tor was determined with *N,N'*-hexamethylenedimaleimide at 360, 334, and 313 nm.

Quenching of the Cyclomerization. Quenching of the cyclomerization with biacetyl was carried out with monochromatic light of 334 ± 7 nm. Solutions of 5×10^{-4} *M* dioxycoumarins in dichloromethane, containing concentrations of biacetyl varying from 0.5×10^{-3} to 1×10^{-1} *M*, were degassed, thermostated at 20°, and irradiated to obtain ±10% conversion. Biacetyl does not absorb light under these conditions. The solutions were stirred vigorously by means of a magnetic stirrer. The conversion was determined after dilution by measuring the optical density at 324 nm. The difference in optical density before and after irradiation was compared with the difference in a reference sample without biacetyl, giving directly a value of Φ^0/Φ.

Preparative Quenching Experiments. A 10^{-2} *M* solution of **1c** in dichloromethane is degassed in the presence of variable biacetyl concentrations and irradiated with a Bausch and Lomb monochromator at 334 ± 7 nm with up to 12 ± 3% conversion. The solvent and biacetyl were evaporated under reduced pressure, and the residue was dissolved in DMSO-d_6. Nmr spectra of the reaction mixture were obtained by accumulation (25 scans) on a XL 100 nmr, and the ratios of the isomer were determined by the ratio of the area of part of the nmr absorptions of the protons H_5, H_6, and H_8 in the head-to-head cyclomer over the area of the absorption of proton H_8 in the head-to-tail cyclomer. The validity of this determination was confirmed by comparison with mixtures prepared from pure cyclomers and starting materials.

Production Distribution. The relative ratios of cyclomer as a function of the excitation wavelength were determined by an analogous method as described in the preparative reaction quenching.

Acknowledgments. The authors thank the National Science Foundation of Belgium for financial support of the laboratory and for two fellowships (J. P. and L. L.) and the IWONL for a fellowship to H. L. Dr. S. Toppet is thanked for assistance by the nmr interpretation.

Supplementary Material Available. A detailed kinetic analysis and data for obtaining Figure 6 will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JACS-74-6994.

A Carbon-13 Nuclear Magnetic Resonance Study of the Visual Chromophores and Model Compounds

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Abstract: The ^{13}C nmr spectra of *all-trans*, 9-*cis*-, 11-*cis*-, and 13-*cis*-retinals, β-ionone, and *cis*- and *trans*-crotonaldehydes have been obtained in acetone-d_6 solution. The ^{13}C nmr spectra are also reported for β-ionone and *all-trans*-, 11-*cis*-, and 13-*cis*-retinals in cyclohexane-d_{12} solution. Striking differences in chemical shifts were found among the protonated carbons of the polyene chain portion of the different retinal isomers. Many of these differences are attributed to the steric polarization effect. In comparing the chemical shifts of the olefinic carbons of each cis isomer relative to the corresponding carbons of *all-trans*-retinal, the 11-cis isomer was found not to follow the pattern set by 9-*cis*- and 13-*cis*-retinals. Spin–lattice relaxation times T_1 are reported for β-ionone, *all-trans*- and 13-*cis*-retinal, and *cis*- and *trans*-crotonaldehydes. The T_1 values imply that (a) the methyl groups of the retinal polyene chain rotate rapidly compared with the overall tumbling of the molecule, and (b) the rotational diffusion of 13-*cis*-retinal and presumably *all-trans*-retinal is considerably anisotropic, with $D_{\parallel} = 4.6D_{\perp}$ for 13-*cis*-retinal.

The conjugated polyene aldehydes 11-*cis*- and *all-trans*-retinal play a crucial role in vision. In the only photochemical reaction in the vision process 11-*cis*-retinal is isomerized to the trans isomer, while attached through an imine linkage to the protein opsin. This initiates a sequence of reactions resulting ultimately in the sensation of vision.[1] The polyene aldehyde 9-*cis*-retinal is also able to combine with opsin,[2] and 13-*cis*-retinal has been implicated as the chromophore of the membrane protein bacteriorhodopsin.[3]

As part of a program to establish retinal as a nmr probe of the active site of rhodopsin, we have obtained the ^{13}C nmr spectra of *all-trans*-, 9-*cis*-, 11-*cis*-, and 13-*cis*-retinals free in solution. In addition, β-ionone and *cis*- and *trans*-crotonaldehyde have been studied as model compounds. In particular the protonated carbons of the polyene chain portion of each of the retinal isomers have been independently and unequivocally assigned, without reference to model compounds, additive parameters, or substituent effects. We discuss the observed results in terms of substituent and conformational effects previously observed in systems other than conjugated polyenes. We also present and discuss longitudinal relaxation time measurements for *all-trans*-retinal, 13-*cis*-retinal, and the model compounds.

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Experimental Section

β-Ionone, *all-trans*-retinal, 9-*cis*-retinal, 13-*cis*-retinal, and *trans*-crotonaldehyde were purchased from Eastman. 11-*cis*-Retinal was generously supplied by P. K. Brown. Acetone-d_6 and cyclohexane-d_{12} were purchased from Stohler Isotope Chemical Co. Most samples were prepared in the concentration range 0.35–0.75 *M*. Samples were filtered through sintered glass, degassed with at least five freeze–pump–thaw cycles, and sealed under vacuum. Samples so prepared, protected from light, and stored at −15° were stable for

Table I. ^{13}C Chemical Shifts in Acetone-d_6[a]

Carbon	β-Ionone	*cis*-Crotonaldehyde	*trans*-Crotonaldehyde	All-trans	9-Cis	11-Cis	13-Cis
1C	32.61			32.89	32.81	32.88	32.86
2C	38.36			38.38	38.26	38.38	38.35
3C	17.54			17.88	17.85	17.90	17.85
4C	31.84			31.64	31.61	31.61	31.61
5C	133.43			128.56	128.67	128.49	128.54
6C	134.69			136.49	136.63	136.52	136.44
7C	140.66			127.77	129.28	127.87	127.71
8C	130.74			136.25	128.49	136.52	136.20
9C	195.52			139.37	138.12	139.74	139.49
10C				128.71	127.17	124.96	128.78
11C				131.14	129.88	129.63	132.01
12C				133.81	133.16	129.63	125.84
13C		146.33	152.54	153.02	153.22	153.87	152.60
14C		129.49	133.26	127.77	127.77	128.67	126.55
15C		189.01	191.93	189.10	189.24	189.19	187.92
1,1′CH_3	27.03			27.32	27.30	27.28	27.28
5CH_3	19.74			19.96	19.98	19.89	19.92
9CH_3	25.18			11.03	18.98	10.43	10.95
13CH_3		12.09	16.56	11.03	11.08	15.93	18.97

[a] All shifts ±0.05 ppm, relative to internal HMDS.

many months. For all samples except 9-*cis*-retinal and 11-*cis*-retinal, which were prepared in 5-mm sample tubes, 12 mm tubes were used. In most cases amber sample tubes were used (Wilmad Glass). Gd(fod)$_3$ was purchased from Bio-Rad Labs. Solvents were used as purchased except for the Gd(fod)$_3$ experiments; in this case the C_6D_{12} was dried over previously heated 3 Å molecular sieve (Linde).

cis-Crotonaldehyde was prepared by irradiation of *trans*-crotonaldehyde, which had been purified by distillation, for 24 hr in a water-cooled Ace Glass Co. photoreactor cell, using a 500-W mercury arc source held in a quartz sleeve.[4] The resulting mixture of *cis*- and *trans*-crotonaldehyde was purified by distillation at reduced pressure. The distillate was analyzed by ^1H nmr and found to be 24% *cis*-crotonaldehyde and 76% *trans*-crotonaldehyde, with no evidence of impurities.

