Evidence for Two Binding Sites of Lanthanoid Shift Reagents at the Carbonyl Groups of Camphor and Canthaxanthin

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By observing the shifts and broadening of ^{1}H and [13]C n.m.r. signals of camphor induced by lanthanoid shift reagents, it has been shown that the metal binds at two sites on the carbonyl group. A similar situation has been demonstrated for the binding of shift reagents to the carbonyl groups in the ionone rings of the carotenoid cantha-xanthin. With the aid of a novel computer program (LISCA), employing a least-squares fitting routine, the conformations of the carotenoid have been investigated. The ring-flip forms are equally populated in solution at room temperature. Our results point to, but cannot distinguish between, either an ionone ring–polyene side chain angle of ca. 90° or a 50 : 50 equilibrium mixture of the forms s-trans and 40° away from s-cis.

THE lanthanoid-induced shift (LIS) effect on the n.m.r. of a substrate possessing a suitable donor group[1] has shown great promise as a means of obtaining structural information in solution. In this paper we describe some of the general methods we have devised to help characterise the conformations of retinals and related molecules in solution by the LIS method. We illustrate our methods by their application to camphor and cantha-xanthin. Our analyses of the retinals themselves will be the subject of subsequent papers.

The utility of the LIS method has been amply demonstrated (see, for example, references 2—7). Rigorous empirical tests of the method's validity have been published.[2,3] The types of system for which significant contributions from contact shifts and a non-axial magnetic susceptibility tensor might be expected have been characterised, and methods of testing for these complications have been devised.[8]

The above-mentioned problems aside, structural information descriptive of the substrate relies upon the accurate determination of the location of the lanthanoid atom with respect to it. In principle it should be easier to locate the lanthanoid when bound to a carbonyl group than when bound to a hydroxy- or amino-group. This is because there is no free rotation about the C–O bond of the carbonyl function, so that the orientation of the electron-donating lone pairs is fixed. Further, in the case of the retinals, the carbonyl group is part of an extensive conjugated system and is therefore itself strongly constrained to adopt a planar configuration

with respect to the π-system. For these reasons we have studied the binding of lanthanoid shift reagents to carbonyl groups in some detail.

The majority of LIS work reported to date has used hydroxy- or amino-containing substrates. For carbonyl compounds, despite the expectation that the metal should be easier to locate than for other functionalities, nearly all attempts to do this have met with difficulties (see, for example, ref. 7); these have been attributed to a significant non-axial component in the shifts[9] and to a breakdown of the common assumption that the lanthanoid occupies a single location.[10] Our work supports the latter supposition; we have found no evidence for the former proposal.

COMPUTATIONAL METHODS

As a result of a preliminary investigation into the conformation of β-ionone using the LIS method,[11] it was apparent that a far more general computer program than was then available was required in order to analyse satisfactorily LIS data from retinals and related molecules. In addition to metal atom positioning with respect to the substrate, orientation of the principal magnetic axis of the complex, and rotation about bonds within the substrate, we wanted to be able to treat fractional populations of two or three conformers differing by rotation about single bonds within the substrate (e.g. s-cis–s-trans mixtures), fractional populations of very different geometries for part or all of the substrate (e.g. alicyclic ring-flip forms), and proportions of metal binding at two or three orientations about the binding site (e.g. binding to each of the lone pairs of a carbonyl oxygen atom). Most early attempts at computer fitting of observed to calculated LIS data relied

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

[3] C. D. Barry, C. M. Dobson, D. A. Sweigert, L. E. Ford, and R. J. P. Williams, in 'Nuclear Magnetic Resonance Shift Reagents,' Academic Press, New York, 1973, pp. 173—195.

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[5] M. R. Willcott, R. E. Lenkinski, and R. E. Davis, *J. Amer. Chem. Soc.,* 1972, **94,** 1742.

[6] H. L. Ammon, P. H. Mazzocchi, W. J. Kopecky, H. J. Tamburin, and P. H. Watts, *J. Amer. Chem. Soc.,* 1973, **95,** 1968.

[7] H. J. Schneider and E. F. Weigand, *Tetrahedron,* 1975, **31,** 2125.

[8] 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.

[9] R. H. Newman, *Tetrahedron,* 1974, **30,** 969.

[10] D. J. Chadwick, *Tetrahedron Letters,* 1974, 1375.

[11] B. H. S. Liénard, M.Sc. Thesis, University of East Anglia, 1972.

upon incremental scanning of the variables.[4,5] Our preliminary investigations showed that, even with efficient 'filtering out' of unacceptable conformations,[4] the computing time required for the complex systems we wished to tackle would be prohibitive. We therefore decided to use non-linear, least-squares minimisation [12] of a quasi-R-factor (see later) to match observed and calculated LIS ratios.

LISCA.—A computer program called LISCA (Lanthanoid Induced Shift Conformational Analysis) has been written which is capable of handling systems of this complexity. Over 3 years LISCA has grown into a comprehensive package which provides, in addition to the central function of fitting calculated to observed LIS data, comprehensive facilities for the construction and checking of sets of atomic co-ordinates, graphical representation of relative goodness-of-fit of individual observed nuclei, tabulation of calculated broadening ratios, graphs of goodness-of-fit *versus* variable value, contour plots of the quasi-R-factor surface about the binding site, *etc.* The program is written in A.N.S.I. FORTRAN IV to facilitate transfer to different computers.

Computer Model of the Lanthanoid–Substrate Complex.— LISCA treats the substrate as a set of rigid units connected to one another by rotatable bonds. Each unit consists of the co-ordinates of the points (atoms) in a section of the molecule which we assume to be rigid in solution. These data can be read into the program as fractional crystal lattice co-ordinates, or rectangular Cartesian co-ordinates, or internal molecular co-ordinates. The lanthanoid atom is connected to the co-ordinating atom, X, in the first unit. The model of the lanthanoid–substrate complex thus produced has several degrees of freedom, or facilities, each of which has an associated status.

Degrees of freedom in the model. B and A are the Ln–X bond length and Ln–X–Y angle supplement, respectively (for aldehydes and ketones Y is the carbonyl carbon atom). They can both be set either fixed or variable in status. α and ϕ define the orientation of the principal magnetic axis of the complex with respect to the Ln–X vector. They may be either set so that the magnetic axis is fixed and collinear with the Ln–X vector or allowed to vary.

Each unit has associated with it a rotatable bond facility which defines its relationship with the preceding unit: in the case of the first unit, its relationship with the lanthanoid atom is defined. Each rotatable bond can be set in one of six states; designated 1-site fixed, 2-site fixed, 3-site fixed, 1-site variable, 2-site variable, and 3-site variable. A 1-site rotatable bond has as its parameter the dihedral angle, D_n (n is the rotatable bond number), which may be either fixed or variable. The 2-site states have three parameters; two dihedral angles, $D_n{}^1$ and $D_n{}^2$, defining the two 'sites,' and the fractional population of the first site, written $f_n{}^d$ where d is the angle $D_n{}^1$ or given a descriptive label such as 'cis.' In the 2-site fixed state all three parameters have fixed values. In the 2-site variable state only the fractional population is variable.

