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# Conformational investigation on retinal by PCILO method

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**Abstract.** The conformational preferences of all-*trans* retinal (referred to as retinal) have been investigated by the PCILO (Perturbative Configuration Interaction using Localized Orbitals) method. The results indicate that the all-*trans* isomer (the conformer in which all the double bonds of the polyene chain in *trans* conformation) is relatively higher in energy than the one in which the terminal $C=O$ bond assumes a *cis*-conformation. The $\beta$-ionone ring and the polyene chain are non-planar and the angle between the two planes is 90°. The all-*trans* isomers with $\theta_5 = 180°$ or 0° are energetically more preferred than the other isomers of retinal, the 11-*cis* isomer being the least stable. A rotation, however, around C12–C13 single bond ($\theta_4$) in 11-*cis* retinal predicts two distorted 11-*cis*, 12-*s*-conformers with $\theta_4 = 90°$ and 270° which are as stable as 9-*cis* or 13-*cis* isomers. The theoretically predicted distorted 11-*cis* conformers are in agreement with experimentally observed conformers of 11-*cis* retinal in solution by NMR studies.

**Keywords.** Retinal; all-*trans* retinal; 9-*cis* retinal; 11-*cis* retinal; 13-*cis* retinal; conformational investigation; PCILO method.

## 1. Introduction

Retinal, the aldehyde of vitamin A has been found to occur as chromophore in visual pigments (Wald 1968; Worthington 1974; Hagins 1972) as well as in photosynthetic pigment called bacteriorhodopsin (Stockenius and Rowen 1967; Oesterhelt and Stoeckenius 1971; Stoeckenius 1980). In rhodopsin 11-*cis* retinal (Hubbard and Wald 1952) is attached by a Schift-base linkage to an $\epsilon$-amino group of lysine of the protein, opsin. In the light adapted bacteriorhodospin, all-*trans* form of retinal is believed to be covalently linked to the nitrogen of an $\epsilon$-amino group of lysine forming the Schiff base (Stoeckenius 1980). The Schift base has been demonstrated to be protonated in both pigments (Oesteroff and Callender 1974; Aton *et al* 1977). In addition to the all-*trans* and 11-*cis* isomers, retinal can assume 9-*cis* and 13-*cis* conformational states (Honig 1978) and both of these isomers have been encountered as intermediates of photochemical reactions of rhodopsin and bacteriorhodopsin respectively.

Retinal consists of a $\beta$-ionone ring attached to a polyene chain having five conjugated double bonds (figure 1) and belongs to the class of molecules known

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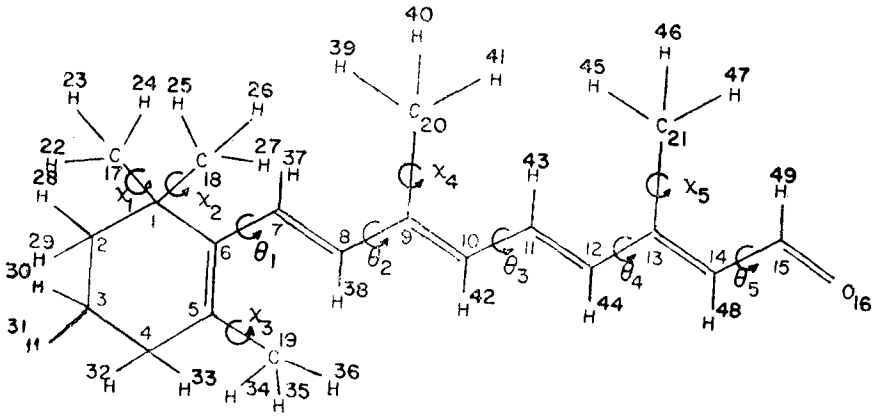

**Figure 1.** Schematic diagram of retinal showing various torsion angles.

as isoprenic polyenes. It is normally expected that the conjugated double bonds in a polyene chain should exist in planar conformation. However, the x-ray crystallographic data on all-*trans* retinal (Hamanaka *et al* 1972 ; Gilardi *et al* 1971) and 11-*cis* retinal (Gilardi *et al* 1971, 1972) indicate departures from the planar conformation around the C6–C7 bond connecting the $\beta$-ionone ring and the polyene chain and also around C12–C13 single bond (figure 1). The rest of the polyene chain is planar. We report in this paper a detailed coformational analysis of retinal and its isomer with an aim to determine the preferred conformation around various single bonds and the relative order of stability of various isomers. This paper on retinal is a prelude to our investigation on the conformational behaviour of the Schiff base of retinal isomers in protonated and deprotonated forms which are known to occur in bacteriorhodopsin and is presented in the following paper.

