JOURNAL

OF THE AMERICAN CHEMICAL SOCIETY

© Copyright 1985 by the American Chemical Society

VOLUME 107, NUMBER 17 AUGUST 21, 1985

High-Resolution Carbon-13 NMR of Retinal Derivatives in the Solid State

G. S. Harbison,[†] P. P. J. Mulder,[‡] H. Pardoen,[‡] J. Lugtenburg,[‡] J. Herzfeld,[†] and R. G. Griffin*[§]

Contribution from the Department of Physiology and Biophysics, Harvard Medical School, Boston, Massachusetts 02115, Department of Chemistry, Rijksuniversiteit te Leiden, 2300 RA Leiden, The Netherlands, and Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139.
Received August 28, 1984

Abstract: ^{13}C NMR spectra of a large number of crystalline retinal derivatives have been obtained by using cross-polarization and magic-angle sample spinning. Most derivatives yield spectra with narrow lines (width around 20 Hz) which can be assigned via their $^{13}C-^{1}H$ dipolar coupling, their chemical shift tensors, comparison with solution spectra, or specific labeling. Measurement of the rotational sideband intensities in the spectra permit calculation of the chemical shielding tensors, and these data have allowed us to analyze variations in isotropic shifts of these compounds in more detail. We show that the tensors exhibit an odd/even effect which results from the steric crowding on one side of the polyene chain, that π-electron perturbations affect primarily the in-plane elements of the tensor, and, conversely, that strong steric interactions due to cis–trans isomerization affect the out-of-plane element. Finally, we observe a downfield shift at the C-5 position on isomerization about the 6–7 bond, and thus deduce from the observed shifts that retinal derivatives are 25–30% 6-s-trans in solution.

The polyene aldehyde *all-trans*-retinal and its geometrical isomers play a central role in rhodospin and other visual pigments[1,2] and in bacteriorhodopsin and other light-driven membrane pumps.[3,4] In both types of systems, photon-induced isomerization is the initial event of light transduction.[5,6] However, in neither case are the spectroscopic properties of the intact protein similar to those of the free aldehydes, or of simple model compounds derived from it. Clearly, therefore, the conformational and electronic properties of the chromophores are strongly modulated by the apoprotein, and an understanding of the differences between bacteriorhodospin and rhodospin on the one hand, and retinal and its derivatives on the other, is crucial to an understanding of the entire system.

For some years, ^{13}C NMR has been used sporadically to investigate both retinal derivatives and retinal-containing proteins. Initially, solution NMR techniques were applied to detergent-solubilized rhodospin and bacteriorhodopsin,[7,8] but the spectra obtained by using this approach were both of poor quality and prone to artifacts. The problems resulted from the extreme measures necessary to achieve short rotational correlation times in solution for what are both intrinsic membrane proteins. A more logical approach is to use the methodology of high-resolution dilute-spin NMR of solids, namely cross-polarization combined with magic-angle sample spinning (MASS),[9] to study the intact membranes. This not only provides higher quality data but also permits examination of the anisotropic NMR interactions which are averaged out in solution. Our recent studies[10–12] of variously labeled dark-adapted bacteriorhodopsins have enabled us to determine both isotropic chemical shifts and chemical shift anisotropies for both all-trans and 13-cis isomers at several positions on the retinal moiety and establish for the first time the configuration of the retinal Schiff base about the C=N linkage.

However, while extensive solution NMR studies have been published on retinals,[13–15] their Schiff bases,[16] and other deriva-

[†] Harvard Medical School.
[‡] Rijksuniversiteit te Leiden.
[§] Massachusetts Institute of Technology.

(1) Wald, G. *Science (Washington, D.C.)* **1967**, *162*, 230–242.
(2) Pitt, G. A. J.; Collins, F. D.; Morton, R. A.; Stok, P. *Biochem. J.* **1955**, *59*, 122–128.
(3) Oesterhelt, D.; Stoeckenius, W. *Nature (London), New Biol.* **1971**, *233*, 149–152.
(4) Lanyi, J. K.; Weber, H. *J. Biol. Chem.* **1980**, *255*, 243–250.
(5) Hubbard, R.; Kropf, A. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *41*, 130–134.
(6) Braiman, M.; Mathies, R. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 403–407.
(7) Shriver, J.; Mateescu, G.; Fager, R.; Torchia, D.; Abrahamson, E. W. *Nature (London)* **1977**, *270*, 271–274.
(8) Yamaguchi, A.; Unemoto, T.; Ikegami, A. *Photochem. Photobiol.* **1981**, *33*, 511–516.
(9) Schaefer, J.; Stejskal, E. O. *J. Am. Chem. Soc.* **1976**, *98*, 1031–1032.
(10) Harbison, G. S.; Herzfeld, J.; Griffin, R. G. *Biochemistry* **1983**, *22*, 1–5.
(11) Harbison, G. S.; Smith, S. O.; Mulder, P. P. J.; Pardoen, H.; Lugtenburg, J.; Herzfeld, J.; Mathies, R.; Griffin, R. G. *Biochemistry* **1984**, *23*, 2662–2667.
(12) Harbison, G. S.; Smith, S. O.; Winkel, C.; Pardoen, H.; Lugtenburg, J.; Herzfeld, J.; Mathies, R.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 1706–1709.

0002-7863/85/1507-4809$01.50/0 © 1985 American Chemical Society

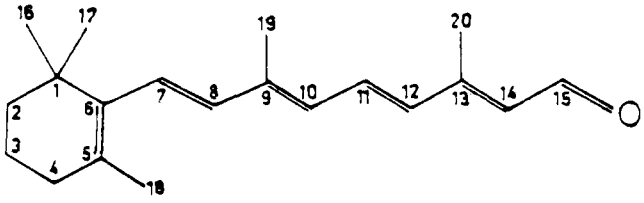


Figure 1. Structure of *all-trans*-retinal, showing the numbering system used in this paper. Note that the conformation shown is 6-s-cis.

tives,[17] neither the isotropic shifts nor the chemical shift tensors of these compounds have been examined in the solid state. More generally, while the overall properties of chemical shift tensors have been studied for many classes of functional groups, there has been no systematic, in-depth investigation of anisotropic shifts for any set of organic compounds. Such studies are commonly performed for isotropic shifts in solution NMR[18] and can be quite informative. In addition, the capacity of high-resolution, solid-state NMR for studying molecular conformations which rapidly equilibrate in solution but which are "frozen out" in the crystalline state (and may also be so in the interior of a protein) has, with limited exceptions,[19] also gone untapped. For these reasons and, in addition, to provide a data base for further studies of bacteriorhodopsin and rhodopsin, we present here an extensive ^{13}C NMR study of retinals, retinal Schiff bases, and other carotenoids in the solid state.

