(54) N. R. Draper and H. Smith, "Applied Regression Analysis," Wiley, New York, N.Y., 1966.
(55) The following assumptions are inherent to the model: the rotational isomeric state model with three states is valid; the free energy of a rotational isomer can be divided into the additive contributions from chain elements; the above contributions can be divided into a set of 1–4 and 1–5 interactions (interactions of higher order are negligible); the conformational energies do not vary with temperature.
(56) Recently, more sophisticated models have been used to investigate the conformational equilibria in polypropylene.[57,58] In the model proposed by Boyd and Breitling,[57] three conformational energies, E_g, E_{SK}, and E_ω are used. E_{SK} corresponds approximately to E_τ, E_g to $(E_\tau - E_\eta)$. E_ω has the same meaning as in this paper. According to this model, chain dimensions in agreement with experimental values have been found using the following values for the conformational energies deduced from potential energy calculations on an isolated 2,4,6-trimethylheptane molecule: $E_g = 400$ cal mol^{-1}, $E_{SK} = 600$ cal mol^{-1}, and $E_\omega = 1300$ cal mol^{-1}. Using the Boyd-Breitling model to fit the epimerization data, however, the relatively high values $E_g = 1100 \pm 500$ cal mol^{-1}, $E_{SK} = 1200 \pm 500$ cal mol^{-1}, and $E_\omega = 2100 \pm 100$ cal mol^{-1} have been found.
(57) R. H. Boyd and S. M. Breitling, *Macromolecules*, **5**, 279 (1972).
(58) F. Heatley, *Polymer*, **13**, 218 (1972).
(59) For the chain length x, the content of isotactic diads $f_{iso,x}$ was calculated with[22]

$$f_{iso,x} = (x-3)^{-1}\frac{[J^*0]\hat{U}_1\hat{U}^{x-3}\hat{U}_{x-1}\begin{bmatrix}0\\J\end{bmatrix}}{J^*\hat{U}_1\hat{U}^{x-3}\hat{U}_{x-1}J}$$

with

$$\mathbf{U}_1 = \begin{bmatrix}U_1 & 0\\ 0 & U_1\end{bmatrix} \text{ and } \hat{\mathbf{U}}_{x-1} = \begin{bmatrix}U_{x-1} & 0\\ 0 & \hat{U}_{x-1}\end{bmatrix}$$

all other matrices being taken directly from Flory.[22]
(60) J. H. Brewster in "Topics in Stereochemistry," Vol. 2, N. L. Allinger and E. L. Eliel Ed., Interscience, New York, N.Y., 1967.
(61) Calculations with varying torsion angles indicate that the calculated optical activity is not very sensitive to this quantity.
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A ^{1}H Nuclear Magnetic Resonance Determination of the Conformations of the Polyene Chain Portions of 9-*cis*- and 13-*cis*-Retinal in Solution

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Abstract: The conformations of the polyene chain portions of 9-*cis*- and 13-*cis*-retinal have been investigated by ^{1}H nmr spectroscopy, including the measurement of long-range nuclear spin-spin coupling constants, chemical shifts, spin-lattice relaxation times, and nuclear Overhauser enhancements. Each isomer is found to exist in solution with a planar s-trans conformation from 7C to 15C. The T_1 data indicate that there is enhanced flexibility about the 14–15 single bond, although the average conformation is planar. ^{1}H nmr spectral parameters for the model compound β-ionone are also presented.

I. Introduction

9-*cis*-Retinal will combine with opsin to form an analogous visual pigment, isorhodopsin.[1] 13-*cis*-Retinal has recently been shown to be the natural chromophore of the bacterial pigment bacteriorhodopsin.[2] Although bacteriorhodopsin, which serves the function of a proton pump,[3] and the bacterial membrane in which it is found, bear little resemblance to rhodopsin and rod disk membranes in their chemical compositions, it is of great interest that bacteriorhodopsin undergoes a bleaching sequence with intermediates analogous to those of rhodopsin.[4] It is thus important to ascertain the solution conformations of the polyene chain portions of these two chromophores. This effort is undertaken in the present paper, using the same ^{1}H nuclear magnetic resonance techniques which were used in earlier work to study *all-trans*- and 11-*cis*-retinal.[5] These include the observation of long-range nuclear spin-spin coupling constants, chemical shifts, spin-lattice relaxation times (T_1's), and nuclear Overhauser enhancements (NOE's). In addition, the ^{1}H nmr spectral parameters for the model compound β-ionone are presented.