## 2. Methodology

The quantum mechanical PCILO method has been utilized to determine the conformational preferences around various single bonds in the retinal molecule. A brief description of the method has been presented in a review by Pullman and Saran (1976). A detailed account of the PCILO method can be found in the original papers (Malrieu *et al* 1969 ; Jordan *et al* 1969). The geometrical input data have been taken from x-ray studies of Starn and McGillavry (1963) on retinal. The various torsion angles which determine the conformation of retinal molecule is shown in figure 1 and they are defined as follows:

$\chi_1$ = C2–C1–C17–H22, $\chi_2$ = C2–C1–C18–H25, $\chi_3$ = C4–C5–C19–H34, $\chi_4$ = C8–C9–C20–H39, $\chi_5$ = C12–C13–C21–H45 ; $\theta_1$ = C5–C6–C7–C8, $\theta_2$ = C7–C8–C9–C10, $\theta_3$ = C9–C10–C11–C12, $\theta_4$ = C11–C12–C13–C14, and $\theta_5$ = C13–C14–C15–O16, with the *cis*-planar arrangement of the terminal bonds being taken as torsion angle equal to zero. The values of torsion angle equal to 60°, 180° and 300° are, respectively, designated by symbols $g^+$, $t$ and $g^-$. The computations have been carried out in 30° intervals of the torsion angles. Conformational

**Table 1.** Energies of various conformers of retinal.

| Conformer | | Energy in kcal/mole | Relative energy* in kcal/mole |
|---|---|---|---|
| All-*trans* | $\theta_5 = 180°$ | −114576·58 | 0·84 |
| | $\theta_5 = 0°$ | −114577·42 | 0·00 |
| 9-*cis* | $\theta_5 = 180°$ | −114575·36 | 2·06 |
| | $\theta_5 = 0°$ | −114576·20 | 1·22 |
| 13-*cis* | $\theta_5 = 180°$ | −114574·97 | 2·45 |
| | $\theta_5 = 0°$ | −114576·20 | 1·22 |
| 11-*cis* $\theta_4 = 180°$ | $\theta_5 = 180°$ | −114563·21 | 14·21 |
| | $\theta_5 = 0°$ | −114563·96 | 13·46 |
| 11-*cis* $\theta_4 = 0°$ | $\theta_5 = 180°$ | −114494·13 | 83·29 |
| | $\theta_5 = 0°$ | −114494·79 | 82·63 |
| 11-*cis* $\theta_4 = 90°$, $\theta_4 = 270°$ | $\theta_5 = 180°$ | −114575·04 | 2·38 |
| | | −114574·96 | 2·46 |
| 11-*cis* $\theta_4 = 90°$, $\theta_4 = 270°$ | $\theta_5 = 0°$ | −114576·04 | 1·38 |
| | | −114575·96 | 1·46 |

* Relative energy by taking the energy of the most stable conformer ($E = -114577·42$ kcal/mole) as energy zero.

energy maps have been constructed as a function of two torsion angles and iso-energy curves on the maps have been limited to 5 kcal/mole. To determine the relative stability of various conformers of retinal, the conformational energies of 9-*cis*, 11-*cis* and 13-*cis* isomers have also been computed using torsion angles obtained for the minimum energy conformer and have been listed in table 1.

## 3. Results

As stated earlier, a retinal molecule consists of a $\beta$-ionone ring to which is attached a polyene chain containing five double bonds (figure 1). It is of prime interest to determine the relative orientation of the polyene chain with respect to the ring and to do this it becomes pertinent to examine the orientation of the methyl groups attached to the ring. Thus, we have first constructed the $(\chi_1 - \chi_2)$ conformational energy map and utilized the results from this map for the subsequent computations to construct the other maps.