Natural abundance ^{13}C nmr spectra were obtained using a Varian-XL-100-15 spectrometer equipped with a Varian 620i computer and operating in the Fourier transform mode. Depending on the sample from 128 to 9000 transients were accumulated, with acquisition time and spectral digitization typically 0.8 sec and 1.25 Hz/pt, respectively. T_1 measurements were made under conditions of full ^1H noise decoupling using both progressive saturation $(90\text{-}\tau)_n$[5] and inversion-recovery $(T\text{-}180\text{-}\tau\text{-}90)_n$[6] sequences. The π pulse length averaged 76 μsec, corresponding to $\gamma H_1 = 6500$ Hz. The maximum spectral width for relaxation time experiments was 5000 Hz for progressive saturation and 3000 Hz for inversion-recovery. Freeman and Hill[5] have shown that for offsets up to $0.8\gamma H_1$, and properly calibrated tip angle, the T_1 measured by progressive saturation deviates not more than 5% from the actual T_1. This was verified in the present experiments by measuring the T_1 of a given peak at both small and maximum offset from the carrier frequency. The relaxation time measurements were analyzed using either a two or a three parameter non-linear least-squares program with the equilibrium peak height, spin-lattice relaxation time T_1, and sometimes the effective tip angle as parameters. The ambient temperature in the probe was maintained at 31° during all experiments including decoupling.

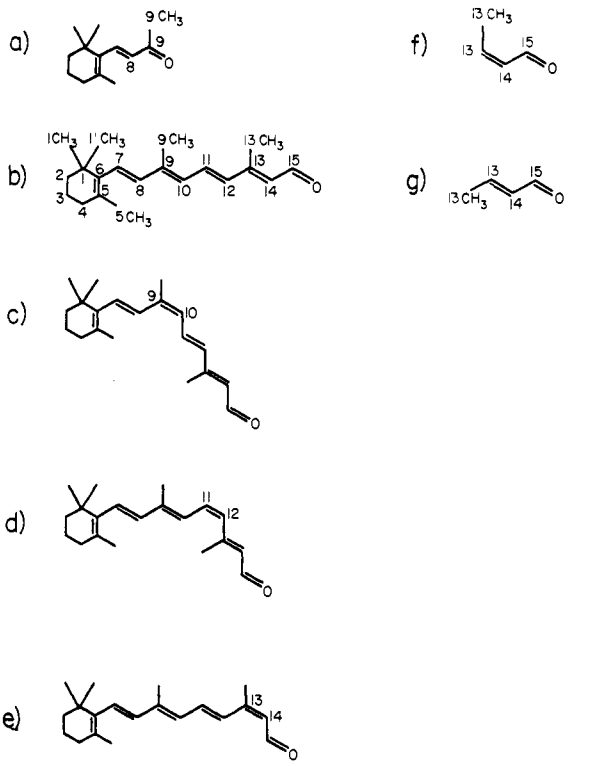

Figure 1. Visual chromophores and model compounds showing the numbering scheme used in the text and in the tables: (a) β-ionone, (b) *all-trans*-retinal, (c) 9-*cis*-retinal, (d) 11-*cis*-retinal, (e) 13-*cis*-retinal, (f) *cis*-crotonaldehyde, (g) *trans*-crotonaldehyde.

Results

Using a variety of techniques including (1) single-frequency and noise-modulated resonant and off-resonant proton decoupling, (2) T_1 relaxation time measurements, and (3) the use of the relaxation reagent Gd(fod)$_3$, all of the carbons in *all-trans*-retinal, 9-*cis*-retinal, 11-*cis*-retinal, 13-*cis*-retinal, and in the model compounds β-ionone and *cis*- and *trans*-crotonaldehyde have been assigned. The ^{13}C nmr chemical shifts of all of these compounds in acetone-d_6 solution are listed in Table I (see Figure 1 for numbering of carbons). The assigned ^{13}C nmr spectra of the polyene chain carbons of the four retinal isomers, excluding the aldehyde carbon, are shown in Figure 2. Since there is some disagreement between these assignments and those reported earlier for β-ionone and *trans*-retinyl acetate,[7] a compound which might be expected to have some

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(5) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, **54**, 3367 (1971).
(6) R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, **48**, 3831 (1968).
(7) M. Jautelat, J. B. Grutzner, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **65**, 288 (1970).

Table II. ^{13}C Spin–Lattice Relaxation Times[a]

Carbon	β-Ionone[d]	β-Ionone[b,c]	*all*-trans-Retinal[d]	*all-trans*-Retinal[c]	13-*cis*-Retinal[c]	*trans*-Croton-aldehyde[c]	*cis*-Croton-aldehyde[c]
1C	17.1[e]	23.7	9.46	17.2	14.7		
2C	1.32	1.85	0.75	1.59	1.22		
3C	0.99[e]	1.66	0.65	0.69[e]	0.93[e]		
4C	1.29	1.94	0.82	1.56	1.36		
5C	15.7	29.8	8.70[e]	17.1	18.4		
6C	23.5[h]	43.0	11.3	23.1[g]	30.7		
7C	3.85	5.26	1.07	2.39[i]	2.37		
8C	3.64	4.94	1.05	2.36	2.56		
9C	14.8[f]	44.0	9.36[e]	21.6[e]	27.4		
10C			0.97	2.18	2.26		
11C			1.00	2.18	2.45		
12C			1.02	2.36	2.53		
13C			9.80[e]	25.2	16.8	22.5	29.2
14C			1.10	2.39[i]	1.52	28.7	27.2[e]
15C			1.42	3.43	2.14	25.7[e]	33.0
1,1′CH_3	1.44	2.07	0.87	1.35	1.65		
5CH_3	4.94	6.17	3.57	4.99	3.85		
9CH_3	7.70	10.2	4.05[k]	6.00[k]	6.44		
13CH_3			4.05[k]	6.00[k]	3.97	36.2	46.3[i]

[a] Relaxation times T_1 in seconds. Samples were degassed unless otherwise noted. Error limits given are the standard deviations of the nonlinear least-squares fits. Most values are $\pm<5\%$. All values are $\pm<10\%$ unless otherwise noted. [b] Not degassed. [c] In acetone-d_6. [d] In C_6D_{12}. [e] $\pm<15\%$. [f] ±5.1 sec. [g] ±4.6 sec; corrected for partial overlap of 8C resonance. [h] ±3.8 sec. [h] ±20.9 sec. [i] 7C and 14C are coincident. [k] 9CH_3 and 13CH_3 are coincident.