II. Methods

9-*cis*-Retinal was obtained from Sigma Chemical Co.; β-ionone and 13-*cis*-retinal were brought from Eastman Organic Chemicals Co. All three compounds were used as obtained without purification. Acetone-d_6 was purchased from Stohler Isotope Chemicals Co. Samples were prepared in acetone-d_6 solution and degassed using at least five freeze-pump-thaw cycles. The approximate concentrations of the samples were: β-ionone, 0.5 *M*; 9-*cis*-retinal 0.3 *M*; and 13-*cis*-retinal, 0.5 *M*. Hexamethyldisiloxane (HMDS), 1–5% (v/v), was used in each case as the internal reference signal. ^{1}H nmr measurements were made as previously described[5] using an XL-100 nmr spectrometer operating in the Fourier transform mode at 100.1 MHz. For the NOE measurements, the peak intensities were obtained by manual planimetry if the decoupling was continuous, or the peak heights were used if the decoupler was gated off during the acquisition of the free induction decay. For the T_1 measurements, the peak intensities were taken as the peak heights.

For the analysis of the olefinic region of the spectrum of each isomer, the program LAOCN3[6] was used. For each isomer, the two spin sets 7H, 8H and 10H, 11H, 12H, 14H, and 15H were used to fit the olefinic and aldehyde chemical shifts and vicinal coupling constants. The long-range couplings reported for 13-*cis*-retinal were estimated with the aid of the interactive spectral simulation program SIMEQ.[7] Nuclear Overhauser enhancements and spin-lattice relaxation times were calculated using the program GENOE.[8] This program calculates NOE's using the formula of Noggle and Schirmer,[9] assuming predominantly intramolecular dipole-dipole relaxation. T_1's were calculated assuming 100% intramolecular dipole-dipole relaxation. For these calculations the olefinic and methyl protons from 8H to the end of the chain were included. The interproton distance input for GENOE was computed using the program ROCOR.[8] For both isomers, the geometry assumed for the polyene chain

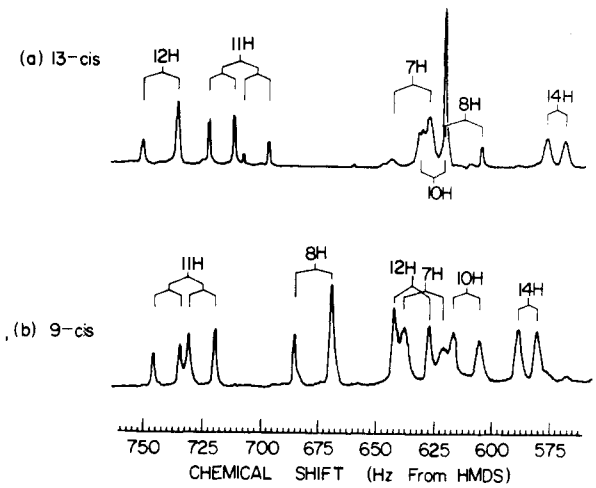

Figure 1. ^{1}H nmr (100.1 MHz) spectra of retinal isomers. Olefinic region of (a) 13-*cis*-retinal and (b) 9-*cis*-retinal, in acetone-d_6.

Table I. ^{1}H Nmr Chemical Shifts and Coupling Constants for β-Ionone

Proton	Chemical shift[a]	J	Coupling[b]
1,1′CH_3	1.000	4H, H′, 5CH_3	0.85[c]
5CH_3	1.682	4H, H′, 7H	1.60[c]
4H, H′	1.977	5CH_3, 7H	0.85[c]
9CH_3	2.179	7H, 8H	16.40
7H	7.200	7H, 9CH_3	≤0.10[c]
8H	5.996	8H, 9CH_3	0[c]

[a] Chemical shifts in acetone-d_6 solution in ppm from HMDS, at 32 ± 1°. [b] Coupling constants in Hz. [c] Honig, *et al.*[13]

Table II. ^{1}H Spin–Lattice Relaxation Times for β-Ionone[a]