### 3·1 $(\chi_1 - \chi_2)$ *conformational energy map*

Figure 2 shows the $(\chi_1 - \chi_2)$ conformational energy map for retinal which has been constructed by fixing the protons of the remaining methyl groups in the stag-

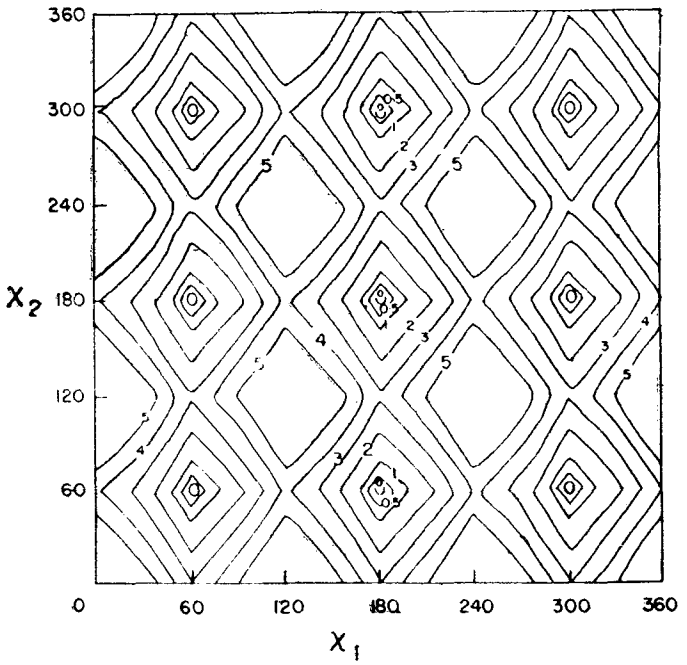


**Figure 2.** $(\chi_1 - \chi_2)$ conformational energy map constructed with $\chi_3 = \chi_4 = \chi_5 = 60°$, $\theta_1 = 40°$ and $\theta_2 = \theta_3 = \theta_4 = \theta_5 = 180°$. Isoenergy curves in kcal/mole with the global minimum taken as energy zero.

gered positions ($\chi_3 = \chi_4 = \chi_5 = 60°$). The double bonds of the polyene chain were kept in *trans* ($\theta_2 = \theta_3 = \theta_4 = \theta_5 = 180°$) conformation with $\theta_1 = 40°$. This choice of $\theta_1$ stems from the x-ray crystallographic results of Gilardi *et al* (1971) on retinal. The map shows nine global minimal having the same energy corresponding to the various staggered conformations for $\chi_1$ and $\chi_2$ and they occur at $(\chi_1, \chi_2) = (g^+, g^+), (g^+, t), (g^+, g^-), (t, g^+), (t, t), (t, g^-), (g^-, g^+), (g^-, t)$ and $(g^-, g^-)$. The value of $\chi_1 = \chi_2 = 60°$ has, thus, been adopted for the subsequent computations.

Next the variations in energy as a function of $\chi_4$ and $\chi_5$ were obtained and the results again indicated staggered conformations. We have, then, adopted $\chi_4 = \chi_5 = 60°$ in the rest of the computations.

### 3.2. $(\theta_1 - \chi_3)$ *conformational energy map*

The $(\theta_1 - \chi_3)$ conformational energy map constructed with $\chi_1 = \chi_2 = \chi_4 = \chi_5 = 60°$ and $\theta_2 = \theta_3 = \theta_4 = \theta_5 = 180°$ has been presented in figure 3. It can be seen that there are six global minima which correspond to the three different conformations of the methyl protons : $\chi_3 = 60°$, 180° and 300° with two values of $\theta_1 = 90°$ and 300°. In fact the global minima associated with $\theta_1 = 300°$ is about 0·14 kcal/mole higher in energy than those associated with $\theta_1 = 90°$ and one can also see in the map that the area encompassing the global minima associated with $\theta_1 = 90°$ are relatively larger than those with $\theta_1 = 300°$. Thus, the results presented in figure 3 clearly indicate two preferred conformations around C6–C7

