Determination of the Solution Conformation of Retinals by using Lanthanoid Shift Reagents

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The conformations of all-*trans*-. 9-*cis*-. 11-*cis*-. and 13-*cis*-retinals and 8'-apo-β-carotenal in solution have been examined by observation of lanthanoid-induced shifts (LIS) in their ^{1}H n.m.r. spectra. Analysis has been carried out with the aid of a computer program. LISCA. in which rotamer averaging and consideration of more than one position for the bound lanthanoid can be tried. When this is done several structures may be found equally good as far as agreement between the experimental and calculated LIS ratios is concerned. In this case the choice becomes dependent upon other evidence such as gadolinium-induced broadening or nuclear Overhauser enhancements (NOEs). We have shown that the lanthanoid binds to both lone pairs of the carbonyl oxygen atom for all four isomers. However. the metal occupies the *cis*-site for less than one fifth of the time spent in the *trans*-site. The angle between the ionone ring and the side chain. D_R. ranges from 108 to 120° for the retinals. This reflects a real, average ring orientation with the skewed s-*cis*-form predominating. This angle is in agreement with the value determined by other techniques. For the angle. D_{12-13}. about the single bond C12—C13 in 11-*cis*-retinal. a value of 90 ± 20° has been found. This agrees with determinations by the NOE technique and represents an average of at least two conformers probably with values of D_{12-13} equal to +30 and −150°. However. the LIS data appear to exclude mixtures of conformers which are s-*trans* and s-*cis* about this bond.

THE retinal molecule consists of a relatively rigid trimethylcyclohexene attached to a side chain of alternating double and single bonds. There is a relatively low barrier to rotation about the formal single bonds.[1] Thus a number of solution conformations are possible for any given isomer, and predictions of the stable one under given conditions are uncertain. Recently two groups of workers have studied retinal conformations in solution by a variety of techniques. These have included ^{1}H n.m.r. chemical shifts, coupling constants, and nuclear Overhauser enhancements (NOEs); [2-4] ^{1}H n.m.r. spin-lattice relaxation times; [3,4] ^{13}C n.m.r. chemical shifts and spin-lattice relaxation times; [5-7] vibrational spectroscopy; [8,9] and semi-empirical potential energy calculations.[2,3,8,9]

In this paper we report the results obtained from conformational analysis of the n.m.r. lanthanoid-induced shifts (LIS) of all-*trans*, 9-*cis*, 11-*cis*, and 13-*cis* retinals and 8'-apo-β-carotenal. The molecular formulae and numbering scheme of these compounds are illustrated in Figure 1. There is steric hindrance in all the isomers to the planarity of C5—8, so that the angle of the ring to the side chain is unknown. Twisting about this formal single bond will reduce the π-conjugation between the ring and the side chain, and presumably have a marked effect on the electronic spectrum. The conformations about other formal single bonds in the chain, whether they are planar s-*cis*, planar s-*trans*, or twisted away from these positions, are also of interest in the interpretation of the electronic structure.[10]

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[1] R. Hubbard and G. S. Wald, in ' Structural Chemistry and Molecular Biology,' eds. A. Rich and N. Davidson, San Francisco, 1968, p. 545.

[2] B. Honig, B. Hudson, B. D. Sykes, and M. Karplus, *Proc. Nat. Acad. Sci., U.S.A.*, 1971, **68**, 1289.

[3] R. Rowan, A. Warshall, B. D. Sykes, and M. Karplus, *Biochemistry*, 1974, **13**, 970.

[4] R. Rowan and B. D. Sykes, *J. Amer. Chem. Soc.*, 1975, **97**, 1023.

[5] R. Rowan and B. D. Sykes, *J. Amer. Chem. Soc.*, 1974, **96**, 7000.

[6] R. S. Becker, S. Berger, D. K. Dalling, D. M. Grant, and R. J. Pugmire, *J. Amer. Chem. Soc.*, 1974, **96**, 7008.

[7] G. Englert, *Helv. Chim. Acta*, 1975, **98**, 2367, and references therein.

[8] A. M. Schaffer, W. H. Waddell, and R. S. Becker, *J. Amer. Chem. Soc.*, 1974, **96**, 2063.

[9] A. Warshall and M. Karplus, *J. Amer. Chem. Soc.*, 1974, **96**, 5677.

[10] See L. Zechmeister, in ' *cis-trans* Isomeric Carotenoids, Vitamins A, and Aryl Polyenes,' Springer-Verlag, Vienna, 1962.

Systems to which conformational analysis by use of LIS data is applicable have been characterised.[11] However, the conformations deduced by the LIS method are those of the lanthanide–substrate complexes, which are not necessarily the same as those adopted by the free substrates in solution. The set of closely related polyenes we have chosen to examine are likely to have
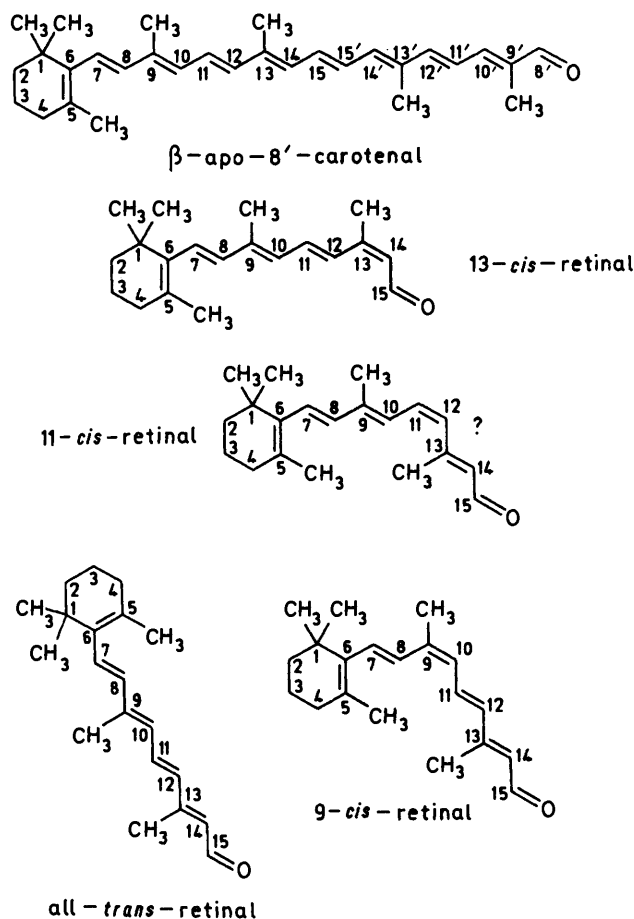

β–apo–8′–carotenal

13–cis–retinal

11–cis–retinal

all–trans–retinal

9–cis–retinal

FIGURE 1 Molecular formulae and numbering

closely similar binding geometries about the carbonyl group and similar conformations about the C13–C14–C15 fragment. Thus they provide an excellent set of compounds for comparative study of the conformation both about the metal ion and beyond the common fragment.

EXPERIMENTAL

The usual precautions were taken to exclude impurities from the shift reagents and the solvent.[12]

Tris(dipivaloylmethanato)ytterbium(III) [Yb(dpm)₃] was purchased from Ryvan Chemicals. Eu(dpm)₃, Gd(dpm)₃, and La(dpm)₃ were prepared by standard methods.[13] all-trans-, 13-cis-, and 9-cis-retinal and 8′-apo-β-carotenal

[11] B. H. S. Liénard and A. J. Thomson, preceding paper.
[12] See, for example, M. D. Johnson, B. L. Shapiro, M. J. Shapiro, T. W. Proulx, A. D. Godwin, and H. L. Pearce, *J. Amer. Chem. Soc.*, 1975, **97**, 542.

were purchased from Hoffmann–La Roche. 11-cis-Retinal was a gift from Mr. P. K. Brown.

