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FT-IR Spectra of All Sixteen Isomers of Retinal, Their Isolation, and Other Spectroscopic Properties.†

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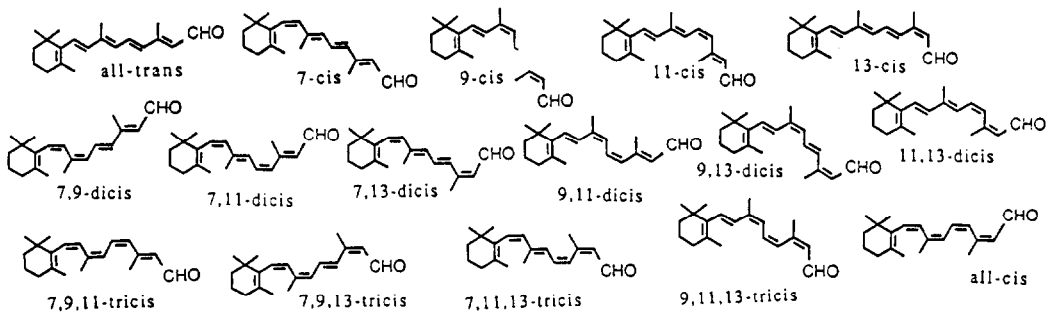
Abstract. The FT-IR spectra of all sixteen isomers of retinal, their synthesis completed only recently, are reported. Characteristic trends of the polycis isomers (in C,C double bond, single bond and HOOP band regions) are discussed and compared with those of the mono-cis isomers. The 7-cis and 7,9-dicis isomers of three deuterioretinals (7D, 19,19,19,-D_3, and 8,19,19,19-D_4) have been prepared, confirming some of vibrational assignments. Conditions for purification of these isomers and other spectroscopic properties (^{1}H-NMR, UV/VIS) are also provided.

Introduction

Following the successful preparation of all-*cis*- and 7,11,13-tri*cis*-retinal,[1] all sixteen possible geometric isomers of retinal are now known. The cis selective coupling of a modified C_5-cyanophosphonate with a C_{15}-aldehyde, the method used for preparation of the last two isomers, while not sufficiently specific to exclude formation of the corresponding 11-trans isomers is, in fact, a facile procedure to all sixteen isomers of retinal. Hence, in addition to the unusual chemical properties of the unstable isomers,[2] we were able to compile spectroscopic and other properties of these isomers. Since the data are potentially useful to future workers for their characterization and other studies of these new isomers,[3] we now report in this

† New Geometric Isomers of Vitamin A no. 18. For previous paper in the series, see: ref. 1.



paper the FT-IR spectra of all sixteen isomers and to update and complete their ^{1}H-NMR and UV/VIS data and hplc separation conditions.

Experimental.

Material. Methods of preparation of all geometric isomers used in this study via the 11-cis stereoselective coupling of the C_5-cyanophosphonate with one of the four isomers of the C_{15}-aldehydes,[4] led to all sixteen isomers of retinonitrile.[1] Their conversion to the corresponding retinal isomer was accomplished via partial reduction with DIBAH.[1] Small quantities of the unstable isomers containing the 11,13-dicis geometry were also prepared by direct irradiation of the corresponding 13-trans isomer.[3]

Procedures for chain elaboration of the 7-cis and 7,9-dicis isomers of three deuterated retinals are the same as those reported for the parent compounds. Method of deuteration and characterization of the final products are briefly described below.

7-Deuterioretinal. A mixture of methyl geranate and nerate was reduced with lithium aluminum deuteride. Oxidation with active MnO_2 afforded 1-deuteriocitral which was condensed with acetone in the presence of KOH to provide isomeric mixture of pseudoionone. Ring closure by acid catalyzed cyclization gave a mixture of 7-deuterio-α- and β-ionones from which the requisite β-isomer was isolated by preparative HPLC. Subsequent C_2-chain extension, selective sensitization, and C_5-chain extension were based on established procedures for retinal synthesis.[5] HRMS for **7-cis**: 285.218, **7,9-dicis**: 285.219 (Calcd for $C_{20}H_{27}OD$ = 285.220). For both isomers, ^{1}H-NMR ($CDCl_3$) signals were identical to those of 7-*cis*-retinal (Table 1) excepting the absence of the H-7 doublet and the appearance of a singlet for H-8.

19,19,19-Trideuterioretinal. The base-catalysed (NaOD in D_2O-pyridine) deuterium exchange of β-ionone was effected twice to afford isotopically pure

trideuterio-β-ionone.[6] Thereafter, chain elaboration followed the same procedures mentioned above giving the desired trideuterio-retinal. All isomers exhibited expected spectral data. HRMS for **7-cis**: 287.232; **7,9-dicis**: 287.233. (Calcd. for $C_{20}H_{25}OD_3$ = 287.233).

