A Coupled Oscillator Calculation of the Induced Circular Dichroism of the Visual Pigment Analog (*S*)-*N*-*all-trans*-Retinylidene-α-(1-naphthyl)ethylamine†

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ABSTRACT: The visual pigment rhodopsin exhibits a Cotton effect in the region of 500 nm. The preceding paper (Johnston, E. M., and Zand, R. (1973), *Biochemistry 12*, 4631) describes the synthesis and CD absorption spectra of (*S*)-*N*-*all-trans*-retinylidene-α-(1-naphthyl)ethylamine, which resembles rhodopsin in possessing a Cotton effect in its main long-wavelength absorption band. Whether a twisted chromophore mechanism (intrinsic mechanism) or a coupled oscillator mechanism (extrinsic mechanism) can best account for the observed optical activity is an unresolved question. In an attempt to answer the question, the rotational strengths to be expected from a coupled oscillator mechanism were calculated for (*S*)-*N*-*all-trans*-retinylidene-α-(1-naphthyl)ethylamine using the Kirkwood theory. All of the calculated rotational strengths agree within a factor of two with those found experimentally. Therefore, the 490-nm Cotton effect of rhodopsin is not *prima facie* evidence for the distortion of the retinaldehyde chromophore. Instead the retinaldehyde transitions could become optically active by coupling with the transitions of aromatic amino acid side chains located within a 15-Å radius of the chromophore.

In the preceding paper (Johnston and Zand, 1973) we reported the preparation and the absorption and circular dichroism (CD) spectra of a number of Schiff's base visual pigment analogs derived from 11-*cis*- or *all-trans*-retinaldehydes and simple optically active amines. Having demonstrated that such simple amine moieties are capable of inducing optical activity into the conjugated chromophoric system of the retinaldehyde molecule, it became desirable to see if a mechanism for the process could be established. The mechanism proposed by Crescitelli and coworkers (1966) for the extrinsic Cotton effect in rhodopsin requires the presence of an imposed geometric asymmetry on the retinaldehyde moiety resulting in an intrinsic mechanism for the origin of the observed CD. Mommaerts (1969) has argued that 11-*cis*-retinaldehyde can assume one of two possible enantiomeric forms and that the observed circular dichroism arises through the preferential stabilization of one of these forms by the protein opsin. Some support for the Mommaerts position has recently appeared and is derived from theoretical considerations (Nash, 1969; Honig and Karplus, 1971; Honig *et al.*, 1973), nuclear magnetic resonance (Honig *et al.*, 1971), and the X-ray crystal structure determination of 11-*cis*-retinaldehyde (Gilardi *et al.*, 1971).

Rhodopsins containing isomers other than the 11-cis isomer also exhibit optical activity. Isorhodopsin (Takezaki and Kito, 1967) contains the 9-*cis*-retinaldehyde isomer while metarhodopsin contains the all-trans isomer and is also optically active (Waggoner and Stryer, 1971). Honig *et al.* (1973) have suggested that the planar chain isomers are twisted about the 6–7 bond and if one enantiomer of the twisted ring-chain bond is selected for the iso- and metarhodopsins then the observed optical activity can be explained by an intrinsic mechanism. In the case of our visual pigment analogs it is difficult to conceive of a mechanism that would preferentially select and stabilize one particular conformer of the *all-trans*-retinaldehyde.

A coupled oscillator mechanism (Kirkwood, 1937) would seem to be a suitable first choice to explain both the rhodopsin data and our visual pigment analog data. To carry through such a calculation we chose to utilize the spectra obtained from the model compound (*S*)-*N*-*all-trans*-retinylidene-α-(1-naphthyl)ethylamine, ((*S*)-RNEA).[1] A coupled oscillator approach has been successfully used to explain the CD of myoglobin (Hsu and Woody, 1969, 1971). In the ideal situation, it would be desirable to perform a similar calculation for either rhodopsin or our model compound. This is not feasible at this time since the X-ray structures have not been determined for either of these substances whereas the X-ray structure for myoglobin is known. Ultimately one might be able to locate proximal aromatic side chains by an analysis of the CD of several transitions of a chromophore. For rhodopsin there are Cotton effects at 340 and 490 nm. If a reliable calculation method was available, these two Cotton effects might yield information about the environment of the chromophore. The rationale for the present calculation was that it might eliminate the coupled oscillator mechanism on an order of magnitude basis if the predicted CD was much weaker than the experimentally observed CD. Not only the rotational strengths but also the wavelengths and signs of the bands could be tested by this approach.

Method of Calculation. In carrying through such a calculation it is preferable to use a rigid molecule for the analysis. Otherwise, the calculated CD must be averaged over the allowed conformations, which introduces an additional component of uncertainty. Rigidity is not possible for a Schiff's

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[1] Abbreviation used is: (*S*)-RNEA, (*S*)-*N*-*all-trans*-retinylidene-α-(1-naphthyl)ethylamine.