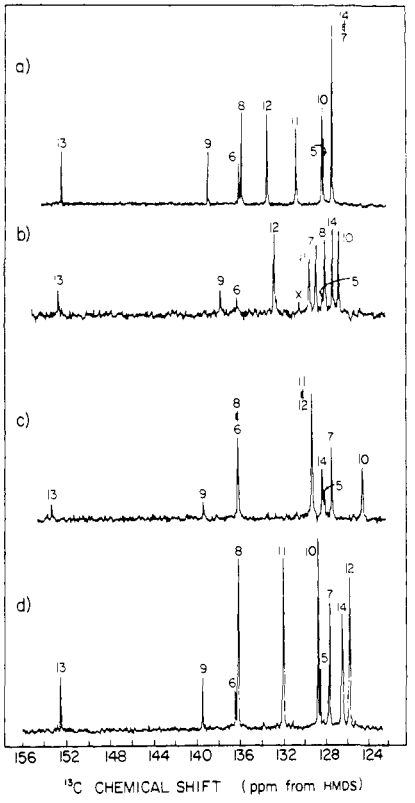

Figure 2. ^{13}C nmr (25.16 MHz) spectra of the four retinal isomers in acetone-d_6, showing the olefinic, nonaldehyde carbon region: (a) *all-trans*-retinal, (b) 9-*cis*-retinal, (c) 11-*cis*-retinal, (d) 13-*cis*-retinal. Peak marked x in spectrum b is an impurity.

spectral similarity to *all-trans*-retinal, our assignments are discussed in detail below.

In the following discussions of ^1H decoupling experiments, reference will be made to characteristics of the corresponding ^1H nmr spectra. These have been reported by several authors and include all of the retinal isomers in $CDCl_3$,[8] *all-trans*- and 11-*cis*-retinal in acetone-d_6,[9] 9-*cis*- and 13-*cis*-retinal in acetone-d_6,[10] all four retinal isomers in C_6D_{12} and ethanol-d_6,[10] β-ionone (neat),[11] and *cis*- and *trans*-crotonaldehyde neat[4] and in acetone-d_6.[12]

We first consider the assignment of the ^{13}C nmr spectrum of β-ionone. The single quaternary carbon, 1C, is identified immediately in the saturated carbon region of the ^{13}C nmr spectrum by its long T_1 value (Table II). Single frequency off-resonance ^1H decoupling distinguishes the resonances of the three methylene carbons 2C, 3C, and 4C from three methyl carbon resonances. 4C is identified by single frequency on-resonance decoupling of 4H,H′. Since the ^1H nmr spectrum of 2H,H′ and 3H,H′ is tightly coupled at 100 MHz, 2C and 3C were only tentatively distinguished from each other by ^1H decoupling. Confidence in this choice of assignments for 2C and 3C is strengthened by comparing the observed ^{13}C nmr chemical shifts with those predicted using the parameters of Savitsky and Namikawa.[13]

The methyl carbon resonances corresponding to 1,1′CH_3, 5CH_3, and 9CH_3 were easily assigned by single-frequency irradiation of the ^1H methyl resonances. Of the five unsaturated carbons, three are unprotonated and thus distinguished by their long T_1's (*cf.* Table II). The carbonyl carbon 9C was immediately identified by its chemical shift. 5C and 6C were assigned on the basis of their T_1 values. Given that the ^{13}C relaxation times are dominated by intramolecular dipole–dipole interactions with neighboring protons,[14] and assuming that the same rotational correlation time characterizes

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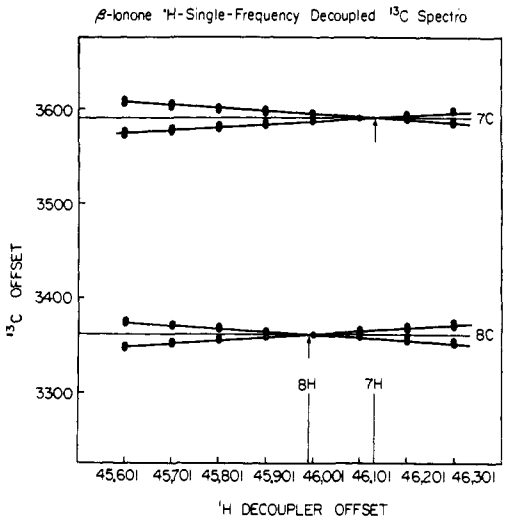

Figure 3. 7C and 8C of single-frequency proton-decoupled ^{13}C nmr spectrum of β-ionone in acetone-d_6. The residual CH coupling is plotted as a function of the proton decoupler frequency. Vertical axis: ^{13}C shifts plotted in Hz relative to arbitrary origin, with the horizontal lines indicating the peak positions observed with full noise decoupling. Horizontal axis: frequency of the proton decoupler, relative to arbitrary origin, vertical lines indicate the chemical shifts of 7H and 8H.

the interactions for both carbons, the ratio of T_1's for 5C and 6C can be estimated from

$$T_1(6C)/T_1(5C) = \sum_{\text{protons } i} r_{i,5C}^{-6} / \sum_{\text{protons } j} r_{j,6C}^{-6}$$

where $r_{i,kC}$ is the i-proton to k-carbon distance. Including only protons within 2.8 Å, the calculated ratio is 1.75. The observed ratio, assigned as in Table I, is 1.44 in acetone-d_6 and 1.50 in cyclohexane-d_{12}. This assignment is consistent with single frequency decoupling experiments, which by themselves were not conclusive. 7C and 8C were assigned by single frequency decoupling using a graphical technique[5,15] (see Figure 3, where the peak positions in several single frequency off-resonance decoupled spectra of β-ionone are plotted *vs.* the ^1H irradiation frequency). Our assignment of 7C and 8C, which was confirmed by Gd(fod)$_3$ broadening experiments (as discussed below for *all-trans*-retinal), reverses the earlier assignment;[7] assignments for the other carbons are in agreement.

The ^{13}C nmr chemical shifts of 1C, 2C, 3C, 4C, 1,1′-CH_3, and 5CH_3 remain nearly constant in going from β-ionone to the retinal isomers, as confirmed for each isomer using the same techniques as described above for β-ionone. Since the corresponding methyl proton resonances are distinct at 100 MHz, the other methyl carbon resonances, 9CH_3 and 13CH_3, were assigned by decoupling for each retinal isomer except 13-cis. For this isomer these assignments were made on the basis of line-broadening experiments with Gd(fod)$_3$.