7,19,19,19-Tetradeuterioretinal. Condensation of α-cyclocitral with perdeuterated acetone provided the tetradeuterated β-ionone. Following the same sequence of reaction, the tetradeuterioretinal was obtained. All isomers exhibited expected spectral data. HRMS for **7-cis**: 288.238; **7,9-dicis**: 288.237. (Calcd. for $C_{20}H_{24}OD_4$ = 288.239).

Methods. HPLC separations were conducted under two sets of conditions. Most of the runs were carried out using a 25 x .46 cm Dynamax Microsorb Si 60 (5μ) column with 4-8% ether in hexane. To enhance separation of the 7,13-dicis or the 11,13-dicis isomer from the 13-cis, the solvent mixture of .9-1.2% t-butyl methyl ether in 1,1,2-trichloro-1,2,2-trifluoroethane (Freon 112)[7] was also used. Whenever unstable isomers (those containing the 11,13-dicis geometry) were involved, the HPLC unit was placed in a low temperature chromotography refrigirator set at 3°C. Representative chromatograms are shown in Figure 1.

FT-IR spectra were recorded on a Nicolet-740 spectrometer. Samples were deposited on KBr plates by evaporating hexane solutions of HPLC purified isomers. Upon completion of recording each spectrum, the sample was redissolved in hexane and checked for purity by HPLC. In no instances were rearranged products found to constitute more than 5% of the sample. An earlier attempt to obtain spectra on the diffusion reflectance mode, where less sample is needed, was found to give spectra of non-reproducible relative peak intensities.

UV/VIS absorption spectra were recorded on a PE-λ5 absorption spectrometer.

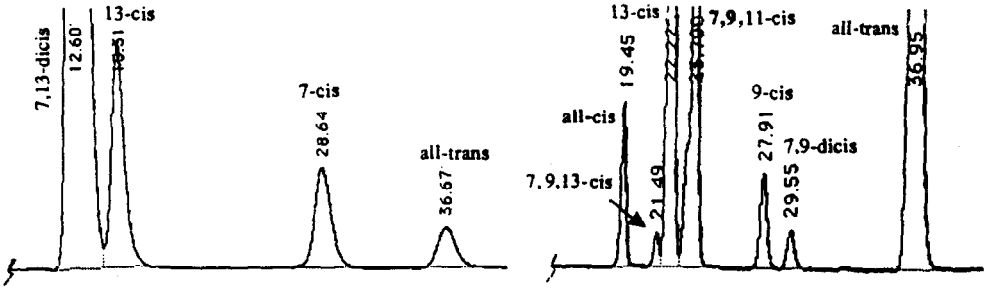


Figure 1. Representative chromatograms (hplc) obtained under two sets of conditions for separation of isomers of retinal. Retention times are in minutes. Right: initial product distribution during direct irradiation of 7,13-di*cis*-retinal, solvent, 5% ether in hexane, 360 nm; left: preparative mixture during direct irradiation of 7,9,11-tri*cis*-retinal, solvent, 1.2 % methyl t-butyl ether in Freon 112, 360 nm.

For the unstable isomers, the best spectra (containing the least amount of rearranged products) were recorded on a diode array detector (HP-100) attached in series with the HPLC column. In these cases, the extinction coefficients were calculated from those of the corresponding rearranged products. For the 11,13-dicis and 9,11,13-tricis isomers, the present values are close to those in the literature[6] (see discussion for the small differences). For 7,11,13-tricis and all-cis, the present values are substantially different from the earlier reported low values.[1] But, the higher values are in better agreement with those of the related 11,13-dicis and 9,11,13-tricis where sufficient materials allowed determination of such values by the direct weighing method.[8]

H-NMR spectra were recorded on a GE QE-300 spectrometer. With the only one exception, the vinyl signals were found readily assignable following first order analysis. In the case of 7,11-di*cis*-retinal, the close chemical shifts of H-10 and H-11 required the assistance of computer simulation for assignment of these signals.

