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Complex Thermal Behavior of 11-*cis*-Retinal, the Ligand of the Visual Pigments

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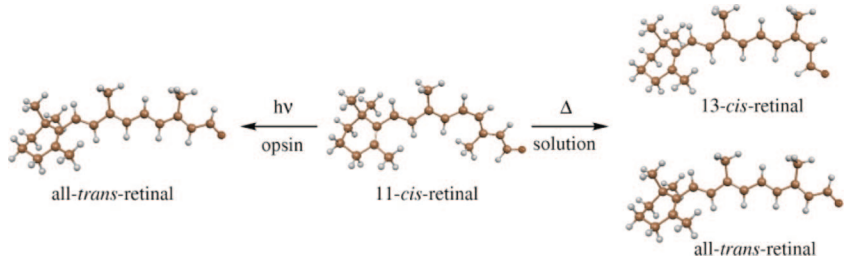

Upon heating to 80 °C, 11-*cis*-retinal yields a mixture of *all-trans*-retinal and 13-*cis*-retinal. This isomerization has been studied by means of density functional theory methods, and the computational results suggest a close competition between two mechanisms of very different nature. A classical internal rotation around the C11−C12 cis double bond, via a diradical transition state, accounts for the formation of the *all-trans* isomer. An intricate sequence of pericyclic reactions, namely a reversible [1,7]-H sigmatropic shift and a reversible 6-π-oxa-electrocyclic reaction, is responsible for the formation of 13-*cis*-retinal. Experiments using 11-*cis*-retinal labeled with deuterium at C19 confirmed the mechanistic proposal and also revealed an unprecedented outcome on the product composition of isotopologues.

Introduction

Rhodopsin, the representative member of the G-protein coupled receptor superfamily (GPCR) in rods, and the cone visual pigments in cone photoreceptors are transmembrane protein complexes formed by the apoprotein (opsin and cone opsins, respectively) and the ligand 11-*cis*-retinal **1**, which is covalently attached via a protonated Schiff base.[1] Light absorption by the bound chromophore 11-*cis*-retinal initiates the visual cycle in vertebrates. Nature has optimized this light-capturing protein complex through evolution to the extent that the photochemically induced isomerization of 11-*cis*-retinal is one of the fastest and most efficient reactions recorded.[1,2] Upon photoisomerization to *all-trans*-retinal **3** in intermediate bathorhodopsin (a highly energetic species that stores 2/3 of the absorbed light energy), the distorted conformation of the chromophore relaxes and generates the metarhodopsin II state through a series of intermediates. The active Meta II binds and transiently activates many copies of the G-protein transducin, which modulates the transmembrane potential within the cell and induces an optical nerve signal that culminates in visual perception in the brain.[3,4]

The chromophore in rhodopsin and cone opsins acts in fact as an inverse agonist since in the dark it suppresses the activity of the receptor to an undetectable level. Crucial to the efficient functioning of 11-*cis*-retinal **1** as an inverse agonist is its thermal