We next consider the olefinic carbons of the retinal isomers. By its chemical shift the aldehyde carbon 15C is immediately assigned. The set of four unprotonated olefinic carbons 5C, 6C, 9C, and 13C can be distinguished from the remaining carbons by their longer T_1's (listed for *all-trans*- and 13-*cis*-retinals in Table II)

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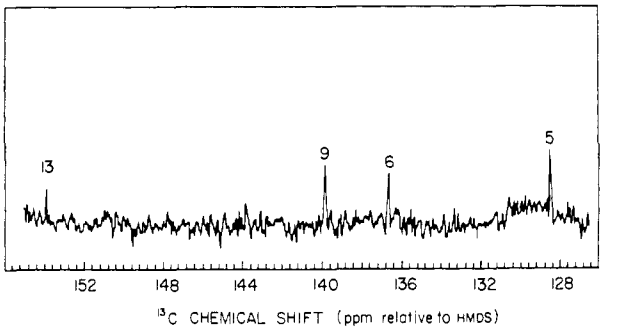

Figure 4. ^{13}C nmr spectrum of the unprotonated olefinic carbons of 11-*cis*-retinal, in acetone-d_6. Proton noise decoupler was centered at, and had a bandwidth just covering, the saturated region of the ^1H nmr spectrum.

which result in a lower intensity for these carbons in the ^{13}C nmr spectra shown in Figure 2. The situation was complicated when the location of these weak resonances was obscured by degeneracies (*e.g.*, 11-cis) or when small impurity resonances were present (*e.g.*, 9-cis; see caption, Figure 6). In these cases relatively low power proton noise decoupling, centered at and with a bandwidth just covering the ring and methyl region of the corresponding proton spectrum, resulted in a carbon spectrum with only four peaks in the olefinic region. Since the olefinic protons were not irradiated on resonance, and since the residual CH coupling is noise modulated, the protonated olefinic carbons appear as much broadened multiplets. However, the unprotonated carbons 5C, 6C, 9C, and 13C have only small ^{13}C–C–H or ^{13}C–C–C–H couplings to any of the protons not being decoupled and thus appear as relatively sharp peaks. In the ^{13}C nmr spectrum of 11-*cis*-retinal shown in Figure 4, the location of one of the unprotonated olefinic carbon resonances was thus found to be at the same frequency as the resonance assigned to 8C (see also Table I and Figure 2). 9C and 13C were assigned for *all-trans*-retinal using Gd(fod)$_3$, as described below. The assignment of 13C as the most downfield olefinic, nonaldehyde carbon resonance for each isomer is consistent with the chemical shifts observed in model compounds.[16] The remaining unprotonated olefinic carbons, 5C and 6C, were distinguished from each other on the basis that their T_1's should have approximately the same ratio as that observed in β-ionone. As assigned in Tables I and III, the ratio $T_1(6C)/T_1(5C)$ for *all-trans*-retinal is 1.35 in acetone-d_6 and 1.30 in cyclohexane-d_{12}. In 13-*cis*-retinal the ratio $T_1(6C)/T_1(5C)$ is 1.67. These values resemble those found for β-ionone (admittedly the retinals are expected to rotate less isotropically than β-ionone making the approximations involved fairly crude). The fact that the chemical shifts of the four saturated carbons of the ring remain invariant among the four isomers suggests that 5C and 6C, always at least three carbons removed from the point of isomerization, should likewise retain nearly the same chemical shifts in all isomers. From Tables I and III, and Figure 2, it can be seen that the two unprotonated olefinic carbons assigned to 5C and 6C have chemical shifts in each isomer of 128.6 ± 0.1 and 136.5 ± 0.1 ppm, respec-

(16) J. B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, New York, N. Y., 1972, pp 189–193, 435 and 436.

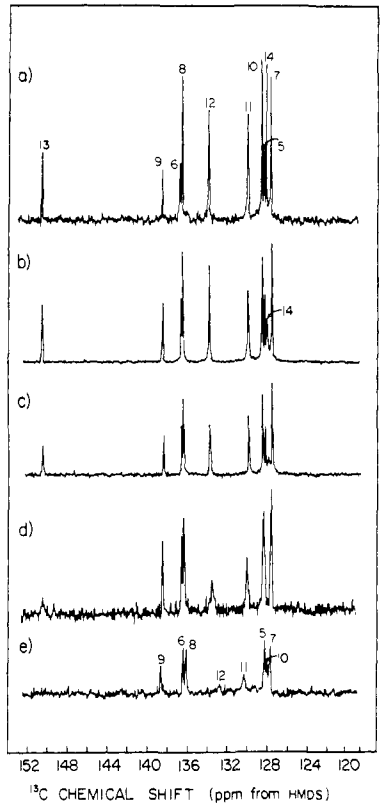

Figure 5. ^{13}C nmr spectra of olefinic region of *all-trans*-retinal in C_6D_{12}, as a function of added Gd(fod)$_3$. [Gd(fod)$_3$]/[retinal] molar ratios: (a) 0, (b) 0.00030, (c) 0.00090, (d) 0.011, (e) 0.048.

Table III. ^{13}C Chemical Shifts in Cyclohexane-d_{12}[a]

Carbon	β-Ionone	*all-trans*-Retinal	11-*cis*-Retinal	13-*cis*-Retinal
1C	32.75	32.92	32.98	32.94
2C	38.56	38.48	38.43	38.47
3C	17.89	18.10	18.14	18.12
4C	32.04	31.78	31.74	31.81
5C	132.16	128.26	128.05	128.29
6C	135.04	136.61	136.67	136.53
7C	139.80	127.56	127.61	127.54
8C	131.12	136.41	136.78	136.41
9C	192.92	138.33	138.87	138.72
10C		128.45	124.75	128.66
11C		129.83	128.80	131.12
12C		133.74	129.30	125.86
13C		150.12	151.15	150.35
14C		128.02	128.80	126.69
15C		186.33	186.41	185.78
1,1′CH$_3$	27.23	27.31	27.34	27.36
5CH$_3$	19.93	19.96	20.00	20.03
9CH$_3$	25.16	10.99	10.48	11.01
13CH$_3$		10.99	15.97	19.05

[a] All shifts ±0.05 ppm, relative to internal HMDS.

tively (relative to HMDS in acetone-d_6). Having assigned 5C, 6C, and 13C for each isomer, 9C was assigned by elimination.

The assignment of the protonated olefinic carbons remains, starting with *all-trans*-retinal. To make these assignments by single frequency decoupling was more difficult than was the case for the methyl carbons. However, when the relatively isolated ^{1}H resonance of 11H in *all-trans*-retinal was irradiated, a single sharp carbon resonance appeared at the position of one of the peaks in Figure 2 and was identified as 11C. When 7H was irradiated (putting the decoupler frequency almost on resonance for 12H, 10H, and 8H), several ^{13}C peaks were fairly sharply defined but one much more so than the others; this was identified as 7C. Further single frequency decoupling experiments with *all-trans*-retinal in acetone-d_6 were inconclusive.