Signals for the vinyl hydrogens of isomeric retinonitriles are, in the order of δH-7, 8, 10, 11, 12 and 14 and $J_{7,8,\ 10,11,\ 11,12}$ (all in $CDCl_3$): **all-trans,** 6.35, 6.16, 6.12, 6.97, 6.31, 6.59 ppm, 16.0, 11.3, 14.9 Hz; **7-cis**, 5.99, 6.14, 6.90, 6.88, 6.26, 5.19 ppm, 12.5, 11.6, 15.0 Hz; **9-cis**, 6.33, 6.64, 6.01, 7.05, 6.24, 5.19 ppm, 16.0, 11.4, 14.9 Hz; **11-cis**, 6.35, 6.16, 6.15, 6.65, 5.88, 5.30 ppm, 16.0, 11.4, 14.9 Hz; **13-cis**, 6.35, 6.18, 6.23, 7.01, 6.82, 5.07 ppm, 16.0, 11.2, 15.0 Hz; **7,9-dicis**, 5.98, 6.58, 6.13, 6.99, 6.21, 5.19 ppm, 12.5, 11.3, 15.0 Hz; **7,11-dicis**, 5.91, 6.03, 6.58, 6.54, 5.84, 5.24 ppm, 12.5, 11, 12 Hz; **7,13-dicis** (100MHz spectrometer) 5.99, 6.14, 6.27, 6.85, 6.80, 5.08 ppm, 12, 10, 15 Hz; **9,11-dicis**, 6.33, 6.64, 6.38, 6.72, 5.80, 5.28 ppm, 15.9, 12.3, 11.9 Hz; **9,13-dicis**, 6.34, 6.64, 6.14, 7.08, 6.76, 5.09 ppm, 16.0, 11.5, 15.0 Hz; **11,13-dicis**, 6.13, 6.35, 6.50, 6.71, 6.36, 5.10 ppm, 15.9, 12.6, 12.2 Hz; **7,9,11-tricis**, 6.33, 6.50, 6.12, 6.60, 5.79, 5.27 ppm, 12.5, 12.2, 11.8; **7,9,13-tricis**, 6.08, 6.58, 6.12, 7.02, 6.73, 5.09 ppm, 12.2, 11.8, 15.0 Hz; **7,11,13-tricis**, 5.91, 6.03, 6.27, 6.58, 6.51, 5.04 ppm, 12.6, 9.4, 11.6 Hz; **9,11,13-tricis** (not isolated); **all-cis**, 6.15, 6.54, 6.34, 6.72, 6.28, 5.13 ppm, 12.7, 12.4, 12.0 Hz.

Results and Discussion.

Much information on chromatographic separation conditions, UV/VIS and H-NMR data are available in a 1984 Tetrahedron Report[9] on retinal isomers. The present paper presents new FT-IR data and also completes information on the missing isomers, and revises some of the earlier less precise assignments of the ^{1}H-NMR data extracted from spectra taken on a lower field spectrometer.

FT-IR spectra. This part of the study was prompted by the recent success in applying vibrational spectroscopy to structural studies of protein bound chromophores[10] and the possible existence of isomeric rhodopsins[11] and other retinal binding proteins[12] from poly-cis retinal isomers.

Raman spectra of the more readily available isomers of retinal (all-trans, 9-cis, 11-cis, 13-cis and 9,13-dicis) have been analyzed in detail by Mathies, Lugtenburg and coworkers.[13] A subsequent Raman and FT-IR study by Koyama and coworkers[14] extended similar studies to three more isomers (7-cis, 9,11-dicis and 11,13-dicis). We have now recorded the FT-IR spectra of all remaining isomers of retinal. For comparison, we have also repeated the spectra of the earlier known isomers. In general our spectra are in agreement with those of Koyama et al.,[14] although the 11,13-dicis and 9,11-dicis spectra of theirs appear to show extra peaks, possibly due to the presence of different amounts of isomeric impurities. The spectra of all sixteen isomers from 1,750-500 cm^{-1} are shown in Figure 2. A few notable trends are discussed below.

First, for the double bond region (1750-1500 cm^{-1}), the strong C=O signals are generally not sensitive to geometric variation. For the C=C region, the most noticeable changes are the peaks for the four unstable isomers (11,13-dicis, 7,11,13-tricis, 9,11,13-tricis and all-cis) both in term of their relative intensity and a simultaneous appearance of a second peak at slightly higher frequency. These trends have been noted along with a discussion of mechanism of thermal isomerization of these compounds.[2] The IR characteristics were attributed to the highly twisted conformation of these isomers, making the 11,12 and 13,14 double bonds not co-planar with the carbonyl group.