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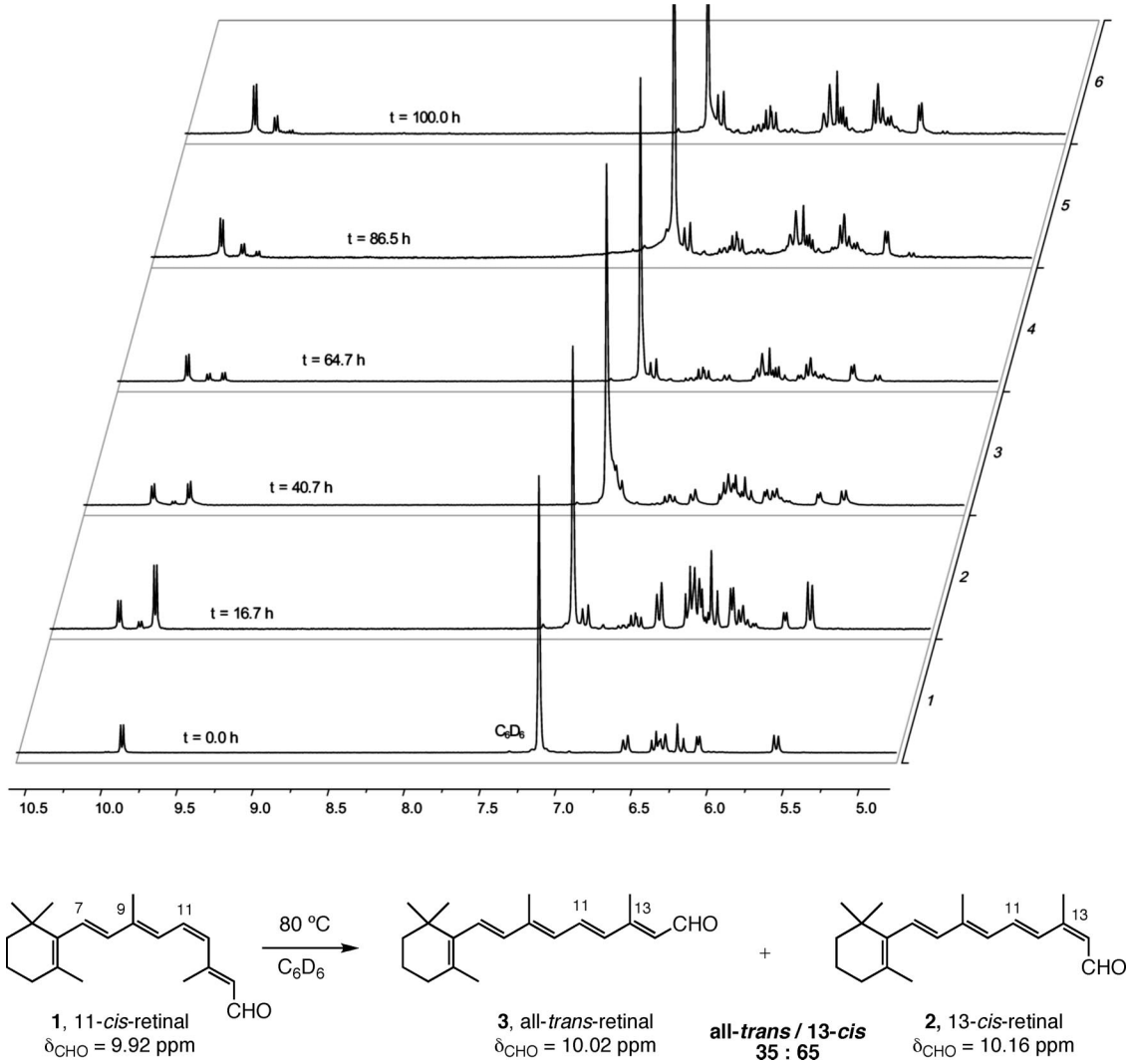

FIGURE 1. ^{1}H NMR spectra recorded over time for the thermal isomerization of 11-*cis*-retinal **1** at 80 °C (C_6D_6). Characteristic ^{1}H NMR chemical shifts of the aldehyde group were used to track the reactant and products over time.

stability. The protein−ligand complex shows a very low level of spontaneous thermal isomerization, a process that would produce the agonist-activated protein complex. Previous reports had indicated photoreceptor noise production due to thermal isomerization of the unprotonated Schiff base.[5] More recently, a thermal denaturation study of rhodopsin at 55 °C showed that the protein−ligand complex is more thermolabile than the free chromophore.[6]

The first report on the thermal isomerization of 11-*cis*-retinal **1** in solution was due to Hubbard in 1966.[7] A rate constant of $k = 1.02 \times 10^{-5}$ s^{-1} was determined at 80 °C, and Arrhenius parameters of $A = 1 \times 10^{11}$ s^{-1} and $\Delta G^{\ddagger} = 26.2$ kcal/mol were measured in *n*-heptane solutions. UV monitoring led to the proposal that the product was the *all-trans*-retinal isomer. This reaction has not been revisited during the 40 year period of intense and stimulating research in the field of vision in vertebrates and contrasts with the comprehensive experimental[8,1] and theoretical[9] studies on the alternative photoisomerization event.

We hereby provide a combined experimental-theoretical (density functional theory, DFT) study of this thermal isomerization which proves that *all-trans*-retinal **3** is not the unique product of the reaction but is instead the minor component (∼35%) of a mixture with the predominant 13-*cis*-retinal **2** (∼65%). Moreover, we show that the mechanistic pathway yielding the latter product most intriguingly involves a cascade of pericyclic reactions completely avoiding any diradical species along the course of the formal double-bond isomerization steps.