N.m.r. measurements were made at constant substrate concentration (ca. 0.15M) in carbon disulphide by the incremental dilution method.[14] Me₄Si (ca. 0.5%) was used as internal standard and frequency lock. In runs designed to yield LIS ratios, molar ratios of shift reagent to substrate in the range 0 to 0.8 : 1 were used. Molar ratios up to 7 : 1 were employed for stoicheiometric measurements. Samples were prepared in a dry nitrogen atmosphere, under subdued light, and at room temperature, except those containing 11-cis-retinal, which were prepared at ca. 0 °C.

All n.m.r. spectra were run on a Varian HA-100 spectrometer operating in the frequency sweep mode. Ambient probe temperature (ca. 31 °C) was used for all samples except those containing 11-cis-retinal, for which the probe was cooled to −5, −33, or −50 °C (methanol calibrated). Twenty min was allowed to elapse between insertion of the sample in the probe and the taking of spectra.[12] A Hewlett Packard 5212A frequency counter was used to measure line positions (±0.01 p.p.m.).

RESULTS AND DISCUSSION

Spectral Analysis.—The general method is illustrated for 13-cis-retinal. For this substrate 11 spectra were obtained from two incremental dilution runs, both with Yb(dpm)₃ as shift reagent. The signal from the 9-methyl protons (9M) was chosen as standard because it was unambiguously identifiable in all the spectra, and because it moved a measurable amount under the influence of the shift reagent. In the higher molar ratio spectra signals belonging to multiplets were readily identified, and their chemical shifts and coupling constants were determined by first-order analyses. We could not detect changes in any of the coupling constants resulting from the presence of the shift reagent. The chemical shifts were used to construct a correlation diagram in which spectra were positioned vertically so that the 9M signals lie on a straight line (see Figure 2). Providing neither the stoicheiometry nor the stereochemistry of the lanthanoid–substrate complex (LSC) alters when the molar ratio of the free components is changed, the signals from each of the other nuclei whose n.m.r. is observed will also fall on straight lines. This was the case in all the systems studied. Thus, further assignments of signals to specific lines on the correlation diagram can be made. Since we know that the lanthanoid binds to the carbonyl oxygen atom, that (in general) the closer nuclei experience bigger shifts, and we can anticipate the multiplet patterns for specific nuclei, the assignment of the lines on the correlation diagram to particular nuclei is straightforward. Linear, least-squares regressions of the signal positions for each observed nucleus against those of the standard nucleus were next undertaken. The results are given in Table 1. Correlation coefficients are >0.99, and the predicted, unshifted chemical shifts (i.e. the chemical shifts in the absence of shift

[13] K. J. Eisentraut and R. E. Sievers, *J. Amer. Chem. Soc.*, 1965, **87**, 5254.
[14] B. L. Shapiro and M. D. Johnson, *J. Amer. Chem. Soc.* 1972, **94**, 8185.
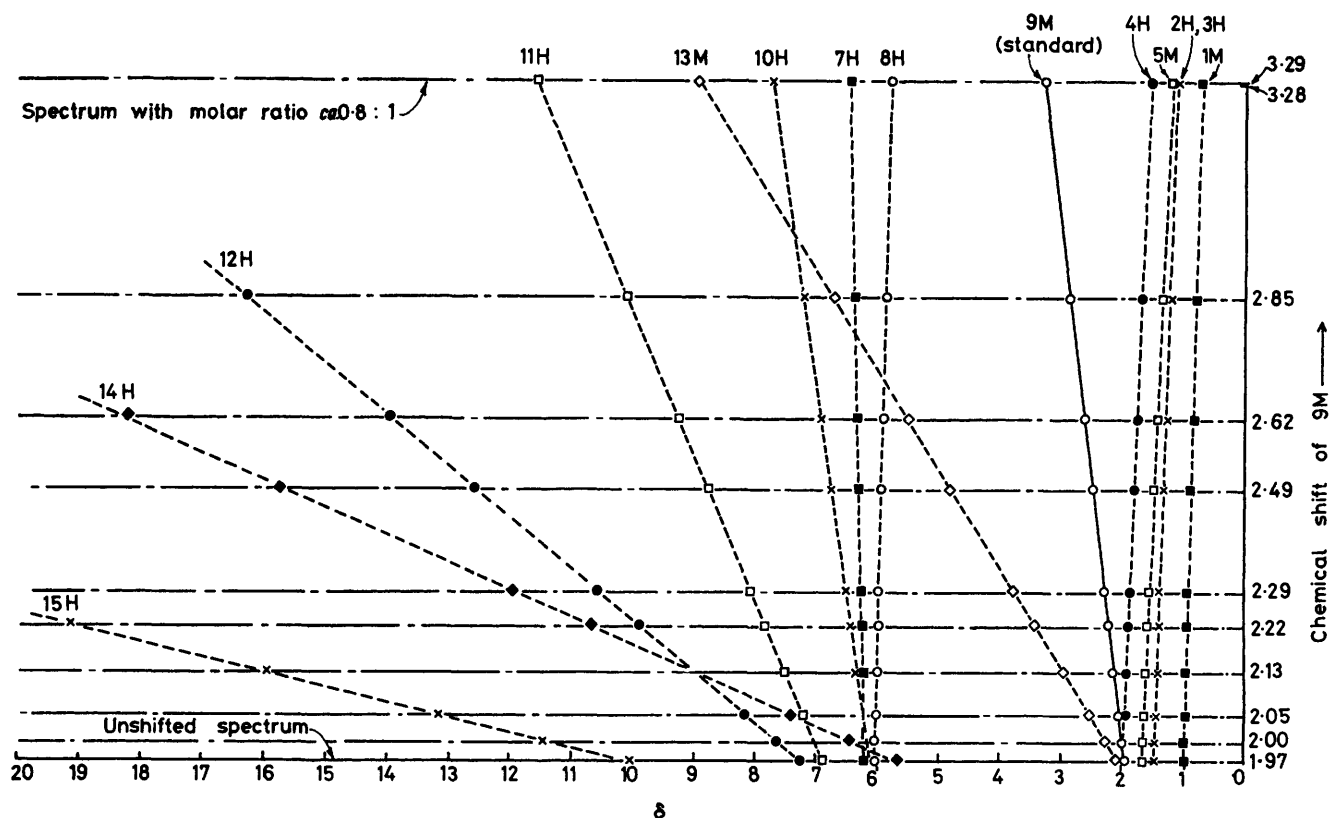

FIGURE 2   Correlation diagram for Yb(dpm)$_3$ LIS of 13-cis-retinal

TABLE 1

Linear regression data for 13-cis-retinal.   Regressions are against the observed line positions of 9M

	15H	14H	13M	12H	11H	10H	8H	7H	5M	4H	2H,3H	1M
Number of observations	5	7	11	8	11	10	11	11	11	11	10	11
Correlation coefficient	0.997 5	0.999 8	0.999 9	0.999 9	0.999 8	0.999 9	−0.999 2	0.998 1	−0.999 0	−0.992 2	−0.998 6	−0.995 1
Slope (LIS ratio)	35.7	19.2	5.21	10.2	3.52	1.22	−0.19	0.19	−0.35	−0.28	−0.26	−0.20
Predicted δ	10.09	5.64	2.09	7.29	6.91	6.13	6.07	6.19	1.67	1.98	1.49	1.00
Experimental δ	10.02	5.68	2.09	7.24	6.90	6.12	6.05	6.21	1.67	1.98	1.49	1.00

TABLE 2

Observed chemical shifts for retinals and 8'-apo-β-carotenal in carbon disulphide solution (δ values ±0.02 p.p.m.)