The expanded region of 1,250-1,000 cm^{-1}, corresponding to C-C stretchings, for the 16 isomers is shown in Figure 3 (upper). Through a detailed study of isotopically labelled isomers of the all-trans and mono-cis isomers of retinals, Mathies,

Lugtenburg and co-workers showed that this region is highly sensitive to the polyene geometry.[15] A diagram correlating the four sets of single bonds (14,15,

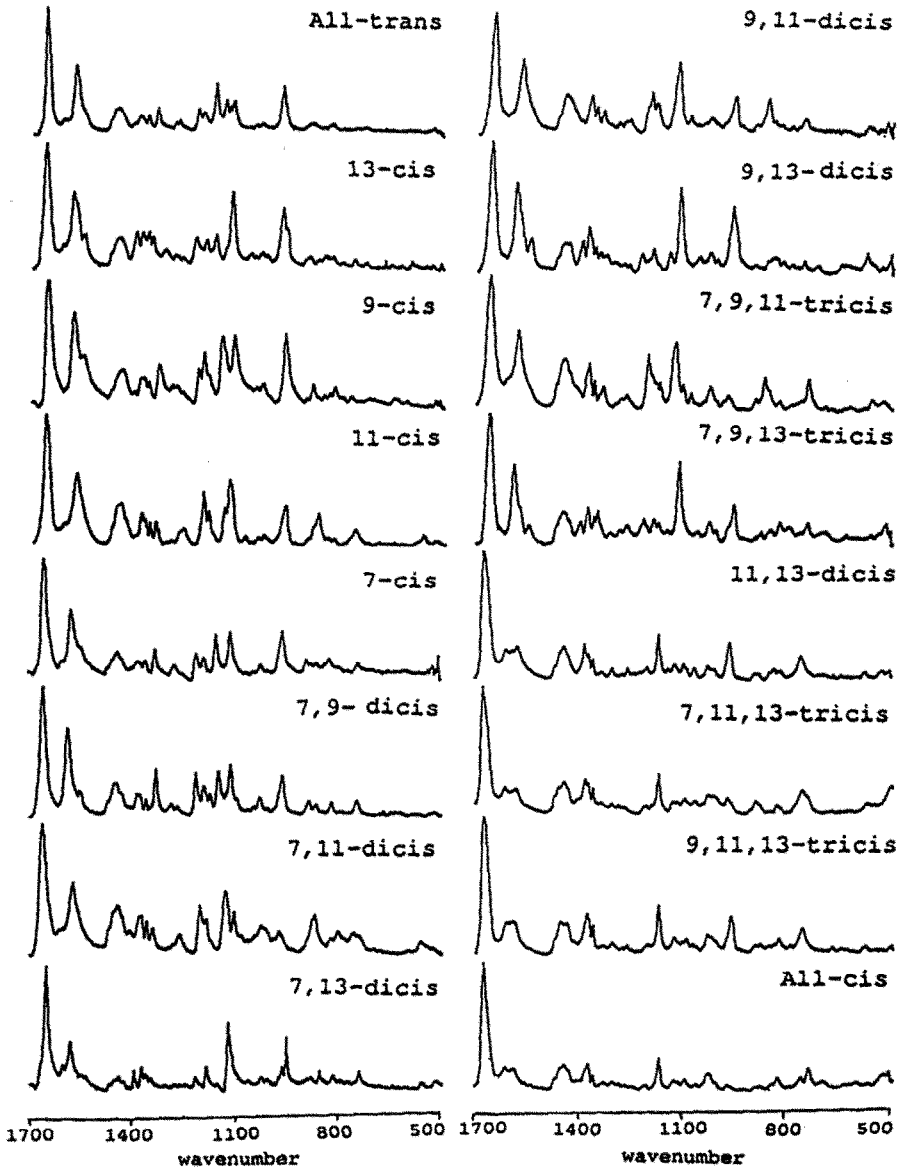


Figure 2. The FT-IR spectra (1,750-500 cm^{-1}) of all sixteen isomers of retinal obtained after depositing each sample on a KBr plate.