The alternative unit facility allows for the treatment of alternative portions of the substrate whose geometries differ radically, for example, alicyclic ring flip forms. This is achieved by designating two units as exchangeable. The degree of freedom is the fractional population of one of these units.

Input of Experimental LIS Data and the Computation of Calculated Values.—Experimental LIS data are fed into LISCA in the form of ratios with respect to the LIS of a 'standard' nucleus. Weights, w_i, for each nucleus i may also be read in.

In the general case a molecule may be represented by several distinct conformations in the model. The total fractional population, $f_m{}^t$, of a single contributing structure, m, is the product of the fractional populations of the component parts of that structure, each equivalent to a degree of freedom in the model. The calculated LIS ratios are obtained by first computing the population weighted geometric factors given by equation (1). In

$$G_i{}^t = \sum_m \{f_m{}^t(3\cos^3\theta_i - 1)r_i{}^{-3}\} \qquad (1)$$

equation (1) the sum is over all contributing structures m. For each observed nucleus, i, θ_i is the angle between the principal magnetic axis of the LSC and the line joining the lanthanoid to nucleus i, and r_i is the length of that line. The calculated LIS ratios are computed by dividing each geometric factor by that of the standard nucleus. We may note that the geometric factors for twelve-point, rotationally averaged, methyl groups are calculated by using a modified form of equation (1) in which $f_m{}^t$ is replaced by 1/12 and the sum is over $m = 1$—12.

Calculated broadening ratios are computed in like manner except that the $(3\cos^2\theta_i - 1)r_i{}^{-3}$ term is replaced by $r_i{}^{-6}$.

Conformational Analysis.—By setting up different combinations of the degrees of freedom in the model, various hypotheses as to the nature of the lanthanoid–substrate complex can be tested, by non-linear, least-squares fitting of the observed LIS ratios to a set of calculated ones.

In order to assess the relative goodness-of-fit to the model hypotheses we use a measure which we call the total quasi-R-factor (TQRF), defined in equation (2), in which

$$\text{TQRF} = \left\{\frac{\sum_i (\text{CalcLISR}_i - \text{ExptLISR}_i)^2 w_i}{\sum_i (\text{ExptLISR}_i)^2 w_i}\right\}^{\frac{1}{2}} \qquad (2)$$

w_i is the weight assigned to each observation, CalcLISR is the calculated LIS ratio, and ExptLISR is the experimental value. The prefix 'total' implies that the summations are over all observed nuclei i. We also use partial quasi-R-factors (PQRFs) in which the summations are over a subset of the observed nuclei. The 'quasi' prefix acts as a reminder that we are using ratios to define our goodness-of-fit measure. This means that when $\text{TQRF} \gg 0$ its absolute value is dependent upon the choice of the standard nucleus. For this reason statistical significance tests are not strictly applicable.[13,14] As $\text{TQRF} \longrightarrow 0$, however, so it also tends towards the true R-factor [5] in which the calculated data are least-squares scaled to the experimental data. For $\text{TQRF} < 0.15$ the location of minima in the true and quasi-R-factor surfaces are not very different. The use of LIS ratios in the goodness-of-fit measure reduces the number of variables to be optimised by one, with a consequent reduction in the computing time required. It also means, however, that we are giving infinite weight to one of our observations, namely the LIS of the standard nucleus.

[12] M. J. D. Powell, *Computer Journal*, 1965, **7**, 303.
[13] W. C. Hamilton, in 'Statistics in Physical Science,' Ronald Press, New York, 1964, pp. 157—162.

[14] R. E. Davis and M. R. Willcott, *J. Amer. Chem. Soc.*, 1972, **94**, 1744.

Each hypothesis is tested three times by starting the fit from widely different initial values of the variables. This greatly reduces the risk of locating ' false minima ' with higher TQRF than the true minimum.

Use of LISCA.—We have found, after using LISCA on four retinals and five related molecules, that TQRF is, at best, dependent within ± 0.02 upon the co-ordinates used to describe the observed nuclei in the rigid units alone. Thus, values of facilities which give TQRF < 0.03 greater than the minimum are acceptable solutions to the structure. Ideally we would hope to achieve a perfect fit, *i.e.* TQRF $=$ 0, for one particular hypothesis. Many factors may contribute to the non-occurrence of perfect fits in practice. Apart from the errors in the structure of the rigid units, these factors include deviations from axial symmetry, contributions to the observed shifts from the contact mechanism, failure of the point dipole approximation at short distances, and the inexactness of our simple hypotheses about the conformationally mobile parts of the structure. Consideration of these sources of error leads us to believe that, as far as the LIS data alone are concerned, only solutions having TQRF > 0.15 can definitely be ruled out. Although this eliminates many hypotheses it is not difficult to obtain a wide variety of solutions which satisfy this criterion. It is therefore essential to examine other experimental data to reduce further the range of acceptable solutions. Principal among these are line broadenings induced by gadolinium. These are dependent upon the inverse sixth power of the distance separating the gadolinium and the observed nucleus. Other evidence to be considered, where available, comes from coupling constants and their dependence on dihedral angle,[15] nuclear Overhauser enhancements,[16] and steric interaction between different parts of the substrate and between the substrate and the lanthanoid complex.[4] Consideration of both shifts and broadening induced by lanthanoids is often sufficient to define a unique solution to the structure. Coupling constants, Overhauser enhancements, and steric hindrance considerations can confirm this. However, any unique structure obtained represents an ensemble average of many similar conformations present in solution and not the much more rigid conformations seen in the solid state by X-ray crystallography. This is so for two reasons: first, the number of data is often 100 times smaller from LIS measurements, and secondly, n.m.r. techniques can only distinguish details on a time-scale greater by many powers of ten than that of rotation about chemical bonds and that of complex formation and dissociation.

EXPERIMENTAL

The usual precautions to exclude impurities from shift reagents and solvent were taken (see, for example, ref. 17). 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.[18] Camphor and canthaxanthin were purchased from B.D.H. and Hoffmann–La Roche, respectively, and used without further treatment.

For the n.m.r. measurements the solvent was carbon disulphide, with *ca.* 0.5% Me₄Si added as internal standard

and frequency lock. Series of spectra were obtained at constant substrate concentration (*ca.* 0.15M) by the incremental dilution method.[19] Molar ratios of shift reagent to substrate in the range 0 to 0.8 : 1 were used. Eu(dpm)₃ was used to separate the signals for observation of broadening due to Gd(dpm)₃.