Peak[b]	T_1	Peak[b]	T_1
HMDS	11.2 ± 0.21	8H dnfld	13.1 ± 0.18
1,1′CH_3	2.4 ± 0.04	7H upfld 2	8.49 ± 0.10
Ring	2.6 ± 0.12	7H upfld 1	8.39 ± 0.13
5CH_3	4.7 ± 0.05	7H dnfld 2	7.87 ± 0.12
9CH_3	7.6 ± 0.07	7H dnfld 1	7.99 ± 0.18
8H upfld	13.8 ± 0.21		

[a] T_1's in seconds. Error limits are standard deviations of the nonlinear least-squares fit. Measurements in acetone-d_6 solution at 32 ± 1°. [b] Peak designation: 7H and 8H split each other into doublets. Dnfld and upfld refer to the downfield and upfield peaks, respectively, of these doublets. 7H is further split by long-range coupling to 5CH_3 and 4H,H′.[13] The two largest peaks in each of the two 7H multiplets were designated 1 and 2, starting from downfield.

was planar s-trans, with 120° C–C–C and C–C–H bond angles, and using C–C bond lengths of 1.35 and 1.46 Å, which are the average conjugated double bond and single bond lengths, respectively, reported in the X-ray crystal structure[10] for *all-trans*-retinal. Point methyl groups (the "methyl proton centroid" model) were assumed, located at 1.86 Å from the corresponding chain carbon nucleus. The C–H bond lengths used were 1.08 Å for the olefinic[11] and 1.11 Å for aldehyde bonds.[12]

III. Results

In Table I are presented the ^{1}H nmr chemical shifts and coupling constants for β-ionone in acetone-d_6 solution. In Table II are given in ^{1}H T_1's for β-ionone. In Table III are listed the ^{1}H chemical shifts for 9-*cis*- and 13-*cis*-retinal, and in Table IV the coupling constants. In Figure 1 is shown the olefinic proton region of the nmr spectrum of 9-*cis*- and 13-*cis*-retinal. The spectrum of 9-cis (Figure 1) shows evidence, here by the presence of a low-intensity doublet upfield of 14H with similar but not identical chemical shift to that of 14H in 13-*cis*-retinal (Figure 1a), of an isomeric impurity to the extent of about 10%. The ^{1}H chemical shift differences of the two cis isomers of retinal relative to trans, in acetone-d_6, are given in Table V. Table VI presents the observed and calculated ^{1}H T_1's for 9-*cis*- and 13-*cis*-retinal. In Table VII are given the results of a number of ^{1}H homonuclear Overhauser enhancement experiments for 9-*cis*-retinal, while predicted NOE's for this isomer are listed in Table VIII. Observed and calculated NOE's for 13-*cis*-retinal are presented in Tables IX and X, respectively. For the predicted NOE's, an enhanced internal relaxation of the methyl groups was assumed, and an additional relaxation pathway ρ^* for all spins was also included (equivalent to about 10% of the calculated intramolecular dipole–dipole relaxation rate of 11H for 9-*cis*-retinal, or about 20% of 11H for 13-*cis*-retinal). Figure 2 represents spectra observed in a NOE experiment on 13-*cis*-retinal.

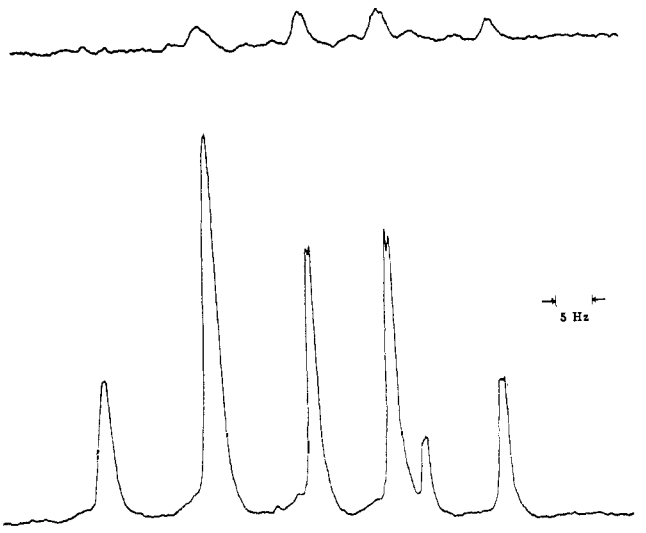

Figure 2. ^{1}H Fourier transform homonuclear Overhauser experiment with 13-*cis*-retinal in acetone-d_6. Bottom trace: 12H and 11H region with 13CH_3 saturated. Top trace: difference between 13CH_3-iradiated and control-irradiated spectrum. Decoupler was gated off during the free induction decay.

