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The Temperature Dependence of Carbon-13 Chemical Shifts of Retinal Isomers and Their Related Compounds

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The temperature dependence of carbon-13 chemical shifts has been studied for retinal isomers, *N-all-trans*-retinylidenebutylamine(*all-trans*-NRB) and *N-all-trans*-retinylidenebutylammonium chloride(*all-trans*-NRB·HCl). The change in the chemical shift was discussed in terms of the temperature dependences of the polarizability and conformation of the polyene-chain. In 11-*cis*-retinal, the coplanarity between the two parts of polyene-chain separated by the cis bond increases with a lowering of the temperature. The equilibrium between 12-*s*-*cis* and 12-*s*-*trans* conformers in 11-*cis*-retinal was studied and it was shown the latter conformer is preferable at low temperatures. In *all-trans*-NRB and *all-trans*-NRB·HCl, the temperature dependences of the chemical shifts are similar to those in *all-trans*-retinal. The extents of the chemical shift changes are in the following order: NRB·HCl<NRB <retinal. This trend can be correlated with the order of the red shifts of the absorption spectra.

The carotenoid compound, 11-*cis*-retinal, acts as a photoreceptor in a visual system. The primary event in the visual process in vertebrate retinas is the photochemical isomerization of the bound 11-*cis*-retinal to the *all-trans* form (Fig. 1). Other succeeding transformations in the retina are known to occur in the dark.[1,2]

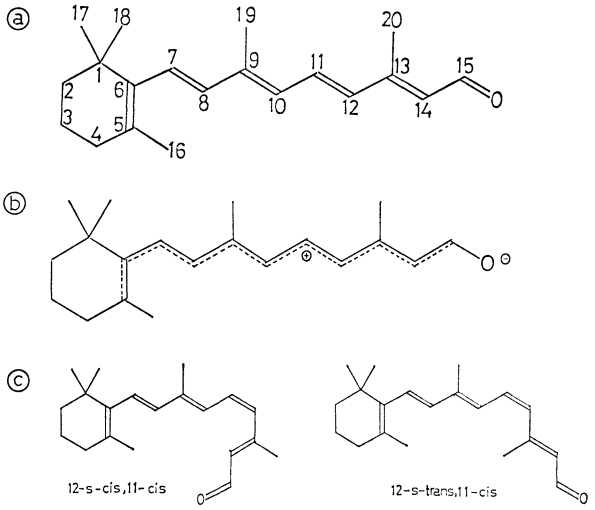

Fig. 1. a) Structural formula of *all-trans*-retinal.
b) Resonance structure of *all-trans*-retinal.
c) Structural formulas of 12-*s*-*cis* and 12-*s*-*trans* conformers in 11-*cis*-retinal.

The conformations of retinal isomers and related compounds have been studied by means of the UV spectra at lower temperatures because of the instability of the transient intermediates in the visual process at ambient temperatures. The absorption maxima of the retinal isomers and their Schiff bases reveal a red (bathochromic) shift upon the lowering of the temperature.[2-4]

The conjugated polyene-chain of 11-*cis*-retinal twists around the C12–C13 single bond as a result of the severe steric hindrance, while that of *all-trans*-retinal is planar. The 11-*cis*-retinal may exist as an equilibrium mixture of distorted 12-*s*-*cis* and 12-*s*-*trans* conformers as is shown in Fig. 1c; this indicates much larger absorption and intensity changes in the UV spectra than in the cases of the other retinal isomers for the same range of temperatures and solvent polarity.[3] Such a conformational equilibrium is a possible key to interpreting the temperature dependence of the spectroscopic parameter in the molecular level. We will study the temperature dependence of the carbon-13 NMR spectra of retinal and its related compounds. Both proton and carbon-13 NMR spectra of these compounds have been widely studied by many including the present authors.[5-10] Rowan *et al.*[7] have investigated the temperature dependence of the proton NMR spectrum of 11-*cis*-retinal; they concluded the 12-*s*-*trans* to be the preferred conformer in the 11-*cis* isomer in acetone at lower temperatures. Since the carbon-13 chemical shift is related to the electron density of the corresponding carbon atom, it makes possible a study of the charge delocalization in conjugated polyene carbons of the retinal Schiff base.[8,10]

In this investigation, the carbon-13 chemical shifts of retinal isomers and their Schiff bases were observed in the temperature range from −50 °C to +30 °C. The temperature dependences of the chemical shifts will be discussed in terms of the changes in the electronic structure. The degree of the delocalization of the positive charge (Fig. 1b) will be related with the conformational changes in the conjugated polyene-chain of 11-*cis*-retinal at lower temperatures. This relation will be used to explain the degree of the red shift of the absorption maximum in the absorption spectra of corresponding compounds at lower temperatures. It has been widely accepted that, in the rhodopsin, a protonated Schiff-base linkage exists between the retinal chromophore and the protein opsin *via* an ε-amino group of the lysin residue.[11] Therefore, the protonated Schiff base with *n*-butylamine was chosen as a pertinent model for the rhodopsin.