All n.m.r. spectra were run on a Varian HA-100 spectrometer operating in the frequency sweep mode and at the ambient probe temperature of 31 ± 1 °C. As has been pointed out,[17] samples must be given time to come to thermal equilibrium after insertion. We have observed small movements of the signals 10 min after insertion. Accordingly, we allowed 20 min to elapse before taking spectra. Line positions (± 0.01 p.p.m.) were measured by using a Hewlett–Packard 5212A frequency counter.

CAMPHOR

Because camphor is a rigid, cage molecule it is an excellent candidate for the illustration of our methods. The structural formula is shown in Figure 1. The labels
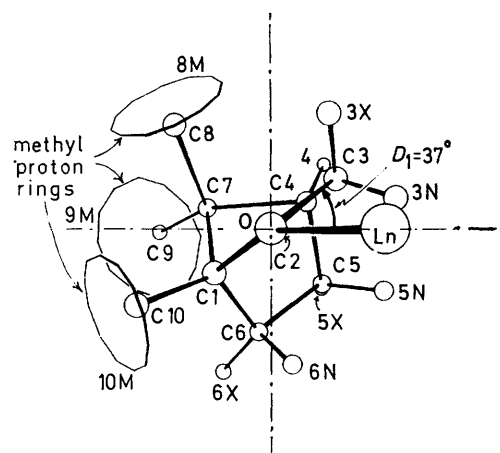

FIGURE 1 ' Ball-and-stick ' representation of camphor viewed directly along the O–C2 bond

which we use to describe the various atoms are also given here: ' X ' and ' N ' refer to *exo* and *endo*, respectively.

LIS Data.—Yb(dpm)₃-shifted ¹H spectra of camphor were recorded at nine different molar ratios. Resonance frequencies from these spectra were used to construct a correlation diagram of one particular signal (that of the methyl protons on C10) *versus* all the others. If we arrange the observed frequencies for these methyl protons so that they lie on a line of slope 1.0, the shift ratios of the other nuclei are just the slopes of the least-squares, linear regression lines through their respective sets of points. We have used the ytterbium shift reagent to obtain our ¹H LIS ratios because this lanthanoid has been shown to produce the smallest contact contribution.[20] A disadvantage of ytterbium, however, is its tendency to broaden the resonances, thus

[15] J. Feeney, *Proc. Roy. Soc.*, 1975, **A345**, 61.
[16] B. Honig, B. Hudson, B. D. Sykes, and M. Karplus, *Proc. Nat. Acad. Sci. U.S.A.*, 1971, **68**, 1289.
[17] M. D. Johnson, B. L. Shapiro, M. J. Shapiro, T. W. Prolx, A. D. Godwin, and H. L. Pearce, *J. Amer. Chem. Soc.*, 1975, **97**, 542.

[18] K. J. Eisentraut and R. E. Sievers, *J. Amer. Chem. Soc.*, 1965, **87**, 5254.
[19] B. L. Shapiro and M. D. Johnson, *J. Amer. Chem. Soc.*, 1972, **94**, 8185.
[20] O. A. Gansow, P. A. Loeffler, R. E. Davis, M. R. Willcott, and R. E. Lenkinski, *J. Amer. Chem. Soc.*, 1973, **95**, 3389.

obscuring the multiplet patterns. We have used $Eu(dpm)_3$ to observe the splitting patterns of the camphor ^{1}H resonances. Assignments are based on the expected splittings by analogy with those of nor-camphor [21] and are in agreement with previous ^{1}H LIS assignments for camphor.[22] The ^{1}H LIS ratios obtained from the $Eu(dpm)_3$-shifted spectra are, in fact, very similar to those obtained with ytterbium. This is good evidence that the magnetic susceptibility has axial symmetry and that contact contributions are small.[8]

We have observed the diamagnetic shifts due to the binding of $La(dpm)_3$ to camphor. Not surprisingly, diamagnetic shifts could only be measured for nuclei close to the binding site, namely, 10M, 3X, and 3N. The shifts are linearly related to the amount of $La(dpm)_3$ added at low molar ratios. Hence we can calculate the diamagnetic contributions to the $Yb(dpm)_3$-induced LIS. For 10M, 3X, and 3N these are $+0.3$, $+0.6$, and $+0.9\%$, respectively. Contributions of this magnitude have negligible effect on derived conformational parameters.

simplify the discussion which follows we state here that all the following ^{1}H analyses were performed also with the magnetic axis facility variable. In all cases which fit reasonably well (TQRF < 0.15), α optimised to $<5°$. This may be interpreted as evidence that the binding site is indeed the oxygen atom (this is obvious for camphor but may not be for polyfunctional substrates). In fact, we have tried telling LISCA that the binding site is C9! Discounting some wild solutions, with high TQRF, LISCA then gives metal positions very similar to those found for oxygen binding and with the magnetic axis directed roughly towards the oxygen atom.

LISCA Analysis.—In the following analyses the magnetic axis is always aligned along the Ln–O bond. Unit weights, w_i, were used for all observed nuclei except for C2, which was zero weighted in case it suffers a large contact shift.[2]

Figure 1 illustrates the definition of the dihedral angle D_1. $D_1 = 0°$ corresponds to the lanthanoid being in the plane of the carbonyl group on the C3 side. A positive value of D_1 implies an anticlockwise rotation of the

TABLE 1 *

LISCA solutions for camphor with rotatable bond number 1 = 1-site; fitting to 9 Yb-induced ^{1}H LIS ratios; 10M as standard

Solution no.	$B/\text{Å}$ Initial	$B/\text{Å}$ Optimum	$A/°$ † Initial	$A/°$ † Optimum	$D_1/°$ Initial	$D_1/°$ Optimum	TQRF
1a	3.0	4.1	30	41	180	258	0.103
1b	3.5	3.0	50	40	300	37	0.106
1c	2.5	3.0	10	40	60	37	0.106
2a		[3.0]	30	0	180	—	0.288
2b		[3.0]	50	40	300	38	0.106
2c		[3.0]	10	39	60	37	0.106
3a	3.0	3.2	30	50		[0]	0.111
3b	3.5	3.2	50	49		[0]	0.111
3c	2.5	4.4	10	0		[0]	0.135

* Square brackets denote values of facilities kept fixed during fitting.   † Note that A is defined as the supplement of the conventional bond angle.

[13]C LIS ratios for camphor, induced by tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyloctane-4,6-dionato)ytterbium(III) [Yb(fod)$_3$] in CCl_4 solution, were taken from the literature.[7]

The LIS ratios used in our conformational analyses are given in Tables 5 and 6.