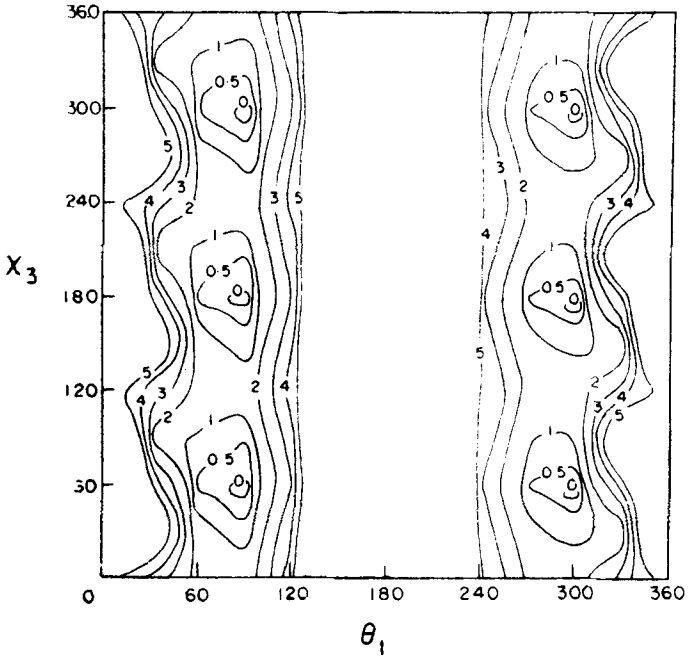


**Figure 3.** $(\theta_1 - \chi_3)$ conformational energy map constructed with $\chi_1 = \chi_2 = \chi_4 = \chi_5 = 60°$ and $\theta_2 = \theta_3 = \theta_4 = \theta_5 = 180°$. Isoenergy curves in kcal/mole with the global minimum taken as energy zero.

bond i.e. $\theta_1 = 90°$ and $300°$ of which $\theta_1 = 90°$ is relatively preferred over $\theta_1 = 300°$. For the subsequent computations we have adopted $\theta_1 = 90°$.

### 3.3. $(\theta_2 - \theta_1)$ *conformational energy map*

Figure 4 shows the $(\theta_2 - \theta_1)$ conformational energy map which has been constructed by fixing $\chi_1 = \chi_2 = \chi_3 = \chi_4 = \chi_5 = 60°$ $(g^+)$ and $\theta_3 = \theta_4 = \theta_5 = 180°$. Simliar to the results of the map in figure 3, there are two global minima at $\theta_1 = 90°$ and $300°$ and both associated with $\theta_2 = 180°$. In this case again the global minimum at $\theta_1 = 300°$ is about 0·14 kcal/mole higher in energy over that at $\theta_1 = 90°$. We have, therefore, utilized $\theta_1 = 90°$ and $\theta_2 = 180°$ in the subsequent computations. It can also be seen from the map shown in figure 4 that although $\theta_2$ spans through out from 0°–360° by the 5 kcal/mole isoenergy curve, the region $\theta_1 \approx 130°$ to 230° is strictly forbidden. A similar result for $\theta_1$ can also be seen in the map shown in figure 3. This is probably due to the interaction of H37 with the protons of the methyl group attached to C5 atom of the $\beta$-ionone ring.

### 3.4. $(\theta_3 - \theta_2)$ *and* $(\theta_4 - \theta_3)$ *conformational energy maps*

These two maps have been constructed consequently by adopting the results of previous maps and they indicate *trans* conformations with $\theta_2 = \theta_3 = \theta_4 = 180°$ to be the most preferred ones.