Results and Discussion

Upon heating to 80 °C in C_6D_6, 11-*cis*-retinal **1** experienced an unimolecular isomerization process affording signals in the ^{1}H NMR spectra corresponding to both *all-trans*-retinal **3** and 13-*cis*-retinal **2** (see Figure 1). These isomers were identified by comparison of their ^{1}H NMR data with those of commercial samples and by HPLC coelution with standards. Reproducible

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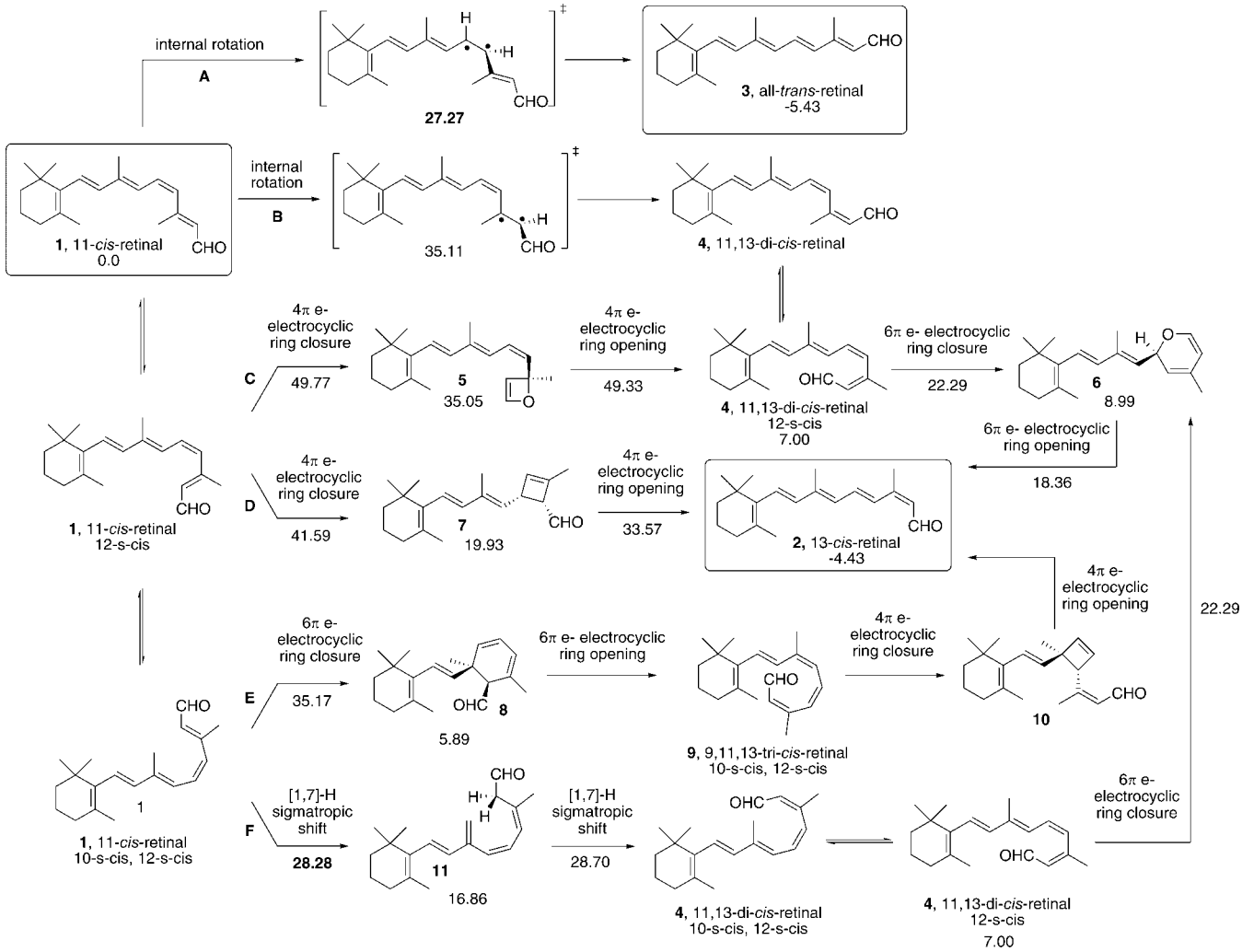

FIGURE 2. Isomerization pathways of 11-*cis*-retinal **1** to *all-trans*-retinal **3** and 13-*cis*-retinal **2** explored in this work. All the free energies (in kcal/mol) are relative to 11-*cis*-retinal **1**.