Table III. Chemical Shifts of Retinal Isomers[a]

Proton	9-Cis	13-Cis
1,1′CH_3	0.981	0.974
5CH_3	1.686	1.650
9CH_3	1.964	1.960
13CH_3	2.272	2.089
7H	6.307	6.305
8H	6.752	6.146
10H	6.109	6.249
11H	7.310	7.097
12H	6.347	7.403
14H	5.843	5.712
15H	10.033	10.163

[a] In ppm relative to internal HMDS. Samples in acetone-d_6 solution at 32 ± 1°. Olefinic proton chemical shifts fit with LAOCN3.

IV. Discussion

The β-ionone spectral parameters are presented in Tables I and II to supplement the work of Honig, *et al.*,[13] and the ^{13}C nmr studies reported in ref 14. Of some interest are the T_1 data listed for the individual lines of the 7H and 8H resonances (Table II), in which the T_1's for two different lines of each of the two 7H multiplets are equal (within experimental error) within the multiplet, but not equal to the T_1's of the lines of the other multiplet. Also the T_1's of the two

Table IV. ^1H Spin–Spin Coupling Constants for Retinal Isomers[a]

Coupling	9-Cis[b]	13-Cis[c]
7H, 8H	16.2[d]	16.2[d]
$9CH_3$, 10H		1.15[d]
$9CH_3$, 11H		0.05
10H, 11H	11.44[d]	11.5[d]
10H, 12H		−0.6
11H, 12H	15.06[d]	15.0[d]
11H, 14H		0.6[d]
12H, $13CH_3$		0.2
12H, 14H		−0.6
12H, 15H		0.3[d]
13H, 14H		1.25[d]
13H, 15H		0
14H, 15H	7.95[d]	7.83[d]

[a] Coupling constants in Hz. Samples in acetone-d_6 solution at 32 ± 1°. Vicinal couplings fit with LAOCN3. [b] Relatively poor resolution of the lines prevented the observation of or estimation of long-range couplings. [c] The presence of the long-range couplings listed was confirmed where possible by decoupling. Couplings listed are those which gave best spectral simulation. [d] Directly observed.

Table V. Chemical Shifts of *cis*-Retinal Isomers Relative to *all-trans*-Retinal[a]

Peak	9-Cis	13-Cis
$1,1'CH_3$	0.007	0.000
$5CH_3$	0.037	0.001
$9CH_3$	−0.022	−0.026
$13CH_3$	−0.022	−0.205
7H	−0.012	−0.014
8H	0.608	0.002
10H	−0.097	0.043
11H	0.084	−0.129
12H	−0.052	1.004
14H	0.005	−0.126
15H	−0.016	0.114

[a] Differences in ppm (cis–trans), in acetone-d_6 at 32 ± 1°. Trans shifts reported in ref 5.

Table VI. ^1H Spin–Lattice Relaxation Times for Retinal Isomers

	9-Cis		13-Cis	
Proton	Obsd[a]	Calcd[b,c]	Obsd[a]	Calcd[c,d]
HMDS	9.4 ± 0.6		10.2 ± 0.2	
$1,1'CH_3$	1.1 ± 0.01		1.0 ± 0.01	
Ring	1.0 ± 0.02			
$5CH_3$	2.1 ± 0.03		2.1 ± 0.02	
$9CH_3$	1.6 ± 0.03		1.8 ± 0.03	
$13CH_3$	2.1 ± 0.02		1.9 ± 0.02	
7H	2.2 ± 0.04		2.0 ± 0.1	
8H	1.5 ± 0.03		1.8 ± 0.03	
10H	2.5 ± 0.06	2.1	1.5 ± 0.04	1.5
11H	1.4 ± 0.03	0.5	1.7 ± 0.05	0.8
12H	2.0 ± 0.02	2.0	1.6 ± 0.04	0.4
14H	3.4 ± 0.08	3.4	7.5 ± 0.1	2.8
15H	4.3 ± 0.14	1.9	3.2 ± 0.1	0.4

[a] T_1's in seconds. Error limits are standard deviations of the nonlinear least-squares fit. Measurements in acetone-d_6 at 32 ± 1°. [b] Normalized to 12H. [c] Calculated assuming geometry described in text. [d] Normalized to 10H.