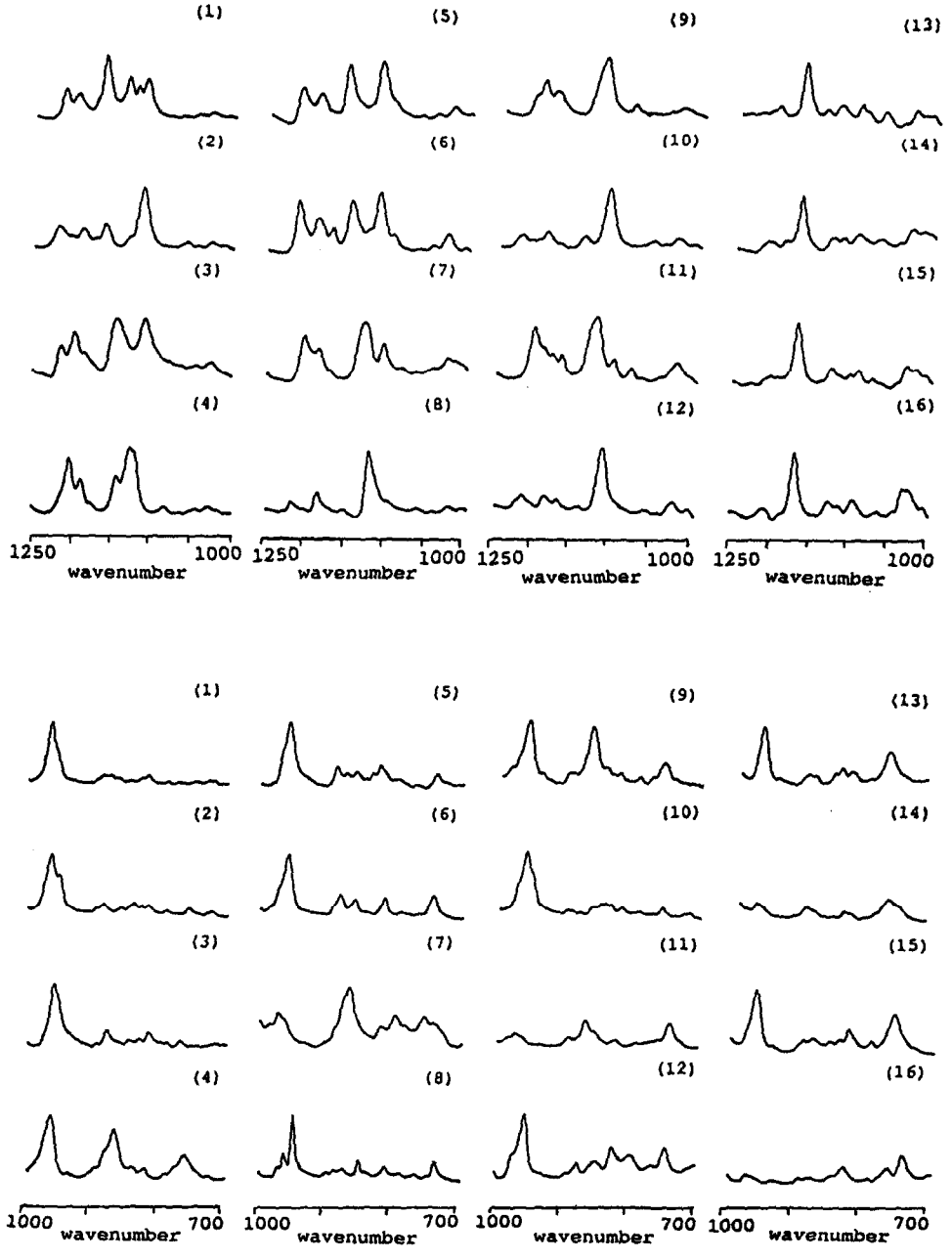


Figure 3. Expanded FT-IR spectra of all sixteen isomers of retinal: upper, the 1,250-1,000 cm^{-1} region; lower, the 1,000-700 cm^{-1} region. (1) All-trans; (2) 13-cis; (3) 9-cis; (4) 11-cis; (5) 7-cis; (6) 7,9-dicis; (7) 7,11-dicis; (8) 7,13-dicis; (9) 9,11-dicis; (10) 9,13-dicis; (11) 7,9,11-tricis; (12) 7,9,13-tricis; (13) 11,13-dicis; (14) 7,11,13-tricis; (15) 9,11,13-tricis; (16) all-cis.

12,13, 10,11, 8,9) is available,[15] which we found most useful in assisting the assignment of similar bands in the poly-cis isomers.

For 7,9-di*cis*-retinal, three of the five peaks in this region are readily assignable to the C10-C11, C12-C13 and C14-C15 stretchings by comparison with those in 9-*cis*-retinal[15,16] which is expected to be similar in shape with these two isomers for this portion of the molecule. The remaining two peaks (1189 and 1171 cm^{-1}) must then be due to C6-C7 and C7-C8 stretchings. The deuterated analogs proved to be most useful for identifying the 1189 cm^{-1} peak being that of the C8-C9 stretch. This band shifts to 1204, 1195 and 1156 cm^{-1} in respectively the 7-D-, 19,19,19-D_3- and 8,19,19,19-D_4-analogs while 1171 cm^{-1} (C5-C6 stretch) along with those assigned to C10-C11, C12-C13 and C14-C15 are relatively insenstive to isotopic substitution (Figure 4).