Experimental

All of the retinal compounds used were prepared by a procedure described elsewhere.[9]

The UV spectra were observed in methanol with a Beckman-25 spectrometer. The carbon-13 NMR spectra were measured in the dark, using a JNM JEOL PS-100 spectrometer equipped with a PFT-100 Fourier transform system at 25.15 MHz and in the temperature range of −50 °C—+30 °C. The 90°-pulse recycle times were chosen to be at least five times the longest spin-lattice relaxation time of the carbon atoms. The number of accumulations were chosen to be 128—1024. The concentrations of the retinal

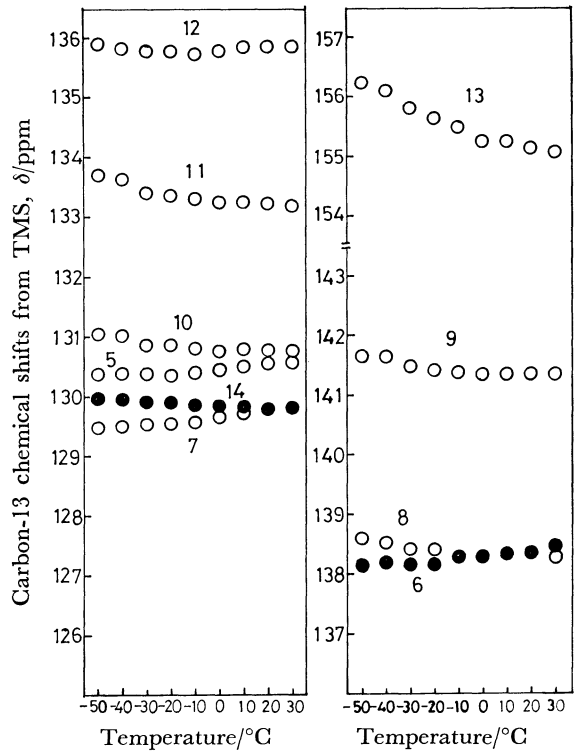

Fig. 2. Temperature dependence of chemical shifts for each of conjugated polyene-chain carbons of *all-trans*-retinal in acetone.

compounds were 0.3—0.5 mol dm^{-3} in a solution of acetone-d_6 and chloroform-*d*. The chemical shifts were expressed in ppm as downfield shifts from internal tetramethylsilane (TMS). The maximum experimental error of the chemical shift was within ±0.06 ppm.

Results

The carbon-13 NMR spectra have been assigned by the use of various techniques shown in previous papers.[6,9,10] Figure 2 shows the temperature dependence of the chemical shift for each of the conjugated polyene-chain carbons in *all-trans*-retinal in an acetone solution. Similar temperature dependences were also obtained for the same isomer in a chloroform solution and for the 11-*cis* isomer in acetone and chloroform solutions. The carbon atoms are numbered as is shown in Fig. 1a; the numbers indicated in Fig. 2 correspond to this numbering.

Tables 1 and 2 are tabulated the chemical shifts at room and lower temperatures and the differences, Δδ, between the chemical shifts at these two temperatures for identical carbons. The plus and minus signs in Δδ indicate the downfield and upfield shifts respectively upon a lowering of the temperature. According to the UV analysis[3,4] and solvent theory,[12] the conformational change of the retinal isomer increases with an increase the polarity of the solvent. Therefore, acetone and chloroform were used in this work as representative polar and non polar solvents respectively.