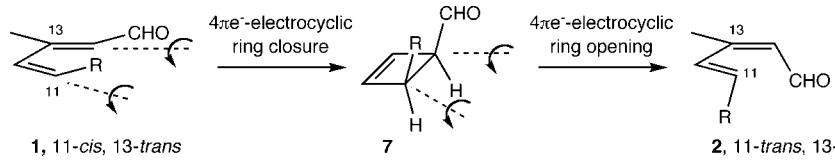

FIGURE 3. Sequential (and torquoselective) four-electron ring-closure/ring-opening process for the isomerization of both double bonds of the terminal dienal fragment of 11-*cis*-retinal **1** (see Figure 2, path **D**).

first-order kinetics ($k = 1.02 \times 10^{-5}$ s^{-1} and $t_{1/2}$ of 22 h) were experimentally determined in C_6D_6 at 80 °C. The mixture of isomers obtained after subjecting 11-*cis*-retinal **1** to thermal conditions (see Figure 1) contrasts with the equilibrium composition found for retinal isomers obtained with CF_3COOH (61.8:23.9:10.9:4.0:0.1 *all-trans*/13-*cis*/9-*cis*/9,13-di-*cis*/11-*cis* from *all-trans*-retinal)[10] and also with the photostationary mixture of retinal in hexane solution (54:5:41 *all-trans*/9-*cis*/13-*cis* from *all-trans*-retinal).[8] It is, however, very similar to that obtained upon treatment of 11-*cis*-retinal **1** with iodine at 40 °C (65.8:31.6:2.6 *all-trans*/13-*cis*/11-*cis*).[10]

The proposed 11-*cis* **1** to *all-trans*-retinal **3** isomerization might involve a simple internal rotation about the C11−C12 double bond via a diradical transition state (Figure 3, path **A**).[11] Our DFT calculations provided an activation barrier ($\Delta G^{\ddagger} =$ 27.3 kcal/mol) for this process in close agreement with the experimental data.[7] Given the feasibility of the direct olefin isomerization process, we then envisioned that *all-trans*-retinal **3** could undergo further *E*/*Z* isomerization at C13−C14 to yield 13-*cis*-retinal **2**. Control experiments, however, indicated no significant interconversion between the final isomers upon heating isolated *all-trans*-retinal **3** or 13-*cis*-retinal **2** at the same temperature for extended reaction times.[12] Therefore, the pathways for formation of the single- (*trans*-) and double-isomerization (13-*cis*) retinal isomers do not appear to converge

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at a common intermediate. This requirement could however be met if the order of the double-bond isomerization steps was reversed: first, a 13*E* to 13*Z* rotation yielding 11,13-di-*cis*-retinal **4** and then an 11*Z* to 11*E* rotation to obtain **2**, thus avoiding *all-trans*-retinal **3** in the reaction path. The computed activation energy for the first *E* to *Z* isomerization (see reaction path **B** in Figure 2) was found to be 35.11 kcal/mol, rendering this possibility out of competition (compared with 27.3 kcal/mol). The increase in activation energy can be attributed to the drastic reduction in conjugation for the functionalized radical moiety in the transition state (see Figure 2, path **B**).[13]

Since double-bond isomerization via diradical pathways is clearly out of the energy range for competition at the working temperatures, nonradical alternatives for the rearrangement of **1** to **2** had to be surveyed. A considerable number of pericyclic reactions are compatible with the high degree of unsaturation, the cis geometry, the allylic methyl groups, and the conjugated aldehyde of 11-*cis*-retinal **1**.[14] This pentaenal structure can accommodate, in addition to the already explored diradical structures, a wide variety of pericyclic transition states (for example, electrocyclic, heteroelectrocyclic, sigmatropic reactions, etc.) that could operate in domino sequences to afford different isomers of 11-*cis*-retinal **1**. Pericyclic processes involving derivatives of other retinal isomers are known. An electrocyclic reaction of a formal dieniminium ion takes place at physiological temperature in the retina during the formation of the A2E pigment (a fluorescent amphiphilic pyridinium bis-retinoid involved in age-related macular degeneration) from two molecules of *trans*-retinal.[15,16] Furthermore, 13-*cis*-retinal Schiff bases afford at ambient temperature the corresponding dihydropyridines by heteroelectrocyclic ring closure.[17]

Based on these precedents, we systematically considered typical low energy unimolecular pericyclic reactions[14] for the pentaenal structure of retinal, including six- and four-electron electrocyclizations, six- and four-electron heteroelectrocyclic reactions, and [1,7] sigmatropic hydrogen shifts. We envisioned four different pericyclic cascades that could afford 13-*cis*-retinal **2** from 11-*cis*-retinal **1** (see Figure 2). They are labeled **C**, **D**, **E**, and **F** and characterized by the increasing number of electrons involved in the pericyclic reaction initiating the cascade (4 in **C** and **D**, 6 in **E**, and 8 in **F**).