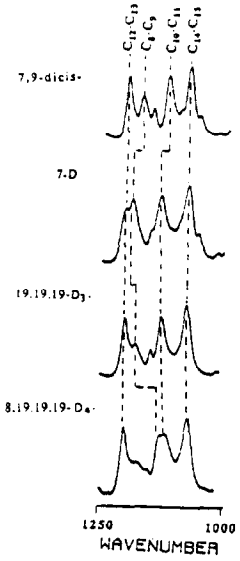


Figure 4. Correlating the C-C stretches in 7,9-di*cis*-retinal (top), the deuterated analogs.

For 7,11-di*cis*-retinal, the C10-C11 bond order is expected to decrease upon introduction of the 11-cis geometry with a simultaneous decrease of the stretching frequency. Thus, we have assigned the 1105 cm^{-1} band to this bond. The remaining bands paralled to those in the corresponding mono-cis isomers. Similar analysis led to the tentative assignment of these signals in all-dicis, tri-cis and the all-cis isomers (data listed in Table 1 and also partly correlated in Figure 5).

The 1000-700 cm^{-1} region contains transitions corresponding to hydrogen out-of-plane (HOOP) vibrations, a region most informative of the stereochemistry of the disubstituted double bonds. Thus, trans olefinic hydrogens couple strongly giving rise to bands at 959 and 966 cm^{-1}, clearly discernible in all-*trans*-retinal.[13] For the cis coupled hydrogens these bands are replaced by a single band of lower wavenumber: 761 cm^{-1} for HC11=C12H in 11-*cis*-retinal and 743 cm^{-1} for HC7=C8H

in 7-*cis*-retinal,[14] and 746 cm^{-1} in 7,9-di*cis*-retinal. The latter two expectedly are sensitive to isotopic substitution, shifting to 674 and 680 cm^{-1} for 7D-retinal and

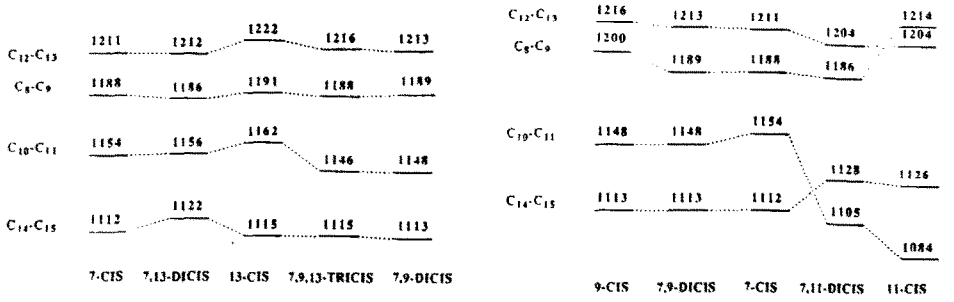


Figure 5. Correlation diagrams for the C-C stretches of selected dicis and tricis isomers of retinal with those of the corresponding mono-cis isomers.

Table 1. 1250-1000 cm^{-1} C-C stretching frequencies of the sixteen retinal isomers

Isomers	C_{14}-C_{15}	C_{12}-C_{13}	C_{10}-C_{11}	C_8-C_9
all-trans[a]	1111	1215	1163	1197
13-cis[a]	1115	1222	1162	1191
11-cis[a]	1126	1204	1084	1214
9-cis[a]	1113	1216	1148	1200
9,13-dicis[a]	1115	1224	1145	1194
7-cis	1112	1211	1154	1188
7,9-dicis	1113	1213	1148	1189
7,11-dicis	1128	1204	1105, 1089	1186
7,13-dicis	1122	1212	1156	1186
9,11-dicis	1124	1202	1088	1187
7,9,11-tricis	1125	1204	1104, 1082	1181, 1169
7,9,13-tricis	1115	1216	1146	1188, 1172
11,13-dicis	1126	w[b]	w[b]	1171
7,11,13-tricis	1122	w[b]	w[b]	1170
9,11,13-tricis	1128	w[b]	w[b]	1171
all-cis	1127	w[b]	w[b]	1170

a. Ref. 13a. b. Weak signals or degenerate with others.

Table 2. Hydrogen-out-of-plane (HOOP) bending vibration in sixteen isomers of retinal: 1000-900 cm^{-1}, trans disubstituted; 800-700 cm^{-1}, cis disubstituted

Isomers	trans mode 11+12,	7+8	cis mode 11+12,	7+8	Isomers	trans mode 11+12,	7+8	cis mode 11+12,	7+8
all-trans	967,	967	757(w),	-	7,13c	951,	-	-,	741
13-cis	954	966	757(w),	-	9,11c	-,	961	758,	-
11-cis	-,	965	761,	-	7,9,11c	-,	-	760(w),	743
9-cis	962,	962	760(w),	-	7,9,13c	957	-	760(w),	746
9,13c	962,	062	w,	-	11,13c	-,	966	761,	-
7-cis	961,	-	-,	743	7,11,13c	-,	-	760,	740
7,9c	961,	-	-,	746	9,11,13c	-,	964	759,	-
7,11c	-,	-	758,	740	all-cis	-,	-	765,	743