From these two tables, a general trend was observed for downfield shifts to be observed with a lowering of the temperature for the conjugated polyene-chain carbons of both *all-trans*- and 11-*cis*-retinal. This trend

Table 1. The chemical shifts of *all-trans*-retinal and 11-*cis*-retinal temperatures in acetone

Carbon number	*all-trans*-Retinal			11-*cis*-Retinal		
	29 °C	−50 °C	Δδ[a]	28 °C	−50 °C	Δδ[a]
1	34.82	34.70	−0.12	34.82	34.64	−0.18
2	40.28	39.55	−0.73	40.22	39.61	−0.61
3	19.83	19.65	−0.18	19.83	19.59	−0.24
4	33.61	33.36	−0.25	33.55	33.30	−0.25
5	130.56	130.38	−0.18	130.62	130.32	−0.30
6	138.45	138.14	−0.31	138.26	138.20	−0.06
7	129.77	129.47	−0.30	129.77	129.59	−0.18
8	138.26	138.57	+0.31	138.51	138.81	+0.30
9	141.36	141.66	+0.30	141.66	142.33	+0.67
10	130.74	131.04	+0.30	126.92	127.28	+0.36
11	133.17	133.71	+0.54	131.59	132.44	+0.85
12	135.84	135.90	+0.06	131.59	131.53	−0.06
13	155.07	156.22	+1.15	155.93	156.89	+0.96
14	129.77	129.95	+0.18	130.62	131.04	+0.42
15	191.11	192.20	+1.09	191.17	192.02	+0.85
16	21.96	22.38	+0.42	21.90	22.26	+0.36
17, 18	—	—	—	—	—	—
19	12.98	12.98	±0	12.25	12.31	+0.06
20	12.98	12.98	±0	17.95	17.77	−0.18

a) The change in the chemical shift in going from room temperatures to −50 °C.

Table 2. The Δδ of polyene-chain carbons of retinal isomers in $CHCl_3$

Carbon number	*all-trans*-Retinal	*all-trans*-NRB	*all-trans*-NRB·HCl
	Δδ[a]	Δδ[a]	Δδ[b]
1	−0.30	−0.24	−0.18
2	−0.79	−0.79	−0.48
3	−0.43	−0.37	−0.25
4	−0.24	−0.24	−0.06
5	+0.18	+0.18	±0
6	−0.49	−0.43	−0.19
7	−0.30	−0.31	−0.12
8	+0.06	−0.19	+0.12
9	+0.36	+0.24	±0
10	±0	−0.18	−0.06
11	+0.30	+0.06	+0.12
12	−0.30	−0.18	−0.12
13	+1.21	+0.60	+0.06
14	−0.12	−0.49	−0.25
15	+0.91	+0.48	±0

a) The chemical-shift changes between room temperature and −40 °C. b) The chemical-shift changes between room temperature and −20 °C.

is clear in acetone, though it is rather ambiguous in chloroform. The β-ionone-ring carbons indicate a upfield shift with a lowering of the temperature in both solvents. The relatively large upfield shift of C2 is remarkable. Among conjugated polyene-chain carbons, the downfield shifts are remarkable for the odd-numbered carbons, while they are not so clear for the even-numbered carbons. The same experimen-

tal result, that the chemical-shift changes of polyene-chain carbons can be classified into two types according to whether the numbering on the carbon is even or odd, is also found in the chemical-shift changes on going from retinal isomers to their Schiff bases and from Schiff bases to protonated Schiff bases.[9,10]

Table 2 also shows the chemical-shift changes, $\Delta\delta$, of the conjugated polyene-chain carbons of *all-trans*-NRB and *all-trans*-NRB·HCl in chloroform. The chemical shift changes in the carbons of *all-trans*-NRB are generally smaller than those in the carbons of *all-trans*-retinal, while those of *all-trans*-NRB·HCl are smaller than those of *all-trans*-NRB.

Discussion

Origin of the Temperature Dependence of Chemical Shifts. Several contributions can be pointed out as the causes of the temperature dependences of chemical shifts. Firstly, the effect of the temperature-dependent polarizability of the solvent must be examined, since the changes in the carbon-13 chemical shifts of polyene carbons of retinal are connected with the changes in electron density.[8–10] Wyman[13] has reported that the dielectric constant and the polarizability of the solvent increase with a lowering of the temperature. An increase in the polarizability of the solvent induces an increase in that of the retinal carbonyl group.[14] As has been shown in previous papers,[9,10] the positive charge at the polyene-chain-end atom tends to be distributed onto the polyene-chain carbons(Fig. 1b); this delocalization of the positive charge results in the downfield shifts for these carbons. Thus, the downfield shifts(Table 1) of the odd-numbered polyene carbons in *all-trans*- and 11-*cis*-retinal with a lowering of the temperature may be attributed to the delocalization of the positive charge at the polyene-chain end resulting from an increase in the polarizability of the retinal carbonyl group. Such downfield shifts are especially clear in the C13 and C15. The large $\Delta\delta$'s of the C9 and C11 in 11-*cis*-retinal imply a larger delocalization of the positive charge compared with that of the same carbons in *all-trans*-retinal.