Experimental Section

all-trans-Retinal was purchased from Aldrich. *all-trans*-Retinoic acid, retinol acetate, and β-carotene were obtained from Sigma. 9-*cis*- and 13-*cis*-retinal were obtained by irradiating *all-trans*-retinal, dissolved in hexane, with sunlight for 2 h and purifying with HPLC (silica gel column, 5% ether in hexane eluant). Retinal Schiff bases were prepared by reacting retinal with a 3-fold excess of the anhydrous amine in methanol over 3-Å molecular sieves for 2 h in the dark at 0 °C, followed by recrystallization from acetonitrile. Schiff base salts were prepared by reacting the base in diethyl ether at −40 °C with a large excess of the anhydrous acids and recrystallizing the filtered product from chloroform by adding two volumes of diethyl ether (chloride, bromide) or by treating the Schiff base in hexane with a 10% excess of the acid at −40 °C and recrystallizing the filtered product from hexane or pentane (trichloroacetate, dichloroacetate). 2,4,6-Octatrienal was prepared from crotonaldehyde by published methods[20] and converted to its butylimine Schiff base as for retinal. The triclinic modification of *all-trans*-retinoic acid was obtained by evaporation of an isooctane solution at 90 °C. The monoclinic modification was prepared by allowing a saturated, filtered solution of the acid in hexane at 45 °C to cool to room temperature. Both forms were checked by comparing the crystals microscopically with published morphologies.[21,22] All other materials were crystallized from hexane before spectroscopy. [10-^{13}C]-, [11-^{13}C]-, and [12-^{13}C]-*all-trans*-retinal, and [12-^{13}C]-13-*cis*-retinal were prepared as previously described[23] and crystallized from hexane before use. Spectra were obtained at 6.9 T by using MASS rotors of an Andrew-Beam design with a home-built spectrometer. Cross-polarization from ^{1}H to ^{13}C spin systems was achieved with rotating fields of 25 and 100 G, respectively, and a mixing time of 2 ms. The ^{1}H-decoupling field was equivalent to 65 kHz, and a flipback pulse[24] was applied to accelerate data acquisition. All chemical shifts were referenced to external (neat) tetramethylsilane (TMS), and no correction was made for bulk susceptibility effects, which we expect to be small.

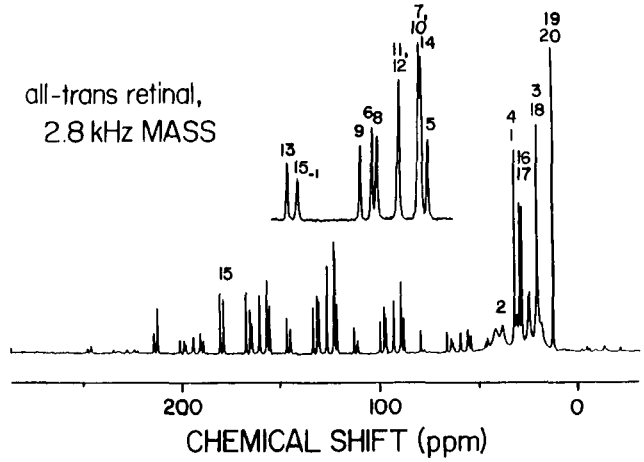


Figure 2. Magic-angle spinning spectrum of crystalline *all-trans*-retinal, at 2.81-kHz rotation frequency, showing the assignment of rotational centerbands obtained as described in the text.

Results

1. Spectra. Figure 1 depicts the structure of and the numbering system conventionally used for *all-trans*-retinal, while Figure 2 shows the MASS spectrum of crystalline *all-trans*-retinal obtained at a rotation frequency of 2.8 kHz. This spectrum is typical of the quality obtained from compounds of this sort. Aliphatic resonances in the upfield part of the spectrum exist as strong single lines with almost no sideband intensity, while the downfield region of the spectrum is comprised of centerbands and two sets of rotational sidebands deriving from the 11 unsaturated carbons of the molecule. The inset shows an expanded view of the olefinic centerband region, displaying the excellent resolution typical of many retinal derivatives in the solid state ($\Delta\nu_{1/2}$ = 0.15 ppm). The line width appears largely to be due to magnetic susceptibility anisotropy,[25] as it is limited neither by field inhomogeneity, decoupling power nor magic-angle misadjustment. It is considerably less in substances such as *all-trans*-retinal, where the conjugated chains are packed herring-bone fashion in the crystal,[26] than in triclinic *all-trans*-retinoic acid, where they are aligned parallel.[21] This spectrum, as is true for most of the others discussed in this paper, has been completely assigned, as shown in the figure.

2. Assignment. The problem in assigning chemically similar resonances in MASS spectra of crystalline organic compounds of even moderate molecular weight can be difficult. The interactions typically used to assign carbon spectra in solution (proton–proton and proton–carbon scalar couplings) are of limited utility in solids because they are dwarfed by the larger proton–proton dipolar interactions. Nevertheless, using a combination of other approaches, we have been able to assign nearly all the resonances in the retinal derivatives which we have examined. We delineate these approaches now in detail, first to describe how our results were obtained and second because, to the best of our knowledge, no comprehensive assignment of a class of compounds of this sort has been made, and the strategies potentially available for such work have not been discussed.

a. Analogy with Solution Spectra. As a general rule, ^{13}C chemical shifts of compounds in the crystalline state are not identical with those observed in solution. This is seen by comparing the data in Tables IV–VI with solution results. Small configurational distortions caused by crystal packing, differences in steric interactions, local charge distributions, and diamagnetic shielding all contribute to deviations in isotropic chemical shifts of up to 4 ppm in the crystalline state. It is therefore obvious that an assignment based solely on solution ^{13}C chemical shifts, particularly

(13) Patel, D. J. *Nature (London)* **1969**, *221*, 825–828.
(14) Rowan, R.; Sykes, B. D. *J. Am. Chem. Soc.* **1974**, *96*, 7000–7008.
(15) Becker, R. S.; Berger, S.; Dalling, D. K.; Grant, D. M.; Pugmire, R. J. *J. Am. Chem. Soc.* **1974**, *96*, 7008–7014.
(16) Tokito, Y.; Inoue, Y.; Chujo, R.; Miyoshi, Y. *Org. Magn. Reson.* **1975**, *7*, 485–488.
(17) Englert, G. *Helv. Chim. Acta* **1975**, *58*, 2367–2390.
(18) MacDonald, J. C. *J. Magn. Reson.* **1979**, *34*, 207–222 and references therein.
(19) Munowitz, M.; Bachovchin, W. W.; Herzfeld, J.; Dobson, C. M.; Griffin, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 1092–1196.
(20) Mukaiyama, T.; Ishida, A. *Chem. Lett.* **1975**, 1201–1201.
(21) Stam, C. H. *Acta Crystallogr., Sect. B* **1972**, *B28*, 2936–2945.
(22) Stam, C. H.; McGillivary, C. H. *Acta Crystallogr.* **1963**, *16*, 62–68.
(23) Pardoen, J. A.; Neijentesch, H. N.; Mulder, P. P. J.; Lugtenburg, J. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, 341–346.
(24) Tegenfeldt, J.; Haeberlen, U. *J. Magn. Reson.* **1979**, *36*, 453–457.
(25) Garroway, A. N.; VanderHart, G.; Earl, W. L. *Philos. Trans. R. Soc. London, A* **1981**, *A229*, 609–628.
(26) Hamanaka, T.; Mitsui, T.; Ashida, T.; Kakudo, M. *Acta Crystallogr., Sect B* **1972**, *B28*, 214–222.

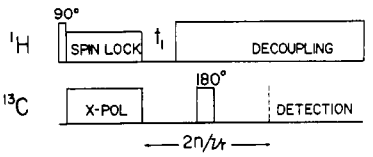


Figure 3. Delay-without-decoupling pulse sequence of Munowitz et al.[33] used to assign unprotonated and methyl carbon resonances of retinal derivatives. The essential features are the delay period, t_1, during which ^{1}H decoupling is absent and the 180° refocusing pulse which removes phase shifts of the sidebands.