Consequently a second solvent was used, cyclohexane-d_{12}, in which it was possible to use the relaxation enhancement reagent Gd(fod)$_3$. Increasing the molar ratio (always $\ll$ 1) of this reagent to *all-trans*-retinal would be expected to successively broaden the resonances of carbons more distant from the Gd, which binds at the aldehyde oxygen.[17] Given the linear structure of the *all-trans*-retinal polyene chain,[9,18] this means that the carbon resonances should successively broaden in decreasing numerical order as a function of added Gd(fod)$_3$, starting with 15C. The results of such a series of additions are shown in Figure 5. The top trace (a) is the spectrum with no Gd(fod)$_3$. At a [Gd]/[*all-trans*-retinal] ratio of 0.0003 (Figure 5b), the 15C resonance (not shown) broadens almost beyond detection. One of the other protonated carbon peaks broadens substantially at this molar ratio (and practically disappears in Figure 5c) and is assigned to 14C. The assignment of 13C is confirmed in these spectra, as it broadens and disappears at slightly higher Gd(fod)$_3$ concentration than does 14C. A protonated carbon broadens next (and is therefore assigned as 12C) and then 11C which has been assigned by decoupling. Of the peaks remaining at the highest molar ratio of [Gd]/[*all-trans*-retinal] used (Figure 5e), 12C has almost disappeared, 11C is very broad, and 10C can be assigned as the next most broadened protonated carbon (note that 10C has shifted to between 5C and 7C in Figure 5e).[19] Among the peaks shown to be the unprotonated carbons 5C, 6C, and 9C by a decoupling experiment like that shown in Figure 4, but on the Gd broadened sample, 9C is identified as being the broadest of these peaks. The only remaining peak is assigned to 8C, thus completing the assignments for *all-trans*-retinal.

In the case of 9-*cis*-retinal, the available quantity of this compound limited us to decoupling experiments in acetone-d_6. 7C and 11C were easily identifiable by single-frequency decoupling. 14C was distinguished from the other carbons by a decoupling experiment, the result of which is shown in Figure 6. The proton decoupler was centered in the proton spectrum in the region 10H to 11H, and a bandwidth for noise modulation was used which just covered this region, excluding both 14H and 15H. 14C was identified (Figure 6) as the protonated carbon resonance with reduced intensity, since 14C was not fully decoupled from 14H or 15H. 8H, 10H, and 12H are sufficiently separated from each other that a series of single frequency decoupling experiments could unequivocally distinguish

(17) G. N. LaMar and J. W. Faller, *J. Amer. Chem. Soc.*, **95**, 3817 (1973).

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

(19) The ^{13}C chemical shifts observed in the presence of Gd(fod)$_3$ (Figure 5) indicate a contact interaction with the Gd. The resultant scalar line broadening is expected to be small with respect to the dipolar relaxation and to approximately decrease with carbon number since the *magnitude* of the shifts decreases down the chain,[20] therefore not affecting any of the assignments made on the basis of line-broadening experiments.

(20) D. J. Patel and R. G. Shulman, *Proc. Nat. Acad. Sci. U. S.*, **65**, 31 (1970).

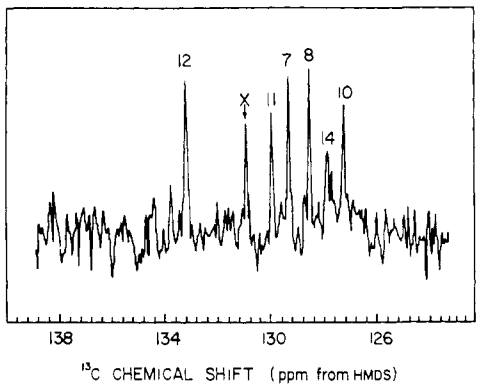

Figure 6. Partially ^{1}H noise-decoupled ^{13}C nmr spectrum of 9-*cis*-retinal. Relatively low-power proton noise decoupler was centered in the proton spectrum in the region from 10H to 11H, with a bandwidth for noise modulation excluding both 15H (farther downfield) and 14H (farther upfield). The peak labeled x is an impurity peak, thought to arise from 9,13-*cis*,*cis*-retinal, which appears from the ^{1}H nmr spectrum to be present about 10%. This peak is of greatly reduced intensity in the full noise-decoupled spectrum (Figure 2b) and is not a quaternary carbon since it fails to appear in a decoupling experiment spectrum similar to that shown in Figure 4.

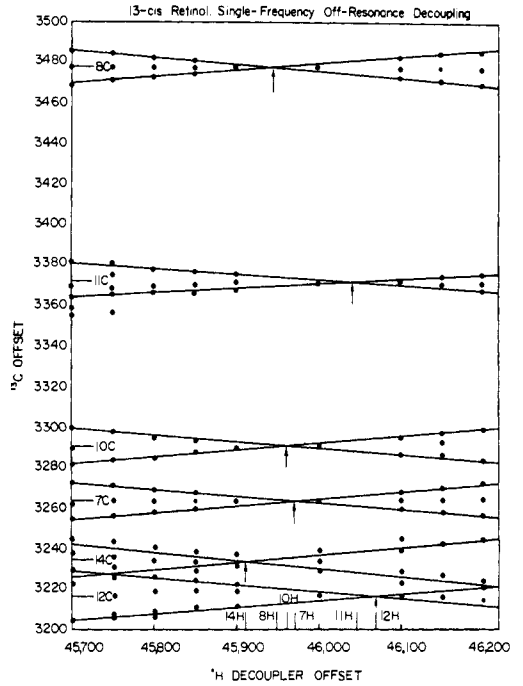

Figure 7. Single frequency proton decoupled ^{13}C nmr spectrum of 13-*cis*-retinal in acetone-d_6. Axes labeled as in Figure 3. ^{14}C is usually a doublet of doublets because of coupling to 15H.

the corresponding carbons, completing the assignments for 9-*cis*-retinal.

Turning to 11-*cis*-retinal, 7C and 11C were immediately identified by single-frequency decoupling, as were 8C and 10C. The assignment for 12C and 14C was made difficult by the near degeneracy of 14H and 12H in the proton spectrum and by the fact that one of these carbons is degenerate with 11C in acetone-d_6 solution. Therefore Gd(fod)$_3$ in C_6D_{12} was used to distinguish between 12C and 14C, and it has been assumed in Table I that these carbons retain the same relative positions in acetone-d_6 as in cyclohexane-d_{12}. The ^{13}C chemical shifts of 11-*cis*-retinal in cyclohexane-d_{12} are listed in Table III.