The overall decrease in the electron density of the polyene-chain can be explained in terms of the intramolecular polarizability, which produces an increase in the electron density in the β-ionone-ring carbons. These carbons show upfield shifts. Thus, at least a part of the origin of the chemical-shift changes, $\Delta\delta$'s, can be explained by the changes in polarizability. However we have to consider other factors besides polarizability, since the polyene carbons of 11-*cis*-retinal show very different $\Delta\delta$'s from those of *all-trans*-retinal, as is indicated in Fig. 3. The peculiarity of $\Delta\delta$ of 11-*cis*-retinal can be explained by the conformational changes of its conjugated polyene-chain.

The following two conformational changes can be induced by lowering the temperature. One is due to a decrease in the skewness in the conjugated polyene-chain, while the other is due to the rotation around the C12–C13 single bond.

In 11-*cis*-retinal, the steric hidrance between the C10-hydrogen and C20-methyl group is relieved by a skew geometry around the C12–C13 single bond at

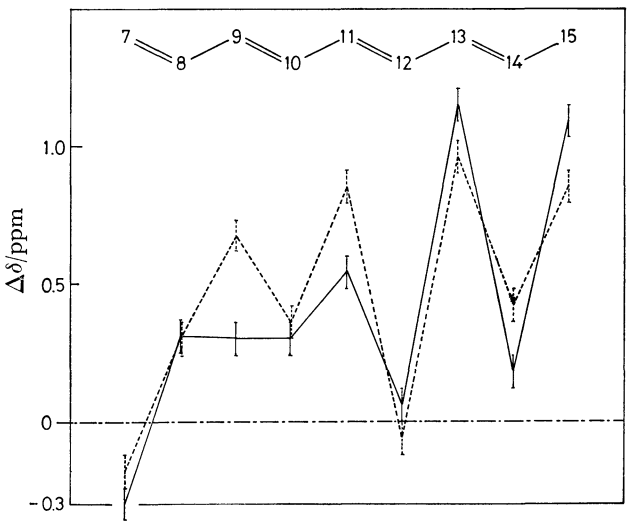

Fig. 3. The plot of the chemical-shift differences $\Delta\delta$ *vs.* carbon number of *all-trans*-retinal (—) and 11-*cis*-retinal (---) in acetone.

room temperature.[5,7] Patel[5] suggested that it is more difficult to distribute the positive charge in 11-*cis*-retinal than in *all-trans*-retinal, judging from the proton chemical-shift differences in the two retinal isomers at room temperature. In Table 1, the $\Delta\delta$'s in acetone of the C9 and C11 in 11-*cis*-retinal are +0.67 and +0.85 ppm respectively, while those in *all-trans*-retinal are +0.30 and +0.54 ppm. The $\Delta\delta$'s of the C15 and C13 in *all-trans*-retinal are about 1.1 ppm, while those in 11-*cis*-retinal are about 0.9 ppm. These trends indicate an increase in the degree of the delocalization of the positive charge on C15 upon a lowering of the temperature. The decrease in the skewness produces an increase in planarity(an increase in the resonance structure) of the 11-*cis* polyene-chain and results in an increase in the delocalization of the positive charge. Therefore, the larger downfield shitfs of C9 and C11 of 11-*cis*-retinal as compared with those of *all-trans*-retinal may be due to a decrease in the skewness of the conjugated polyene-chain upon a lowering of the temperature. This explanation is consistent with that of UV analysis at lower temperatures.[3,15]

The other origin of chemical-shift change is the change in equilibrium between the 12-*s-cis* and 12-*s-trans* conformers of 11-*cis*-retinal. It has been reported that the relative stabilities of the two conformers in 11-*cis*-retinal change with temperature[7] and the kind of solvent.[12] The chemical shifts of sterically perturbed carbon atoms are generally found at higher magnetic fields than those of similar carbons which are not spatially crowded. This steric perturbation of the carbon-13 chemical shifts is known as the γ-effect.[16] The chemical shifts of C10, and C20 may be affected by the γ-effect resulting from the steric hindrance between the C10-hydrogen and C20-methyl or C14-hydrogen, depending on the equilibrium between the two conformers. As is shown in Table 1, the $\Delta\delta$ of C10 in 11-*cis*-retinal in acetone is nearly equal to that of *all-trans*-retinal. The 14 and 20 carbons of 11-*cis*-retinal show a downfield shift of 0.42 ppm and an upfield shift of 0.18 ppm respectively, while those of