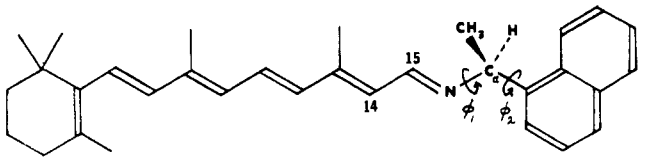

FIGURE 1: The rotation angles ϕ_1, the N–C_α rotation, and ϕ_2, the C_α–C_1 rotation, in a molecule of (*S*)-*N-all-trans*-retinylidene-α-(1-naphthyl) ethylamine.

base of retinaldehyde. However, steric hindrances may restrict the allowed conformation into a narrow range. In our calculation we sought to produce two maps. The first map would show the conformational energy as a function of the two rotation angles shown in Figure 1. The second map would show the rotational strength of a particular induced band as a function of these two angles. Thus, the CD spectrum is predictable from the sterically allowed conformations. If some flexibility remains, in spite of the steric restrictions, one may try to average the rotational strength over the allowed regions.

Hard Sphere Map. As a preliminary test of our method, a hard sphere map for (*S*)-RNEA was calculated. The bond lengths and bond angles used are given in Table I. The nonbonded contact distances used are given in Table II. The two rotation angles ϕ_1, the N–C_α rotation, and ϕ_2, the C_α–C_1 rotation, are indicated in Figure 1 and each of these angles is defined so that $\phi = 0$ in the (*S*)-cis conformation. A positive rotation turns the group at the far end clockwise as seen from the near end. The convention employed in that of Sugeta and Miyazawa (1967). A computer program was written in Fortran IV to calculate the position of each relevant atom as a function of the two rotation angles. Successive coordinate transformations using the transformation matrices of Sugeta and Miyazawa (1967) were used to carry out this calculation.

TABLE I: Bond Lengths and Angles Used in the Calculation.

	Retinylidene Group	
C=C	1.36 Å	Inuzuka and Becker (1968)
C—C	1.46 Å	Inuzuka and Becker (1968)
All C–C–C– angles	120°	Inuzuka and Becker (1968)
	Naphthyl Group	
C_1=C_2	1.365 Å	Abrahams *et al.* (1949), as recalculated by Ahmed and Cruickshank (1952)
C_1=C_9	1.425 Å	Abrahams *et al.* (1949), as recalculated by Ahmed and Cruickshank (1952)
C_9=C_{10}	1.393 Å	Abrahams *et al.* (1949), as recalculated by Ahmed and Cruickshank (1952)
C_2=C_3	1.404 Å	Abrahams *et al.* (1949), as recalculated by Ahmed and Cruickshank (1952)
$<C_9$–C_{10}–C_5	120°	Assumed
These five quantities completely determine the structure if one assumes D_{2h} symmetry. The other two angles will differ slightly from 120°.		
	Ethylamine Linkage	
N—C_α	1.48 Å	Sutton (1958), p S-16 (from CH_3N=NCH_3)
C_α—C_{methyl}	1.54 Å	Sutton (1958)
C_α—C_{ring}	1.52 Å	Sutton (1958), p S-13 (from toluene)
C—H	1.08 Å	Sutton (1958)
$<C_{14}$–C_{15}=N	120°	Bürgi and Dunitz (1969) give 122.7° for *N*-benzylideneaniline and 120.2° for *N-p*-methylbenzylidene-*p*-nitroaniline
$<C_{15}$=N—C_α	120°	Bürgi and Dunitz (1969) give 119.9° for *N*-benzylideneaniline
All angles at C_α and C_{methyl}	109.47°	Assumed tetrahedral

TABLE II: Nonbonded Contact Distances Used for the Hard-Sphere Map of (*S*)-RNEA (Figure 3).[a]

C···H	2.20 Å
N···H	2.20 Å
H···H	1.90 Å
C···C	3.00 Å

[a] These distances are the inner limits given by Ramakirshnan and Ramachandran (1965).

The retinylidene group is assigned its own coordinate system as is the naphthyl group. The positions of the atoms within each group are fixed by the bond lengths and angles of Table I. The resulting coordinates for the retinylidene and naphthyl atoms are given in Tables III and IV. A syn configuration at the C to N double bond was assumed.

Conformational Energy Map. An improvement on the hard-sphere calculation is to use a conformational energy calculation. The nonbonded potential energy functions are those of Bartell (1966) except for one change. The H···H function was modified slightly for computer use, to avoid a spurious minimum at $r = 0$. The functions used are mostly repulsive and do not include the electrostatic attractions that have been used in many polypeptide calculations such as those of Brant and Flory (1965). Most of the conformations that are forbidden by the energy map have very high energies and the inclusion of electrostatic attractions would not alter the outer limits significantly. Also, there is some doubt whether the attractive forces in such a highly conjugated system can be accurately represented as a sum of pairwise interactions. The attractive forces between polyenes calculated by Haugh and Hirschfelder (1955) cannot be reduced to a sum of pairwise forces.