Geometric Data and Degrees of Freedom in the Model.— The fractional crystal lattice co-ordinates of the ten carbon atoms and the oxygen atom were taken from the X-ray crystal structure determination of (+)-8-bromo-camphor.[23] Twelve equivalent points were used to represent each methyl group. In the LISCA model, therefore, camphor is represented by 55 points in a single rigid unit. The only unknowns in the structure of the lanthanoid–substrate complex are those which describe the relationship between the substrate and the lanthanoid, namely, A, B, α, ϕ and rotatable bond number 1.

Direction of the Principal Magnetic Axis.—In order to

* This is the reverse of the most frequently employed convention.

[21] J. L. Marshall and S. R. Walter, *J. Amer. Chem. Soc.*, 1974, **96**, 6358.

substrate with respect to a fixed lanthanoid atom.* Thus, $D_1 = 90°$ places the camphor molecule such that the metal is 'below' the six-membered ring, *i.e. endo*; $D_1 = 180°$ puts C10 near to the metal, and $D_1 = 270°$ brings C8 close to it, *i.e. exo*.

1-Site Metal Binding Models.—We first consider the hypothesis that the lanthanoid occupies a unique position anywhere in space. The status of the LISCA facilities which correspond to this hypothesis are: B variable, A variable, and rotatable bond number 1 = 1-site and variable. The results, using the ^{1}H LIS ratios, are given in Table 1 as solutions 1a—c. Solution 1a illustrates a common phenomenon found by us, and others,[8] when fitting LIS data. This is the elongation of B when a false minimum is found with respect to another variable, in this case D_1.

Hypothesis 2 is the same as 1 except that we have fixed $B = 3.0$ Å. This time the compensation for the false minimum in D_1, for solution 2a, manifests itself in a reduction of A to zero, making D_1 undefined. As one

[22] P. V. Demarco, D. Doddrell, and E. Wenkert, *Chem. Comm.*, 1969, 1418.

[23] C. A. Bear and J. Trotter, *Acta Cryst.*, 1975, **B31**, 903.

might expect, solutions 2b and c are very similar to 1b and c.

These solutions are wholly reasonable. Figure 1 illustrates the geometry of solution 1c. The lanthanoid is bound to the carbonyl lone pair away from the bulky methyl group on C10. B, at 3.0 Å, is wholly reasonable for a solution structure, and A is reduced, from a maximum orbital overlap value of 60°, to 40° (included

to, the binding site. Such a map, for the plane of the camphor carbonyl group, is shown in Figure 2. Bearing in mind our comments regarding the acceptability of TQRF values up to ca. 0.15, the map indicates the precision with which we can define structural parameters, for a single hypothesis, using LIS data alone.

Table 2 gives the calculated broadening ratios, tabulated by LISCA, for the ^1H nuclei close to the binding
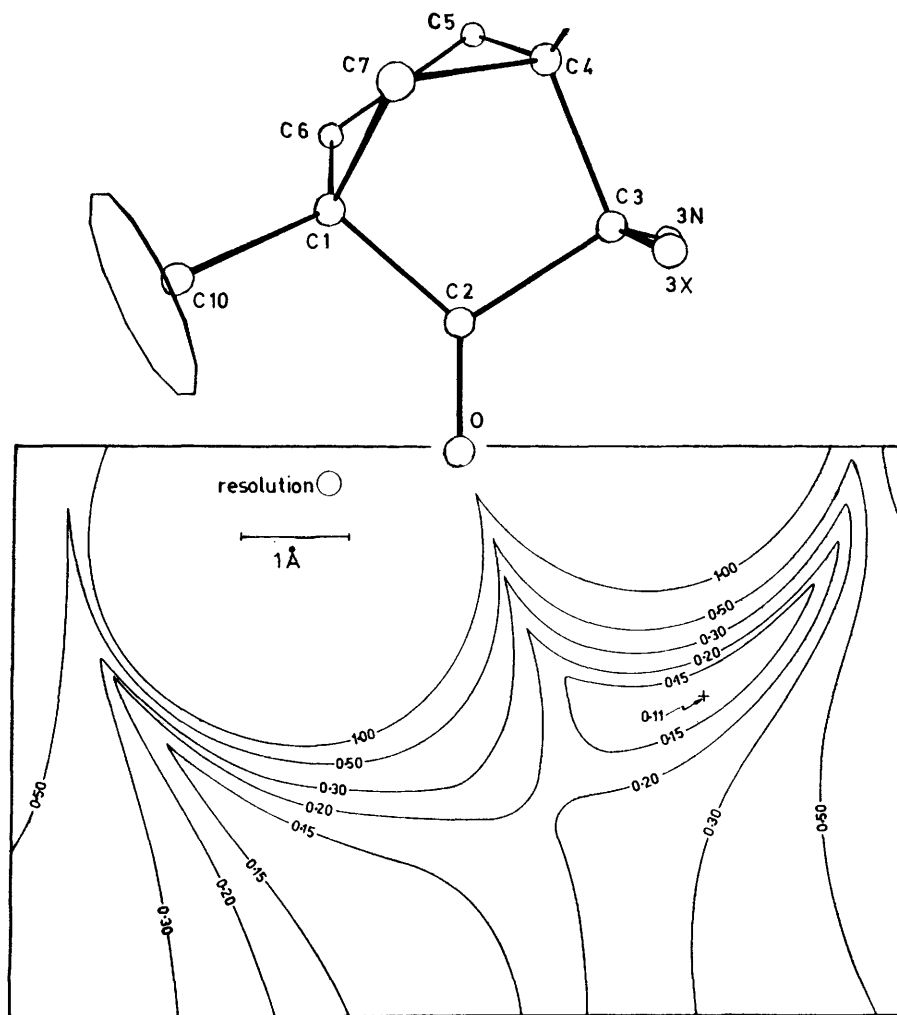

FIGURE 2   Map of the TQRF surface for camphor in the plane of the carbonyl group. ^1H data, Yb LIS ratios, standard = 10M.
The methyl groups on C7 have been omitted for clarity

angle = 140°), presumably by steric hindrance. Distance tabulations by LISCA show that steric hindrance from 6X and 5X is comparable with that due to the C8 methyl group when the metal is in the plane of the carbonyl group. Our solutions show a preference for a lanthanoid position 37° out of this plane on the exo-side, but the data are not sufficiently sensitive to confirm this.

Hypothesis 3 has A and B variable but D_1 fixed at 0°. The results (Table 1) show an increase of 0.005 in TQRF over that for 1 and 2. A and B have increased to 50° and 3.2 Å, respectively.

LISCA incorporates an instruction which produces maps of TQRF in real-space planes containing, or near

site. These data show that the 1-site hypotheses are not supported by the experimental broadenings, also given in Table 2. Note that the experimental errors (uncertainties) quoted are estimated maximum possible errors. They are greater for 3X and 3N than for 8M and 9M because the former are multiplets of area 1 whereas the latter are singlets of area 3.

1-Site hypotheses using the ^{13}C data give results essentially similar to those described above.