For 13-*cis*-retinal, the ^{1}H and ^{13}C spectra in acetone-d_6 were both reasonably well resolved so that the ^{13}C assignments could be made on the basis of decoupling experiments alone. The previously mentioned graphical method[5,15] was used, as shown in Figure 7, to establish the assignments of all of the protonated carbons of the olefinic chain for 13-*cis*-retinal.

Our assignments for *trans*- and *cis*-crotonaldehyde (Table I) were made by single frequency on- and off-resonance decoupling. The assignments for the trans isomer in acetone solution are in complete agreement with those reported earlier for the neat liquid.[21]

Discussion

The pronounced differences found among the geometrical isomers of retinal can be seen in general to depend in a regular way upon the location of the cis bond. We first consider the carbons of the cyclohexene ring. As noted previously, the chemical shifts for carbons 1, 2, 3, and 4 are to a first approximation the same for β-ionone and for the four retinal isomers. Looking more closely, slight (<0.4 ppm) shifts can be discerned for β-ionone, undoubtedly due to the close proximity of the carbonyl group. Some very slight shifts of 1C, 2C, 5C, and 6C are also seen for 9-*cis*-retinal relative to the mean shifts for all the other retinal isomers, which may derive from the nearness of the 9-cis bond to the ring. In general, as pointed out in the previous section, 5C and 6C retain very nearly the same chemical shifts in all four retinal isomers.

Turning next to the ring methyl groups, we find that 1,1′CH_3 and 5CH_3 have almost identical chemical shifts in β-ionone and the retinals, with a slight shift of 5CH_3 in β-ionone. The shieldings of these groups might be expected to reflect any similarities or differences in the torsional angle about the 6–7 single bond, which has been shown to be a distorted s-cis bond in β-ionone and *trans*- and 11-*cis*-retinals.[22] A change in the steric interaction between these methyl groups and the 7H and 8H protons should be reflected in an altered ^{13}C chemical shift for the methyl carbons.[23] Since no differences in the shifts of the methyl carbons are observed, we conclude that the average position of the ring about the 6–7 bond is essentially the same for all five compounds. Of additional interest is the fact that the two methyl groups attached to Cl are isochronous. Coincidence of the 1,1′CH_3 is also observed in the ^{1}H nmr spectrum of β-ionone, the retinals, and a number of derivatives.[11,24] Since accidental degeneracy seems unlikely to be occurring in both the ^{1}H and ^{13}C spectra, we conclude that the reorientation of the ring about the 6–7 single bond and the interconversion of the ring conformers are rapid on the nmr time scale at ambient temperature (31°).

Considering next the methyl carbons of the chain, 9CH_3 in β-ionone is α to a carbonyl group and has a chemical shift very similar to that of acetone.[25] For the retinals, we find a dramatic dependence of the chain

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(25) L. M. Jackman and D. P. Kelly, *J. Chem. Soc. B*, 102 (1970).

Table IV. Shifts of Cis Isomers Relative to *trans*-Retinal[a]

Isomer	7C	8C	9C	10C	11C	12C	13C	14C	15C
9-Cis	1.51	−7.76	−1.25	−1.54	−1.26	−0.65	0.20	0.00	0.14
11-Cis	0.10	0.27	0.37	−3.75	−1.51	−4.18	0.65	0.90	0.09
13-Cis	−0.06	−0.05	0.12	0.07	0.87	−7.97	−0.42	−1.22	−1.18

	1C	2C	5C	6C	1,1′CH_3	5CH_3	9CH_3	13CH_3
9-Cis	−0.08	−0.12	0.11	0.14	−0.02	0.02	7.95	0.05
11-Cis	−0.01	0.00	−0.07	0.03	−0.04	−0.07	−0.60	4.90
13-Cis	−0.03	−0.03	−0.02	−0.05	−0.04	−0.04	−0.08	7.94

[a] Relative shifts (cis–trans), in ppm, in acetone-d_6 solution. Positive shift means downfield.

methyl group chemical shifts on the location of the cis bond. Isochronous with $13CH_3$ in *all-trans*-retinal (as well as in retinyl acetate and *trans*-β-carotene),[7] $9CH_3$ moves downfield 7.95 ppm in 9-*cis*-retinal; $13CH_3$ stays about the same as in *all-trans*-retinal (see Table IV). However, in 13-*cis*-retinal, $13CH_3$ moves downfield 7.94 ppm, while $9CH_3$ remains the same as in all-trans. A similar effect of isomerization is noted in the crotonaldehydes. In the trans isomer, an analog of the terminal end of 13-*cis*-retinal, the methyl carbon resonance is shifted 4.47 ppm downfield from the position of the methyl resonance in *cis*-crotonaldehyde, a model for the terminal end of *all-trans*-retinal. In 11-*cis*-retinal, however, although neither methyl group is attached to a carbon which has a cis double bond, both the $9CH_3$ and $13CH_3$ resonances are shifted relative to *all-trans*-retinal. $9CH_3$ in 11-cis shows a small but significant upfield shift of 0.60 ppm, while $13CH_3$ moves downfield 4.90 ppm.

The shifts in 9-*cis*- and 13-*cis*-retinal follow the generally observed trend that α carbons of cis isomers in monoolefins absorb about 6 ppm upfield of trans isomers.[26,27] An explanation for this well-known γ effect has been offered by Grant and Cheney[23] on the basis of a steric polarization of the affected CH bonds: the steric interaction of the hydrogens attached to two carbon atoms results in a decrease in the electron density at the hydrogen nuclei concomitant with an increase in density at the affected carbon nuclei. The result is a shielding effect on the carbon which depends both on the interproton distance and the angle between the CH bond and the interproton vector. The observed relative shifts in the retinals obey the Grant and Cheney rule at least qualitatively.

In the present case, steric compression on one of the methyl groups $9CH_3$ or $13CH_3$ is *relieved* by cis isomerization at the corresponding chain carbon. In 9-*cis*-retinal, the $9CH_3$–11H interaction is removed, so $9CH_3$ shifts downfield. $13CH_3$ in 9-*cis*-retinal, still experiencing nonbonded repulsion from both 11H and 15H, remains unchanged. Similar arguments rationalize the downfield shift of $13CH_3$ in 13-*cis*-retinal, with $9CH_3$ remaining the same, relative to all-trans.[28] The situation is only slightly less straightforward in the case of 11-*cis*-retinal. A recent conformational calculation has found that the 11H–11C–10C angle is smaller in 11-*cis*-retinal than in all-trans, probably resulting in a slightly increased steric interaction between $9CH_3$ and 11H in 11-*cis*-retinal.[9] This would account for the slight shielding of $9CH_3$ in 11-*cis*- relative to *all-trans*-retinal. $13CH_3$ in 11-*cis*-retinal no longer interacts with 11H and consequently shifts downfield. The downfield shift is not so large as that in 13-*cis*-retinal, because in 11-cis, $13CH_3$ interacts significantly with 10H (confirmed by a ^1H homonuclear Overhauser enhancement of 10H upon irradiation of $13CH_3$[9]).