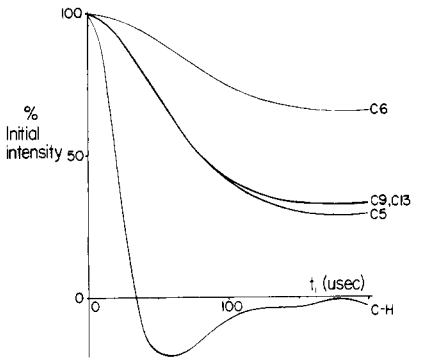


Figure 4. Calculated dipolar evolution of the olefinic resonances of *all-trans*-retinal, as a function of the delay without decoupling (t_1).

where there are many closely spaced lines,[27] is likely to be incorrect. As an example, it was recently shown that in crystalline glucose, the order of the chemical shifts is different from that observed in solution.[28] Therefore, we have been very cautious in applying this method, employing it only where the assigned lines differ by 5 ppm or more, where no other method was readily available, and where there was no reason to expect a significantly different conformation in solution from that of the crystalline material.

b. ^{13}C–^{1}H Dipolar Interactions. Opella and Frey[29] first proposed a delay-without-decoupling (DWD) pulse sequence as a means of differentiating between protonated and nonprotonated carbons in the solid state (Figure 3). We have employed the improved version of Munowitz et al.,[30] which eliminates frequency-dependent phase shifts. The essential feature is the period t_1, during which ^{1}H decoupling is removed and the ^{13}C magnetization precesses in the dipolar field of the nearby protons. For a protonated carbon a t_1 of 40–50 μs results in a null in the signal intensity. Because C–H bond lengths are almost invariant, the dipolar evolution of all C–H groups is essentially identical, and the DWD sequence will not discriminate among them. However, nonprotonated carbons, whose C–H dipolar interactions are derived from protons two or more bonds distant, have a wide range of dipolar couplings, and the DWD pulse sequence can be of considerable value in differentiating between them. Methyl groups also display a range of dipolar couplings for somewhat similar reasons. Specifically, motional averaging due to 3-fold hops of the methyl group, which is always in the fast limit at ambient temperatures (cf. ref 31), causes the interaction with directly bonded protons to be comparable to unaveraged interactions with more distant protons, which, as already stated, vary widely.

Ignoring, for the moment, proton–proton couplings, the rate of evolution of a ^{13}C signal in the field of distant protons may be calculated explicitly by summing the individual dipolar tensors in a common reference frame for all combinations of states, calculating the sideband patterns of the summed states by the method of Herzfeld and Berger[32] and Fourier transforming to give the time evolution. The results of such a calculation are diagrammed in Figure 4, for the nonprotonated olefinic carbons of *all-trans*-retinal, where it is apparent that the effective dipolar interaction decreases in the order C-5 ≥ C-9 = C-13 ≫ C-6. Additionally, the relative orientation of dipolar tensors and the chemical shift tensor of C-5 is different from those of C-9 and C-13, and, therefore, the dipolar evolution of the chemical shift sidebands also varies somewhat. In most organic solids, the ^{13}C–^{1}H interaction in DWD is strongly modulated by ^{1}H–^{1}H coupling, leading to considerable divergence between actual and calculated rates of dipolar evolution. However, at least in retinal derivatives, proton spin–flip rates are sufficiently similar to preserve the relative rates of evolution of the nonprotonated carbons. As an example of the use of DWD for the assignment of such carbons, we show in Figure 5 a series of spectra of the downfield region of *all-trans*-retinal, obtained with values of t_1 from 0 to 180 μs in increments of 45 μs. The spinning frequency is nearly identical with that of Figure 2. Signals from protonated carbons disappear almost completely within 45 μs, and 180 μs, the signal from the downfield sidebands of C-5 has also almost vanished, while that of C-6 remains strong. In most cases, we have found it unnecessary to obtain an entire series of such spectra; a single t_1 of 135–180 μs is usually sufficient.

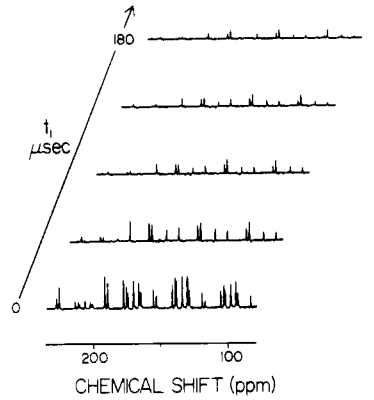


Figure 5. Experimental dipolar evolution of the centerbands and sidebands of the olefinic resonances of *all-trans*-retinal. Each slice represents an increment of 45 μs in t_1.

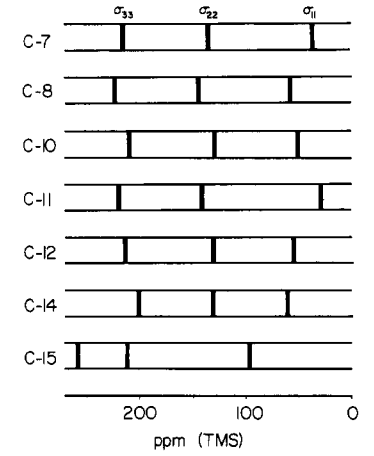


Figure 6. Chemical shift tensor principal values for the protonated olefinic carbons of *all-trans*-retinal, obtained from the sideband intensities of spectra of labeled and unlabeled retinal by the method of Herzfeld and Berger.[32]

c. Specific Isotopic Labeling. Specific isotopic labeling is obviously an unambiguous means to distinguish between resonances in MASS spectra, when specifically labeled compounds can be synthesized. We have used ^{13}C-10-, ^{13}C-11-, and ^{13}C-12-labeled *all-trans*-retinals, and their protonated and unprotonated Schiff bases, to obtain definitive assignment of these

(27) Hexem, J. G.; Frey, M. H.; Opella, S. J. *J. Am. Chem. Soc.* **1983**, *105*, 5717–5719.
(28) Pfeffer, P. E.; Hicks, K. B.; Frey, M. H.; Opella, S. J.; Earl, W. L. *J. Magn. Reson.* **1983**, *55*, 344–346.
(29) Opella, S. J.; Frey, M. H. *J. Am. Chem. Soc.* **1979**, *101*, 5854–5856.
(30) Munowitz, M. G.; Griffin, R. G.; Bodenhausen, G.; Huang, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 2529–2533.
(31) Alemany, L. B.; Grant, D. M. Alger, T. D.; Pugmire, R. J. *J. Am. Chem. Soc.* **1983**, *105*, 6697–6704.
(32) Herzfeld, J.; Berger, A. E. *J. Chem. Phys.* **1980**, *73*, 6021–6030.