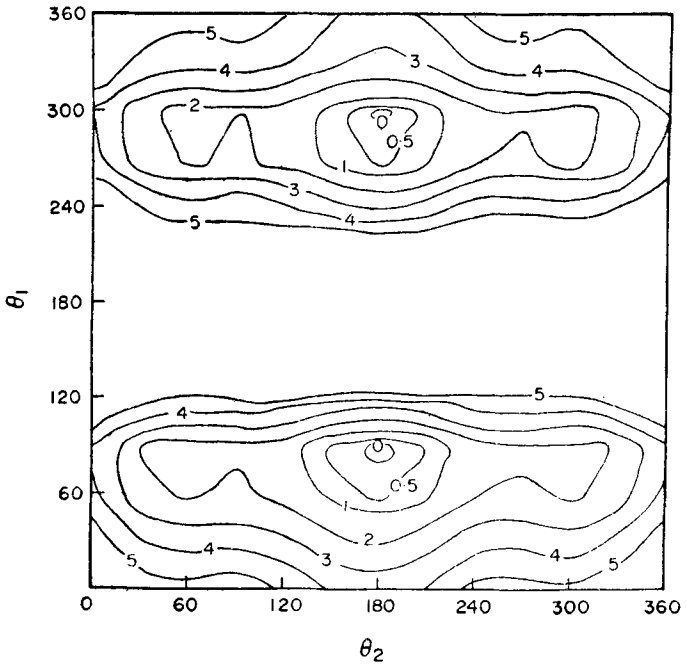


**Figure 4.** $(\theta_2 - \theta_1)$ conformational energy map constructed with $\chi_1 = \chi_2 = \chi_3 = \chi_4 = \chi_5 = 60°$ and $\theta_3 = \theta_4 = \theta_5 = 180°$. Isoenergy curves in kcal/mole with the global minimum taken as energy zero.

### 3.5. $(\theta_5 - \theta_4)$ *conformational energy map*

Figure 5 shows the $(\theta_5 - \theta_4)$ conformational energy map which indicates a global minimum for $\theta_5 = 0°$ and $\theta_4 = 180°$. The preferred value of $\theta_5 = 0°$ is different than the *trans* conformation (*i.e.*, $\theta_4 = \theta_5 = 180°$) which is about 1 kcal/mole higher in energy. In fact the conformation $\theta_4 = \theta_5 = 180°$ is 0·84 kcal/mole higher in energy than the most preferred conformation $\theta_4 = 180°$ and $\theta_5 = 0°$ (table 1).

## 4. Discussion

The PCILO computations on retinal presented above, thus, indicate the preferred values of the various torsion angles as $\chi_1 = \chi_2 = \chi_3 = \chi_4 = \chi_5 = 60°$, $\theta_1 = 90°$ (also 300°) $\theta_2 = \theta_3 = \theta_4 = 180°$ and $\theta_5 = 0°$. There are subtle differences in the conformation of retinal predicted by the present calculations and the experimentally observed conformation of all-*trans* retinal by x-ray crystallography. The magnitude of non-planarity of the $\beta$-ionen ring and the polyene chain predicted by the theoretical computations ($\theta_1 = 90°$) is slightly larger than that observed in the crystal structure of all-*trans* retinal ($\theta_1 = 40°$ by Gilardi *et al* 1971; $\theta_1 = 62°$ by Hamanaka *et al* 1972). In order to probe this difference, the energy of retinal was computed as a function of $\theta_1$ by fixing the other torsion angles in their preferred values as mentioned above. Figure 6 shows the results of such

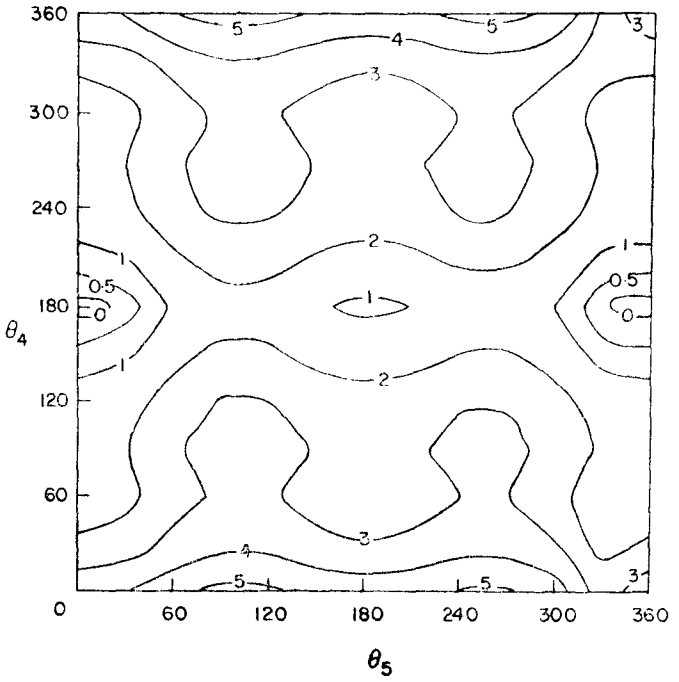


**Figure 5.** $(\theta_5 - \theta_4)$ conformational energy map constructed with $\chi_1 = \chi_2 = \chi_3 = \chi_4 = \chi_5 = 60°$, $\theta_1 = 90°$, $\theta_2 = \theta_3 = 180°$. Isoenergy curves in kcal/mole with the global minimum taken as energy zero.