	15H	14H	13M	12H	11H	10H	9M	8H	7H	5M	4H	2H,3H	1M
all-trans-Retinal	9.95	5.77	2.27	6.29	6.99	6.07	1.99	6.03	6.18	1.66	1.99	1.49	1.00
13-cis-Retinal	10.02	5.68	2.09	7.24	6.90	6.12	1.97	6.05	6.21	1.67	1.98	1.49	1.00
11-cis-Retinal	9.93	5.79	2.31	5.86	6.58	6.38	1.92	6.03	6.18	1.65	†	†	0.98
9-cis-Retinal	9.94	5.79	2.25	6.20	7.06	6.02	1.96	6.55	6.21	1.69	2.00	1.50	1.00

8'-Apo-β-carotenal													
	6.62	6.26	1.93	†	†	†	1.90	†	†	1.66	†	1.48	0.98
	8'H	9'M	10'H	11'H	12'H	13'H	14'H	15'H					
	9.31	1.81	6.71	6.56	6.61	1.95	6.39	6.51					

† Not assigned.

TABLE 3

Observed coupling constants (Hz) for retinals in dilute solution in carbon disulphide

|	$J_{14H,\ 15H}$	$J_{13M,\ 14H}$	$J_{11H,\ 12H}$	$J_{10H,\ 11H}$	$J_{9M,\ 10H}$	$J_{7H,\ 8H}$	$J_{4H,\ 5M}$	Errors
all-trans-Retinal	8		15	11.5		16		±0.5
13-cis-Retinal	7.2	1.2	14.9	11.3	1.1	15.9	0.8	±0.2
11-cis-Retinal	8		10	12		15.5		±0.5
9-cis-Retinal	7.8	1.0	14.9	11.4	1.1	15.7	1.0	±0.2

reagent) are in good agreement with the directly observed values.

In like manner we have measured and assigned the chemical shifts and major coupling constants of all the ^{1}H nuclei in all-*trans*-, 11-*cis*-, and 9-*cis*-retinals. Most of the signals from 8'-apo-β-carotenal have also been assigned in this way. The data are collected in Tables 2 and 3.

LIS Ratios.—The experimental LIS ratios, which we require for our conformational analyses, are simply the slopes of the regression lines in the correlation diagrams. These data are given later, together with calculated values, in Tables 5—7. In our conformational analyses we use LIS ratios determined from Yb(dpm)$_3$-shifted spectra because this lanthanoid produces the smallest contact contribution.[15] It is evident that contact contributions to the shifts are generally small, and that the magnetic susceptibility has near axial symmetry, from the similarity between the LIS due to Yb(dpm)$_3$, Eu(dpm)$_3$, and Pr(dpm)$_3$.[16,17]

We have observed the diamagnetic shifts caused by the binding of La(dpm)$_3$ to 13-*cis*-retinal. This substrate was used because the 14H signal is conveniently positioned to low frequency of the other vinylic resonances. At a molar ratio of 0.32 : 1, diamagnetic shifts were observed in the 14H and 15H signals only. The 15H signal moves to lower frequency by 0.02 p.p.m., whereas the 14H signal moves the other way by 0.05 p.p.m. At the same molar ratio, the Yb(dpm)$_3$ LIS of 14H is 11.20 p.p.m. The diamagnetic contribution is, therefore, <0.5% for 14H and that for 15H is even smaller. Changes of this magnitude have insignificant effects upon the values of derived conformational parameters.

Stoicheiometry of the LSC.—Equilibria which may be involved in a solution containing a substrate (S), and a lanthanoid shift reagent (L) are represented by equations (1)—(3). There is now good evidence that self-associ-

$$L + S \rightleftharpoons LS \qquad (1)$$

$$LS + S \rightleftharpoons LS_2 \qquad (2)$$

$$2L \rightleftharpoons L_2 \qquad (3)$$
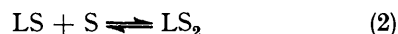
ation [equation (3)] is not significant for Ln(dpm)$_3$ shift reagents.[16,18] The analysis of Shapiro *et al.*[12] shows that equilibrium (1) is predominant for ketones binding to Ln(fod)$_3$. We may expect this to be so also for Ln(dpm)$_3$ complexes, which are weaker Lewis acids.

For equilibrium (1), under conditions of fast exchange, the lanthanoid-induced shift, $\Delta_{calc.}$, is given by equation (4), and the equilibrium constant, K, by equation (5).[19] The subscript t implies total concentrations, *i.e.* bound plus free. Substituting for [LS] from (5) into (4) gives an expression for $\Delta_{calc.}$ in terms of K and $\Delta_{max.}$, the

[15] O. A. Gansow, P. A. Loeffler, R. E. Davis, M. R. Willcott, and R. E. Lenkinski, *J. Amer. Chem. Soc.*, 1973, **95**, 3389.

[16] B. H. S. Liénard and A. J. Thomson, unpublished work.

[17] B. Bleaney, C. M. Dobson, B. A. Levine, R. B. Martin, R. J. P. Williams, and A. V. Xavier, *J.C.S. Chem. Comm.*, 1972, 791.

chemical shift of totally bound substrate. We have solved this expression iteratively, using a non-linear,

$$\Delta_{calc.} = \Delta_{max.} \frac{[LS]}{[S]_t} \qquad (4)$$

$$K = \frac{[LS]}{([S]_t - [LS])([L]_t - [LS])} \qquad (5)$$

least-squares technique, for 12 observations each of the 9M, 5M, and 1M signals of all-*trans*-retinal. The values of [L]/[S] employed extended over the range 0.8 to 7.0. The results are illustrated in Figure 3. The symbols represent $\Delta_{obs.}$ values and the dotted lines are the best fit $\Delta_{calc.}$ curves. $K = 28.0$ l mol^{-1} for all three sets of
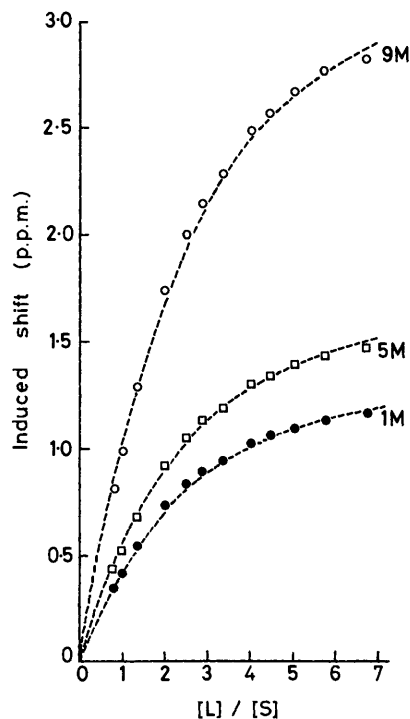

FIGURE 3 Plot of $\Delta_{obs.}$, the LIS of 9M, 5M, and 1M in all-*trans*-retinal *versus* the molar ratio of Yb(dpm)$_3$ to retinal

data. The shifts of totally bound substrate are $\Delta_{max.}$ 9M = 3.73, $\Delta_{max.}$ 5M = 1.95, and $\Delta_{max.}$ 1M = 1.54 p.p.m. In each case the correlation coefficient is >0.99.

Equation (6) defines an agreement factor, R. Figure 4

$$R = \left\{ \frac{\sum_i (\Delta_{obs.}{}^i - \Delta_{calc.}{}^i)^2}{\sum_i (\Delta_{obs.}{}^i)^2} \right\}^{\frac{1}{2}} \qquad (6)$$

shows contours of R for various K and $\Delta_{max.}$ values. Figure 3 leads us to expect that additional data at still higher molar ratios would lower the predicted $\Delta_{max.}$ values and (from Figure 4) raise the value of the equilibrium constant. The fact remains, however, that complex formation between aldehydes and Ln(dpm)$_3$ in CS$_2$ is weak with $K < 100$ l mol^{-1}.