8H lines are unequal to an extent greater than experimental error. The T_1 of each of the two inner lines of the overall 7H–8H four-line system, relative to the T_1 of the outer line of the same proton, tends in the direction of the T_1 of the *other* proton. Thus, the T_1 of the downfield line of 8H (which is closer to the 7H frequency) is shorter than the T_1 of the upfield line of 8H. This phenomenon has been discussed in some detail by Freeman and Campbell[15] for a two-spin system, and has some relevance to the phenomenon reported below for 13-*cis*-retinal, in which unexpected NOE's are observed for one spin upon irradiation of a second, when a third spin which does experience a large NOE from the second is moderately strongly coupled to the first.

Table VII. 9-*cis*-Retinal Nuclear Overhauser Enhancements[a]

	Obsd						
Irradiated	7H	8H	10H	11H	12H	14H	15H
$1,1'CH_3$	12	5	0	0	0	0	0
$5CH_3$	0	8	0	0	0	0	0
$9CH_3$	14	2	15	0	0	0	0
$13CH_3$	0	0	0	11	0	0	35
8H				20			

[a] Enhancements in %. Expected error ±2%. Measured at 100.1 MHz in acetone-d_6 solution at 32 ± 1°.

Table VIII. Calculated NOE's for 9-*cis*-Retinal[a]

	Obsd					
Irradiated	8H	10H	11H	12H	14H	15H
$9CH_3$	1	16	0	−2	0	0
$13CH_3$	−3	0	10	2	2	32
8H			34			

[a] Calculated NOE's in %. See text for details of geometry assumed and external relaxation included.

Table IX. 13-*cis*-Retinal Nuclear Overhauser Enhancements[a]

	Obsd						
Irradiated	7H	8H	10H	11H	12H	14H	15H
$9CH_3$	11	6	0	17	8	0	0
$13CH_3$	0	0	0	14	4	24	0
12H							36
14H							1
15H				7	20		

[a] Enhancements in %. Expected error ±2%. Measured at 100.1 MHz in acetone-d_6 solution at 32 ± 1°.

Table X. Calculated NOE's for 13-*cis*-Retinal[a]

	Obsd					
Irradiated	8H	10H	11H	12H	14H	15H
$9CH_3$	4	2	19	0	0	0
$13CH_3$	0	0	18	1	22	0
12H						43
14H						1
15H			−1	38.2		

[a] Calculated NOE's in %. See text for details of geometry assumed and external relaxation included.

The chemical shift assignments for 9-*cis*- and 13-*cis*-retinal in acetone-d_6 are in agreement with those reported by Patel[16] in his 220-MHz study of all known retinal isomers in $CDCl_3$. However, in acetone-d_6, 7H of 9-*cis*-retinal is upfield of 12H, not downfield of 12H as in $CDCl_3$.

The chemical shifts are of primary interest as they relate to the chemical shifts of *all-trans*-retinal, which was shown[5] to have an effectively s-trans structure for its olefinic chain in solution. Referring to Table V, it can be seen that the largest shift for 9-*cis*-retinal relative to the trans isomer is the huge downfield shift of 8H. This is interpreted in terms of the steric polarization mechanism presented by Cheney.[17] This model proposes that the downfield shifts observed for sterically hindered protons are caused by a mutual polarization of the two interacting protons' C–H bonds, resulting in decreased electron density at the protons and enhanced electron density at the attached carbon nuclei. Thus 9-cis isomerization brings 8H and 11H into close proximity, and both nuclei experience significant downfield shifts relative to the trans isomer. The downfield shift exhibited by 11H is less substantial, since it was in steric contact with $9CH_3$ in the trans isomer. Conversely, $9CH_3$, with