Table 3. Uv/vis absorption maxima (nm) and extinction coefficient data of all sixteen isomers of retinal (solvent, hexane)

Isomer	λ_{max} (ϵ)	ϵ_{360}	Isomer	λ_{max} (ϵ)	ϵ_{360}
all-trans[a]	368 (48,000)	45,400	9,11-c[e]	352 (30,600)	29,830
7-cis[b]	359 (45,100)	45,100	9,13-c[a]	359 (34,170)	34,100
9-cis[a]	363 (37,660)	36,600	11,13-c[f]	302 (7,000)	17,800
11-cis[a]	365 (26,360)	24,300	7,9,11c[g]	345 (22,000)	18,900
13-cis[a]	363 (38,770)	37,500	7,9,13c[b]	346 (36,600)	30,580
7,9-c[c]	351 (42,500)	39,800	7,11,13c[h]	289 (17,600)	9,973
7,11-c[c]	355 (18,800)	18,360	9,11,13c[f]	302 (15,500)	6,090
7,13-c[d]	357 (36,000)	36,000	all-cis[g]	287 (16,800)	5,072

a. R. Hubbard, P. K. Brown, D. Bownds, Methods Enzymol., 18, 628 (1971). b. W. DeGrip, R. S. H. Liu, A. E. Asato and V. Ramamurthy, Nature (London), 262, 416 (1976). c. A. Kini, H. Matsumoto and R. S. H. Liu, J. Am. Chem. Soc., 101, 5078 (1979). d. ref. 1. e. A. Kini, H. Matsumoto and R. S. H. Liu, Bioorg. Chem., 9, 406 (1980). f. Ref. 8. g. A. E. Asato, A. Kini, M. Denny and R. S. H. Liu, J. Am. Chem. Soc., 105, 2923 (1983). h. this work.

665 and 669 cm^{-1} for 8D-retinal. The expanded HOOP band region for the sixteen isomers are shown in Figure 3 (lower). These features detected by previous workers with the all-trans and mono-cis isomers are also noticeable in the poly-cis isomers. The assignments in this region are listed in Table 2.

Other spectroscopic data. Selected UV/VIS absorption spectra are shown in Figure 6 with the corresponding data listed in Table 3. The extinction coefficient values of 11,13-dicis and 9,11,13-tricis isomers (two of the four unstable isomers) in this paper are close but not identical to those reported.[8] The lower intensity of the shoulder at ~350 nm in the current spectra probably partly reflects the less time delay in recording the spectra of such unstable isomers when a diode array detector was employed. However, the differences between the two sets of extinction coefficients at λ_{max} are probably not experimentally meaningful. The earlier reported low extinction coefficients for 7,11,13-tricis and all-cis isomers (~5,500),[1] on the other hand, need to be revised upward, The current values (17,000 and 17,500 respectively) are close to those of the similarly twisted 11,13- and 9,11,13-tricis isomers.

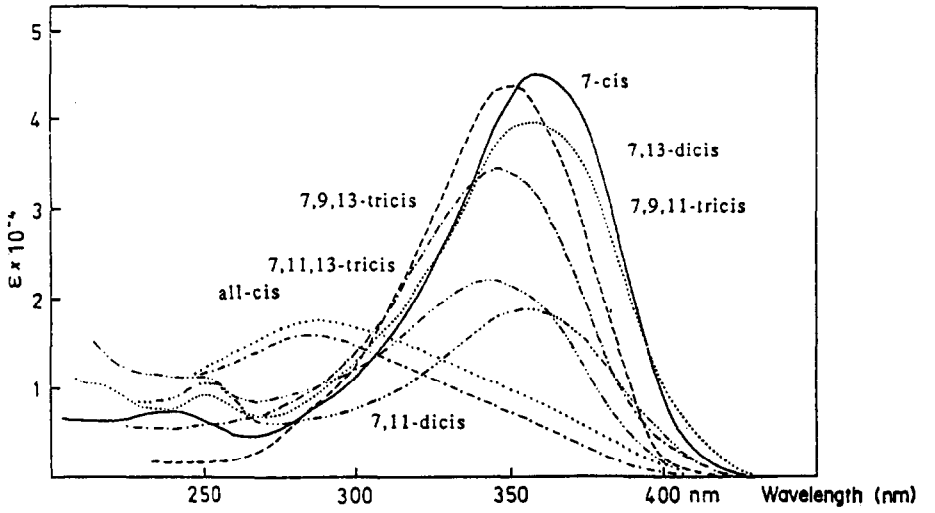


Figure 6. UV/VIS absorption spectra of selected hindered isomers of retinal in hexane. Complete data are listed in Table 3.