[18] J. S. Ghotra, F. A. Hart, G. P. Moss, and M. L. Staniforth, *J.C.S. Chem. Comm.*, 1973, 113.

[19] I. Armitage, G. Dunsmore, L. D. Hall, and A. G. Marshall, *Canad. J. Chem.*, 1972, **50**, 2119.

Similarity of Lanthanoid Binding Sites.—Because the carbonyl ends of all the retinal isomers are similar, it was anticipated that the lanthanoid binding would be similar also. 15H and 14H are the only protons which are in the same position with respect to the carbonyl group in the retinal isomers which we have studied. The 15H : 14H LIS ratios for all-*trans*-, 9-*cis*-, 11-*cis*-, and 13-*cis*-retinal is 1.81, 1.80, 1.80, and 1.87, respectively. These values are so similar that we can say that, within the accuracies to which conformational parameters can be determined by the LIS method,[11] the natures of the binding in the four complexes are identical.

Method of Conformational Analysis.—The computer program LISCA, written by one of us (B. H. S. L.), was used to perform the conformational analyses. The
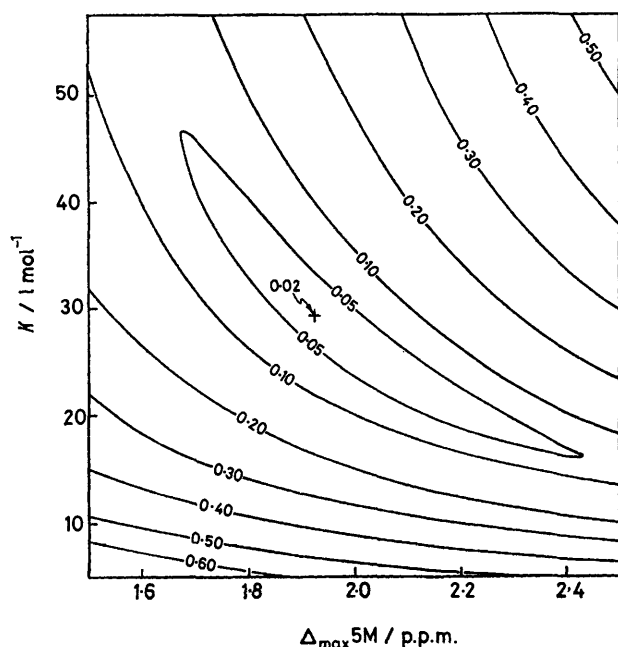

FIGURE 4　Contours of the agreement factor, R (see text), for a 1 : 1 binding model, plotted for various values of the equilibrium constant, K, and the fully complexed LIS of 5M, Δ_{max}. 5M

method of computation and the terminology used here are briefly described in the preceding paper.[11] Copies of the program and the instruction manual are available.*

Nature of the Retinal Side Chains.—Despite severe steric interaction between the methyl and vinylic hydrogen atoms on the all-*trans* retinal side chain (C7—15), the conjugated π-system maintains the planarity of this part of the molecule in the crystal.[20] The coupling constants reported here (see Table 3), and elsewhere,[3-5,21] and NOEs [3-5] strongly suggest that the side chains of all-*trans*-, 9-*cis*-, and 13-*cis*-retinals are also planar in solution

* ACL/SRC NMR Computer Program Library, c/o Dr. R. K. Harris, School of Chemical Sciences, University of East Anglia, Norwich NR4 7TJ

[20] T. Hamanaka, T. Mitsui, T. Ashida, and M. Kakudo, *Acta Cryst.*, 1972, **B28**, 214.

[21] D. J. Patel, *Nature*, 1969, **221**, 825.

and that all the side-chain single bonds are s-*trans*. In fact, we have attempted to fit our LIS data from all-*trans*-, 13-*cis*-, and 9-*cis*-retinals to structures involving non-planar geometry about the C14–C15, C12–C13, and C14–C15, and C10–C11 bonds, respectively. In all cases we obtained markedly inferior fits when the side-chain bonds were twisted more than 20—30° out of plane.

all-trans-*Retinal: LIS Data.*—We have used LISCA to set up a model of all-*trans*-retinal from the fractional crystal lattice co-ordinates.[20] The model consists of three units: one representing the side-chain, and two representing the two ring-flip forms of the ionone ring (C1—6). The models of 13-*cis*- and 9-*cis*-retinals are based on the same set of co-ordinates, but rotated by 180° about the C13–C14 and C9–C10 bonds, respectively. We have not attempted to compensate for increased steric crowding near the *cis*-double bonds by altering bond angles. Minor inaccuracies of this sort in the models result in small compensatory changes in the derived conformational parameters. However, these changes are small in comparison with the estimated accuracies. For each methyl group LISCA sets up groups of 12 equivalent points, on the locus of rotation of the methyl hydrogens, over which the calculated LIS are averaged. We feel that this is the most realistic way to treat methyl groups known to be freely rotating from spin-lattice relaxation time studies.[5]

In all the following analyses we have kept the fractional populations of each of the ring-flip forms equal. Because the cyclohexene ring is *ca.* 15 Å away from the lanthanoid atom, TQRF and PQRF for the ring nuclei are nearly independent of the proportions of the ring

TABLE 4

LISCA solutions for all-*trans*-, 13-*cis*-, and 9-*cis*-retinals. In all cases $A = 57°$, $B = 3.0$ Å, and $f_1{}^{cis} = 0.15$

	$D_R/°$	TQRF	PQRF
all-*trans*	108	0.093	0.181
13-*cis*	116	0.125	0.114
9-*cis*	108	0.065	0.443

forms. For the initial analysis we have also assumed that the principal magnetic axis of the lanthanoid-substrate complex is collinear with the Ln–O bond. This assumption has been shown to be valid in previous studies.[11,22,23] The remaining degrees of freedom in the model are as follows: the Ln–O–C15 angle supplement (A), the Ln–O bond length (B), the geometry of rotatable bond number 1 (the O–C15 bond), and the dihedral angle (D_R) about the C7–C6 bond (which defines the orientation of the cyclohexene ring with respect to the side chain). Note that A is 180° minus the conventional (included) bond angle. The definitions of D_1 and D_R are illustrated in Figures 5(a) and 6, respectively. For rotatable bond number 1, we refer to the planar position with $D_1 = 0°$ as the ' *cis* site,' and that with $D_1 = 180°$

[22] G. E. Hawkes, D. Leibfritz, D. W. Roberts, and J. D. Roberts, *J. Amer. Chem. Soc.*, 1973, **95**, 1659.

[23] R. M. Wing, J. J. Uebel, and K. K. Anderson, *J. Amer. Chem. Soc.*, 1973, **95**, 6046.

as the ' *trans* site ' [see Figure 5(b)]. For the ring–side chain angle, $D_R = 0°$ is equivalent to C5–C6–C7–C8 being s-*trans* planar, and $D_R = 180°$ is equivalent to s-*cis* planar.

1-*Site metal binding*. We first ask LISCA to find the best (lowest TQRF),[13] unique position for the lanthanoid using all 17 observed nuclei. TQRF = 0.037 is the lowest value, for which $A = 58°$, $B = 2.9$ Å, $D_1 = 204°$

(i.e. 24° away from the *trans* site), and D_R is either 84 or 232°.