Calculation of Rotational Strengths. For a retinylidene transition at frequency ν_R and a given naphthyl transition of frequency ν_N, the rotational strength is given by the equation

TABLE III: Coordinates of Selected Retinylidene Atoms.[a]

Atom	*x*	*y*	Atom	*x*	*y*
C_5	0.00000	0.00000	C_{12}	8.49173	−0.70397
C_6	1.16363	−0.70397	C_{13}	9.77080	0.00000
C_7	2.44270	0.00000	C_{14}	10.93443	−0.70397
C_8	3.60633	−0.70397	C_{15}	12.21350	0.00000
C_9	4.88540	0.00000			
C_{10}	6.04903	−0.70397	H_{11}	10.93129	−1.78397
C_{11}	7.32810	0.00000	H_{15}	12.21664	1.08000

[a] See Figure 2 for atom numbers. The origin of the retinylidene coordinate system is at C_5. The positive *x* axis passes through C_7, C_9, C_{11}, C_{13}, and C_{15}. The *xy* plane is chosen to pass through the remaining retinylidene atoms, with the positive *y* axis pointing away from C_6. Coordinates are given in ångstroms.

TABLE IV: Coordinates of the Naphthyl Atoms.[a]

Atom	x	y	Atom	x	y
C_1	0.0000	0.0000	H_2	0.1670	−2.1029
C_2	0.7070	−1.1676	H_3	2.6510	−2.1029
C_3	2.1110	−1.1676	H_4	3.8980	0.0000
C_4	2.8180	0.0000	H_5	3.8980	2.4682
C_5	2.8180	2.4682	H_6	2.6510	4.5711
C_6	2.1110	3.6358	H_7	0.1670	4.5711
C_7	0.7070	3.6358	H_8	−1.0800	2.4682
C_8	0.0000	2.4682			
C_9	0.7125	1.2341			
C_{10}	2.1055	1.2341			

[a] See Figure 2 for atom numbers. The origin of the naphthyl coordinate system is at C_1. The xy plane is the plane of the naphthyl group. The positive x axis passes through C_4; the positive y axis passes through C_8. Coordinates are in ångstroms.

$$R = \frac{2\pi}{C}\frac{V_{OR;ON}\nu_R\nu_N\mathbf{R}_{RN}\cdot(\boldsymbol{\mu}_R \times \boldsymbol{\mu}_N)}{h(\nu_N^2 - \nu_R^2)} \quad (1)$$

where $V_{OR;ON}$ is the coulomb matrix element between the retinylidene transition and the naphthyl transition (Tinoco, 1962). That is

$$V_{OR;ON} = \int\int \phi_{RO}(\mathbf{r}_1)\phi_{R_1}\frac{2e^2}{r_{12}}\phi_{N_0}(\mathbf{r}_2)\phi_{N_1}(\mathbf{r}_2)d\tau_1 d\tau_2 \quad (2)$$

For our purposes all the group wave functions can be taken to be real. Equation 1 is valid for the interaction of two nondegenerate, electrically allowed, magnetically forbidden transitions, where the interaction energy V is a small perturbation on the total energy of the groups. The transition dipole moments and frequencies were obtained from the experimental spectra. The dipole moment of *all-trans*-retinaldehyde was calculated to be 9.67 D by integrating the 360-nm peak of the absorption spectrum of *all-trans*-retinaldehyde in isooctane. The dipole moments for the three naphthyl transitions were calculated from the oscillator strengths reported by Klevens and Platt (1949). The evaluation of $V_{OR;ON}$ was carried out by using the monopole approximation of London (1942) and free-electron molecular orbital (FEMO) wave functions for the ground and excited state for both the retinylidene group and the naphthyl group.

FEMO Wave Functions. The original shape of the polyene was used for these calculations rather than the flattened out polyene representation of Haugh and Hirschfelder (1955). The conjugated chain of the retinylidene group includes carbons C_5 through C_{15}, plus the nitrogen atom so the chain of twelve atoms is an even atom chain. The single bond lengths are taken as 1.46 Å and the carbon–carbon double bond lengths are taken as 1.36 Å. According to Salem (1966), even atom chains should be extended by half a bond length at each end to obtain the length of the bond. If 1.27 Å is allowed for the length of the C=N— bond and if the box is extended by 1.46 Å/2 at one end and 1.27 Å/2 at the other end the total length of the box is 16.84 Å. The shape of the one-dimensional box is shown in Figure 2. The FEMO orbitals for this box are

$$\phi_n(x) = \sqrt{\frac{2}{16.84}}\sin\left(\frac{n\pi x}{16.84}\right) \quad \text{for } n = 1, 2, \ldots 6 \quad (3)$$

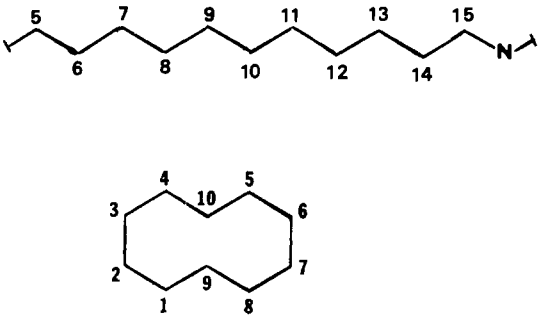

FIGURE 2: The one-dimensional box representation of the retinylidene and naphthyl moieties of (*S*)-*N*-*all-trans*-retinylidend-α-(1-naphthyl)ethylamine.

where x is a length coordinate that follows the box rather than going in a straight line.