The reaction path **C** implies a four-electron heteroelectrocyclic ring closure at the carbonyl end of the polyenal to afford 2*H*-oxete derivative **5**, followed by the four-electron ring opening of this ring in the same rotatory direction yielding 11,13-di-*cis*-retinal **4**.[18] 11,13-Di-*cis*-retinal **4** would then undergo a six-electron heteroelectrocyclic ring closure to form 2*H*-pyran **6**,[19] finally converted to 13-*cis*-retinal by ring opening.[20]

Reaction path **D** represents perhaps the shortest pericyclic pathway since a combined four-electron ring-closure/ring-opening process in the same rotatory directionality (see Figure 3) has the overall effect of isomerizing both double bonds of the involved diene. The directionality of the conrotation would moreover be enforced by the strong CHO-*in* torquoselectivity inherent to the ring opening of 3-formylcyclobutenes.[21]

Pathway **E** implies two pairs of ring-closing/opening electrocyclizations. A six-electron ring closure (**1** to **8**),[14a] involving the C9−C14 triene, followed by the corresponding ring opening of **8** in the same rotatory direction to furnish 9,11,13-tri-*cis*-retinal **9**, for which conversion to 13-*cis*-retinal can be attained by double isomerization of the *cis* double bonds at C9 and C11 through a four-electron electrocyclic ring closing/opening sequence as illustrated above.

These three different mechanistic options **C**−**E** show rate-limiting steps with activation energies much higher than 27 kcal/mol (see values in Figure 2), thus rendering them noncompetitive with path **A**.

A mechanistic alternative (labeled as **F** in Figure 2) for the isomerization of the terminal double bond in conjugated polyene **1** would be a reversible antarafacial [1,7]-H sigmatropic shift[22] (**1** → **11** → **4**) between the methyl group at C9 and H at C14. The migration of diastereotopic hydrogens at C14 in 10-*cis*,12-*cis*-19,14-*retro*-retinal **11** or the back migration of the same atom after helix inversion would both effect the isomerization of the C13−C14 double bond of **1**. This step would be followed by

(13) In the restricted environment of the opsin protein (as has been found in bacteriorhodopsin, the proton pumping device of halobacteria, which uses a protonated Schiff base of *all-trans*-retinal as chromophore) or in the solid state, retinoids have been reported to undergo double-bond isomerizations via concerted motions (hula twist and bicycle pedal); see: (a) Liu, R. S. H.; Browne, D. T. *Acc. Chem. Res.* **1986**, *19*, 42–48. (b) Baudry, J.; Crouzy, S.; Roux, B.; Smith, J. C. *Biophys. J.* **1999**, *76*, 1909–1917. The same studies accept that, in solution, the most favored isomerization mechanism is the sequential pathway, where each double-bond rotation occurs as a single and independent step. We thoroughly explored this possibility and reached similar conclusions: the concerted isomerization about C11−C12 and C13−C14 lies very high in energy (ca. 48.6 kcal/mol) and the stationary point associated with such a transformation was found to be a second-order saddle point (see the Supporting Information). Other theoretical studies rule out the bicycle pedal motion of the photoexcited chromophore, which moreover appears to restrict its movement to the C10−C11−C12−C13 fragment (a so-called "photochemical hot spot"), whereas the rest of the molecule begins to rotate only upon relaxation to the ground state; see: (c) Weingart, O. *J. Am. Chem. Soc.* **2007**, *129*, 10618–10619.

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(17) (a) Okamura, W. H.; Lera, A. R. D.; Reischl, W. *J. Am. Chem. Soc.* **1988**, *110*, 4462–4464. (b) de Lera, A.; Reischl, W.; Okamura, W. *J. Am. Chem. Soc.* **1989**, *111*, 4051–4063.

(18) The ring-closure activation energy computed (49.77 kcal/mol) is in good agreement with previous computations of analogous systems at the MP4SDTQ/6-31G(d) level; see: Yu, H.; Chan, W. T.; Goddard, J. D. *J. Am. Chem. Soc.* **1990**, *112*, 7529–7537.

(19) The mechanism of isomerization of di-*cis*-dienals and dienones to the monocis isomers through formation of α-pyrans had been previously studied by Kluge and Lillya: (a) Kluge, A. F.; Lillya, C. P. *J. Org. Chem.* **1971**, *36*, 1977–1988. (b) Kluge, A. F.; Lillya, C. P. *J. Org. Chem.* **1971**, *36*, 1988–1995.

