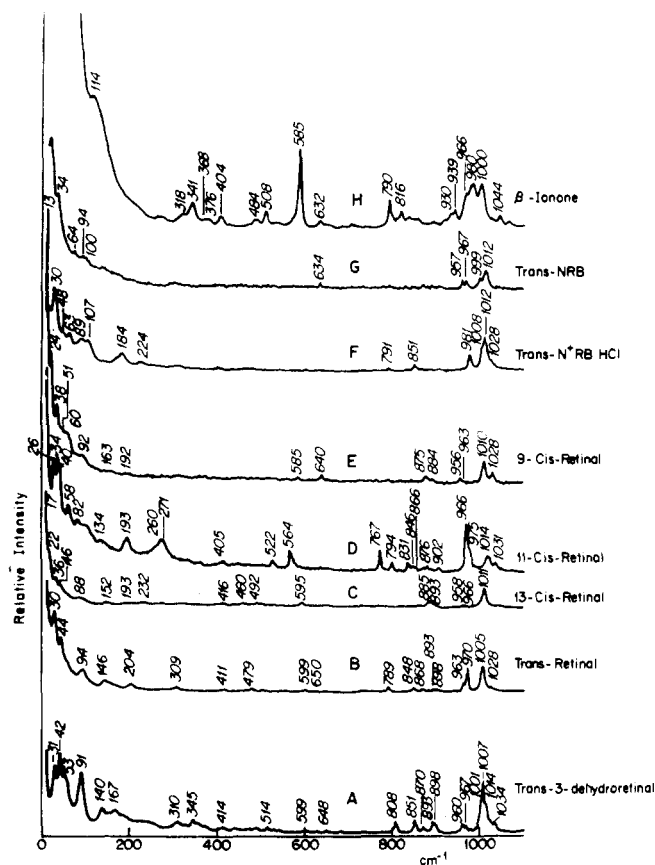


FIGURE 15: Resonance Raman spectra of (A) *trans*-3-dehydroretinal, (B) *trans*-retinal, (C) 13-*cis*-retinal, (D) 11-*cis*-retinal, (E) 9-*cis*-retinal, (F) *trans*-N<sup>+</sup>RB-HCl, (G) *trans*-N-retinylidene-*n*-butylamine (*trans*-NRB), and (H)  $\beta$ -ionone. All samples are crystalline except for  $\beta$ -ionone, which is a liquid at room temperature. The spectral resolution is 2 cm<sup>-1</sup>, and band positions are accurate to  $\pm 2$  cm<sup>-1</sup>.

The region below 100 cm<sup>-1</sup> for each retinal isomer (Figure 15B–15E) is unique. The relative intensities of the bands, the frequencies of the bands, and the number of bands composing the spectrum of each isomer are different. There do seem to be some common features, such as at least one very strong band between 22 and 30 cm<sup>-1</sup> in each isomer and a second band between 34 and 44 cm<sup>-1</sup>. One other band occurs at about 90 cm<sup>-1</sup> in all of the isomers. The remainder of the spectrum to 700 cm<sup>-1</sup> is very weak, with a few bands occurring at the same frequency in each isomer. 11-*cis*-Retinal has a much stronger spectrum and will be discussed in detail later. Because of the lack of infrared spectra and analogue Raman spectra, very few definite assignments can be made. In general, calculations (Cookingham, 1978) predict C=C–C bending modes from about 400 to 150 cm<sup>-1</sup> and torsional modes coupled with skeletal modes below about 150 cm<sup>-1</sup>. These calculations generally agree with previous calculations (Warshel and Karplus, 1974; Warshel and Dauber, 1977).