Figure 7 shows a map of the TQRF surface in the plane

of the carbonyl group of all-*trans*-retinal with 9M as the standard nucleus. TQRF = ∞ along a line on which
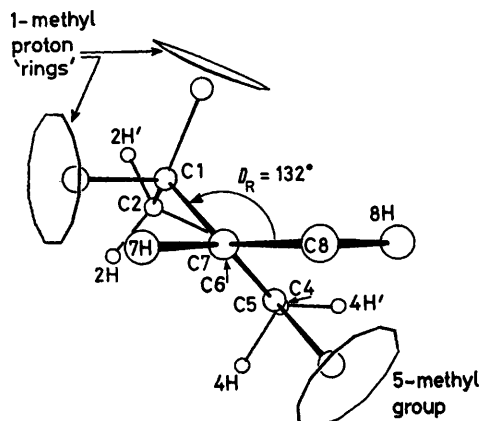
FIGURE 6 The junction between the side chain and the cyclohexene ring viewed directly along the C7–C6 bond, illustrating the definition of D_R; C3, 3H, and 3H' have been omitted for clarity

$θ_{9M}$, the 9M–Ln–O angle, is 54.7°. At this angle the geometric factor, $G_{9M} = (3\cos^2θ_{9M} - 1)r_{9M}^{-3}$, is zero, and therefore all the calculated LIS ratios are infinite. A different choice of standard nucleus results in a map with altered contours at high TQRF, but the positions of the minima are virtually unchanged. As can be seen, in addition to a minimum at $A = 58°$ and $B = 2.9$ Å, there

FIGURE 7 Map of the TQRF surface in the plane of the carbonyl group of all-*trans*-retinal; the standard nucleus is 9M
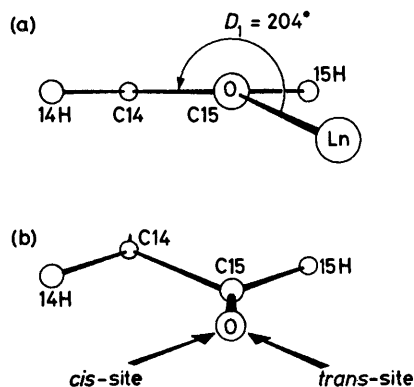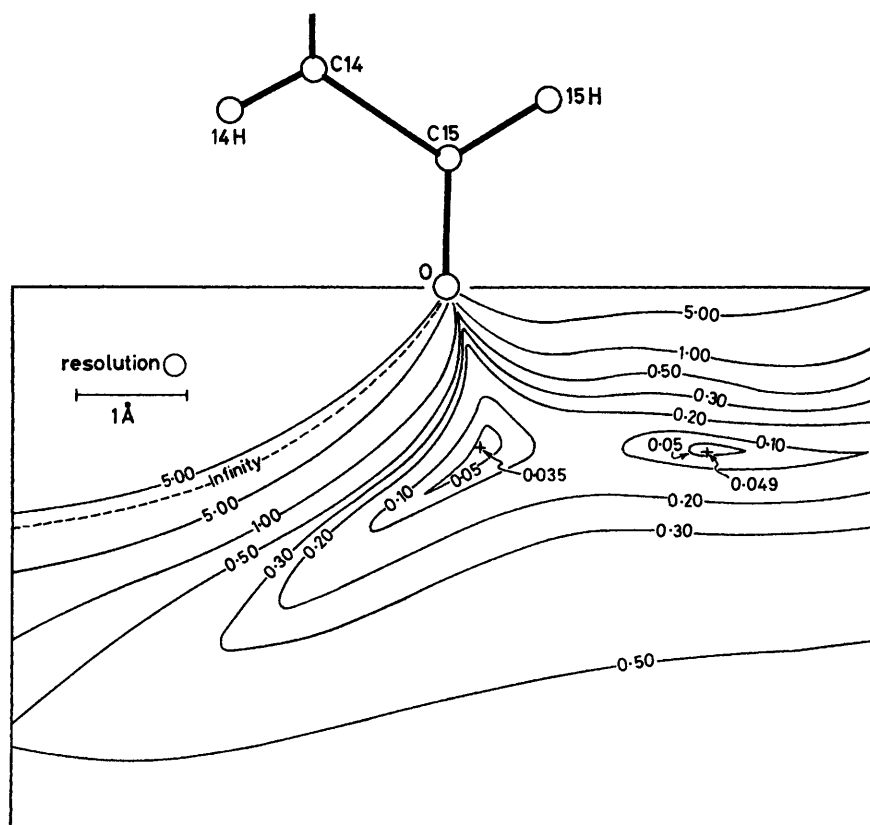
FIGURE 5 Geometry about the carbonyl group in the retinals: (a) view of the side chain, looking directly along the O–C15 bond, illustrating the definition of D_1; and (b) the location of the *cis*- and *trans*-binding sites

is another, slightly lower, minimum at $A = 12°$ and $B = 1.6$ Å. We reject the latter solution primarily because B is too small.

cis–trans-*Mixture about* C14–C15. There will be a two-fold barrier to rotation about this bond [24] dominated by the energy of the π-system. Accordingly, we used two sites for $D_{14–15}$ at planar s-cis, and planar s-trans. Some solutions with TQRF <0.15 were found; however, each of these disagrees with one or more of the following observations: (a) the value of $J_{14H,15H}$ implies that a high proportion of the s-trans-conformer is present, (b) $B \leqslant 1.8$ Å is unlikely, and (c) the experimental and calculated broadening ratios do not agree (see later).

2-*Site metal binding.* We have shown previously [11] that lanthanoid shift reagents bind to both the lone pairs of the carbonyl oxygen atoms of camphor and canthaxanthin. When we allow binding at both cis- and trans-sites in the model of all-trans-retinal, the degrees of freedom describing the relationship between the lanthanoid and the side chain become A, B, and f_1^{cis} (the fraction binding at the cis site). For this hypothesis we obtain TQRF $= 0.050$ when $A = 56°$, $B = 3.1$ Å and $f_1^{cis} = 0.16$.

13-cis-*Retinal: Gadolinium-induced Broadening.*—We have observed the broadening of the 15H and 14H signals due to the addition of Gd(dpm)$_3$ to 13-cis-retinal solutions. Again, we used this substrate because the 14H signal is positioned away from the other vinylic proton resonances. The experimental 15H to 14H broadening ratio is $2.0 (\pm 1.0) : 1$. The best 1-site binding solution to the LIS ratios for 13-cis-retinal, with $A = 57°$, $B = 2.0$ Å, and $D_1 = 207°$, gives a calculated 15H : 14H broadening ratio of $8.1 : 1$. With the metal in the trans site $(D_1 = 180°)$, $A = 56°$, and $B = 3.0$ Å, we get a calculated 15H : 14H broadening ratio of $10.5 : 1$; whereas fixing the lanthanoid in the cis-site $(D_1 = 0°)$, with the same A and B, gives $0.06 : 1$. For the 2-site case, with $A = 56°$, $B = 3.1$ Å, and $f_1^{cis} = 0.16$, the calculated ratio is $1.45 : 1$. We consider the above results good evidence that the lanthanoid binds at both the lone pairs of the oxygen atom.

Lanthanoid–Retinal Binding: Conclusion.—9-cis-Retinal produces results similar to those described for 13-cis- and all-trans-retinal.

The stereochemistry of lanthanoid binding to the carbonyl group of retinals appears to be dominated by (a) the disposition of the lone pairs of the oxygen atom and (b) steric interaction with atoms adjacent to the functional group. Hindrance by 15H of a Ln(dpm)$_3$ complex in the trans-site is clearly less than that by 14H when the metal occupies the cis site [see Figure 5(b)]. The groups adjacent to the carbonyl group in the retinals, for which we get $A = 57°$, are less bulky than those in camphor and canthaxanthin (methyl and CH$_2$ groups) where $A = 42°$.[11] Combining LIS and broadening data we estimate $f_1^{cis} = 0.15 \pm 0.06$, and for the major (trans) binding site, A and B values of $57 \pm 10°$ and 3.0 ± 0.2 Å, respectively.