(20) (a) 11,13-Di-*cis*-retinal was first shown to convert to 13-*cis*-retinal by Wald et al. in 1955: Wald, G.; Hubbard, R.; Brown, P. K.; Oroshnik, W. *Proc. Natl. Acad. Sci. U.S.A.* **1955**, *41*, 438–451. (b) The isomerization of the 11-cis double bond of 11,13-di-*cis*-retinal and 9,11,13-tri-*cis*-retinal was studied by Okamura: Knudsen, C. G.; Chandraratna, R. A. S.; Walkeapaa, L. P.; Chauhan, Y. S.; Carey, S. C.; Cooper, T. M.; Birge, R. R.; Okamura, W. H. *J. Am. Chem. Soc.* **1983**, *105*, 1626–1631. (c) In addition, Liu described the thermal rearrangement of the four labile isomers of retinal (11,13-di-*cis*-, 7,11,13-tri-*cis*-, 9,11,13-tri-*cis*-, and *all*-*cis*-retinal) presumably occurring via consecutive 6πe$^-$-heteroelectrocyclization reactions and determined the kinetic parameters of these processes: Zhu, Y.; Ganapathy, S.; Liu, R. S. H. *J. Org. Chem.* **1992**, *57*, 1110–1113.

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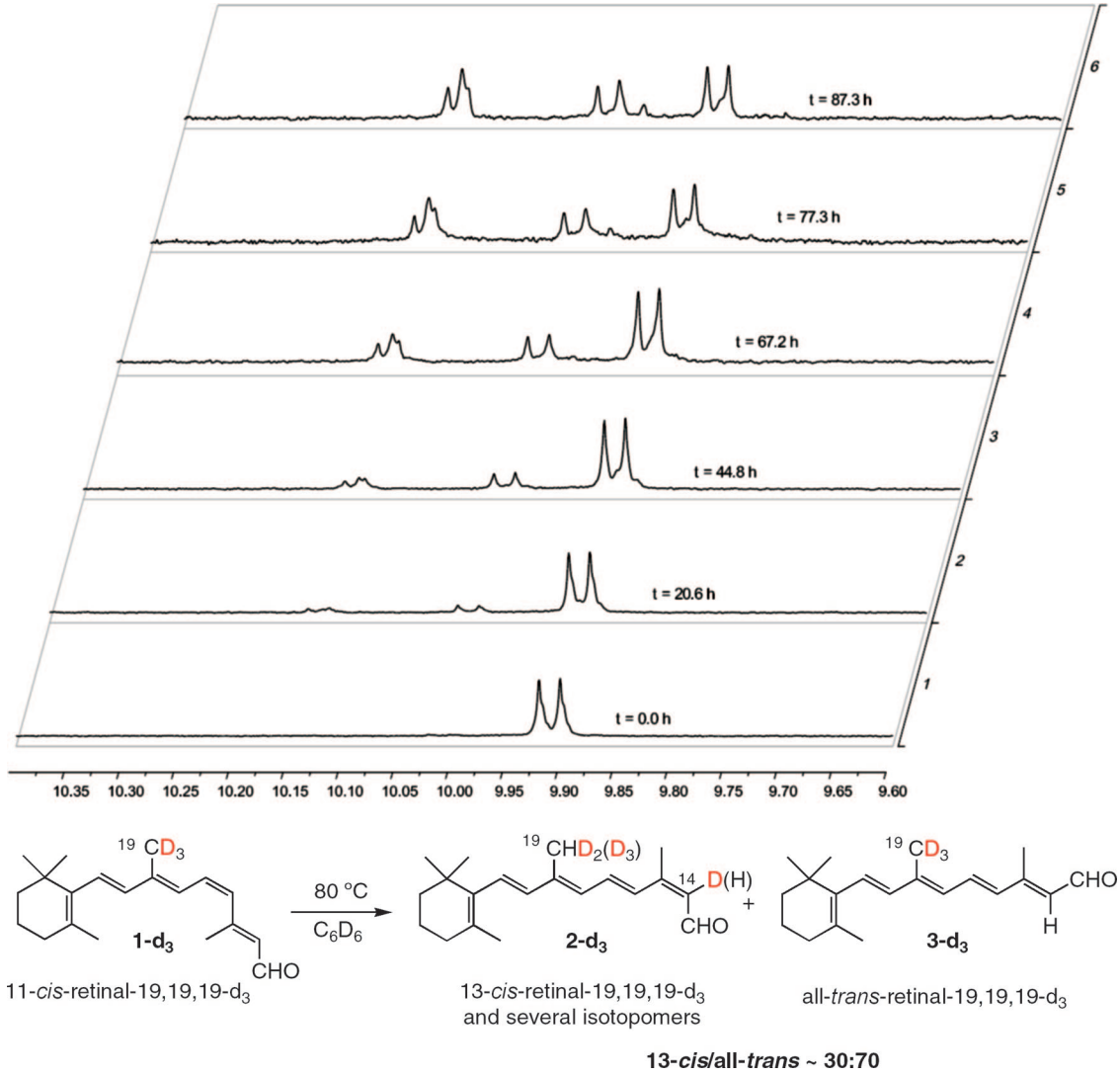