2-Site Metal Binding Models.—Results for these are given in Table 3 from the ^1H data. Hypothesis 4, with B free, gives rise to solutions for which either B is too long or $f_{C3}^1 = 1.0$ (equivalent to the 1-site case).

Hypothesis 5 is the 2-site equivalent to 2. Hypotheses 4 and 5 show that, as far as the ^1H LIS data are concerned, the 1-site solutions are preferred. Hypothesis 6

TABLE 2

Calculated and experimental broadening ratios for ^1H nuclei of camphor; 1-site metal binding hypotheses; 10M as standard

Solution no.	3X	3N	8M	9M
1a	0.5	0.2	0.7	0.1
1b	3.5	6.4	0.6	0.1
1c	3.4	6.4	0.6	0.1
2a	0.7	0.7	0.3	<0.1
2b	3.4	6.4	0.6	0.1
2c	3.3	6.1	0.6	0.1
3a	12	12	1.5	0.2
3b	11	11	1.5	0.2
3c	0.7	0.7	0.4	<0.1
Experimental	2.3	2.3	0.6	0.2
Max. error	±1.0	±1.0	±0.2	±0.2

forces the metal to spend 5% of its time on the 10M side. A and B are also fixed at 42° and 3.0 Å, re-

2-Site solutions for the ^{13}C data are in good agreement with those for the ^1H data. The best solution has $B = 3.0$ Å, $A = 42°$, and $f_{C3}^1 = 0.88$, with TQRF = 0.056. The minima in TQRF are not so sharply defined for the ^{13}C as they are for the ^1H data. This is because the carbon atoms are not spread out in space as much as the hydrogen atoms are. The lower sensitivity of the ^{13}C data to changes in the structural parameters implies that structural parameters so derived may not be as accurate as those derived from ^1H measurements, despite the fact that the ^{13}C LIS ratios can be determined with greater precision.

Conclusion.—Our picture of the $Ln(dpm)_3$–camphor complex derived from the foregoing analyses is as follows. The lanthanoid tries to bind to the lone pairs of the carbonyl oxygen atom with an Ln–O bond length of 3.0 ± 0.2 Å. Steric hindrance by 10M reduces the fraction binding on that side to 0.08 ± 0.06. The Ln–O–C2 bond angle is $140 \pm 10°$. The dihedral angle, D_1, for the major binding site is $20 \pm 25°$, *i.e.* nearest to 3N.

TABLE 3 *

LISCA solutions for camphor with rotatable bond number 1 = 2-site; fitting to 9 Yb-induced ^1H LIS ratios; 10M as standard

Solution no.	$B/Å$ Initial	Optimum	$A/°$ † Initial	Optimum	f_{C3}^1 ‡ Initial	Optimum	TQRF
4a	3.0	4.6	30	0	0.50	—	0.131
4b	3.5	4.2	50	37	0.20	0.46	0.093
4c	2.5	3.1	10	24	0.80	1.0	0.175
5a	[3.0]		30	35	0.50	0.99	0.127
5b	[3.0]		50	95	0.20	0.44	0.672
5c	[3.0]		10	10	0.80	0.92	0.239
6	[3.0]		[42]		[0.95]		0.142

*,† See footnotes to Table 1. ‡ f_{C3}^1 Is the fractional population for the first site for which $D_1^1 = 0$; the second site is at $D_1^2 = 180°$.

spectively (the relevance of these particular parameter values will become clear when we examine canthaxanthin).

Table 4 gives the calculated broadening ratios for hypotheses 4—6. Hypothesis 6 is the only one which

TABLE 4

Calculated and experimental broadening ratios for ^1H nuclei of camphor; 2-site metal binding hypotheses; 10M as standard

Solution no.	3X	3N	8M	9M
4a	0.7	0.7	0.4	<0.1
4b	0.3	0.3	0.1	<0.1
4c	2.6	2.6	0.7	<0.1
5a	4.4	4.4	0.9	0.1
5b	<0.1	<0.1	<0.1	<0.1
5c	1.0	1.0	0.4	<0.1
6	3.0	2.9	0.5	0.1
Experimental	2.3	2.3	0.6	0.2
Max. error	±1.0	±1.0	±0.2	±0.2

gives agreement with *both* the LIS (Table 3) and broadening data.

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

CANTHAXANTHIN

Canthaxanthin is stereochemically much more complicated than camphor. The molecular formula is shown in Figure 3 together with the numbering scheme. It will be seen that canthaxanthin has a centre of symmetry midway between C15 and C15′. For convenience, the half-molecule can be described as consisting of two parts: C1 to C6 is known as ' the ring,' and C7 to C15 ' the side chain.' Despite steric crowding of methyl and vinylic hydrogen atoms in the side chain, the energy of the π-system maintains the planarity of this part of the molecule to a high degree in the crystal structure.[24] High resolution n.m.r., nuclear Overhauser enhancements,[25] and LIS analyses[26] of retinals show that polyene side chains remain planar in solution. We have therefore assumed that the canthaxanthin side chain is also planar in solution. The ring, which contains three

[25] D. J. Patel, *Nature*, 1969, **221**, 825; R. Rowan, A. Warshel, B. D. Sykes, and M. Karplus, *Biochemistry*, 1974, **13**, 970; R. Rowan and B. D. Sykes, *J. Amer. Chem. Soc.*, 1974, **96**, 7000; 1975, **97**, 1023; R. S. Becker, S. Berger, D. K. Dalling, D. M. Grant, and R. J. Pugmire, *J. Amer. Chem. Soc.*, 1975, **98**, 2367; G. Elglert, *Helv. Chim. Acta*, 1975, **58**, 2367.

[26] B. H. S. Liénard and A. J. Thomson, following paper.

sp^2-hybridised carbons, is relatively rigid with carbon atoms 1 and 3—6 in one plane. Conformational disorder in the crystal[24] shows that 'ring flipping' can occur by movement of C2 from one side to the other of the plane containing the rest of the ring carbon atoms. Framework models show that motion of C2 will be accompanied by 'rocking' of the methyl groups on C1,

made up of a π and a non-bonding term which are large and nearly equal in magnitude but opposite in sign.