An implicit assumption in our method of conformation

analysis is that the degrees of freedom in the model are independent of one another. This assumption is an approximation, the magnitude of which must be judged for individual cases. We take the above results as an example. When the proportions of the metal binding at the two sites are significantly different, as is the case here, we may expect that the nature of the binding will also be different, *i.e.* A and B will be different for the two sites. Thus, the values derived from our analyses will be some (population weighted) average of the actual values for the two sites. This is the reason why we have given values of A and B specifically for the major binding site. Values for the minor site may be different, but this does not invalidate the method because the contributions to the calculated (and the observed) LIS ratios from the metal at the minor site are small.

Contact Shifts.—We have used unit weights, w_i, for the observed LIS ratios in all the LISCA analyses presented in this paper. Because of this the large ratios, particularly those of 15H and 14H, to some extent dominate the TQRF of each conformation. From the work of Patel and Shulman [25] we know that unpaired spin density attenuation is marked as we move away from the carbonyl group. For example, the unpaired spin density at C13 is $<5\%$ of that at C15 in the retinyl-N-methyl Schiff's base bound to the acetylacetonate complex of nickel(II).

We have repeated the 1-site and 2-site metal binding analyses of all-trans-, 9-cis-, and 13-cis-retinals with the 15H and 14H ratios zero weighted. The best values of the degrees of freedom were little different from those previously described. The main differences, in fact, lie not in the actual values but in the accuracy with which the values can be defined. This is so because the TQRF surfaces all exhibit broader minima when 15H and 14H are omitted.

From the above, we conclude that contact shifts, if present, have little effect on the conformational parameters derived from our conformational analyses.

Combined Retinal Side Chains.—Because all-trans-, 13-cis-, and 9-cis-retinals give similar, but nevertheless slightly different, optimum values of A, B, and f_1^{cis}, we have combined these three side chains into a single model with the carbonyl groups occupying the same position in space. We have not used the 11-cis-side chain because in this case the conformation beyond C12 was not certain.

Using the 9M shift of all-trans-retinal as standard, we have 26 observed nuclei in the three side-chains, and just three variables: A, B, and f_1^{cis}. The best solution has $A = 57°$, $B = 3.0$ Å, and $f_1^{cis} = 0.15$, for which TQRF $= 0.120$.

*Individual all-*trans-, 13-cis-, *and* 9-cis-*Retinal Solutions.*—Since the side chains of these isomers are planar units, and the relationship between each of them and the lanthanoid was fixed using the values determined for the combined structure, there is only one degree of freedom,

[24] E. B. Wilson, *Chem. Soc. Rev.*, 1972, **1**, 293.

[25] D. J. Patel and R. G. Shulman, *Proc. Nat. Acad. Sci., U.S.A.*, 1970, **65**, 31.

D_R, the ring–side chain angle. In each case there are 17 observed nuclei although only 13 distinct shifts were measured: on the cyclohexene ring four pairs of equivalent groups have the same LIS values. Table 4 presents the values of D_R, TQRF, and PQRF (for the cyclohexene ring nuclei only) for the three isomers. The TQRF values are higher than those obtained when A, B, and f_1^{cis} are allowed to vary for each molecule individually. This is to be expected because the individual solutions do not agree exactly. Experimental and calculated LIS ratios are given in Table 5.

were able to measure the LIS of 1M, *ca.* 25 Å away from the lanthanoid. The 8'H/1M LIS ratio is 515.0!

Assuming 2-site metal binding and a rigid, planar side chain, the degrees of freedom in the model are A, B, f_1^{cis}, and D_R. D_R has little effect on the LISCA solution to the structure because the cyclohexene ring is far removed from the site of complexation of the lanthanoid. Accordingly, we have fixed D_R, arbitrarily at 100°. The best solution has TQRF = 0.048 and PQRF (for the ring only) = 0.708, and is characterised by $A = 58°$, $B = 3.1$ Å, and $f_1^{cis} = 0.15$.

TABLE 5

Experimentally measured and calculated LIS ratios for the individual LISCA solutions to the structures of all-*trans*-, 13-*cis*-, and 9-*cis*-retinals reported in Table 4. In each case the ratios are with respect to the appropriate 9M induced shift

Observed nucleus	all-*trans*		13-*cis*		9-*cis*	
	ExptLISR	CalcLISR	ExptLISR	CalcLISR	ExptLISR	CalcLISR
15H	27.7	30.4	35.8	36.6	30.9	34.9
14H	15.3	16.6	19.2	20.6	17.1	19.1
13M	5.24	5.06	5.21	4.46	5.85	5.82
12H	4.68	4.38	10.2	8.22	5.29	5.04
11H	2.69	2.56	3.52	3.07	3.04	2.94
10H	1.90	1.80	1.22	1.33	2.17	2.07
8H	0.963	0.986	−0.188	−0.055	1.44	1.43
7H	0.644	0.641	0.188	0.245	0.754	0.743
5M	0.529	0.608	−0.354	−0.290	0.684	0.710
4H	0.346	0.381	−0.281	−0.249	0.304	0.266
4H′	0.346	0.445	−0.281	−0.438	0.304	0.289
3H	0.293	0.324	−0.260	−0.135	0.281	0.287
3H′	0.293	0.362	−0.260	−0.198	0.281	0.264
2H	0.293	0.332	−0.260	−0.272	0.281	0.179
2H′	0.293	0.373	−0.260	−0.394	0.281	0.274
1M	0.414	0.367	−0.198	0.003	0.491	0.418
1M′	0.414	0.494	−0.198	−0.257	0.491	0.513
TQRF	0.093		0.125		0.065	

8'-Apo-β-carotenal.—Our interest in this molecule stems from the different arrangement of the ^{1}H nuclei close to the carbonyl group from that in the retinals, but with a chemically similar binding site for the lanthanoid shift reagent. Thus, conformational analysis of the mode of lanthanoid binding here acts as a check that the relationship we have found in the retinal complexes is not due to a fortuitous agreement between experimental and calculated LIS ratios.

Since our aim is not detailed conformational analysis, an approximate model was constructed as follows. The crystal structure co-ordinates of all-*trans*-retinal [20] were rotated by 180° about an axis, perpendicular to the plane of the side chain, passing through a point 0.68 Å along the C15–O bond. This produced co-ordinates for the side chain from C15′ to C8′ with a C15–C15′ bond length of 1.36 Å, similar to that observed in canthaxanthin.[26] The C–CHO fragment of all-*trans*-retinal was translated so that C15 was coincident with C8′, and C14 as near as possible to C9′.

Our spectral analysis method gave LIS ratios for all the methyl groups and for the vinylic protons between 8'H and 14H. Because of the large distance involved we could not separate the signals from 7H to 12H. The observed LIS ratios are given in Table 6. Note that we

[26] J. C. J. Bart and C. H. MacGillavry, *Acta Cryst.*, 1968, **B24**, 1587.

These results are in good agreement with those for the retinals, unexpectedly so because a smaller proportion of metal binding at the *cis*-site would reflect the presence of the nearby 9'-methyl group. We consider that the

TABLE 6

Experimental and calculated LIS ratios for 8'-apo-β-carotenal. Experimental values are Yb(dpm)₃-induced shift ratios. Calculated values are for the structure with $A = 58°$, $B = 3.1$Å, and $f_1^{cis} = 0.15$, for which TQRF = 0.048 and PQRF = 0.708 for the cyclohexene ring nuclei only. The standard nucleus is 13′M

Observed nucleus	Expt-LISR	Calc-LISR	Observed nucleus	Expt-LISR	Calc-LISR
8'H	16.46	16.24	13 M	0.155	0.159
9'M	6.49	6.32	9 M	0.055	0.066
10'H	4.55	5.29	5 M	0.045	0.069
11'H	2.67	2.58	1 M	0.036	0.041
12'H	1.52	1.77	1 M′	0.036	0.069
14'H	0.664	0.919	2 H	0.032	0.047
15'H	0.473	0.566	2 H′	0.032	0.052
15 H	0.332	0.345	3 H	0.032	0.057
14 H	0.264	0.245	3 H′	0.032	0.070

results support our view that contact shifts are small for the ^{1}H nuclei, and that 2-site metal binding to carbonyl groups is correct.