11H removed by cis isomerization, experiences a slight *upfield* shift in 9-*cis*-retinal. The bond anisotropy mechanism of ApSimon *et al.*,[18] invoked by Patel[16] to rationalize some of the downfield shifts in cis isomers relative to trans, would predict downfield shifts for the sterically hindered carbon atoms as well. However, as discussed in detail in ref 14, the sterically hindered carbon atoms such as 8C are shifted dramatically *upfield* from their positions in the trans isomers; these ^{13}C nmr shifts are predicted by the steric polarization model of Cheney and Grant[19] and Cheney.[17] The fact of the large downfield shift of 8H in 9-*cis*-retinal is evidence that such single bonds as 8–9 and 10–11, which could theoretically twist to remove 11H from such close proximity to 8H, actually remain planar s-trans. On the other hand, the modest upfield shift of 10H in 9-*cis*-retinal may be due to the anisotropies of C–C and C–H bonds, since 10H is in a different orientation with respect to several bonds in 9-cis as compared with *all-trans*-retinal. 14H of 13-*cis*-retinal, which experiences an identical geometrical substitution of an adjacent methyl group at a 60° angle for a parallel C–H bond, also experiences a significant upfield shift relative to *trans*-retinal.

Considering next the chemical shifts of 13-*cis*-retinal relative to *all-trans*-retinal, we see that the most dramatic shift is the enormous downfield excursion of the resonance of 12H. This nucleus is forced into close contact with 15H by 13-cis isomerization, so the downfield shifts of both are predicted by the steric polarization model. 15H is somewhat sterically hindered by the $13CH_3$ group in the all-trans isomer, so the relative downfield shift of 15H is not as great as that of 12H, which is unhindered in the trans isomer. Aside from the upfield shift of 14H, mentioned above, the other substantial relative shifts for 13-*cis*-retinal seen in Table V are the large upfield shifts of $13CH_3$ and 11H. The carbonyl C=O bond is in a different orientation with respect to $13CH_3$ in this isomer. Thus $13CH_3$ is affected differently by the anisotropy of that bond, which, in addition to the removal of the steric crowding by 15H, causes the upfield shift of $13CH_3$ in 13-*cis*-retinal relative to the trans isomer. The upfield shift of 11H may reflect the easing of the steric crowding from $13CH_3$, which, with 15H removed from the other side, is free to bend away from $11CH_3$. All of these relative shifts for 13-*cis*-retinal are indicative of, or consistent with, a planar s-trans conformation of its olefinic chain.

Turning next to the coupling constants for 13-*cis*-retinal (Table IV), the most significant value in the present context is $J_{12H,15H}$, which is observed as a splitting in the spectrum of 15H and confirmed by decoupling. The 12H spectrum (Figure 1a) is broadened by a superposition of many lines and exhibits long-range couplings only by virtue of increased line width. This value for $J_{12H,15H}$ is to be compared with 0.15 Hz for *all-trans*-retinal[5] which was inferred from the line width of 15H, but not observed as a splitting in either the 15H or 12H resonances. The large value for this coupling in 13-*cis*-retinal is presumably reflective of a direct through-space interaction[20,21] and is further confirmation of the s-trans planarity of the 12–13 and 14–15 single bonds.

In the spectrum of 9-*cis*-retinal (Figure 1b), no long-range splittings are observable (except those of 7H to the ring). The resolution may be somewhat impaired by (a) the presence of the isomeric impurity and (b) the presence of a very small trace of paramagnetic impurity, contributing slightly to the line widths. (Evidence for this hypothesis is that the T_1 of HMDS in the 13-cis sample is 10.2 sec *vs.* 9.4 sec for the 9-*cis*-retinal sample (Table VI).) Despite these difficulties, evidence of a significant increase in the value of $J_{8H,11H}$ in 9-*cis*- compared with *all-trans*- retinal is given by the relative increase in line widths of the 8H and 11H resonances in 9-*cis*-retinal compared with either *all-trans*-[5] or 13-*cis*-retinal (Figure 1a). This is again an indication of the close proximity of 8H and 11H in this isomer, caused by the s-trans planarity of its conformation.