The ^{1}H-NMR data listed in Table 4 is a more complete and updated version of that in the 1984 review. The chemical shifts of the vinyl hydrogens, particularly useful for configuration assignments, follow trends dictated by the well recognized parameters: down-field shifts of H-8 and H-12 in respectively 9-cis and 13-cis isomers due to steric depolarization, and high field shift of H-7 and H-12 in respectively 7-cis and 11-cis isomers due to increased single bond twist with a simultaneous increase of electron density at the nearby carbons. Additional useful trends are the upfield shift (.15-.2 ppm) of the 5-methyl signals for all 7-cis isomers and

Table 4. ^{1}H-nmr data of all sixteen isomers of retinal in $CDCl_3$[a]

Isomer	CH_3-1	CH_3-5	CH_3-9	CH_3-13	H_7	H_8	H_{10}	H_{11}	H_{12}	H_{14}	H_{15}
all-t	1.04	1.72	2.03	2.33	6.36	6.18	6.19	7.14	6.37	5.98	10.11
7-c	1.05	1.52	1.93	2.31	5.99	6.11	6.25	7.08	6.33	5.96	10.10
9-c	1.05	1.76	2.02	2.32	6.31	6.64	6.06	7.20	6.27	5.94	10.11
11-c	1.03	1.72	1.99	2.35	6.32	6.14	6.54	6.69	5.92	6.07	10.08
13-c	1.04	1.72	2.02	2.14	6.35	6.18	6.23	7.05	7.28	5.85	10.20
7,9-c	1.06	1.48	1.88	2.32	6.11	6.60	6.03	7.15	6.26	5.96	10.10
7,11-c	1.03	1.51	1.89	2.34	5.96	6.10	6.61	6.59	5.90	6.03	10.10
7,13-c[c]	1.05	1.54	1.94	2.12	5.93	6.11	6.24	6.90	7.27	5.74	10.10
9,11-c	1.04	1.74	2.01	2.35	6.35	6.68	6.44	6.80	5.88	6.10	10.11
9,13-c	1.04	1.73	2.02	2.13	6.36	6.65	6.15	7.13	7.28	6.02	10.23
11,13-c	1.01	1.57	1.97	2.08	6.26	6.05	6.18	6.75	6.09	5.96	9.69
7,9,11-c	1.03	1.44	1.84	2.32	6.08	6.48	6.34	6.66	5.83	6.04	10.07
7,9,13-c	1.04	1.46	1.87	2.11	5.97	6.58	6.13	6.99	7.12	5.83	10.11
7,11,13-c	1.01	1.48	1.90	2.06	5.91	6.03	6.36	6.74	6.22	5.89	9.63
9,11,13-c	1.04	1.71	1.96	2.05	6.22	6.59	6.05	6.79	6.01	5.86	9.58
all-c[d]	1.04	1.50	1.51	1.77	6.06	6.41	6.13	6.54	5.56	5.90	9.89

Isomer	$J_{7,8}$	$J_{10,11}$	$J_{11,12}$	$J_{14,15}$	Isomer	$J_{7,8}$	$J_{10,11}$	$J_{11,12}$	$J_{14,15}$
all-t	16.5	12.1	15.2	8.0	9,11-c	15.8	13.0	13.4	7.2
7-c	11.3	11.5	15.0	8.1	9,13-c	16.0	11.4	14.9	7.4
9-c	15.9	11.5	15.4	8.1	11,13-c	16.2	12.0	12.3	8.2
11-c	16.0	12.6	11.7	8.0	7,9,11-c	12.5	12.2	12.7	8.1
13-c	16.0	11.1	15.0	8.0	7,9,13-c	12.1	10.5	15.0	8.0
7,9-c	12.5	11.5	15.2	8.2	7,11,13-c	12.5	11.7	11.4	8.0
7,11-c	12.5	10.5	11.5	8.2	9,11,13-c	15.8	11.9	11.3	7.9
7,13-c	12.5	11.3	15.0	7.6	all-c	12.6	10.8	11.6	7.9

a. Based on spectra obtained on 300 MHz spectrometer. b. Based on simulated spectra. c. In acetone-d_6. d. In C_6D_6.

the upfield shift (~.3 ppm) of the aldehydic hydrogens in all 11,13-dicis isomers. The same trends are also present in the retinonitriles of which the vinyl signals are listed in the experimental section.

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