The ground state of the retinylidene can be constructed as a Slater determinant over the above six orbitals, containing the coordinates of twelve electrons. Then, the first excited state involving the promotion of one electron from the 6th to 7th orbital is an f → g transition, in Platt's notation (Platt, 1961), and the electric dipole moment for this transition reduces to

$$\boldsymbol{\mu}_{01} = \sqrt{2}e\int\phi_6^*\mathbf{r}\phi_7 d\tau \quad (4)$$

which involves single-particle orbitals rather than Slater determinants; formulas for this reduction are given by Murrell (1963).

Transition Densities. It is convenient to define a transition density for the $\psi_0 \rightarrow \psi_1$ transition of the retinylidene, namely, $\rho_{01} = (2)^{1/2}e\phi_6^*\phi_7$. Since ϕ_6 is real here, this becomes $\rho_{01} = (2)^{1/2}e\phi_6\phi_7$. Substitution into eq 4 results in the expression

$$\boldsymbol{\mu}_{R_{01}} = \int\rho_{R_{01}}\mathbf{r}d\tau \quad (5)$$

For a given naphthyl transition a similar expression can be written

$$\boldsymbol{\mu}_{N_{01}} = \int\rho_{N_{01}}\mathbf{r}d\tau \quad (6)$$

These transition densities may be substituted into eq 2 for the coulomb matrix element to yield:

$$V_{OR;ON} = \int\int\rho_{R_{01}}(\mathbf{r}_1)\frac{2e^2}{r_{12}}\rho_{N_{01}}(\mathbf{r}_2)d\tau_1 d\tau_2 \quad (7)$$

The value of the coulomb matrix element is thus reduced to the interaction energy between two "transition charge" distributions.

Monopole Approximation. The monopole approximation of London is an approximation of the matrix element V as a sum of the interactions of point charges, or transition monopoles:

$$V_{OR;ON} = \sum_j\sum_k\frac{G_{Rj}G_{Nk}}{r_{RjNk}} \quad (8)$$

where G_{Rj} is the jth monopole of the retinylidene group, G_{Nk} is the kth monopole of the naphthyl group, and r_{RjNk} is the distance between the two monopoles.

Evaluation of Retinylidene Monopoles. Since the retinylidene

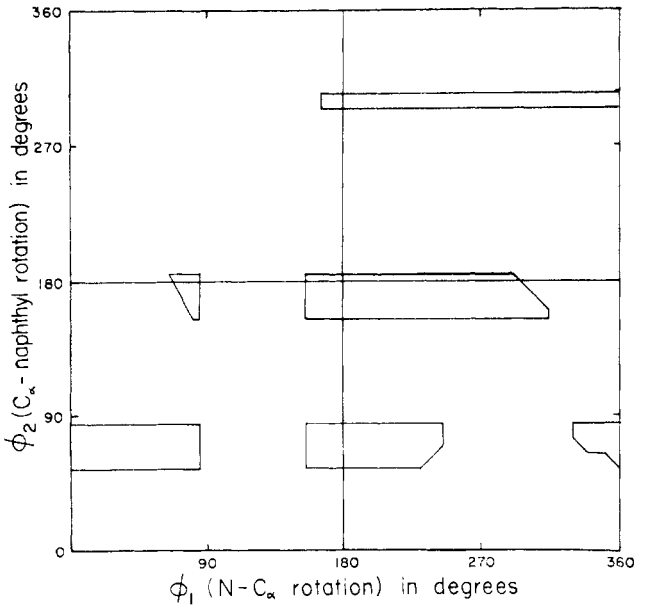

FIGURE 3: The hard sphere map calculated with C_{15}—N=C_α equal to 114° and the C_α methyl group represented as a bare carbon atom.

group has been represented as a zigzag box along the polyene skeleton with a kink at every carbon atom, the transition charge density $\rho_R(x) = (2)^{1/2}e\phi_6(x)\phi_7(x)$, contains the length coordinate x that follows the box rather than going in a straight line. The "nodal surfaces" used in the monopole approximation are here points, the zeros of the function

$$\rho = \frac{2\sqrt{2}}{l} e \sin\frac{6\pi x}{l} \sin\frac{7\pi x}{l} \qquad (9)$$

TABLE V: FEMO Monopoles for Retinylidene Group.