Table I. Chemical Shift Tensors of Retinal and Derivatives

position	σ_{33}	σ_{22}	σ_{11}
	all-trans-Retinal		
5	215.9	141.1	30.9
6	230.4	141.5	51.0
7	213.8	134.5	38.8
8	224.2	144.4	59.2
9	228.0	168.5	32.7
10	211.2	129.7	50.2
11	220.9	142.2	29.3
12	215.6	130.9	55.2
13	249.9	187.6	31.2
14	200.7	131.0	60.9
15	259.9	212.9	97.6
	Unprotonated Retinal Schiff Bases		
	(Mean of < Five 6-s-cis Values Given as ±1 Std Dev)		
15	241.6 ± 5.1	154.2 ± 6.9	85.7 ± 6.1
14	204.9 ± 1.7	124.7 ± 4.1	63.9 ± 3.0
13	237.0 ± 7.4	165.8 ± 4.8	28.65 ± 7.5
12	210.1	130.1	67.0
11	213.3 ± 8.9	136.6 ± 1.7	28.8 ± 5.9
10	207.6 ± 3.6	127.3 ± 0.9	61.4 ± 1.7
9	221.7 ± 7.9	155.4 ± 3.3	30.25 ± 6.7
8	224.1	132.2	62.7
7	214.9 ± 11.0	131.6 ± 4.2	30.3 ± 13.0
6	235.45	134.55	41.7
5	202.2	143.2	33.0
	6-*s*-*trans*-*all-trans*-Retinoic Acid		
5	234.9	143.1	28.1
6			
7	225.6	132.8	34.3
8			
9	235.2	159.7	24.3
10			
11			
12			
13	257.0	183.3	25.9
14	211.8	104.3	51.2
15	234.8	169.4	113.7

lines and accurate values of their chemical shielding tensors, in a few retinal derivatives. These results support our use of the ^{13}C chemical shielding tensors of these carbons as a general means of assignment of the rest of the materials studied.

d. Chemical Shielding Tensors. Using the method of Herzfeld and Berger,[32] principal values of the chemical shielding tensor can be reconstructed from the rotational sideband intensities. The chemical shielding tensors of two carbons may be sufficiently different to permit us to differentiate between them, even where the isotropic shifts are insufficient to do so. In Figure 6, we present the tensors of the protonated carbons of *all-trans*-retinal, obtained from spectral sideband intensities of labeled and unlabeled retinals. Discounting the tensor of the aldehyde carbon C-15, which is very similar in principal values to previously published aldehyde shift tensors,[33] it is apparent that the other tensors can be divided into two classes. In the first class are the tensors of even-numbered carbons—C-8, C-10, C-12, and C-14—which have σ_{11} of 50–65 ppm and $\eta = 1$, while the second consists of the odd-numbered carbons—C-7 and C-11—which have σ_{11} upfield-shifted to 30–40 ppm with $\eta = 0.75$. The σ_{11} element which is most affected is aligned perpendicular to the plane of the double-bonded system.[35] As is seen in Table I, this phenomenon is repeated in the *all-trans*-retinoic acids, in retinol acetate, and in unprotonated retinal Schiff bases. It has also been reported in dark-adapted purple membrane.[11] Aside from this odd–even effect, σ_{11} varies little among these compounds. In contrast, no odd–even effect is evident in the shielding tensor principal values of the unsubstituted linear polyene Schiff base *N*-butyl-2,4,6-octatrienimine (Table II). We therefore surmise that the odd–even effect in σ_{11} is a result of methyl substitution on the polyene chain and is largely independent of the nature of the functional group on C-15.

Table II. Chemical Shielding Tensors in *N*-Butyl-2,4,6-octatrienylidineimine

position	σ_{33}	σ_{22}	σ_{11}
1	245.9	168.9	78.8
2	207.6	128.2	61.8
3	230.9	131.1	61.6
4	202.9	141.2	54.8
5	217.4	132.2	65.0
6	209.3	134.9	57.8
7	225.0	131.6	49.2

There are two possible sources for this effect. One is a change in the diamagnetic shielding caused by the presence or absence of the electrons of the methyl group. However, it appears somewhat unlikely that changes of this magnitude could be brought about two or three bonds distant from the substitution. A more likely explanation is that the phenomenon is steric in origin, and there are several arguments in favor of this hypothesis. First, it is known from X-ray crystallography that the polyene chain in *all-trans*-retinal derivatives is sterically strained on the methyl group side. This crowding results in a 20–30° bending of the chain along its length in *all-trans*-retinal[26] and in retinoic acids.[21,22] Second, sterically-induced changes on cis–trans isomerization are much larger than those in simple olefins. These changes can be attributed to the so-called γ effect and can be as great as 9 ppm,[11,17] as opposed to the 4–6 ppm changes observed in less-crowded systems. A 9 ppm isotropic shift corresponds to a 27 ppm change in the tensor elements, which is sufficiently large to account for changes in the tensors of the magnitude observed in *all-trans*-retinal.

e. Assignments. Using a combination of the above four methods, we have assigned the compounds listed in Tables IV–VI. Where residual uncertainly remains about the assignment, we have put the chemical shift in question in parentheses. The chemical shielding tensors for some of these compounds are listed in Table I; these data are much less complete, since resonances which partially overlap may show sufficient differences in sideband intensity to be assigned, while not permiting accurate tensor calculation. Additionally, we note that the tensors of non-protonated carbons show larger errors than those of protonated ones, presumably because of anisotropic cross-polarization.

Discussion

1. Factors Influencing Polyene Chemical Shielding Tensors. Aside from the odd–even effect on σ_{11}, and perhaps local diamagnetic shielding at C-15 and, to a lesser extent, at C-14, all the differences in isotropic chemical shifts observed between *all-trans*-retinal derivatives, and between positions on the molecule close to or far away from the prosthetic group, are a result of differences in the in-plane tensor elements σ_{22} and σ_{33}. When one considers the source of these differences, this fact is entirely unsurprising. According to Karplus-Pople theory,[35] which has been applied to ^{13}C chemical shifts in conjugated polyenes,[36] the changes in chemical shift on protonating a polyene Schiff base, or on substituting a more- for a less-electronegative group, derive in large part from changes in the local paramagnetic contribution to the chemical shielding. According to Salem,[37] these effects appear only within the plane of the conjugated system.

In other work on ^{15}N tensors in protonated Schiff bases,[38] we showed that the hydrogen-bonding affects σ_{22} and σ_{33} proportionately and that the effect on σ_{22} was relatively greater. Although the ^{13}C data are insufficiently accurate to draw so close a correlation, even for the C-13 carbon which has the largest chemical shift changes for this family of compounds, there does

(33) Pines, A.; Gibby, M. G.; Waugh, J. S. *Chem. Phys. Lett.* **1972**, *15*, 373.
(34) Wolff, E.; Griffin, R. G.; Waugh, J. S. *J. Chem. Phys.* **1977**, *67*, 2387.
(35) Karplus, M.; Pople, J. A. *J. Chem. Phys.* **1963**, *38*, 2803–2805.
(36) Pugmire, R. J.; Grant, D. M. *J. Am. Chem. Soc.* **1971**, *93*, 1880–1887 and references therein.
(37) Salem, L. In "The Molecular Orbital Theory of Conjugated Systems"; W. A. Benjamin: New York, **1967**; pp 202–204.
(38) Harbison, G. S.; Herzfeld, J.; Griffin, R. G. *Biophys. J.* **1983**, *41*, 338a.