The observed and calculated T_1's listed in Table VI for 9-*cis*- and 13-*cis*-retinal provide still further indications of the planar s-trans configuration assumed by both isomers. For 9-*cis*-retinal, the calculated T_1's predicted on the basis of the idealized geometry described in the Methods section, and normalized to 12H, are in good agreement for 10H and 14H. As we have pointed out,[22] the "centroid" model for the relaxation of an adjacent proton by a rapidly rotating methyl group utilizes an effective methyl group–other proton distance which is too short. This is particularly evident for 15H. The 11H T_1 is also predicted too short. This is not too surprising, since 11H is relaxed by both $13CH_3$ (overemphasized by the "centroid" model) and 8H, and the 8H–11H distance calculated on the basis of the idealized geometry is only 1.73 Å (far less than twice the hydrogen van der Waals radius of 1.2 Å[23]). Obviously, in the actual 9-*cis*-retinal molecule, some relaxation of the bond angles occurs which increases the 8H–11H distance to a reasonable value. Putting in an 8H–11H distance of the van der Waals contact value of 2.4 Å, and keeping the other distances the same plus using the same normalization as in Table VI (*i.e.*, the same choice of rotational correlation time), yields an 11H T_1 of about 1.6 sec, compared with the experimental value of 1.4 sec. However, on the basis of the other evidence presented, this relaxation of the idealized geometry probably does not include substantial deviations from planarity. The good agreement for the calculated T_1 of 14H implies that the rotational correlation time governing both its relaxation and that of 12C is the same. For 15H, the very short calculated T_1 relative to that observed raises the possibility of oscillations about the 14–15 bond, which would serve to shorten the effective correlation time for the relaxation of 15H and lengthen its T_1.

For 13-*cis*-retinal, the agreement between observed and calculated T_1's is poor, for the same reasons as in 9-cis, except that it appears worse because in this case both of the protons involved in the unrealistically close approach (12H and 15H, with the 12H–15H distance assumed to be 1.72 Å) are now included in the list of the calculated T_1's. Also, the T_1's of 11H and now 14H suffer from the unrealistic "centroid" model for the methyl groups. In addition, there was evidence of an opening up of the 11H–$13CH_3$ distance from the chemical shift results, which was not included in the T_1 calculation. Replacing the 12H–15H distance by the more reasonable value of 2.4 Å, but leaving all other distances the same and keeping the same normalization, the T_1's become 1.7 sec for 12H and 2.4 sec for 15H. This is in good agreement for 12H, but 15H is again calculated too short. One possibility is that there is some flexibility about the 14–15 bond, although the average conformation is planar. This possibility is strengthened by ^{13}C T_1 measurements for 13-*cis*-retinal,[14] which show that the T_1 of 15C in 13-*cis*-retinal is longer than would be predicted assuming the same correlation time for the 15CH interaction as for the rest of the chain C–H groups.

Finally, we discuss the experimental and calculated NOE results. Considering Table VII, we see that for 9-cis the relaxation of 7H is dominated by $1,1'CH_3$ and $9CH_3$, that of 11H by 8H and $13CH_3$, and that of 15H by $13CH_3$. The enhancements of 7H upon irradiation of the ring methyl groups $5CH_3$ and $1,1'CH_3$ are nearly equal to those reported earlier[13] for β-ionone and *all-trans*-retinal. However, 8H shows a significantly reduced enhancement from saturation of $1,1'CH_3$ (12% in all-trans[13] and 5% in 9-cis

(Table VII)). This difference is due to the additional relaxation of 8H by 11H in 9-*cis*-retinal, and does not necessarily imply an altered 6–7 torsion in this isomer. Information about the conformation of the polyene chain of 9-cis can be gained by considering Table VIII, which lists the calculated NOE's assuming a planar s-trans geometry, in comparison with the experimental results listed in Table VII. The ring methyl groups were not included in the calculation, but for the rest of the experiments there is excellent agreement between the calculated and the observed enhancements. The overestimation of f_{11H} (8H) ($f_d(s)$ = fractional enhancement of spin d when spin s is saturated) is due to the previously discussed unrealistically short 8H–11H distance assumed. These results confirm the conclusion that the 9-*cis*-retinal olefinic chain assumes a planar s-trans structure in solution.