As mentioned above, 11-*cis*-retinal has additional intense bands in the low-frequency region. We see relatively strong vibrations at 26, 34, 40, 58, 82, 134, 193, 260, 271, 405, 522, and 564 cm<sup>-1</sup>. The vibrations at 193, 260, 271, and 564 cm<sup>-1</sup> are also present in solution spectra (cyclohexane solvent) and are observed at 181, 248, 259, and 560 cm<sup>-1</sup>. Bands of this intensity were not observed in any of the other retinal isomers. Warshel and Karplus (1974) explain the featureless absorption spectrum of 11-*cis*-retinal, using torsional modes with ground-state frequencies at 286, 202, 83, 55, 29, and 17 cm<sup>-1</sup> to demonstrate how low-frequency progressions could produce

an absorption spectrum with no structure. The principal 11-*cis*-retinal bands that we observe are in excellent agreement with their predictions.

The resonance Raman spectrum of *trans*-retinal in this region shares several common features with those of the protonated and unprotonated Schiff bases of *trans*-retinal and *trans*-3-dehydroretinal. The spectra of *trans*-retinal and *trans*-3-dehydroretinal are almost identical, with the exception of a 53-cm<sup>-1</sup> band, a stronger 91-cm<sup>-1</sup> band, and 167-cm<sup>-1</sup> band in *trans*-3-dehydroretinal. The Schiff bases both have a strong 30-cm<sup>-1</sup> band but no strong bands between 40 and 60 cm<sup>-1</sup>. In addition to a 64-cm<sup>-1</sup> band, they have a doublet structure near 100 cm<sup>-1</sup>, in contrast to the retinals. The protonated Schiff base also has a band at 184 cm<sup>-1</sup>. Few additional definite assignments can be made, but there is the suggestion that the differences between the *trans*-retinal spectrum and *trans*-3-dehydroretinal spectrum may be due to the enhancement of several ring torsional modes. The differences between retinal and the Schiff bases are probably due to the presence of the Schiff base and butyl group instead of the aldehyde group.

The  $\beta$ -ionone fragment has some very interesting structures, although extremely low-frequency modes are obscured by Rayleigh scattering. The bands at 585, 404, 341, and 114 cm<sup>-1</sup> agree remarkably well with the predictions of Warshel and Karplus (1974) of 567, 391, 285, and 102 cm<sup>-1</sup>. The strong 88-cm<sup>-1</sup> band predicted was not observed because it may have been masked by the Rayleigh line.

## Conclusions

The results of this study have revealed considerable information on the particular nature of the normal modes of vibration of retinal and related models for visual pigments and bacteriorhodopsin. By studying the combined information available from the resonance Raman and infrared spectra of all the isomers of retinal and the chemically modified retinals, reasonable assignments for all of the vibrational features observed in the resonance Raman spectra were made. Table II contains a brief summary of the suggested assignments of the bands observed in the resonance Raman spectra of the isomers of retinal. In several instances, our results were applied to interpret the spectral features of the Schiff bases of retinal. Many of these same features were also reflected in the resonance Raman spectra of visual pigments. Thus, the results of this study should enable future workers studying visual pigments and bacteriorhodopsin to understand in greater detail the information present in the resonance Raman spectra of these pigments.

Finally, the data presented in this paper should also be of considerable interest to those workers who are attempting to calculate resonance Raman spectra of retinylidene chromophores (Warshel and Karplus, 1974; Warshel and Dauber, 1977). Many of their predictions of expected low-frequency modes were verified by our experimental data (Figure 15). Further theoretical calculations on some of the butyl or desmethyl analogues may prove useful in providing additional information for refining these calculations.

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plied 11-*cis*-retinal, 3-dehydroretinal, and the sulfur analogue. Dr. Henri Crespi prepared and gave us totally deuterated *Halobacterium halobium* from which we extracted fully deuterated retinal.

Michael Marcus assisted in performing the infrared measurements on the retinal isomers. We also thank Dr. Bishun Khare for the use of his infrared spectrometer and for his help in making the measurements.

#### Supplementary Material Available

Figure 16: resonance Raman and infrared spectra of *trans*-retinal, 13-*cis*-retinal, 11-*cis*-retinal, 9-*cis*-retinal, and *trans*-3-dehydroretinal (6 pages). Ordering information is given on any current masthead page.

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