FIGURE 4. ^{1}H NMR spectra of the aldehyde region recorded over time for the thermal isomerization of 11-*cis*-retinal-19,19,19-d_3 at 80 °C (C_6D_6).

consecutive six-electron heteroelectrocyclic reactions[19] of 11,13-di-*cis*-retinal **4** through the corresponding 2*H*-pyran **6** to the final 13-*cis*-retinal **2** as indicated above. Examination of the activation energies for the sequence reveals that this alternative pathway to 13-*cis*-retinal from 11-*cis*-retinal is highly competitive (a rate-limiting step of 28.70 kcal/mol for the second [1,7]-H sigmatropic migration) with that to the *all-trans* isomer described before (27.27 kcal/mol).

The computations led us to propose that the thermal isomerization of 11-*cis*-retinal **1** yields *all-trans*-retinal **3** via internal rotation around the double bond at C11−C12 involving a diradical transition structure and 13-*cis*-retinal **2** via [1,7]-H sigmatropic shifts between the methyl group at C9 and H at C14, followed by a six-electron electrocyclic ring closure of the resulting 11,13-di-*cis*-retinal **4** and the reverse ring opening of the corresponding 2*H*-pyran **6**.[20]

The implication of a [1,7]-H sigmatropic migration reaction in the thermal isomerization of 11-*cis*-retinal was empirically investigated through deuterium-labeling experiments. If, as predicted by our calculations, such rearrangement is operating, the introduction of deuterium at the C9-CH_3 position should provide experimental evidence of the hydrogen migration. 11-*cis*-Retinal-19,19,19-d_3 **1**-d_3 was prepared and subjected to the thermal conditions (80 °C in C_6D_6) and the reaction monitored by ^{1}H NMR. Two relevant observations could be derived from this study: first, as expected, reaction times increased considerably with respect to those of the protium isotopologue due to the primary isotope effect involved in the sigmatropic shift step (cf. Figures 1 and 4); second, the product distribution is inverted in the thermal isomerization of **1**-d_3 when compared to that of **1** (see Figure 4). To our knowledge, such extreme reversal in product distribution upon isotope labeling is unprecedented. This observation could only be explained by cooperatively combining our experimental and computational results.

In the parent system **1**, a product distribution of 65/35 under kinetic control at 80 °C implies[23] that the activation energy of the [1,7]-H sigmatropic shift step is just 0.45 kcal/mol lower than the activation of the double bond for the isomerization along the diradical route **A**. Additionally, we computed through harmonic and thermochemical analysis a large value of k_H/k_D= 5.5 for the initial [1,7]-H sigmatropic shift. This KIE is in very good agreement with the values found by Okamura et al. in the vitamin D field.[24] The computed KIE of 5.5 is due to a 1.2 kcal/mol increase in the activation energy of the [1,7]-H

(23) Using the Maxwell−Boltzmann distribution.

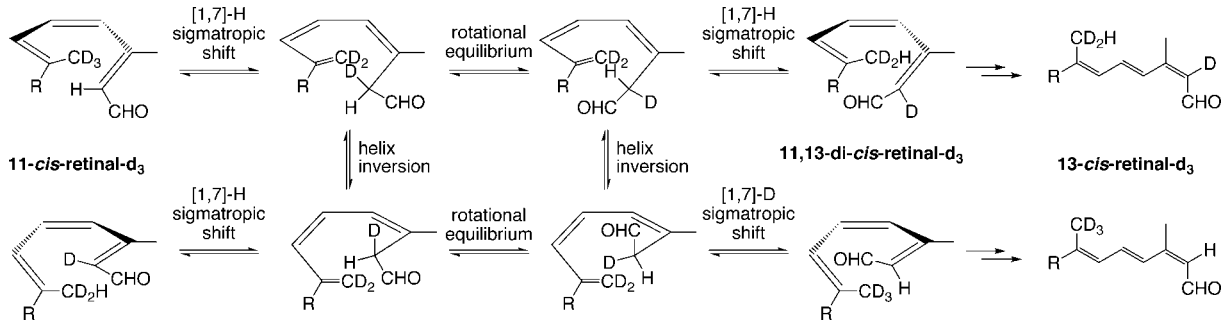
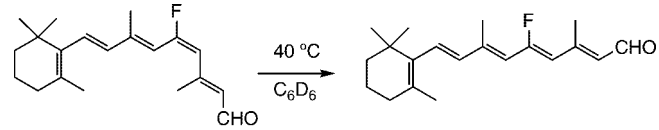