For 13-*cis*-retinal, the NOE results also serve to confirm the planarity of the polyene chain. Comparison of Table IX, the experimental NOE's, with Table X, the NOE's calculated on the basis of a planar s-trans conformation, shows excellent agreement for the large enhancements: $f_{11H}(9CH_3)$, $f_{11H}(13CH_3)$, $f_{14H}(13CH_3)$, $f_{15H}(12H)$, and $f_{12H}(15H)$. It is interesting in light of the previously postulated opening up of the 11H–$13CH_3$ distance to note that the actual $f_{11H}(13CH_3)$ is slightly smaller than that predicted on the basis of the idealized geometry, whereas $f_{11H}(9CH_3)$ is more nearly equal to the predicted value. The 12H–15H interaction is overemphasized in the calculated NOE's because of the unrealistically short 12H–15H distance assumed, but the predicted $f_{12H}(15H)$ is affected more than the converse experiment, since 12H has the additional relaxation pathway of 10H, which is probably equidistant with 15H from 12H in the open structure. Even in the more open structure, and with possibly some oscillation about the 14–15 single bond, the principal relaxation of 15H is from 12H, so the observed $f_{15H}(12H)$ is large. The agreement of the observed and calculated $f_{8H}(9CH_3)$ is probably fortuitous for this isomer. Irradiation of the ring methyl groups shows that the relaxation of 8H is dominated by them plus presumably the adjacent nucleus 10H. The observed enhancement $f_{8H}(9CH_3)$ may be due to the phenomenon discussed below, rather than to direct relaxation of 8H by $9CH_3$.

This interesting, not well understood feature of the 13-*cis*-retinal NOE's was alluded to earlier in this section: the observation of NOE's in resonances which cannot reasonably be caused by direct relaxation between the two spins involved. This effect can be seen in Table IX, where 11H is shown to exhibit a 7% enhancement upon irradiation of 15H. No reasonable single-bond torsions, consistent with all of the other data reported in this paper, bring 15H into proximity with 11H. This phenomenon is exhibited only by 11H and 12H. That is, when one of these spins is strongly relaxed by a third spin, thus showing a large enhancement when the third spin is saturated, the second of the 11H, 12H pair also shows an enhancement. Thus $f_{12H}(13CH_3)$ = 4% and $f_{12H}(9CH_3)$ = 8%. An example of this effect is shown in Figure 2, which shows spectra from the $13CH_3$ irradiated experiment. Note that only the large half of the 12H doublet closest to 11H shows an enhancement. This was also observed for $f_{12H}(9CH_3)$. In the case of $f_{11H}(15H)$, only the two large downfield lines of the 11H quartet, closest to 12H in frequency, show the enhancement. It is noteworthy that despite this NOE behavior, all of the lines in the 11H multiplet show the same T_1, and both peaks in the 12H doublet have the same T_1, but this may be because the T_1's of 11H and 12H are essentially equal (Table VI).

This effect appears to be limited to closely coupled nuclei and presumably is a result of the mixing of nuclear spin states. As noted above, the enhancements of 8H upon irradiation of $9CH_3$ seen in β-ionone[13] and all isomers other than 9-*cis*-retinal may then result from the AB character of 7H and 8H, combined with the substantial NOE of 7H upon saturation of $9CH_3$. The phenomenon was also noted with 11-*cis*-retinal, where a reproducible 2% enhancement of 11H upon saturation of $13CH_3$ was seen,[5] even though 11H was sufficiently distant from $13CH_3$ in all conformations that it was not expected to be relaxed at all by $13CH_3$. However, 11H in 11-*cis*-retinal is closely coupled to 10H, which is relaxed by $13CH_3$, as evidenced by the 11% enhancement $f_{10H}(13CH_3)$. The observation of this phenomenon suggests that (a) caution must be observed when interpreting NOE's when one of two closely coupled spins is being observed, and (b) further work should be done to obtain a sound theoretical basis for interpreting the NOE's in such cases.

V. Conclusion

The polyene chain portions of 9-*cis*- and 13-*cis*-retinal have been shown to exist in solution with planar, completely s-trans conformations from 7C to 15C. This conclusion is in agreement with that of Patel and Shulman[24] based on spin delocalization studies. These results are in marked contrast to the situation for 11-*cis*-retinal which was found to exist as a rapidly exchanging equilibrium between at least two conformers involving distortions around the 10–11 and 12–13 single bonds of the polyene chain.

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