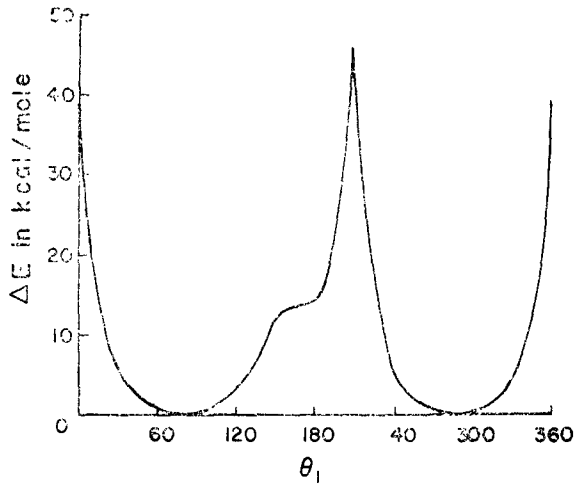


**Figure 6.** The variation of conformational energy as a function of torsion angle $\theta_1$. The global minimum has been taken as energy zero.

computations and one can see flat regions at $\theta_1 \approx 60$–$90°$ and $\theta_1 \approx 270$–$300°$ corresponding to the global minima. The x-ray crystallographic value of $\theta_1 = 62°$ by Hamanaka *et al* (1972) is quite close to the theoretical results. The present results also broadly agree well with Pullman *et al* (1969) results on retinal by

EHT. The major difference between the x-ray structure of all-*trans* retinal and the one predicted by the present theoretical calculations is that the all-*trans* conformation is about 0·84 kcal/mole higher in energy than the conformation in which $\theta_5 = 0°$. This is true not only for the all-*trans* retinal but for all other isomers of retinal (table 1). The *cis*-conformation around C14–C15 ($\theta_5 = 0°$) is, probably, stabilized by the close proximity of carbonyl oxygen and the protons of the methyl attached to C13.

The various other isomers of retinal namely : 9-*cis*, 11-*cis* and 13-*cis* have been schematically shown in figure 7. We have computed the energies of these