No.	Charge (Electrons)	x (Å)	y (Å)
1	0.027176	−0.16868	−0.09284
2	0.176380	0.53488	−0.32359
3	0.006785	1.24954	−0.65669
4	−0.002575	1.61564	−0.45519
5	0.043836	2.23807	−0.11263
6	0.127419	2.85238	−0.24785
7	−0.000781	3.57773	−0.68667
8	−0.018380	3.88191	−0.55230
9	0.048898	4.65655	−0.12595
10	0.063873	5.15141	−0.16093
11	−0.022651	2.89131	−0.60855
12	−0.034450	6.27365	−0.58034
13	0.040112	7.07000	−0.14205
14	0.016988	7.45866	−0.07898
15	−0.072713	8.19374	−0.52369
16	−0.040058	8.69331	−0.59302
17	0.019131	9.43704	−0.18369
18	0.000029	9.77526	−0.00270
19	−0.136853	10.48686	−0.43320
20	−0.034403	11.11565	−0.60423
21	0.002575	11.69140	−0.28735
22	−0.011298	12.10649	−0.05890
23	−0.179217	12.81203	−0.36210
24	−0.019826	13.43931	−0.56051

The dipole moment of this transition charge distribution is parallel to the long axis of the retinylidene group.

In order to locate the monopoles, the integration of every peak between a pair of successive zeros was modified by the addition of cuts in the charge distribution at the position of the atom, *i.e.*, kinks. The integral $\int_{C_i}^{C_i+1}\rho(x)dx$ was taken between every pair of successive cuts, C_i and C_i^{+1}, and a monopole was placed at the center of gravity of each segment. By this procedure all of the monopoles were placed on the polyene skeleton. The position and charges of the retinylidene monopoles are given in Table V. Before being used in the rotational strength calculation, the monopole strengths were multiplied by a factor so that the dipole moment of the whole monopole distribution became equal to the experimental dipole moment for the transition being considered.

Evaluation of Naphthyl Monopoles. For naphthalene, the one-dimensional FEMO box is taken as the perimeter. The C_9–C_{10} bridge is neglected. The box is kinked as with the retinylidene group, but now runs in a closed loop around the carbon skeleton as illustrated in Figure 3.

The free electron orbitals to be used for the naphthyl group have the same form as those used for benzene by Salem (1966). The ten naphthyl electrons will go into the lowest five orbitals, those for which $\Delta = 0, \pm 1$, and ± 2.

The singlet excited state wave functions can be obtained as combinations of Slater determinants in the following way

$$\psi_1 = \frac{1}{\sqrt{2}}(\chi_{2\to 3} + \chi_{-2\to -3})$$

$$\psi_2 = \frac{1}{\sqrt{2}}(\chi_{2\to 3} - \chi_{-2\to -3})$$

$$\psi_3 = \frac{1}{\sqrt{2}}(\chi_{2\to -3} + \chi_{-2\to 3})$$

$$\psi_4 = \frac{1}{\sqrt{2}}(\chi_{2\to -3} + \chi_{-2\to 3})$$

where

$$\chi_{2\to 3} = \frac{1}{\sqrt{2}}\{|\phi_{-2}\bar{\phi}_{-2}\phi_{-1}\bar{\phi}_{-1}\phi_0\bar{\phi}_0\phi_1\bar{\phi}_1\phi_2\bar{\phi}_3| + |\phi_{-2}\bar{\phi}_{-2}\cdots\phi_3\bar{\phi}_2|\}$$

and ψ_0 is the ground state.

The transition densities for these electrons can be obtained using the following expressions

For

$$\psi_0 = \frac{1}{\sqrt{2}}(\chi_{j\to k} + \chi_{-j\to -k})$$

$$\rho = \sqrt{2}e\left(\frac{1}{\sqrt{2}}\right)(\phi_j^*\phi_k + \phi_{-j}^*\phi_{-k})$$

where

$$\phi_j = \frac{1}{\sqrt{2\pi}}e^{ij\theta}$$

$$\rho = \frac{e}{2\pi}[e^{i\theta(k-j)} + e^{-i\theta(k-j)}]$$

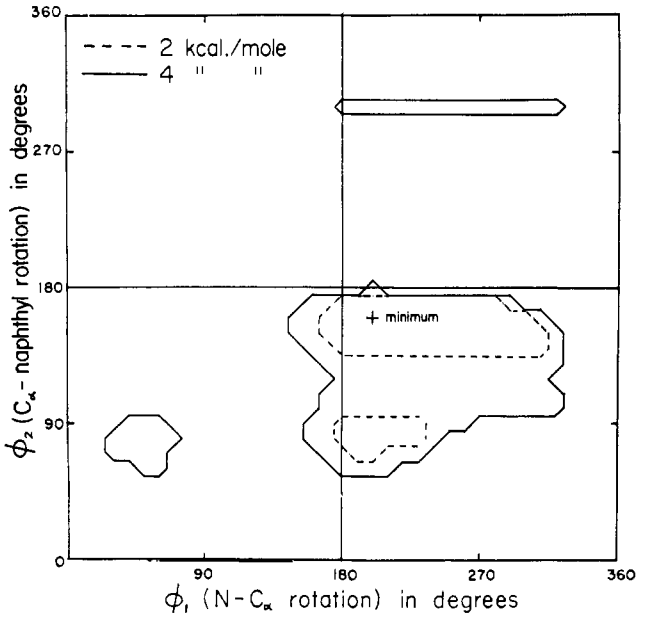

FIGURE 4: The conformational energy map calculated with C_{15}—N=C_α equal to 120° and the C_α methyl group represented as an actual methyl group.