LIS Data.—The ^{1}H LIS ratios with respect to the 1-methyl signals were determined, by methods already described for camphor, from nine $Yb(dpm)_3$-shifted spectra. They are given in Figure 4. Because canthaxanthin has a centre of symmetry and the two carbonyl

TABLE 5

Camphor, ^{1}H data, Yb LIS ratios; solution for hypothesis 6; 10M as standard

Nucleus	ExptLISR	CalcLISR	Weighted residue *	Weighted frac. res.	CalcBR †
3X	1.100 0	1.065 3	−0.034 7	−0.031 6	2.95
3N	1.050 0	1.030 1	−−0.019 9	−0.019 0	2.94
4	0.416 0	0.423 2	0.007 2	0.017 2	0.21
5X	0.367 0	0.437 0	0.070 0	0.190 6	0.11
5N	0.448 0	0.588 5	0.140 5	0.313 6	0.38
6X	0.596 0	0.661 8	0.065 8	0.110 3	0.16
6N	0.943 0	1.170 5	0.227 5	0.241 2	0.65
8M	0.609 0	0.715 5	0.106 5	0.174 9	0.50
9M	0.381 0	0.395 5	0.014 5	0.038 0	0.06

Weighted total quasi-R-factor = 0.1432

* ExptLISR − CalcLISR.   † Calculated broadening ratio.

TABLE 6

Camphor, ^{13}C data from ref. 7; solution for hypothesis 6; C10 as standard

Nucleus	ExptLISR	CalcLISR	Weighted residue *	Weighted frac. res.	CalcBR †
C1	1.460 0	1.367 8	−0.092 2	−0.063 1	1.13
C2	3.870 0	3.463 1	−0.000 0	−0.000 0	7.13
C3	1.610 0	1.499 0	−0.111 0	−0.068 9	3.97
C4	0.760 0	0.687 2	−0.072 8	−0.095 8	0.61
C5	0.540 0	0.569 4	0.029 4	0.054 4	0.37
C6	0.830 0	0.823 8	−0.006 2	−0.007 5	0.51
C7	0.800 0	0.786 2	−0.013 8	−0.017 2	0.47
C8	0.600 0	0.670 6	0.070 6	0.117 7	0.57
C9	0.360 0	0.510 0	0.050 0	0.138 8	0.13

Weighted total quasi-R-factor = 0.068 4
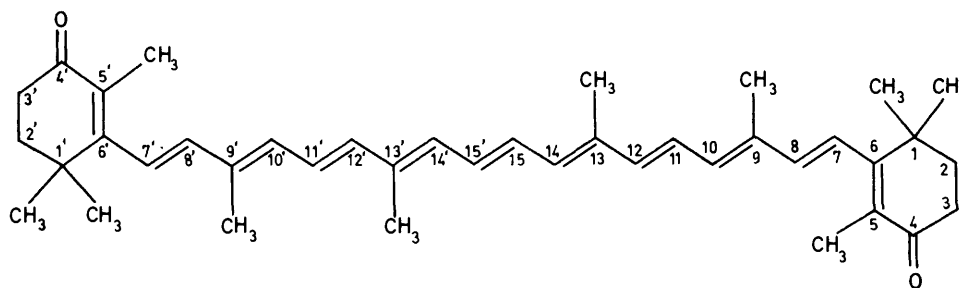
*·† See Table 5.

FIGURE 3   Molecular formula for canthaxanthin

and of the hydrogen atoms on C3, while the remainder of the ring atoms move only slightly, if at all. Of special interest is the orientation of the ring and side chain with respect to one another. Severe steric hindrance between the hydrogen atoms on C7 and C8 and the ring methyl groups for some orientations (particularly planar, s-*cis*) make these conformations very unfavourable. Semiempirical potential energy calculations have been undertaken for this type of molecule.[16,27] The accurate determination of the relative energies of the minima in such calculations is fraught with difficulty because the total potential energy is

groups are *ca.* 30 Å apart, we can treat it as a half-molecule of twice the actual molar concentration. If we think of the observed shifts as being due solely to the unprimed half of the molecule (see Figure 3), we can estimate the contribution that the lanthanoid binding at the primed end will make to the shifts induced by the unprimed metal in nuclei close by. This contribution turns out to be less than 2% for the ring nuclei. If the standard nucleus is one of those on the ring the effect of the primed metal on the *ratios* will

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

be $\ll 2\%$. As we move along the side chain the influence of the unprimed metal decreases while that of the primed one increases. At 7H and 8H the maximum contribution to the shifts is 3%. At 9M it is 7%; and at 10H, 9%. In our conformational analyses, therefore,
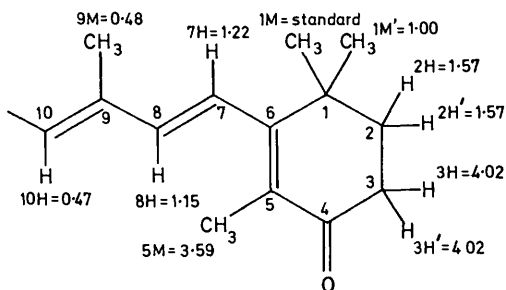

FIGURE 4 Yb-induced, ^{1}H LIS ratios for canthaxanthin used in the conformational analyses. The labels used to distinguish the observed nuclei are also shown. Here we use primes to denote atoms ' below ' the plane of the ring

we have excluded all the observed nuclei nearer the centre of the molecule than 10H.

Geometric Data and Degrees of Freedom in the Model.—Fractional crystal lattice co-ordinates were taken from the crystal structure of canthaxanthin.[24] These were used to construct the LISCA model of the system

incorporating twelve-point methyl groups throughout. The model consists of three units: two, each of 51
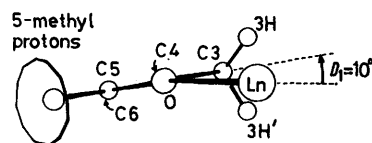

FIGURE 5 The canthaxanthin ring viewed directly along the O–C4 bond illustrating the definition of D_1. Atoms at the ' top ' of the ring are not shown, for clarity
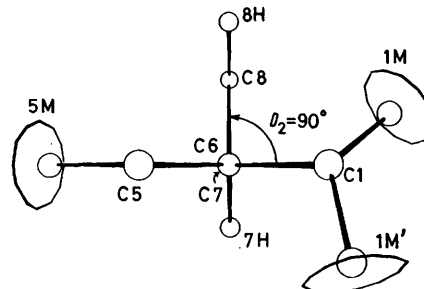