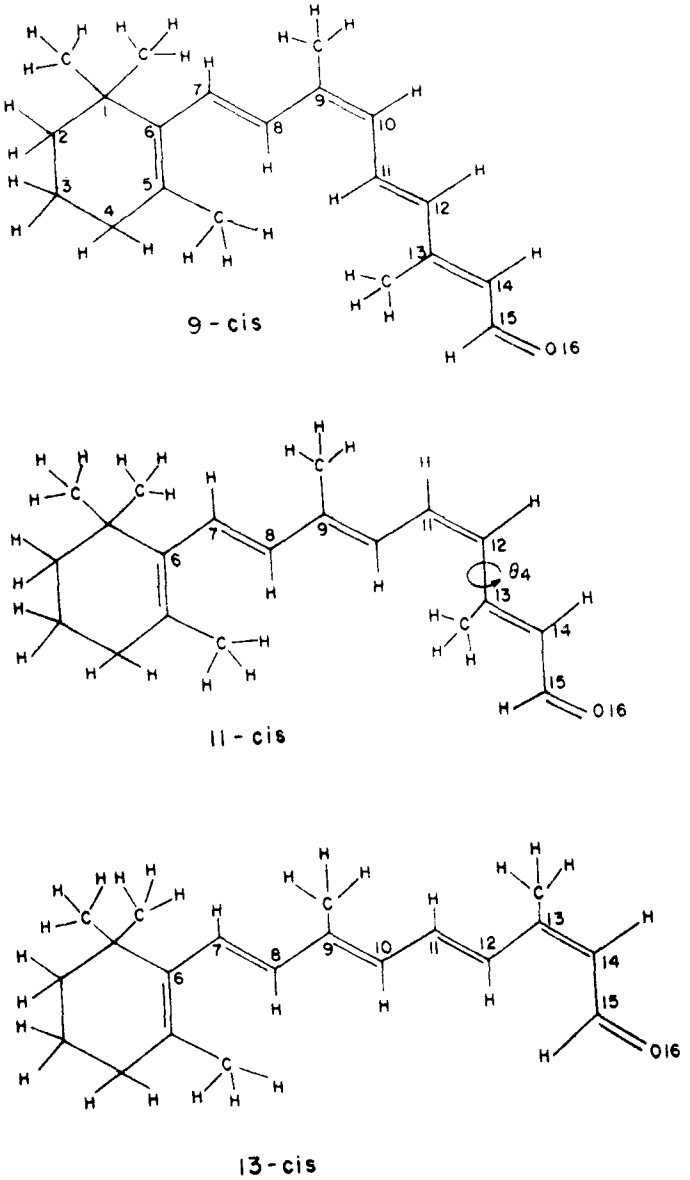


**Figure 7.** Schematic diagrams of 9-*cis*, 11-*cis* and 13-*cis* retinals.

isomers by fixing $\theta_5 = 180°$ and $0°$ and these are listed in table 1. It can be seen from this table that all-*trans* isomer with $\theta_5 = 0°$ is the most stable conformer of retinal followed by all-*trans* isomer with $\theta_5 = 180°$ which is about 0·84 kcal/mole higher in energy. The next stable isomers are 9-*cis* and 13-*cis* isomers with energy upto 1 to 2·5 kcal/mole depending upon the value of $\theta_5$. The least stable conformer is 11-*cis* with $\theta_5 = 180°$ which is about 14·21 kcal/mole higher in energy than the most preferred conforation (table 1). This isomer, which is also designated as 11-*cis*, 12*s*-*trans* (*i.e.*, *trans* orientation around C12–C13 single bond, $\theta_4 = 180°$) is realatively unstable due to interaction of H42 with one of the protons of methyl group attached to C13.

To alleviate this strain, rotations around C10–C11 or C11–C12 or C12–C13 bonds have been suggested (Honig and Ebrey 1974). Out of these, the rotation around C10–C11 can be easily ruled out because this rotation will not relieve the strain (figure 7). The rotation around C11–C12 is hard to accomplish because of the large potential barrier associated with a double bond. Furthermore, this rotation will destory the very definition of 11-*cis* retinal. The rotation around C12–C13, however, can relieve the strain. We have carried out energy calculations on 11-*cis* retinal with $\theta_5 = 180°$ as well as $0°$ by varying the torsion angle $\theta_4$ around C12–C13 single bond in 30° intervals. Both computations with $\theta_5 = 180°$ and $0°$ show similar results, only the energies with $\theta_5 = 0°$ are lower