when $j = 2, k = 3$

$$\rho = \frac{e \cos \theta}{\pi}$$

If the zero point for θ is taken at C_{10}, then this density function has loops at the atoms, and this is the ^1B_a transition density. By similar methods we obtained the other three transition densities. The four expressions are

$$\rho_{^1B_a} = \frac{e \cos \theta}{\pi}$$

short axis polarized

$$\rho_{^1L_a} = \frac{e \sin 5\theta}{\pi}$$

$$\rho_{^1B_b} = \frac{e \sin \theta}{\pi}$$

long axis polarized

$$\rho_{^1L_b} = \frac{e \cos 5\theta}{\pi}$$

The ^1L_b transition was not included in the calculation because of its low intensity.

The procedure for computing naphthyl charges and locations, starting from the transition densities was the same as that described for the retinylidene group. The positions and charges of these monopoles are listed in Table VI.

TABLE VI: FEMO Monopoles for the Naphthyl Group.

No.	Charge (Electrons)	x (Å)	y (Å)
	1. ^1L_a Transition		
1	0.098363	0.53935	0.93419
2	0.091986	0.18678	0.32352
3	0.067169	0.16472	−0.27203
4	0.045053	0.50868	−0.84007
5	0.015739	0.94178	−1.16760
6	−0.015739	1.87622	−1.16760
7	−0.045053	2.30932	−0.84007
8	−0.067169	2.65328	−0.27203
9	−0.091986	2.63122	0.32352
10	−0.067169	2.27865	0.93419
11	−0.098363	2.27865	1.53401
12	−0.091986	2.63122	2.14468
13	−0.067169	2.65328	2.74023
14	−0.045053	2.30932	3.30827
15	−0.015739	1.87622	3.63580
16	0.015739	0.94178	3.63580
17	0.045053	0.50868	3.30827
18	0.067169	0.16472	2.74023
19	0.091986	0.18678	2.14468
20	0.098363	0.53935	1.53401
	2. ^1B_b Transition		
1	0.063186	0.24080	0.41708
2	0.156271	0.37964	−0.62698
3	0.197707	1.40900	−1.16760
4	0.156271	2.43836	−0.62698
5	0.063186	2.57720	0.41708
6	−0.063186	2.57720	2.05112
7	−0.156271	2.43836	3.09518
8	−0.197707	1.40900	3.63580
9	−0.156271	0.37964	3.09518
10	−0.063186	0.24080	2.05112
	3. ^1B_a Transition		
1	0.190349	0.36897	0.63908
2	0.112222	0.30281	−0.50008
3	0.015739	0.94178	−1.16760
4	−0.015739	1.87622	−1.16760
5	−0.112222	2.51519	−0.50008
6	−0.190349	2.44903	0.63908
7	−0.190349	2.44903	1.82912
8	−0.112222	2.51519	2.96828
9	−0.015739	1.87622	3.63580
10	0.015739	0.94178	3.63580
11	0.112222	0.30281	2.96828
12	0.190349	0.36897	1.82912

Results

The hard-sphere map resulting from our calculations and embodying the restrictions listed in Table II is shown in Figure 4. The molecular geometry given in Table I was used, with the following two exceptions. The angle C_{15}—N=C_α was set equal to 114° and the methyl group on C_α was represented as a bare carbon atom. The energy was calculated for all combinations (ϕ_1, ϕ_2) using ten-degree increments. If the hard-sphere map is valid, the steric restrictions are rather severe and only about 10% of the map area is permitted.

The conformational energy map used for the CD calculations is shown in Figure 5. It is based on the potential functions of Table VII and uses a value of 120° for the C_{15}—N=C_α rather than 114° as was used in the hard sphere map. Also the methyl group on C_α was represented as such rather than a bare carbon atom. It was allowed to rotate freely to its minimum energy position for each pair of values given to ϕ_1 and ϕ_2. The region inside the 4-kcal/mol contour represents about 15% of the area of the map. There is an allowed area