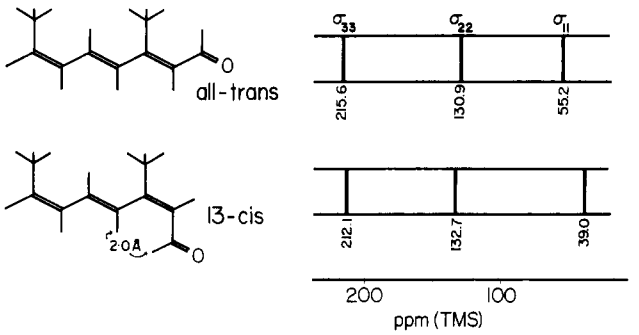


Figure 7. Chemical shift tensor principal values for the C-12 resonance of *all-trans*- and 13-*cis*-retinal, showing the effect of steric interaction on the σ_{11} element of the shift tensor.

appear to be a greater effect on σ_{22} than on σ_{33} on protonating a Schiff base.

2. Effect of Isomerization on the Chemical Shielding Tensor. As already mentioned, it has been known for some time that cis–trans isomerization causes upfield shifts of the ^{13}C resonances one bond distant from the isomerized bond itself.[14,15] The phenomenon has been ascribed to steric interactions between hydrogens across cis linkages, causing mutual repulsion of the electrons of the C–H bonds toward the carbons. As a consequence, there is an increase in the diamagnetic shielding of the latter, and an upfield shift is observed.[39] Solution spectra of several retinal derivatives have shown the ^{13}C-12 of 13-cis isomers to be upfield-shifted 5–9 ppm from the corresponding all-trans compounds.[17]

In order to determine which elements of the chemical shielding tensor are involved in the γ effect, we have compared spectra of [12-^{13}C]-*all-trans*- and [12-^{13}C]-13-*cis*-retinals. The former compound has a single series of sidebands from which the tensor could directly be obtained, while the latter gives two sets of sidebands from the two nonidentical molecules in the unit cell.[40] Since no significant difference could be detected between the 13-cis sideband patterns, their combined intensities were used to calculate a tensor.

The shielding tensors of [12-^{13}C]-*all-trans*- and [12-^{13}C]-13-*cis*-retinal are shown in Figure 7. It is seen from the isotropic shifts (134.0 vs. 128.9 ppm) that the γ effect observed in solution is reproduced in the solid state and also that the effect is highly anisotropic, being confined largely to the out-of-plane element[34] of the chemical shielding tensor σ_{11}. *cis*- and *trans*-polyacetylene show a similar, almost exclusive upfield shift of the σ_{11} element.[41] This result permits the γ effect to be experimentally distinguished from other factors which might influence isotropic chemical shifts, since these are manifested largely through σ_{22} and σ_{33}. This observation concerning σ_{11} is also in accord with results previously obtained by us for the 13-cis and all-trans isomers of dark-adapted bacteriorhodospin[11] and confirms our use of the chemical shielding tensor of [14-^{13}C]retinal bacteriorhodopsin to infer that the 13-cis isomer in that protein is syn about the C=N linkage. [14-^{13}C]-13-*cis*,15-*syn*-Retinal bacteriorhodopsin, however, also shows a large upfield shift in σ_{22}. While this σ_{22} effect might result from other factors, such as twisting about single bonds, we are reluctant to pose as a general rule that σ_{11} is the only element subject to a γ effect; however, it is the only element *consistently* affected.

3. Effect of C-6–C-7 Conformation on Retinal Chemical Shielding Tensors. Retinal derivatives are known from X-ray crystallography to exhibit conformational polymorphism in the solid state. While most retinal derivatives crystallize in a skewed 6-s-cis conformation, with an angle between cyclohexene ring and conjugated chain of 40–65°,[22,26,40,42] a small subset has been found

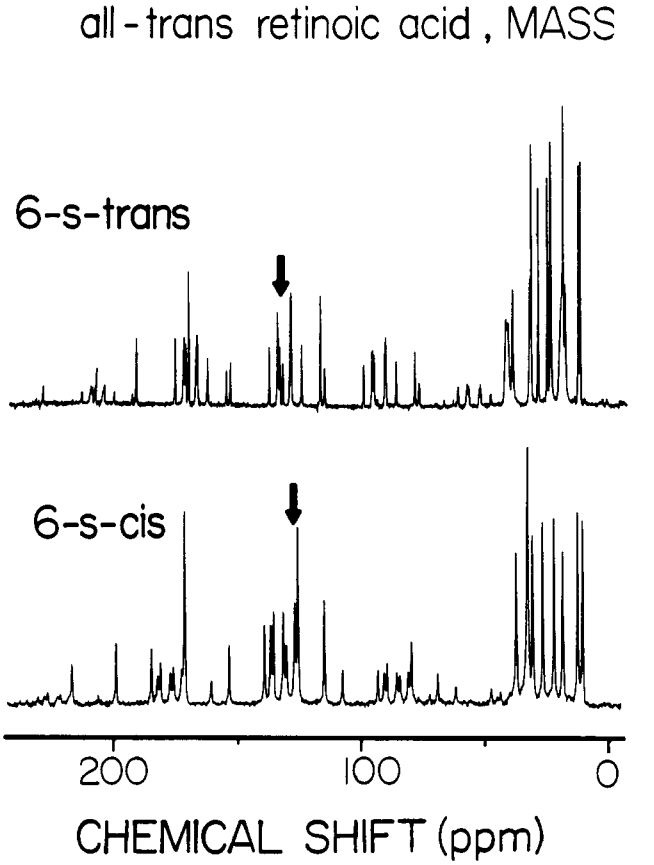


Figure 8. MASS spectra of the two crystalline forms of *all-trans*-retinoic acid, demonstrating the significant differences between the monoclinic (6-s-trans) and triclinic (6-s-cis) structures. The C-5 resonances are arrowed.

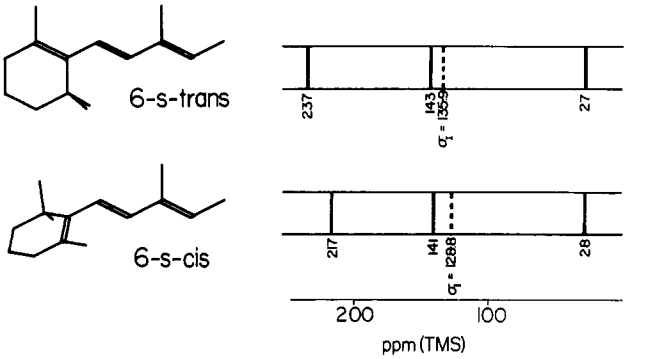


Figure 9. Chemical shift tensor principal values for the C-5 resonance of 6-*s*-*trans*- and 6-*s*-*cis*-retinoic acids; the principal effect of single-bond isomerization is on σ_{33}. The 6-*s*-*cis* molecule is shown in its skewed conformation.

to exist in a 6-s-trans state,[21,40] with ring and chain almost coplanar (see Figure 9). Such behavior is consistent with theoretical analysis of the molecular conformation, which predicts two free-energy minima on rotating about the C-6–C-7 bond: a broad one for the skewed 6-s-cis conformation, and a narrower minimum for the 6-s-trans, which is predicted to be about 10 kJ mol^{-1} less favored than the 6-*s*-cis state. We have been able to obtain ^{13}C MASS spectra of compounds in both conformations and can therefore examine the effect of ring–chain angle on both the isotropic shifts and the chemical shift tensors, neither of which can be done in solution. The most facile case of this sort to analyze is *all-trans*-retinoic acid.