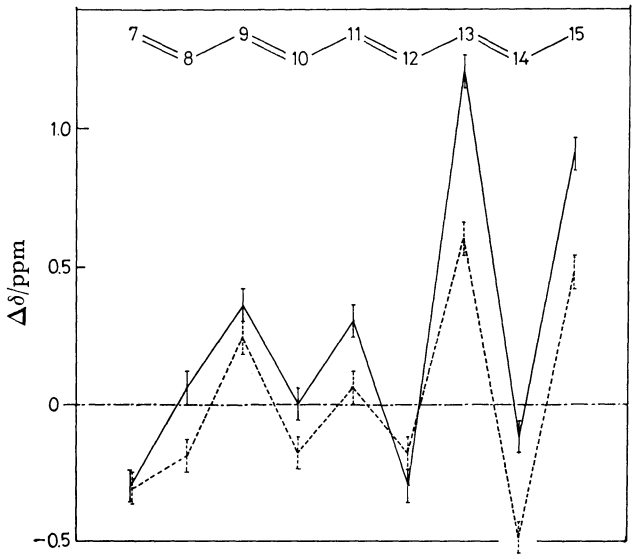

Fig. 4. The plot of the chemical shift differences $\Delta\delta$ *vs.* carbon number of *all-trans*-retinal(—) and *all-trans*-NRB(---) in chloroform.

all-trans-retinal remain in nearly the same positions. These results reveal that, in 11-*cis*-retinal, the 12-*s-trans* conformer is preferred over the 12-*s-cis* one in acetone at low temperatures. These results are consistent with the proton-NMR analysis,[7] which indicates a higher population of the 12-*s-trans* conformer in acetone at −52 °C. At present, however, we can not evaluate the population of each conformer.

In *all-trans*-NRB, the smaller downfield shifts for C13 and C15 as compared with those of *all-trans*-retinal might be due to a decrease in the delocalization of the positive-charge density induced by a decrease in the electronegativity of the terminal group,[9] because $\Delta\delta$'s of the β-ionone ring carbons are approximately equal to those of *all-trans*-retinal(Table 2 and Fig. 4).

Correlation between the UV Red Shift and the Carbon-13 Chemical Shift. It has previously been reported that the blue shift with a magnitude of about 20 nm on going from *all-trans*-retinal to *all-trans*-NRB is due mainly to the delocalization of the π-electron density,[9] and that the large red shift (about 80 nm) by the N-protonation on NRB is due mainly to the collapse of the bond alternation[9] and the delocalization of the positive charge.[9,17,18] Schaffer *et al.*[4] have indicated that 11-*cis*-retinal shows a larger red shift than other retinal isomers and that the magnitudes of the red shifts of retinal isomers are somewhat larger than those of the corresponding Schiff bases on going from room to lower temperatures. These phenomena are explained by the carbon-13 NMR analysis as follows.

The red shifts in retinal compounds at lower temperatures may be due to an increase in the degree of delocalization of the positive charge as a result of an increase in the degree of polarizability. The difference in the magnitude of the red shift in *all-trans*-retinal and 11-*cis*-retinal can be related to the magnitude of the chemical-shift changes of C9 and C11 resulting from the delocalization of the positive charge induced by an increase in planarity in the conjugated polyene-chain of 11-*cis*-retinal upon a lowering of the temperature.

The small chemical-shift changes of *all-trans*-NRB as compared with those of *all-trans*-retinal explain why *all-trans*-retinal shows a larger red shift than does *all-trans*-NRB on going from room to lower temperatures. For *all-trans*-NRB·HCl, the smaller red shift[4] corresponds to the smaller chemical-shift changes. Thus, the chemical-shift changes are correlated with the UV red shift.

From the present work, it is clear that the temperature dependences of the carbon-13 chemical shifts of the retinal isomers and related compounds are explainable in terms of the changes in the polarizability and conformation of the polyene chain, although this explanation is more or less qualitative. To give a quantitative explanation of the experimental results, a theoretical investigation by means of CNDO calculation is now in progress and will be reported elsewhere.

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