FIGURE 6 Canthaxanthin viewed from the centre of the six-membered ring (C2, C3, C4, and O have been omitted for clarity) illustrating the definition of D_2. $D_2 = 0°$ corresponds to planar, s-*trans*-geometry about the C6–C7 bond

points, representing the two ring flip forms, and one of 44 points defining the side chain as far as C15'.
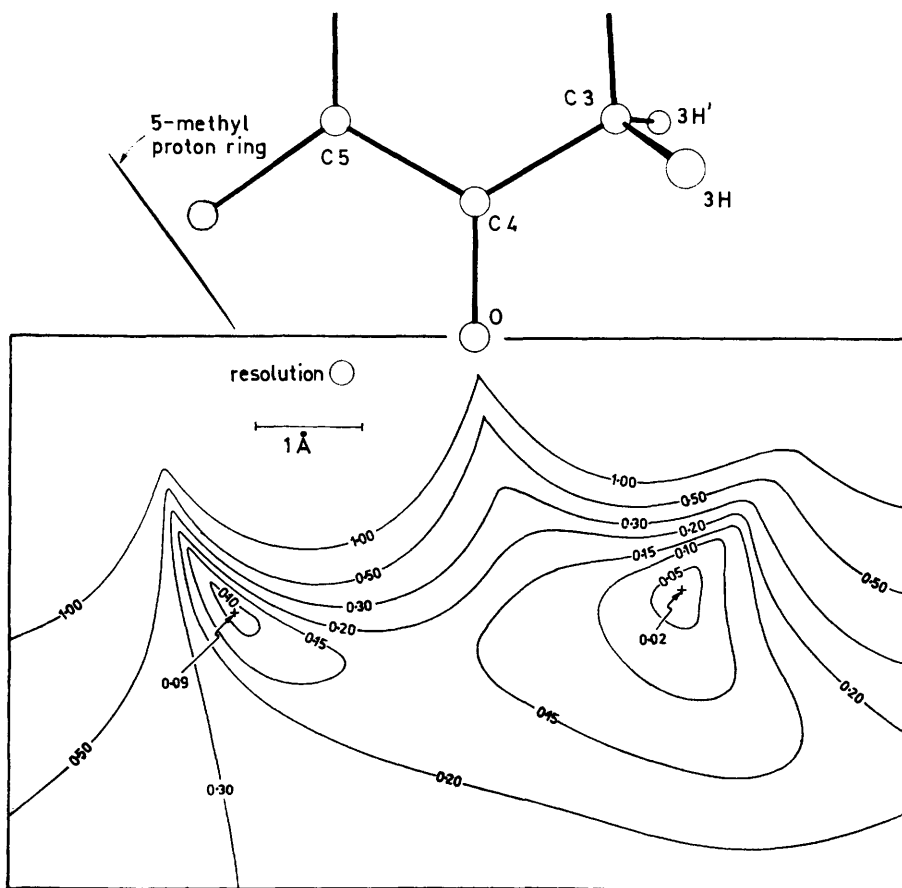

FIGURE 7 Map of the TQRF surface for the canthaxanthin ring only in the plane of the carbonyl group. ^{1}H data, Yb LIS ratios, standard = 1M.

Apart from those defining the relationship between the ring and the lanthanoid atom, the degrees of freedom are the geometry about the C6–C7 bond (rotatable bond number 2) and the fractional population of the first of the two ring flip forms (f^+). The definition of dihedral angle D_1 is illustrated in Figure 5, and that of D_2 is shown in Figure 6.

LISCA Analysis.—In the following the magnetic axis is always aligned along the Ln–O bond. Unit weights were used for all observed nuclei.

TABLE 7 *

LISCA solutions for canthaxanthin, ring only; fitting to 6 Yb-induced ¹H LIS ratios; standard 1M

Solution no.	f^+	$B/Å$	$A/°$ †	$D_1/°$	f_{C3}^1	TQRF
1a	0.50	3.0	37	10		0.015
1b	0.51	3.3	31	30		0.045
1c	0.50	3.0	37	10		0.015
2a	0.30	3.7	27	[0]		0.108
2b	0.30	2.9	28	[0]		0.085
2c	0.42	2.4	0	[0]		0.188
3a	0.48	3.1	1	[180]		0.156
3b	0.43	3.4	40	[180]		0.104
3c	0.55	3.3	22	[180]		0.150
4a	0.50	3.7	38		0.71	0.019
4b	0.52	4.1	41		0.75	0.011
4c	0.49	1.6	1		0.94	0.374
5a	[0.50]	[3.0]	42		0.93	0.026
5b	[0.50]	[3.0]	41		0.94	0.027
5c	[0.50]	[3.0]	43		0.91	0.027

*,† See footnotes to Table 1.

In the unshifted spectrum of canthaxanthin the two 1-methyl groups, and the pairs of protons on C2 and

have a mirror plane of symmetry through the atoms of the ring. If this is so the time-averaged potential energies of the two ring-flip forms will be equal and, therefore, the populations of the ring forms will be equal also.

Ring-only Solutions.—We have performed analyses analogous to those for camphor using only the LIS ratios and geometries of the canthaxanthin ring. The results are given in Table 7, in which we have omitted the initial values of the variables. The starting values follow the same pattern as those given in Tables 1 and 3. Table 7 reveals a very similar pattern to that already described for camphor. The solutions produced by LISCA in this case show a greater range of values of the degrees of freedom, because we have fewer observed LIS ratios and, in general, more degrees of freedom in the model. The conclusions, however, are the same: the lanthanoid binds to the carbonyl lone pairs and, of the two, prefers the sterically less crowded C3 side. A contour map of TQRF in the plane of the carbonyl group is shown in Figure 7. Unlike the map for camphor (Figure 2), there are two distinct minima corresponding to the two binding sites.

Owing to the low solubility of canthaxanthin in carbon disulphide the experimental broadening ratios obtained with $Gd(dpm)_3$ are not as accurate as those measured for camphor. They are sufficiently good to rule out hypotheses in which the metal bonds solely to one lone pair or the other, *i.e.* hypotheses 1—3. They are not accurate enough, however, to distinguish between

TABLE 8 *

LISCA solutions for canthaxanthin including the side chain; fitting to 10 Yb-induced ¹H LIS ratios; standard 1M

Solution no.	f^+	$B/Å$	$A/°$	f_{C3}^1	$D_2/°$	$f_{n\text{-}trans}^2$ †	TQRF	PQRF ‡
6a	[0.5]	3.0	42	0.95	91		0.028	0.057
6b	[0.5]	4.4	42	0.76	112		0.043	0.166
6c	[0.5]	3.0	42	0.96	91		0.028	0.060
7a	[0.5]	3.3	39	0.86	[0]		0.053	0.186
7b	[0.5]	4.3	41	0.77	[0]		0.056	0.220
7c	[0.5]	3.4	40	0.83	[0]		0.053	0.197
8a	[0.5]	[3.0]	42	0.93	[0]		0.053	0.187
8b	[0.5]	[3.0]	42	0.93	[0]		0.053	0.187
8c	[0.5]	[3.0]	42	0.94	[0]		0.053	0.187
9a	[0.5]	4.3	41	0.77	[140]		0.045	0.176
9b	[0.5]	4.3	41	0.77	[140]		0.045	0.175
9c	[0.5]	3.3	42	0.65	[140]		0.090	0.334
10a	[0.5]	[3.0]	36	0.80	[140]		0.085	0.147
10b	[0.5]	[3.0]	43	0.90	[140]		0.047	0.138
10c	[0.5]	[3.0]	43	0.90	[140]		0.047	0.138
11a	[0.5]	3.0	42	0.94		0.49	0.028	0.055
11b	[0.5]	4.6	43	0.77		0.33	0.042	0.164
11c	[0.5]	3.0	42	0.095		0.50	0.028	0.055

* See footnote to Table 1. † First site, $D_2^1 = 0°$; second site, $D_2^2 = 140°$. ‡ Calculated for observed nuclei in the side chain only.