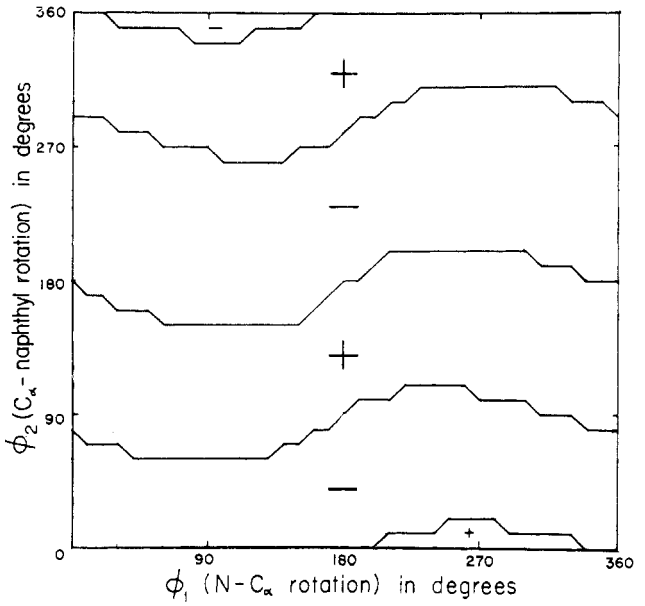

FIGURE 5: The sign of the calculated rotational strength as a function of ϕ_1 and ϕ_2 for the f → g (360-nm) band in the retinylidene group.

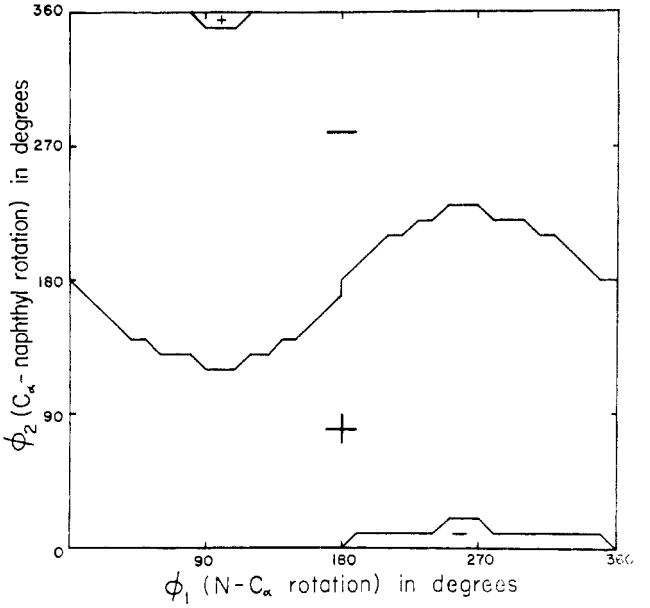

FIGURE 6: Sign of the calculated rotational strengths as a function of ϕ_1 and ϕ_2 for the naphthyl ^1L_a (282-nm) and ^1B_a bands.

in the lower right quadrant, a smaller area to its left and a very narrow slot in the upper right quadrant at $\phi_2 = 300°$.

The calculated rotational strength of the 360-nm transition of the retinylidene group can be mapped as a function of the angles as shown in Figure 5. The map shows the sign of this rotational strength as a function of ϕ_1 and ϕ_2. It is obtained by summing the contributions of the three naphthyl transitions, ^1L_a, ^1B_b, ^1B_a. Sign maps for the induced rotational strengths of the three naphthyl transitions are given in Figures 6 and 7.

The 360-nm transitions of the retinylidene group was observed to have a positive rotational strength. If this fact is used to restrict the allowed pairs of angles (ϕ_1, ϕ_2) then about 50% of the map area is not allowed. A further restriction is that the 282-nm induced band of the naphthyl was observed to have a negative rotational strength. This restricts (ϕ_1, ϕ_2) to lie in the region of Figure 6 marked with a negative sign. Lastly, the CD induced in the ^1B_b transition at 224 nm was observed to be positive. Superposition of Figures 5, 6, and 7 shows that the signs are given correctly only if (ϕ_1, ϕ_2) lies in a narrow twisting region reaching across the map from left to right and centered at $\phi_1 = 180°$ and $\phi_2 = 300°$. The region includes about 5% of the map area. Comparison of these results with the energy map of Figure 4 reveals that no point in the large low-energy region of the lower right quadrant would give the correct set of signs for the three rotational strengths. The only permissible region is the narrow low-energy strip in the upper right quadrant, reaching from (180°, 300°) to (320°, 300°). The points in this region are all between 2 and 4 kcal per mol above the global minimum energy. Only the points (180°, 300°), (190°, 300°), and (200°, 300°) give the correct signs for the three rotational strengths. The conformation (180°, 300°) has been selected as the one that gives the best agreement with the observed spectrum. Theoretical rotational strengths (180°, 300°) are compared graphically in Figure 8.

TABLE VII: Electric Transition Dipole Moments Used in the Calculation.[a]

Retinylidene band	9.669 D
Naphthyl bands	
^1L_a	3.326
^1B_b	8.923
^1B_a	4.619

[a] These dipole moments come from observed adsorption spectra and were used to calculate the theoretical CD spectrum of (*S*)-RNEA. The value for the retinylidene transition was obtained by integrating the peak in the *all-trans*-retinal absorption spectrum. The values for the naphthyl transitions were obtained from the oscillator strengths for naphthalene given by Klevens and Platt (1949).

