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Carbon-13 Chemical Shifts of Retinal Isomers and Their Schiff Bases as Models of Visual Chromophores

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Synopsis. Carbon-13 NMR spectra have been observed for 11- and 13-*cis*-retinals and their Schiff base linkage compounds with butylamine as a model compound of visual chromophore. The chemical shift changes on going from retinal to Schiff base. The changes in chemical shifts of polyene carbons were discussed in correlation with the changes of π-electron densities.

The visual mechanism in the vertebrate is mainly controlled by rhodopsin composed of the vitamin A_1 aldehyde isomer, 11-*cis*-retinal, and the lipoprotein, opsin. Absorption of the photon by a visual pigment induces immediate isomerization of the retinal from the 11-*cis*- to *all-trans*-isomer. This isomerization act as a trigger for stimulating the nerve.

When the 11-*cis*-retinal, which has an absorption maximum at 380 nm, forms the visual pigment by combination with opsin, a large bathochromic shift is observed.[1] In order to understand the origin of this spectral shift, it is necessary to know the electronic structure of polyene chain of retinal. Since experimental results suggest that the retinal is bonded through a Schiff base linkage to the ε-amino group of a lysine residue in a opsin,[2–5] the Schiff base compound of retinal with butylamine, *N*-retinylidenebutylamine (NRB), have been used as a model for the rhodopsin. The change in carbon-13 chemical shifts of the conjugated polyene carbon atoms on going from *all-trans*-retinal to *all-trans*-NRB can be correlated quite well with that in π-electron density.[6,7]

We have investigated the changes in the π-electron densities of polyene carbon atoms on going from 11-*cis* and 13-*cis*-retinals to their Schiff base linkage compounds with butylamine by observing the carbon-13 NMR chemical shifts. The polyene aldehyde 13-*cis*-retinal is considered the chromophores of the membrane protein, bacteriorhodopsin.[8]

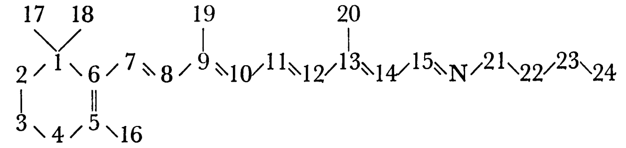
Experimental

11- and 13-*cis*-retinals were prepared by the photoisomerization of *all-trans*-retinals in ethanol in the sunlight. The resulting mixture of retinal isomers was fractionated on a column of sodium aluminosilicate by eluting with petroleum ether. Each crude isomer was purified by repeated crystallization from petroleum ether at −15 °C. Details of the methods of preparation for the *all-trans*-retinal and the Schiff base compound of retinal isomer have been reported.[7]

Carbon-13 NMR spectra were observed on a JEOL JNM PS-100 spectrometer linked with a PFT-100 Fourier transform system at 25.14 MHz. All measurements were carried out immediately after dissolution of sample in CCl_4 in 8 mm o.d. glass tubes in the dark. Chemical shifts were measured as downfield shifts from internal tetramethylsilane. The carbon-13 NMR spectra of 11- and 13-*cis*-retinals were assigned according to the results published.[9,10] All peaks in the carbon-13 NMR spectra of NRB have been assigned by use of various techniques.[7]

Results and Discussion

The chemical shifts of the conjugated polyene chain carbons in *cis*-retinal isomers and their Schiff base linkage compounds are given in Table 1 together with the data of *all-trans*-retinal and *all-trans*-NRB for the sake of comparison. The chemical shifts of the NRB are expressed as the chemical shift differences $\Delta\delta$, the differences between chemical shifts of retinal and those of corresponding NRB, in which the positive and negative signs indicate the downfield and upfield shifts, respectively. The numbering of the carbons, for example in *all-trans*-NRB, is given as shown in the following.



For all isomers the peaks assigned to the odd-numbered carbons show large upfield shifts and the peaks assigned to the even-numbered carbons show small downfield shifts by formation of the Schiff base com-

TABLE 1. THE CARBON-13 CHMICAL SHIEFTS OF CONJUGATED CHAIN CARBONS ON RETINAL ISOMERS AND THEIR SCHIFF BASE COMPOUNDS (NRB)

Carbon No.	11-*cis*		13-*cis*		*all-trans*[c]	
	Retinal[a]	NRB[b]	Retinal[a]	NRB[b]	Retinal[a]	NRB[b]
5	131.5	−0.7	131.7	−0.6	131.7	−1.0
6	139.8	0.1	139.4	0.5	139.8	−0.1
7	130.9	−1.9	130.9	−1.6	130.9	−2.2
8	139.8	0.5	139.8	0.2	139.4	0.3
9	142.6	−2.6	142.3	−2.9	142.1	−3.6
10	128.0	1.3	131.8	0.8	131.7	0.6
11	132.6	−3.8	134.5	−4.0	133.5	−4.9
12	132.6	1.8	129.0	1.0	137.1	1.4
13	155.5	−10.5	154.2	−10.4	154.7	−10.6
14	131.9	0.9	129.8	1.0	131.2	1.1
15	191.5	−30.8	190.2	−30.7	191.5	−31.3

a) Chemical shifts were measured as downfield shifts from internal tetramethylsilane and are expressed in terms of ppm. The estimated error in chemical shifts is less than 0.1 ppm. b) Chemical shifts of NRB are expressed as downfield (plus) and upfield (minus) shifts from the corresponding carbon of retinal. c) The chemical shifts of *all-trans*-retinal and *all-trans*-NRB were taken from Refs. 6 and 7.

pounds. Table 1 also indicates the presence of the gradients in the chemical shifts differences $\Delta\delta$ along the polyene chain from $C(15)$ to $C(5)$.

The carbon-13 chemical shifts of conjugated π-electron systems are generally dominated by the contribution from the paramagnetic term, being related to the π-electron density and π-bond order.$^{11)}$ According to the data of X-ray analysis$^{12)}$ and resonance-enhanced Raman spectra,$^{13)}$ and the results of CNDO/2 MO calculation,$^{7)}$ there are neither collapse of the bond alternation nor remarkable difference of the π-bond order between *all-trans*-retinal and *all-trans*-NRB. The constancy of the π-bond order may be also valid in the *cis*-isomers. Thus the changes of the chemical shifts can be explained by those of π-electron densities on the polyene chain.$^{7,11)}$

When the terminal atom of the polyene chain changes from oxygen to nitrogen, the total π-electron density of the conjugated polyene carbons of NRB increases due to the decrease in electronegativity at the polyene terminal. The carbon-13 chemical shifts differences show that an increase in total π-electron density leads to the increase in π-electron density at the odd-numbered carbons of NRB. It is noticeable that the chemical shifts in the conjugated polyene carbons of NRB are distributed in a relatively narrow range of approximately 30 ppm, whereas those of corresponding retinal isomers are in a range of 60 ppm. The decrease in the chemical shift range indicates that the delocalization of π-electron on the polyene chain is induced by the formation of Schiff base linkage.

The chemical shift differences $\Delta\delta$ of the odd-numbered polyene carbons of *all-trans*-NRB are slightly larger than those of the corresponding carbons in 11- and 13-*cis*-NRB's. This result may reflect the fact that the changes in π-electron density induced by the formation of the Schiff base are slightly larger on the *all-trans* polyene chain than on the *cis*-chain.

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