The most striking similarities and differences in the ^{13}C spectra of the retinal isomers are found for the carbons of the olefinic chain. Table IV presents a listing of the differences between the resonant frequencies of each of the chain carbons of the cis isomers and the corresponding carbon of *all-trans*-retinal. Two strong patterns are evident from this table. First, for carbons far from the point of isomerization (more than two carbons away from either cis-bonded carbon), the chemical shifts are very similar to those of *all-trans*-retinal. Second, considering the six chain carbons making up either side of the cis bond (*e.g.*, 7C to 12C in 9-*cis*-retinal), the shift of each carbon relative to the shift of the corresponding carbon in *all-trans*-retinal forms a trend for 9-*cis*- and 13-*cis*-retinal, which is not followed by the 11-cis isomer. For example, 11C in 9-*cis*- and 15C in 13-*cis*-retinal, each one carbon removed from the respective cis bond, show upfield shifts of 1.26 and 1.18 ppm, respectively, relative to these carbons in *all-trans*-retinal, while the corresponding carbon of 11-*cis*-retinal, 13C, shows a shift of 0.65 ppm *downfield*. This pattern, that the relative shifts for 11-*cis*-retinal deviate from the trend set by the 9-cis and 13-cis isomers, is followed for each series of positions within two carbons of the cis bond, the only exception being the series 9C in 9-cis, 11C in 11-cis, and 13C in 13-cis, where the shift in 11-cis is more similar to the 9-cis shift than is the corresponding shift in 13-*cis*-retinal.

Many of the relative shifts are plausible on the basis of increased or reduced steric crowding upon cis isomerization. For example, the tremendous upfield shift of 8C in 9-*cis*-retinal is most likely due to interaction of 8H with 11H, which was not present in the trans isomer. 11C experiences a more modest upfield shift, because 11H was previously crowded by $9CH_3$ and $13CH_3$; the 11H–8H interaction is added, but the 11H–$9CH_3$ interaction is removed, and the 11H–$13CH_3$ interaction remains. Similar arguments rationalize the shifts relative to *trans*-retinal of 12C and 15C in 13-*cis*-retinal.[30]

(26) Reference 16, p 406.

(27) D. E. Dorman, M. Jautelat, and J. D. Roberts, *J. Org. Chem.*, **36**, 2757 (1971).

(28) Additional evidence for this model is found in the proton chemical shifts. The relief of steric compression should lead to *upfield* proton shifts (of about 1/200 the corresponding downfield ^{13}C shifts).[29] In the proton spectrum $13CH_3$ in 13-*cis*-retinal shifts 0.203 ppm upfield, and $9CH_3$ in 9-cis shifts 0.022 ppm upfield relative to the corresponding resonances in *all-trans*-retinal, in acetone-d_6.[10]

(29) B. V. Cheney, *J. Amer. Chem. Soc.*, **90**, 5386 (1968).

(30) Again, the proton spectra support this model. 8H and 11H in 9-*cis*-retinal show downfield shifts from all-trans, so also do 12H and 15H in 13-*cis*-retinal.[10]

Also the previously mentioned interaction of $13CH_3$ with 10H in 11-*cis*-retinal probably accounts for much of the substantial upfield shift of 10C in this isomer, making it the most shielded olefinic carbon of any of the isomers. (Also contributing to the upfield shift of 10C in 11-*cis*-retinal is the interaction of 10H with 14H, as demonstrated by ^1H homonuclear Overhauser enhancements.[9]) In each cis isomer, the carbons making up the cis bond are shifted significantly upfield relative to the all-trans isomer. This pattern is in accord with the behavior of cis-bonded carbons in monoolefins,[31] for which no definite rationalization has been forthcoming. However, 12C in 11-*cis*-retinal shows a particularly large upfield shift. This, and the unexpected upfield shift of 13C, may be reflective of the conformational mobility which has been shown to exist about the 12–13 single bond in 11-*cis*-retinal.[9] Considering finally the set of relative shifts for 7C in 9-*cis*- and 11C in 13-*cis*-retinal, it is reasonable to suppose that the downfield shifts result from the *relief* of steric strain upon cis isomerization. The removal, by isomerization, of 11H in 9-cis and of 15H in 13-cis from a position adjacent and parallel to the respective methyl group allows the methyl group to bend away from the adjacent hydrogen (7H in 9-cis and 11H in 13-cis) on the other side of it. The failure of the shifts of the carbons of 11-*cis*-retinal to follow the pattern set by the corresponding (relative to the cis bond) carbons of 9-*cis*- and 13-*cis*-retinal may be ascribed in part to the difference in methyl substitution relative to the cis bond for 11-cis and in part to the conformational mobility of this isomer.

Thus far only the effects of cis isomerization within the retinal molecular framework have been considered. Several sets of additive substituent parameters have been devised to calculate ^{13}C chemical shifts in olefins, most notably by Savitsky and Namikawa[13] and Dorman, *et al.*[27] Both of these schemes do poorly in predicting chemical shifts for dienes and longer conjugated systems.[27,32] In the case of *trans*-retinal, there is the additional complication of the aldehyde functional group, the specific effect of which on the chemical shifts is difficult to separate from those of substitution and conjugation. It can be seen that the effect of the carbonyl group is transmitted through the π-electron system, since $9CH_3$ and $13CH_3$ are degenerate in *trans*-retinal, β-carotene, and retinyl acetate. Regarding substituent effects, even so basic a concept as the α effect apparently needs reconsideration in conjugated systems; in retinal three of the nonprotonated olefinic carbons are among the least shielded olefinic nonaldehyde carbons, while the fourth is among the most shielded. A complete study of these effects would necessarily include such additional compounds as retinol, retinyl acetate, and desmethyl retinals. Consequently no attempt at present will be made to devise correction terms for the additivity parameters to extend them to conjugated systems.

The present results cast doubt on some of the assignments previously published by Jautelat, *et al.*,[7] for the olefinic carbons in the carotenoid systems retinyl acetate, β-carotene, 15,15′-*cis*-β-carotene, and 15,15′-dehydro-β-carotene. These systems are much less amenable to single frequency decoupling, since their ^1H spectra are not as well separated. Certainly it appears that the assignments for 7H and 8H in the previous work should be reversed.[33] It is also likely that the assignments for the unprotonated olefinic carbons are in error. In particular, based on the present work one would assign 5C as the most shielded unprotonated olefinic carbon and 13C as the least shielded.

^{13}C spin–lattice relaxation times are of interest for several reasons, among these being their utility as aids to assignment and the information they contain regarding molecular motion. An example of the first case is our use of the T_1's to distinguish 5C and 6C in β-ionone and retinals. We now consider the implications of the T_1's with regard to the motional characteristics of *all-trans*- and 13-*cis*-retinal. These molecules are sufficiently large and asymmetrical that spin–rotation relaxation can be ruled out, and the samples were prepared in such a way that the intramolecular dipole–dipole mechanism from protons should dominate the ^{13}C relaxation. In the nonviscous solutions used here, the extreme narrowing approximation ($[\omega_0\tau_c]^2 \ll 1$) is certainly a good one.