C3 each give rise to 'single' signals (2H and 3H are split by coupling to one another). This chemical equivalence is maintained in the lanthanoid-shifted spectra. Thus, ring flipping must be fast on the n.m.r. time-scale. If the ring and the side chain can rotate rapidly from one mirror image form to the other, then the ensemble average 'seen' by the shift reagent will

hypotheses 4a and 5b, for example, for which f_{C3}^1 is 0.71 and 0.94, respectively.

Solutions Including the Side Chain.—These are tabulated in Table 8. Hypothesis 6 is the one corresponding to the existence of a single value of the ring–side chain dihedral angle, D_2. The calculated LIS ratios, broadening ratios, *etc.* are given in Table 9 for solution 6a.

Solutions **7** and **8** (see Table 8) show the effect of forcing the side chain to adopt a planar, s-*trans*-configuration with respect to the ring. The agreement factors are significantly worse.

Owing to steric hindrance between 8H and 5M the planar, s-*cis*-conformation is of high energy.[16,27] In the crystals [24] $D_2 = 137°$, *i.e.* 43° away from planar, s-*cis*.

Canthaxanthin's two ring-flip forms are equally populated in solution. The LIS data do not distinguish between a single ring–side chain angle of *ca.* 90° and a 50 : 50 equilibrium between s-*trans* and 40° away from s-*cis*. The former would require little conjugation between the π-systems of the ring and side chain, in disagreement with the evidence from u.v. spectra.[28]

TABLE 9

Canthaxanthin, ^{1}H data, Yb LIS ratios; solution for hypothesis 6; standard 1M

Nucleus	ExptLISR	CalcLISR	Weighted residue *	Weighted frac. res.	CalcBR †
5M	3.590 0	3.502 8	−0.087 2	−0.024 3	22.53
3H	4.020 0	4.028 9	0.008 9	0.002 2	42.02
3H′	4.020 0	4.028 9	0.008 9	0.002 2	42.02
2H	1.570 0	1.462 4	−0.107 6	−0.068 5	3.97
2H′	1.570 0	1.462 4	−0.107 6	−0.068 5	3.97
1M′	1.000 0	0.999 5	−0.000 5	−0.000 5	1.01
7H	1.217 0	1.219 8	0.002 8	0.002 3	0.65
8H	1.153 0	1.142 0	−0.011 0	−0.009 5	0.66
9M	0.480 0	0.551 7	0.071 7	0.149 4	0.14
10H	0.467 0	0.539 0	0.072 0	0.154 2	0.17

Weighted total quasi-R-factor = 0.027 6
Weighted partial quasi-R-factor = 0.056 6 for unit 2 onwards

*,† As Table 5.

TABLE 10

Canthaxanthin, ^{1}H data, Yb LIS ratios; solution for hypothesis 11; standard 1M

Nucleus	ExptLISR	CalcLISR	Weighted residue *	Weighted frac. res.	CalcBR
5M	3.590 0	3.485 6	−0.104 4	−0.029 1	25.41
3H	4.020 0	4.028 5	0.008 5	0.002 1	41.95
3H′	4.020 0	4.028 5	0.008 5	0.002 1	41.95
2H	1.570 0	1.465 4	−0.104 6	−0.066 6	3.96
2H′	1.570 0	1.465 4	−0.104 6	−0.066 6	3.96
1M′	1.000 0	0.999 5	−0.000 5	−0.000 5	1.01
7H	1.217 0	1.246 2	0.029 2	0.024 0	0.73
8H	1.153 0	1.154 0	0.001 0	0.000 9	0.81
9M	0.480 0	0.547 5	0.067 5	0.140 6	0.15
10H	0.467 0	0.532 4	0.065 4	0.140 1	0.21

Weighted total quasi-R-factor = 0.028 0
Weighted partial quasi-R-factor = 0.054 5 for Unit 2 onwards

*,† See Table 5.

In hypotheses 9 and 10 D_2 is fixed at 140°. TQRF is marginally better than for the case with $D_2 = 0°$, but B, at 4.3 Å, is too long. Forcing $B = 3.0$ Å (hypothesis 10) leads to little increase in TQRF but it is still significantly worse than for $D_2 = 91°$.

Finally, we try allowing rotatable bond number 2 to be 2-site, variable. The first site is at $D_2{}^1 = 0°$; the second has $D_2{}^2 = 140°$. The results (solution 11 in Table 8) exhibit equal populations at the two sites. The values of TQRF and PQRF are virtually identical with those for the 1-site case with $D_2 = 91°$; hence we cannot distinguish between them on the basis of the LIS data. The calculated LIS ratios are given in Table 10.

Conclusion.—The two-site metal binding found for camphor is confirmed by the canthaxanthin results. The structural parameters A, B, and $f_{C3}{}^1$ are indistinguishable (within the experimental uncertainties) for the two substrates. This is not very surprising in view of the similarity of the camphor and canthaxanthin binding sites (compare Figures 1 and 5).

Both exclusively s-*trans*- and exclusively 40° from s-*cis*-conformations are ruled out.

CONCLUSION

We have found evidence for 2-site metal binding by lanthanoid shift reagents to carbonyl groups in camphor and canthaxanthin. This finding is confirmed by our studies of retinals and related molecules.[26] The fractions binding at each of the two sites, in all cases, appear to be controlled by steric interactions with the group.

Because the n.m.r. time-scale is long compared with that of rotation about chemical bonds it is *essential* to examine hypotheses involving (chemically reasonable) rotamer averaging as well as those in which dihedral angles are single-valued. In general, this leads to several solutions which are acceptable by the LIS agreement criterion. Choice amongst these depends upon other experimental evidence, principally line broadening by gadolinium.

[28] U. Schwieter, G. Englert, N. Rigassi, and W. Vetter, *Pure Appl. Chem.*, 1969, **20**, 365.

We have shown that LISCA can be used to investigate quite complicated conformational descriptions of the lanthanide–substrate complex. For example, cantha-xanthin hypothesis 11 requires a model made up of eight contributing structures. The efficiency of LISCA is illustrated by the following. The entire ^{1}H analysis of camphor presented in Tables 1—4 required LISCA to set up 1 704 individual conformations. The execution time required for this, on an ICL 1906A computer, was 100 seconds. Of course, more complicated hypotheses require more time, both because the number of contributing structures is greater and because the use of additional variables means that the least-squares routine requires more iterations in order to find the minimum in the TQRF surface.

Copies of the computer program listing and the instruction manual are available.*