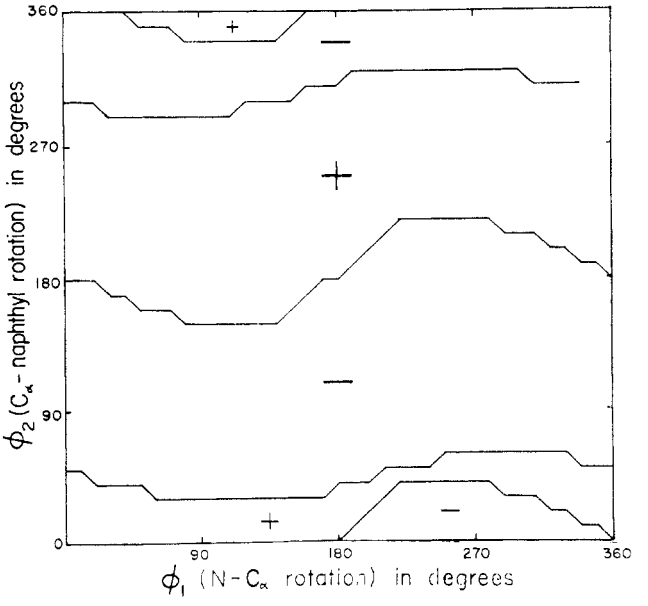

FIGURE 7: Sign of the calculated rotational strengths as a function of ϕ_1 and ϕ_2 for the naphthyl ^1B_b (224-nm) band.

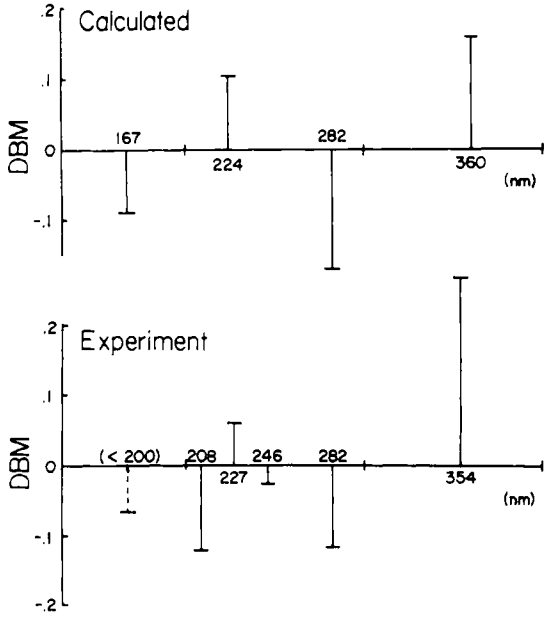

FIGURE 8: Comparison of the calculated and experimental values of the induced rotational strengths, wavelengths, and signs of (*S*)-*N*-*all-trans*-retinylidene-α-(1-naphthyl)ethylamine.

Discussion

Our calculation shows that there exists a conformation for (*S*)-*N*-*all-trans*-retinylidene-α-(1-naphthyl)ethylamine that predicts four rotational strengths within a factor of two, and gives all their signs correctly. The ^1B_a rotational strength taken from the spectrum was an estimate only, since it was obtained as the negative of the sum of the observed rotational strengths. However, it does agree in sign and approximate magnitude with the calculated ^1B_a rotational strength. The wavelengths of the three observed CD bands also agree within 6 nm with the locations of the transitions they are predicted to result from.

An *a priori* prediction of the CD spectrum without having the observed spectrum would have led to a choice of the minimum energy conformation, which lies in the lower right quadrant of the energy map at (200°, 160°). This choice would have resulted in the wrong sign for both the ^1L_a and ^1B_b predicted rotational strengths. Restricting the conformation to the region of the energy map reading from (180°, 300°) to (320°, 300°) would not improve the results obtained. If a Boltzmann average were performed over this allowed region the sign of the retinylidene band would come out wrong, since for the majority of this region the sign is negative.

The fact that reasonable agreement, between the calculated and observed CD spectra, results for a particular conformation is encouraging. The specific factors that cause the conformation of (*S*)-RNEA to be fixed at (180°, 300°) are not known at this time. It may be possible to obtain some answers to this question by applying the method to other molecules having similar conformational restrictions.

The results of this calculation in conjunction with the experimental study in the previous paper (Johnston and Zand, 1973) show that the 490-nm Cotton effect of rhodopsin cannot be viewed as arising necessarily from an imposed distortion of the polyene chain of retinaldehyde. A major contributing mechanism if not the sole mechanism, for this Cotton effect could be the interaction of the polyene transitions with the transitions of nearby (less than 15 Å) aromatic side chains of the protein opsin.

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