(39) Grant, D. M.; Cheney, B. V. *J. Am. Chem. Soc.* **1967**, *89*, 5315–5316.
(40) Simmons, C. J.; Liu, R. S. H.; Denny, M.; Seff, K. *Acta Crystallogr., Sect. B* **1981**, *B37*, 2197–2205.
(41) (a) Mehring, M.; Weber, T.; Muller, W.; Wegner, G. *Solid State Commun.* **1982**, *45*, 1079–1082. (b) Terao, T.; Maeda, S.; Yamabe, T.; Akagi, K.; Shirakawa, H. *Chem. Phys. Lett.* **1984**, *103*, 347.
(42) Oberhansli, W. E.; Wagner, H. P.; Isler, O. *Acta. Crystallogr., Sect. B* **1974**, *B30*, 161–166.

Table III. ^{13}C-5 Chemical Shifts of Retinal Derivatives

	6-s-cis	6-s-trans	soln
all-trans-retinal	128.5		130.3
retinol acetate	127.5		129.3
β-carotene	128.7		129.3
13-*cis*-retinal	126.7	136.8	130.3
all-trans-retinoic acid	127.8	135.9	129.8
all-trans-retinal schiff bases			129.7
methylimine		134.6	assumed
ethylimine	127.2		identical
propylimine	129.4		for
butylimine		135.5	all
pentylimine	127.8		imines
hexylimine	127.2		
cyclohexylimine	127.0		

Stam and McGillivary[22] showed the existence of two crystalline forms of this retinoic acid: a stable, triclinic 6-s-cis form, which is obtained by crystallization above 80 °C, and a monoclinic, metastable 6-s-trans form, which crystallizes from solution at room temperature or slightly above. As described in the Experimental Section, we have prepared both polymorphs in the pure state and as mixtures, and their spectra are compared in Figure 8. The most significant differences exist at C-8, where the strong upfield shift in 6-s-trans is probably steric, and at C-5, a nonprotonated carbon which may be definitively assigned. From the isotropic shift and the chemical shift tensor shown in Figure 9, it is apparent that the chemical shift in the 6-s-trans form is shifted strongly downfield from the 6-s-cis conformer. The source of this shift is unlikely to be steric, because the chemical shifts of quaternary carbons are relatively immune to steric effects. Furthermore, an examination of the chemical shielding tensors shows the effect to be largely confined to σ_{33} and thus is more likely to be a π-electron perturbation than a steric effect. We therefore suggest that the upfield shift in 6-s-cis is due to the breaking of the conjugation between the C-5–C-6 double bond and the rest of the chain, thus making it behave rather more as an isolated double bond than part of a delocalized system. While data on other systems of this sort are unavailable, it is notable that a 4 ppm downfield shift on terminal ^{13}C atoms is noted on going from monoenes to dienes,[43] an effect ascribed to conjugation. Thus,

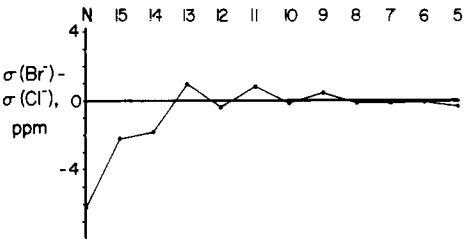


Figure 10. Differences in chemical shift between bromide and chloride salts of *N*-butyl-*all-trans*-retinylidene imine, as a function of position.

Table IV. ^{13}C Chemical Shifts of Retinal Derivatives

position	*all-trans*-retinal	13-*cis*-retinal	6-s-*cis*-*all-trans*-retinoic acid	6-s-*trans*-*all-trans*-retinoic acid	retinol acetate	β-carotene
1	33.7	34.4/34.9	34.65	33.8	34.35	34.7
2	40.2	39.5/43.5	40.5	41.4	34.5	40.4
3	20.4		20.5	20.0	21.4	19.9
4	32.4	34.4/35.6	36.0	34.6	32.2	34.1
5	128.5	126.7/136.8	128.8	135.9	127.5	128.7
6	134.1	136.8/139.5	137.6	135.4	140.0	139.1
7	130.2		127.8	126.6	128.4	131.05
8	135.15	138.9	138.9	130.9	140.0	(139.1)
9	141.4	142.1/141.1	141.4	134.7	137.7	138.1
10	130.2		129.1	130.9	131.8	(131.05)
11	133.9		132.5	130.9	125.7	125.8
12	134.0		133.7	136.3	137.7	(138.1)
13	155.5	155.2/153.7	155.8	155.4	137.7	138.8
14	129.8		117.1	114.1	128.4	(132.6)
15	189.4	191.85/190.1	173.6	172.4	61.9	137.1
16	30.9	29.7/30.5	32.5	30.9	29.3	30.0
17	29.7	28.4/29.2	28.7	27.3	29.3	30.0
18	21.7	23.6/20.8	24.1	20.8	22.1	24.0
19	13.1	13.25/12.6	(13.5)	(13.9)	(11.4)	(11.6)
20	13.1	21.60	(15.6)	(14.7)	(13.4)	(13.4)

Table V. ^{13}C Chemical Shifts of Unprotonated Retinal Schiff Bases

	methylimine	ethylimine	propylimine	butylimine	pentylimine	hexylimine	cyclohexylimine
Cl	34.3	34.45	34.4	34.3	35.1	34.4	34.3
2	43.6	39.8	39.6		40.15	39.6	40.4
3	18.5	20.2	20.3		20.6	20.0	20.2
4	35.0	33.8	32.6	35.5	33.8	33.2	33.9
5	134.6	127.2	129.4	135.5	127.8	127.2	127.0
6	136.4	136.8	137.7	143.2	139.8	139.2	137.5/138.9
7	(125.15)	124.1	(125.3)	125.4	126.0	(125.5)	129.3
8	133.4	139.1	140.0		139.7	138.55	139.5/139.7
9	135.5	134.6	135.1	135.5	135.7	135.5	135.6
10	133.4	131.2	132.7	133.1	132.9	132.3	132.1/132.3
11	(126.0)	126.8	(125.4)	127.2	127.9	(127.0)	126.3
12	139.8	135.7	140.0	138.8	134.7	139.0	138.3
13	144.5	142.1	141.2	143.2	143.8	143.5	144.7
14	131.0	129.8	132.1	132.2	132.6	131.7	131.4
15	160.7	157.6	159.7	159.3	159.8	159.0	158.9
16	30.5	31.2	32.6	29.4	30.6	29.2	29.4
17	28.4	29.8	28.5	29.4	29.0	29.5	24.7
18	21.3	21.9	22.8	22.1	23.0	22.1	22.8/22.3
19	(14.2)	(13.8)	(14.1)	(14.2)	12.3	11.9	13.4/13.8
20	(11.8)	(12.6)	(13.5)	(12.1)	12.3	11.9	12.6

Table VI. ^{13}C Chemical Shifts of Protonated *N*-Butylretinylideneimine Schiff Bases

	chloride	bromide	dichloro-acetate	trichloro-acetate
1	34.4	34.35	35.3	34.7
2	40.0	39.7	39.6	39.7
3	20.5	20.1	21.7	20.0
4	34.2	32.8	34.2	31.9
5	128.75	129.4	130.7	130.9
6	138.85	138.8	139.4	139.2
7	128.8	128.5	129.7	131.5
8	140.8	140.65	138.7	139.3
9	142.1	142.6	144.0	143.2
10	135.0	134.8	130.4	135.9
11	138.9	139.7	135.4	139.0
12	135.0	134.8	135.4	135.9
13	161.8	162.7	160.3	160.8
14	122.55	120.7	121.1	121.7
15	167.0	164.8	164.9	162.0
16	28.9	29.1	31.1	30.8
17	31.7	30.8	31.1	30.8
18	23.3	22.5	24.6	24.6
19	14.0	(13.29)	(14.0)	(14.9)
20	14.0	(14.94)	(11.9)	(12.7)

the magnitude of the effect does not seem unreasonable.