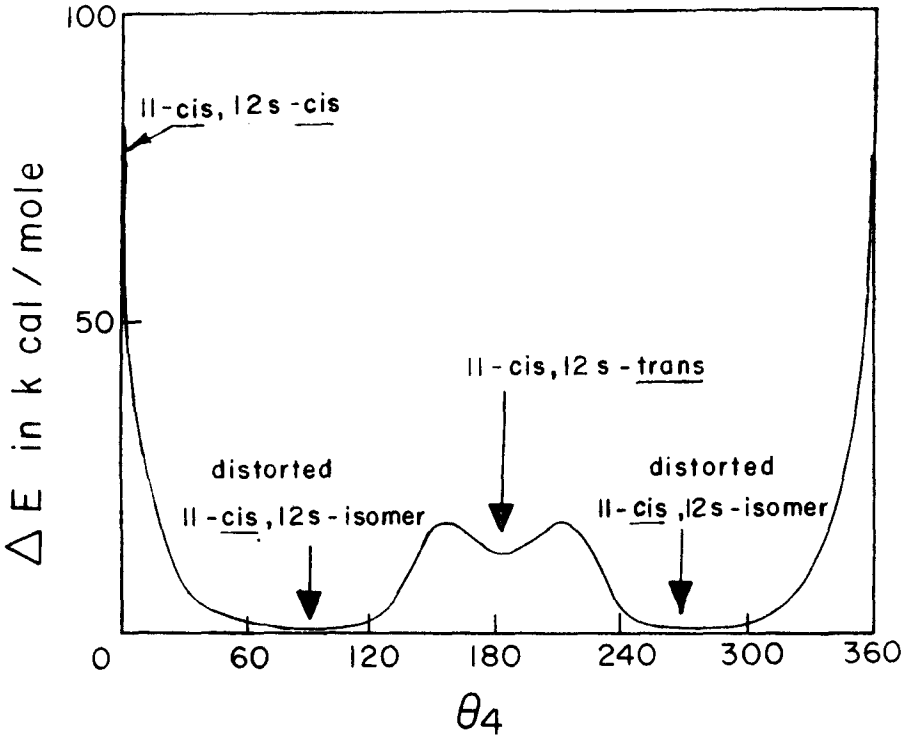


**Figure 8.** The variation of conformational energy of 11-*cis* retinal as a function of torsion angle $\theta_4$ with $\theta_5 = 0°$. The global minimum has been taken as energy zero.

(table 1) than those with $\theta_5 = 180°$. This is in agreement with our results on all-*trans*, 9-*cis* and 13-*cis* retinals. The variation of energy as a function of $\theta_4$ has been plotted and we present in figure 8 the results of the computations carried out by fixing $\theta_5 = 0°$. An exactly similar plot has been obtained for $\theta_5 = 180°$. It can be seen from figure 8 that there are two global minima which occur at $\theta_4 = 90°$ and 270° and their energies (relative to the most stable all-*trans* conformer, i.e., with $\theta_5 = 0$) are 1·46 and 1·38 kcal/mole (table 1). Thus, a rotation of 90° or 270° around C12–C13 single bonds brings down the relative energy from about 14 kcal/mole to about 1·4 kcal/mole and makes 11-*cis* retinal as stable as 9-*cis* or 13-*cis* retinals. Patel (1969) from his NMR studies on 11-*cis* retinal concluded that there is twisting around C12–C13 bond and not around C10–C11 or C11–C12 bonds. The x-ray data on 11-*cis* retinal indicate $\theta_4 = 39°$ (Gilardi *et al* 1972). These experimental results are in good agreement with our theoretical predictions. A more recent NMR and NOE data on 11-*cis* retinal by Rowan *et al* (1974) suggest that 11-*cis* in solution exists as an equilibrium mixture of two low energy conformers, viz., distorted 11-*cis*, 12*s*-*cis* and distorted 11-*cis*, 12*s*-*trans* isomers at room temperature. Our theoretical results on 11-*cis* retinal (figure 8) indicate that 11-*cis*, 12*s*-*cis* planar ($\theta_4 = 0°$) conformation is about 8l kcal/mole higher in energy than the 11-*cis* conformers with $\theta_4 = 90°$ or 270°. This is due to the fact that in this conformer ($\theta_4 = 0°$) the hydrogens on carbon atoms C10 and C14 come very close to each other (~ 0·8Å) and cause substantial repulsive interaction to the total energy. However, there are two low-energy conformers in our predicted results with $\theta_4 = 90°$ and 270° and one can identify one of these to distorted 11-*cis* 12*s*-*cis* and the other to 11-*cis*, 12*s*-*trans* isomers and these results agree excellently with the NMR and NOE results of Rowan *et al* (1974) and theoretical predictions of Honig and Karplus (1971). Of the two conformers, the one with $\theta_4 = 90°$ is slightly more stable (about 0·08 kcal/mole) than the other.

## 5. Conclusion

It can be stated from our present investigation that all the retinal isomers exist in a twisted ring-chain conformation and all-*trans* isomer (with $\theta_5 = 0°$) is the most stable conformer for retinal. The steric hindrance in 11-*cis* isomer (with $\theta_4 = 180°$ or 0°) which makes it the least stable conformer is overcome by twisting around C12–C13 single bond ($\theta_4 = 90°$ and 270°) and this makes 11-*cis* as stable as 9-*cis* or 13-*cis* retinals. This twisting around C12–C13 bond in 11-*cis* retinal might be playing a crucial role in visual pigment which contains this isomer as chromophore.

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