11-cis-*Retinal*.—In the ' classical ' structure of the 11-*cis*-retinal side chain, depicted in Figure 1, there exists severe steric interaction between the 13-methyl

protons and 10H. In the crystal structure [26] this steric crowding is relieved solely by a rotation of 140° (from the classical structure) about the C12–C13 bond: the C9–C10–C11–C12 and C10–C11–C12–C13 sections are planar s-*trans*, and *cis*, respectively. In solution, the similarity between the values of $J_{10H,11H}$ in all-*trans*- and 11-*cis*-retinals (see Table 3) suggests that the C9–C10–C11–C12 section is also planar in solution.[21] Patel has pointed out [21] that the shift to lower frequency of the 12H signal in the 11-*cis*-isomer, in comparison with the other retinals (see Table 2), implies that the conjugation of the π-system is broken to some extent by a non-planar geometry about the C12–C13 bond, as in the crystal structure. Measurements of spin-lattice relaxation times for the carbon atoms suggest that the side chain to C12 is relatively rigid, whereas that from C13 onwards undergoes much more rapid segmental motion.[6]

Conformational analysis. We have constructed our model from the crystal structure co-ordinates of the 11-*cis*-isomer.[27] The model consists of four rigid units, two for the flip forms of the ionone ring, one for the side chain from C6 to C13, and one for C13 to the carbonyl group. Using the values of A, B, and f_1^{cis} obtained for the combined all-*trans*-, 13-*cis*-, and 9-*cis*-structure, and assuming a 50 : 50 mixture of the ring flip forms, we have just two areas of conformational freedom in the model. These are the ring–side chain geometry about C6–C7, and the conformation about the C12–C13 bond. Although the metal binding parameters are from spectra taken at 30 °C, whereas the 11-*cis* data were obtained at −5 °C, we believe that they are not very temperature dependent (see later).

First we set both rotatable bonds 1-site and variable: that is the dihedral angles are single valued. The best

TABLE 7

Experimental and calculated LIS ratios for the best solution to the 11-*cis*-retinal structure in which D_{12-13} has a single value. The calculated ratios are for the structure with $A = 57°$, $B = 3.0$ Å, $f_1^{cis} = 0.15$, $D_{12-13} = 90°$, and $D_R = 117°$, for which TQRF = 0.087 and PQRF (for the cyclohexene ring only) = 0.248. The standard nucleus is 9M

Nucleus	Expt-LISR	Calc-LISR	Residue *	CalcBR †
15H	21.1	19.8	1.3	313.9
14H	11.7	13.3	−1.6	198.5
13M	3.69	4.39	−0.70	20.2
12H	3.21	3.26	−0.05	6.4
11H	1.95	1.99	−0.04	2.8
10H	3.18	2.95	0.23	6.5
8H	1.20	1.20	0.00	2.7
7H	0.59	0.59	0.00	0.6
5M	0.23	0.27	−0.04	0.9
1M	0.19	0.26	−0.07	0.3
1M'	0.19	0.16	0.03	0.8

* Residue = ExptLISR − CalcLISR. † Calculated broadening ratio.

fit, using 11 observed nuclei, has TQRF = 0.087 and PQRF (for the ring only) = 0.248, and is characterised by $D_{12-13} = 90°$ and $D_R = 117°$. The experimental and calculated LIS ratios for this solution are given in Table

7. D_R is defined as for the other retinals (see Figure 6). D_{12-13} is defined such that a value of 0° corresponds to planar s-*trans* (classical structure). Strictly, we should consider a 50 : 50 mixture of positive and negative D_R values here. However, fortuitously D_R has another minimum at 260° (= −100°) and a plot of PQRF *versus* D_R is roughly symmetrical about $D_R = 180°$ in this case. Hence, inclusion of a 50 : 50 mixture of positive and negative D_R values would result in virtually the same value of D_{12-13}, in which we are primarily interested here.

We next tried to fit our data to three models having two sites at C12–C13. These models have pairs of D_{12-13} values of 0 and 180°, 30 and 210°, and 60 and 240°. The LISCA results are summarised in Table 8.

TABLE 8

LISCA results for 11-*cis*-retinal models with two possible orientations about the C12–C13 bond. For all solutions $A = 57°$, $B = 3.0$ Å, $f_1^{cis} = 0.15$

'trans' site (°)	'cis' site (°)	f_{12-13}^{trans}	$D_R/°$	TQRF	PQRF *	PQRF †
0	180	0.80	120	0.111	0.26	3.38
30	210	0.55	35	0.091	0.18	1.27
60	240	0.48	132	0.087	0.06	0.27

* For cyclohexene ring and side chain nuclei up to and including 12H. † For cyclohexene ring nuclei only.

Temperature dependence. We have recorded spectra from a single sample containing 11-*cis*-retinal and Yb(dpm)$_3$ at 5, −33, and −50 °C. As we move to lower temperatures two changes are observed. First, all resonances are broadened by similar amounts. This may be due either to a decrease in the exchange rate of the lanthanoid on the substrate or a reduction in the tumbling rate of the species in solution. Secondly, there are concerted shifts in the signal positions to higher frequencies. For example, the LIS of 13M was 2.44 p.p.m. at −5 °C, and 3.26 p.p.m. at −50 °C. These shifts can be attributed primarily to a change, in favour of the lanthanoid–substrate complex, in the position of equilibrium between the species present.

Discussion.—Of the hypotheses we have tested by the LIS agreement criterion, the one having D_{12-13} single-valued at 90° is the best. Agreement becomes progressively worse as the two sites are moved away from the 90° position. The model which best fits the NOE data [3] also has $D_{12-13} = 90°$. However, theoretical calculations [3] suggest minimum energy conformations with D_{12-13} equal either to +30 and −150° or −30 and +150°. Reasonable agreement between the observed and calculated NOEs was achieved by averaging over two structures with equal populations of $D_{12-13} = 165°$ and −45°.

The temperature dependence of the vibrational spectrum of 11-*cis*-retinal has been attributed to either a change in the ' effective average value ' of D_{12-13} in the ground state of the 11-*cis*,12-s-*cis*-isomer, or a change in the equilibrium between 11-*cis*,12-s-*cis*- and 11-*cis*,12-s-*trans*-conformations.[9] The latter explanation, like that

[27] R. D. Gilardi, I. L. Karle, and J. Karle, *Acta Cryst.*, 1972, **B28**, 2605.

for the temperature dependence of the NOEs,[3] requires that the 11-cis,12-s-trans-isomer predominates at low temperatures. This is in disagreement with the results of potential energy calculations [3] and the crystal structure,[27] which predict that the 12-s-cis-form will be the more stable.