A similar example of a molecule which is found to exist in 6-s-cis and 6-s-trans conformers is 13-*cis*-retinal, but in this case, one molecule of each type is present in the unit cell. Because there are 40 rather than 20 chemically distinct carbons in the solid state, the spectrum cannot be completely assigned. However, we can assign the nonprotonated carbons, and, as is seen from Table III, the two C-5 resonances are again split by 8–9 ppm. Again, we assign these to the 6-s-cis and 6-s-trans conformers.

In Table III, we give the chemical shifts of C-5 in seven unprotonated retinal Schiff bases in the solid state. It can be seen that the shifts partition into two groups, the larger one being similar to that of 6-*s-cis*-retinal derivatives, while the smaller comprising the methylimine and butylimine Schiff bases, is close to those of 6-s-trans conformers. While crystal structures of these compounds have not yet been published, it is seen that the NMR can effectively predict the ring–chain conformation. The propylimine Schiff base is somewhat anomalous, in that it is 2 ppm downfield of the other 6-s-cis derivatives; perhaps it has an unusually low ring–chain angle.

While the data for the C-8 position are less complete, it appears, as already stated, that this position is upfield-shifted in 6-s-trans derivatives. This may reflect steric interactions with the C-16 and C-17 methyls which in 6-*s-trans*-retinoic acid are in close van der Waals' contact with the proton on C-8. However, because of overlap with other resonances, shift tensors are not available for this position; therefore, we cannot compare the anisotropic properties of the chemical shift with those of the C-12 position.

When we compare the shifts of our 6-s-trans and 6-s-cis derivatives with solution chemical shifts, we find that, while solution values are generally close to those of 6-s-cis conformers, they are uniformly somewhat downfield of them, toward the 6-s-trans shifts (see Table III). This suggests either that the ring–chain angles in solution are uniformly less than those measured in solids or, more likely, that an equilibrium population of 6-s-trans and 6-s-cis conformers exists in solution. From the published chemical shifts of retinoic acid, 13-*cis*-retinal[17] and retinal Schiff bases,[16] and our solid state data, we obtain an equilibrium value of 28 ± 5% 6-s-trans in solution at 25 °C. This is in conflict with the stated conclusions of long-range *J*-coupling and nuclear Overhauser enhancement studies which were held to rule out a significant population of 6-s-trans in solution.[44] However, given the indirect nature of the latter methods, and the straightforward character of the present results, we suggest that the assumption that retinal derivatives are purely 6-s-cis in solution be reevaluated.

It has usually been assumed that bacteriorhodopsin and rhodopsin contain 6-s-cis chromophores. However, the present results indicate that the free-energy difference between 6-s-trans and 6-s-cis is far too small to justify such an assumption. Indeed, it has been shown that an external point-charge near the ionone ring would be much more effective in red-shifting the bacteriorhodopsin visible absorption maximum were the ring and chain coplanar.[45] Studies using the ^{13}C-5 chemical shift as a probe of the ring–chain conformation, suggest that the 6-s-trans conformer is present in bacteriorhodopsin.[46]

4. Counterion Effects. We have previously shown[10,38] that ^{15}N chemical shifts in solid protonated retinal Schiff bases are very sensitive to the nature of the counterion and can vary over a range of 20 ppm. We have therefore investigated the effects of counterion substitution on ^{13}C chemical shifts. Figure 10 plots the change in chemical shifts on substituting a chloride for a bromide counterion. It is evident that the effect of the counterion is much smaller than observed for ^{15}N and decreases rapidly with distance from the Schiff base linkage. The regular zig-zag pattern observed is not unexpected; the weaker hydrogen bond in the bromide salt is expected to increase the positive charge density on the odd-numbered carbons of the conjugated chain. Theoretical calculations[47] show that where this occurs, downfield shifts of odd carbons and smaller upfield shifts of even carbons should be observed. This clear trend is only seen on chloride/bromide substitution, where presumably the counterion can be exchanged without significantly perturbing the crystal structure. Behavior in the trichloroacetic and dichloroacetic acid salts is much less regular. In general, however, no chemical shift differences greater than 3 ppm are observed between any protonated Schiff base salts for any position on the molecule.

5. ^{14}N Quadrupole Effects in Retinal Schiff Bases. Lines from spin $^1/_2$ nuclei in close proximity to nuclei with spin 1 or greater are known to undergo broadening or splitting from dipolar and scalar coupling with the higher spin nucleus.[48] This effect is due to quadrupolar effects which are not fully averaged by MASS. In retinal Schiff bases and in *N*-butyl-2,4,6-octatrienylideneimine, the carbons bonded to ^{14}N by both double and single bonds are broadened but not split by the quadrupole nucleus. From the extent of the broadening, we can estimate the quadrupolar coupling constant to be 0.5–1.0 MHz, smaller than those of amino acids (which give a resolved splitting of 25–30 Hz at our field) but large enough to be noticeable, indicating that unprotonated Schiff bases given considerably broader signals in the solid and suggesting that the ^{14}N quadrupole coupling constant is significantly increased on protonation. This is in marked contrast to the nitrogen chemical shift anisotropy, which is diminished by a factor of 2.[10] Similarly broad signals have been observed by us in ^{13}C MASS spectra of [15-^{13}C]retinal bacteriorhodopsin.

Conclusions

To adequately interpret spectroscopic data obtained from a complex system, such as a protein, an extensive and well-understood data base is essential. While solution NMR methods are and will continue to be important, this paper demonstrates that high-resolution NMR spectroscopy of solids is as feasible as, is complementary to, and in many ways is more informative than the more conventional technique. This is true not only for high molecular weight systems, but also molecules of moderate size. By employing and extending the use of well-known separated-local-field experiments, by comparison of solid with solution spectra, by careful correlation of shielding tensors with molecular

(43) Dorman, D. E.; Jautelat, M.; Roberts, J. D. *J. Org. Chem.* **1971**, *36*, 2757–2766.

(44) Honig, B.; Hudson, B.; Sykes, B. D.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 1289–1293.

(45) Honig, B.; Greenberg, A. D.; Dinur, U.; Ebrey, T. G. *Biochemistry* **1976**, *21*, 4593–4599.

(46) Harbison, G. S.; Smith, S. O.; Pardoen, J. A.; Courtin, J. M. L.; Lugtenburg, J.; Herzfeld, J.; Mathies, R. A.; Griffin, R. G. *Biochemistry*, in press.

(47) Shriver, J.; Abrahamson, E. W.; Mateescu, G. D. *J. Am. Chem. Soc.* **1976**, *98*, 2407–2409.