Under these conditions we first consider the T_1's of the methyl carbons. From the work of Wallach,[34] and as pointed out by Allerhand,[35] one can show that for a methyl carbon undergoing rotation about its axis which is rapid relative to the tumbling of the whole molecule

$$T_1(\mathrm{A}) = 3T_1(\mathrm{B})$$

where $T_1(A)$ is the T_1 of the methyl carbon and $T_1(B)$ is the spin–lattice relaxation time of a singly protonated carbon fixed within the molecular framework, whose CH bond is parallel to the threefold axis of the methyl group. For carbon A we take $9CH_3$ and $13CH_3$ of *all-trans*-retinal in acetone-d_6 so $T_1(A) = 6.00$; for $T_1(B)$ we take the average of the T_1's of the protonated carbons 7C to 14C = 2.31. Thus $T_1(A) \simeq 3T_1(B)$, indicating that the correlation time for internal rotation of the chain methyl groups is very rapid compared to the correlation time for the overall rotation of the molecule. On the other hand, the T_1 of the geminal methyl groups attached to 1C is substantially shorter than that of the other methyl groups, implying that there is restricted rotation for $1,1'CH_3$. We note that in general the T_1's for retinal in acetone-d_6 solution are approximately twice the values measured in cyclohexane-d_6; this is consistent with the greater viscosity of cyclohexane compared with acetone.[36]

Implicit in the preceding paragraph is the notion that carbons 7 to 14 are fixed relative to one another, i.e., that the *all-trans*-retinal olefinic chain is conformationally rigid, at least out through 14C. This is consistent with the findings of the ^1H nmr and theoretical study of retinal conformation.[9] However, in both acetone-d_6 and C_6D_{12}, the T_1 of 15C is considerably longer than the mean T_1 of the other protonated chain

(31) Reference 16, p 407.
(32) Reference 16, p 76.

(33) This conclusion was also reached by Dorman, *et al.*,[27] on the basis of trying to improve the fit of predicted and observed spectra using their additivity parameters. Steric interactions of 7H with $1,1'$-CH_3, $5CH_3$, and $9CH_3$ probably cause 7C to be one of the most shielded olefinic carbons in all four retinal isomers. The large upfield shift of 8C, and large downfield shift of 7C, in β-ionone relative to *trans*-retinal are due to the carbonyl group, illustrating the difficulty in separating contributions to the ^{13}C chemical shifts.
(34) D. Wallach, *J. Chem. Phys.*, **47**, 5258 (1967).
(35) A. Allerhand and R. A. Komoroski, *J. Amer. Chem. Soc.*, **95**, 8228 (1973).
(36) E. Washurn, Ed., "International Critical Tables," 1st ed, Vol. VII, 1930.

carbons. Even when correction is made for the slightly longer CH bond length expected for the aldehyde carbon compared to the other olefinic carbons (1.11 *vs.* 1.08 Å, which would account for a 20% increase in the T_1), the T_1 of 15C is still long. 15C must have a shorter correlation time than the rest of the chain carbons, which implies some oscillatory motion about the 14–15 single bond.

We now turn to consideration of the isotropy of the tumbling of retinal in solution. A reasonable assumption is that one of the principal diffusion axes is the axis of the chain. For *all-trans*-retinal, all of the relaxation vectors for the protonated chain carbons are perpendicular to this axis, so it is impossible to determine from these T_1's the relative rates of rotation about this axis compared to an axis perpendicular to the chain. The situation is more informative for 13-*cis*-retinal, which has a bend at the end of the chain. We assume that one of the principal diffusion axes is the axis of the chain from 7C to 13C, but now the relaxation vector of 14C makes a 30° angle with the main axis. We now use the formula of Woessner[37] as transcribed by Noggle[38] for the rotational correlation time in the case of anisotropic motion in a symmetric-top molecule

$$\tau_c(\theta) = \frac{(3\cos^2\theta - 1)^2}{24D_\perp} + \frac{3\sin^2\theta\cos^2\theta}{5D_\perp + D_\parallel} + \frac{{}^3/_4\sin^4\theta}{2D_\perp + 4D_\parallel}$$

where $\tau_c(\theta)$ is the correlation time of a carbon whose CH bond makes an angle θ with the major rotational axis, $D_\parallel$ is the diffusion constant for rotation about this axis, and $D_\perp$ is the diffusion constant for rotation about an axis perpendicular to the main axis. While we do not know the correlation times, we do know that T_1 (90°) = 2.43 (the average value for protonated chain T_1's 7C to 12C) and T_1 (30°) = 1.52 (T_1 of 14C). Under the extreme narrowing condition, one can now obtain the ratio of $D_\parallel$ to $D_\perp$ by taking the ratio of $\tau_c(90°)/\tau_c(30°) = T_1(30°)/T_1(90°)$. One obtains $D_\parallel = 4.6D_\perp$. Presumably something approximating this ratio also obtains in *all-trans*-retinal, where the mean T_1 of the protonated carbons 7C–14C is 2.31, close to the T_1 (90°) of 13-*cis*-retinal, 2.43. It is noteworthy that the T_1 of 15C in 13-*cis*-retinal is also longer than would be predicted if it had the same correlation time as 14C, again implying some oscillatory motion about the 14–15 bond.

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(38) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect," Academic Press, New York, N. Y., 1971, p 27.

Carbon-13 Magnetic Resonance Investigation of Retinal Isomers and Related Compounds

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Abstract: Carbon-13 chemical shift and relaxation data are reported for the *all-trans*-retinal as well as several isomeric and related species. The results demonstrate that *all-trans*-, 13-*cis*-, and 9-*cis*-retinal, as well as the related all-trans- and 9-cis 15-carbon aldehydes, exist as essentially planar structures in the polyene portion of these molecules. However, 11-*cis*-retinal, the chromophore of the visual pigment rhodopsin, is not planar along the entire polyene chain but exists as an intermediate structure. Both chemical shift and relaxation data indicate substantial segmental motion about the C-12, C-13 bond. Woessner's equations for the effect of anisotropic motion on spin relaxation are used to confirm structural details of the rigid parts of these molecules and failure of these expressions to correlate relaxation data can be used to characterize segmental motion of molecular moieties.

The solution conformation of retinal isomers is of value in understanding the mechanism by which the chromophore 11-*cis*-retinal serves as the visual pigment when attached to the protein opsin through an imine linkage. The structures of *all-trans*- and 11-*cis*-retinal have been determined by X-ray crystallography[2,3] in the solid state and proton nmr studies[4–7] have been used to study their conformations in solution.

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