Table 9 gives the LIS ratios, with respect to the 13-methyl LIS, at the three temperatures employed: also

the metal atom in our model are given in Table 10. These must be considered ' worst cases ' because steric hindrance is greatest when the side chain is planar. Bearing in mind that the Ln(dpm)$_3$ is not a ' hard sphere ' (i.e. it has inclusions in its surface), the distances are all very acceptable except those for the case with the cis metal binding and C12–C13 s-cis. However, the calculated population of this species is less than 5%, so

TABLE 9

Temperature dependence of 11-cis-retinal LIS ratios. The signals from 14H and 15H were beyond δ 20 and therefore not observed in this experiment. Calculated values are also given for models with $D_{12-13} = 0$ (s-trans), 90, and 180° (s-cis), in each case using $A = 57°$, $B = 3.0$ Å, $f_1^{cis} = 0.15$, and $D_R = 120°$. The standard in all cases is 13M

	Temp. (°C)	D_{12-13}/°	1M	5M	7H	8H	9M	10H	11H	12H
Observed	−5		0.057	0.070	0.168	0.324	0.275	0.881	0.525	0.869
Observed	−33		0.057	0.071	0.169	0.325	0.287	0.870	0.550	0.932
Observed	−50		0.058	0.071	0.169	0.325	0.294	0.865	0.567	0.969
Change	−5 to −50		0.001	0.001	0.001	0.001	0.019	−0.016	0.042	0.100
Calculated		0	0.034 *	0.042	0.100	0.154	0.191	0.457	0.436	0.862
Calculated		90	0.049 *	0.063	0.135	0.272	0.228	0.669	0.454	0.745
Calculated		180	0.368 *	0.351	0.295	−0.123	0.261	0.769	0.479	0.654
Change		180 to 0	−0.334	−0.309	−0.195	0.277	−0.070	−0.312	−0.043	0.208
Chemical shift change †	32 to −52 °C		−0.005	+0.017	+0.014	+0.076	+0.039	+0.139	+0.117	+0.094
(p.p.m.) for temp. change										

* Average of calculated values for the two 1-methyl groups. † Taken from ref. 2.

given are calculated values for models with single D_{12-13} values of 0, 90, and 180°. When attempting to interpret these data we must consider the possibility that the geometry of the lanthanoid binding is also temperature dependent. The virtually unchanged ratios of 1M, 5M, 7H, and 8H suggest that neither the nature of the lanthanoid binding nor the geometry about the C12–C13 bond undergoes a major change on cooling from −5 to −50 °C, unless fortuitous cancelling of effects takes place. Small changes do occur, however, in the LIS ratios of the nuclei close to the 11-cis part of the side chain. We have been unable to correlate these changes with either our calculated LIS ratio changes brought about by a shift in the s-cis–s-trans equilibrium, or temperature dependent changes in the unshifted chemical shifts [3] (see Table 9).

TABLE 10

Distances (Å) separating the lanthanoid from points in the model structures of 11-cis-retinal. For all cases $A = 59°$ and $B = 3.0$ Å

Metal site	D_{12-13}/°	10H	8H	Ring *	f_t †
cis	0 (s-trans)	7.9	10.3	11.7	0.12
trans	0 (s-trans)	8.5	10.2	10.9	0.68
cis	180 (s-cis)	3.4	2.8	2.9	0.03
trans	180 (s-cis)	6.9	7.7	8.0	0.17

† Distance to the closest point in the cyclohexene ring. * Total fractional population of each structure when $f_1^{cis} = 0.15$, and $f_{12-13}^{trans} = 0.80$.

Modification of the conformation by the shift reagent. In the case of the 11-cis-isomer we must consider this possibility. Ln(dpm)$_3$ complexes are roughly spherical with a maximum radius of ca. 7 Å.[28] In 11-cis-retinal, 10H and 8H are the nuclei closest to the metal atom in the portion of the side chain between C7 and C12. Distances from these nuclei, and from the ionone ring, to

that it does not contribute greatly to the overall calculated LIS ratios. Bearing in mind our comments about the implied independence of the variables in our model (see above), and assuming that the s-cis–s-trans mixture model is correct, the fractional population of the conformation with s-cis-geometry about C12–C13 and with the metal in the cis-site may, in fact, be zero. In any case, non-interaction of the 11-cis-side chain and the lanthanoid complex appears likely from the constancy of the 15H : 14H LIS ratio from one isomer to another (see above). However, we cannot be certain that the presence of the lanthanoid complex does not affect the conformation of 11-cis-retinal.

Due to the change, on rotation about the C12–C13 bond, in the conjugation of the π-system and the altered anisotropic shifts due to the different disposition of the C=C bonds, we expect changes in the unaltered chemical shifts of the side chain nuclei.[21] If the lanthanoid shift reagent does, in fact, alter the conformation, such changes might be observed upon addition of the diamagnetic reagents La(dpm)$_3$ and Lu(dpm)$_3$. We have not yet performed this experiment because of the small quantity of 11-cis-retinal available to us at present.

Conclusion.—On the basis of the LIS agreement criterion and the temperature dependence of the LIS ratios, we conclude that D_{12-13} has an average value of $90 \pm 20°$ in the lanthanoid–substrate complex. This is in agreement with the value determined by NOE.[3] However, this value must represent an average of at least two limiting conformations. A single conformer with $D_{12-13} = 90 \pm 20°$ implies virtually total lack of π-conjugation between the two parts of the side chain. The electronic spectrum does not support this contention.

[28] J. P. R. DeVilliers and J. C. A. Boeyens, Acta Cryst., 1972, B28, 2335.

The maximum of the longest wavelength intense band is more characteristic of a penta- than of a tetra- or tri-ene. This fact, combined with calculation of the shape of the potential energy well as a function of D_{12-13},[12] leads to the conclusion that the value of $90 \pm 20°$ determined represents an average of at least two conformers. However, considerable motion about the mean value may occur, but a rapidly interconverting mixture of two conformations which could be described as s-*trans* and s-*cis* is not supported by the LIS data.

Ring–Side Chain Angle.—The orientation of the ring with respect to the side chain is of special interest in all the retinals. Steric interaction between 7H and 8H and the ring methyl groups is severe for some orientations, particularly planar s-*cis*. Potential-energy calculations by Honig *et al.* on β-ionone, which has an essentially identical ring–side chain linkage, show that a broad minimum from $D_R = 50$ to $150°$, and another, sharper one with $D_R = 0 \pm 10°$ exist.[2] In the solid state the triclinic modification of Vitamin A Acid has $D_R = 145°$, whereas the monoclinic form has $D_R = 6°$.[29] For all-*trans*-retinal, the crystal value of D_R is $118°$.[20] Clearly, the depths of the minima are so similar that crystal packing forces can dictate the conformation adopted in the solid state.

Because the cyclohexene ring is small in comparison with the distance with separates it from the lanthanoid binding site in retinals, we were at first wary of emphasising the ring–side chain angles deduced from our LIS analyses. However, the PQRF curves for D_R in the four

retinal molecules are very similar, with deep minima at 108, 116, 120, and $108°$, for all-*trans*-, 13-*cis*-, 11-*cis*-, and 9-*cis*-isomers, respectively. We note that the cyclo-hexene ring as a whole is in different magnetic environments in the various isomers. In all-*trans* retinal, with the metal in the *trans*-binding site, the ring is *ca.* 16 Å away from the lanthanoid, near the centre of the ' shift cone'; the angle between the ring–lanthanoid line and the principal magnetic axis of the complex is *ca.* 15°. In the 13-*cis*-isomer, the ring is *ca.* 14 Å away and sub-tends an angle of *ca.* 65° with the principal magnetic axis. Also the orientations of the rings in the magnetic field are various: the experimental 1M : 5M LIS ratios for all-*trans*- and 13-*cis*-isomers are 0.78 and 0.56 respectively. For the above reasons we think that our data reflect the real, average ring orientation, namely skewed s-*cis*, which predominates in all four isomers. There may be some, small population of the s-*trans*-conformation; however, the ring is too far away from the metal atom for 2-site ring angle models to be meaningful. Therefore, we must be content with a population weighted, average conformation. The ring–side chain angle found here is in agreement with that found for β-ionone from the NOEs,[2] low resolution microwave spectra,[30] and LIS ratios;[31] for retinals from the analysis of NOEs;[3,4] and for canthaxanthin from LIS ratios.[11]

[29] C. H. Stam, *Acta Cryst.*, 1972, **B28**, 2936, and references cited therein.

[30] W. E. Steinmetz, *J. Amer. Chem. Soc.*, 1974, **96**, 685.
[31] B. H. S. Liénard and A. J. Thomson, in preparation.