(48) Opella, S. J.; Frey, M. H.; Cross, T. A. J. *J. Am. Chem. Soc.* **1979**, *101*, 5856–5857.

structure, and by strategic employment of selective isotopic labeling, we have shown that the problem of assigning chemically similar resonances in the solid is by no means an intractable one. This is exemplified by our almost-complete assignment of a series of 17 retinal derivatives, many of which have provided problems even in solution. The fruits of the endeavor are several. First, we have acquired a much deeper understanding of the chemical shielding tensors of polyene derivatives and the factors which influence them, particularly the quite clear distinction between steric effects, which perturb the out-of-plane tensor element almost exclusively, and π-electron perturbations, which affect the in-plane elements. This distinction probably applies equally to other π-electron systems such as aromatics, purines, pyrimidines, and other heterocycles. Second, we have been able to distinguish between molecular conformations which rapidly equilibrate in solution. In our case, we have shown tnat the 6-s-trans and 6-s-cis conformers in retinal derivatives are demarked by quite different chemical shifts at the C-5 position, enabling us to determine this conformation in molecules of unknown structure. Third, we have demonstrated that the counterion effects noted by us for ^{15}N-labeled retinal Schiff bases penetrate quite deeply along the carbons of the conjugated system but are considerably weaker for ^{13}C than they are for nitrogen. Finally, we have obtained a comprehensive set of shielding tensor data for all positions of a variety of retinal derivatives, which should greatly aid continuing research on retinal containing proteins like rhodopsin and bacteriorhodopsin.[10-12]

Acknowledgment. We thank Drs. D. J. Ruben, M. G. Munowitz, W. P. Aue, and S. K. Das Gupta for their valuable assistance with various parts of this work. The research was supported by the National Institutes of Health (GM-23316, GM-23289, and RR-00995), the National Science Foundation (DMR-8211416), the Netherlands Foundations for Chemical Research (SCN), and the Netherlands Organization for the Advancement of Pure Research (ZWO). J. H. is a recipient of an American Cancer Society Faculty Research Award.

Registry No. *all-trans*-Retinal, 116-31-4; 13-*cis*-retinal, 472-86-6; methylamine, 74-89-5; ethylamine, 75-04-7; propylamine, 107-10-8; butylamine, 109-73-9; pentylamine, 110-58-7; hexylamine, 111-26-2; cyclohexylamine, 108-91-8; retinal methylimine Schiff base, 51424-44-3; retinal ethylimine Schiff base, 96998-37-7; retinal propylimine Schiff base, 53633-90-2; retinal butylimine Schiff base, 36076-04-7; retinal pentylimine Schiff base, 82628-41-9; retinal hexylimine Schiff base, 34882-02-5; retinal cyclohexylimine Schiff base, 96949-06-3; 2,4,6-octatrienal, 17609-31-3; 2,4,6-octatrienal butylimine Schiff base, 96949-07-4; *N*-butylretinylideneimine hydrochloride, 28448-64-8; *N*-butylretinylideneimine hydrobromide, 28448-68-2; *N*-butylretinylideneimine dichloroacetate, 96949-08-5; *N*-butylretinylideneimine trichloroacetate, 28448-65-9; *all-trans*-retinoic acid, 302-79-4; retinol acetate, 127-47-9; β-carotene, 7235-40-7.

Nuclear Spin–Spin Coupling via Nonbonded Interactions. 5. N–F Coupling[1]

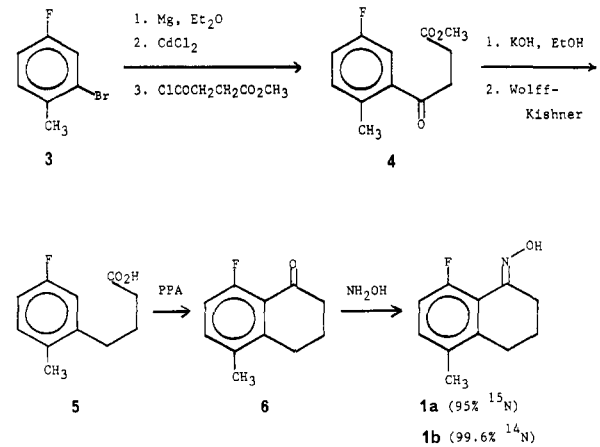
Frank B. Mallory*[2a] and Clelia W. Mallory[2b]

Contribution from the Department of Chemistry, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010, and the Department of Chemistry, University of Pennsylvania, Philadelphia, Pennsylvania 19104. *Received January 14, 1985*

Abstract: On the basis of the lone-pair orbital overlap theory that was developed earlier to account for the phenomenon of "through-space" F–F nuclear spin–spin coupling between intramolecularly crowded fluorine atoms, the existence of "through-space" N–F coupling is predicted. This previously untested prediction is verified experimentally through the observation of a much larger ^{15}N–^{19}F coupling constant for ^{15}N-enriched 3,4-dihydro-8-fluoro-5-methyl-1(2*H*)-naphthalenone oxime (J_{NF} = 22.4 Hz) than for ^{15}N-enriched *o*-fluorobenzaldehyde oxime (J_{NF} = 3.2 Hz).

The phenomenon of "through-space" nuclear spin–spin coupling between fluorine atoms that are crowded against one another intramolecularly is well documented;[3] some extraordinarily large "through-space" F–F coupling constants in the range of 170–200 Hz have been reported.[3a,b,e] Such coupling has been attributed theoretically to direct orbital overlap interactions of the type illustrated in Figure 1.[4] In this particular theoretical formulation,[5] the overlap of two nominally one-center $2p_F$ lone-pair orbitals is imagined to generate two nominally two-center orbitals, a σ_{FF} bonding orbital and a σ^*_{FF} antibonding orbital. Although this overlap interaction would not lead to net *chemical bonding* between the two fluorine atoms, it has been argued[4] that it should provide a four-electron linkage for the transmission of spin information between the two fluorine nuclei.

Scheme I



On the basis of the lone-pair overlap picture[4] of "through-space" F–F coupling presented in Figure 1, we predicted the existence

(1) For Part 4, see: Mallory, F. B.; Mallory, C. W.; Ricker, W. M. *J. Org. Chem.* **1985**, *50*, 457-461.
(2) (a) Bryn Mawr College. (b) University of Pennsylvania.
(3) (a) Servis, K. L.; Fang, K.-N. *J. Am. Chem. Soc.* **1968**, *90*, 6712-6717. (b) Chambers, R. D.; Sutcliffe, L. H.; Tiddy, G. J. T. *Trans. Faraday Soc.* **1970**, *66*, 1025-1038. (c) Mallory, F. B.; Mallory, C. W.; Fedarko, M.-C. *J. Am. Chem. Soc.* **1974**, *96*, 3536-3542 and references cited therein. (d) Hilton, J.; Sutcliffe, L. H. *Prog. Nucl. Magn. Reson. Spectrosc.* **1975**, *10*, 27-39. (e) Mallory, F. B.; Mallory, C. W.; Ricker, W. M. *J. Am. Chem. Soc.* **1975**, *97*, 4770-4771 and references cited therein. (f) Matthews, R. S.; Preston, W. E. *Org. Magn. Reson.* **1980**, *14*, 258-263. (g) Matthews, R. S. *Ibid.* **1982**, *18*, 226-230.
(4) Mallory, F. B. *J. Am. Chem. Soc.* **1973**, *95*, 7747-7752.
(5) Numerous other theoretical approaches have been suggested as well: see ref 3-18 cited by Schaefer and co-workers.[6b]

0002-7863/85/1507-4816$01.50/0 © 1985 American Chemical Society