FIGURE 5. Mechanistic pathway for the formation of several isotopomers of 11-*cis*-retinal **1** and 13-*cis*-retinal **2** through [1,7]-H/D sigmatropic shifts.

sigmatropic migration step. Remarkably, this computed energy difference not only is sufficient to invert the balance of the activation energies for the competing processes but it also justifies the observed inversion in product distribution for the thermal isomerization of the deuterated substrate **1**-d_3. Thus, the **2/3** 65/35 product distribution observed in the thermal isomerization of 11-*cis*-retinal together with the **2**-d_3:**3**-d_3 30/70 distribution for the thermal isomerization of the **1**-d_3 isotopologue is also an indication of a ca. 1.2 kcal/mol energy change of the corresponding barriers, approximately. This difference is in very good agreement with our computed KIE for the [1,7]-H migration reaction. Furthermore, we also computed the expected secondary KIE values for the diradical transition state in path **A** and found this effect to be almost negligible ($k_H/k_D = 1.05$). In conclusion, our experimental and computational data provide consistent evidence suggesting that the dramatic changes in product distribution observed upon deuterium labeling arise entirely from the primary KIE affecting the [1,7]-H sigmatropic shift.

The reversibility of the [1,7]-H sigmatropic shifts together with the presence of deuterium labeling promotes H/D exchanges that produce a complex mixture of isotopomers. The identification through ^{1}H NMR spectroscopy of 11-*cis*-retinal-19,19,19-d_3, 11-*cis*-retinal-14,19,19-d_3, *all-trans*-retinal-19,19,19-d_3, *all-trans*-retinal-14,19,19-d_3, 13-*cis*-retinal-19,19,19-d_3, and 13-*cis*-retinal-14,19,19-d_3 further confirms our mechanistic proposal (see Figure 5).[25] HPLC separation of the different double-bond isomers revealed the extent of deuterium scrambling in the rearrangement manifold (see the Supporting Information).

Another corollary of the drastically different nature of the competing mechanisms detailed above is the possibility to tune the product distribution stabilizing or destabilizing selectively only one of the rate-limiting transition states (i.e., the diradical structure leading to *all-trans*-retinal or the sigmatropic shifts on route to 13-*cis*-retinal). The diradical transition state should be favored by fluorine substitution at C11.[26] 11-*cis*-11-Fluororetinal **12**, prepared as described in previous studies,[27] was subjected to thermal isomerization (Figure 6). The reaction proceeds very rapidly compared to the parent system, undergoing complete conversion at only 40 °C in 4.5 h (see the Supporting Information). This thermal rearrangement yielded all-*trans*-11-fluororetinal **13** as exclusive product, which is in good agreement with the hypothesis based on the mechanistic routes proposed for the thermal isomerization of 11-*cis*-retinal **1**.



12, 11-*cis*-11-fluororetinal **13,** all-*trans*-11-fluororetinal

FIGURE 6. Thermal rearrangement of 11-*cis*-11-fuororetinal **12**.

Conclusions

The thermal isomerization of 11-*cis*-retinal has been shown to afford a mixture of double-bond isomers, namely 13-*cis*- and *all-trans*-retinal in a 65:35 ratio, approximately. On the one hand, the formation of *all-trans*-retinal occurs through direct internal rotation around the double bond at C11 via a diradical transition state. On the other hand, the formation of 13-*cis*-retinal occurs through a complex pericyclic mechanism involving a pair of [1,7]-H sigmatropic shifts followed by a six electrons heteroelectrocyclic ring closure/opening process. Increase of the activation energy for the latter and change of product distribution was enforced by deuterium labeling of the CH_3 group at C9, which moreover proved the occurrence of a [1,7]-H sigmatropic shift in the vitamin A field.

Computational and Experimental Methods

For the thermal studies, dilute solutions of 11-*cis*-retinal **1** in C_6D_6 were heated to 80 °C in the NMR probe, and spectra were collected at the appropriate times. The synthesis of 11-*cis*-retinal-19,19,19-d_3 is described in the Supporting Information.

All of the structures presented in this work were optimized by means of density functional theory methods.[28,29] B3LYP was used in conjunction with a 6-31+G(d,p) basis set.[30] This level of theory is known to provide an excellent balance between cost and accuracy (MAD of 3.9 kcal/mol).[30] The nature of all the stationary points was determined by harmonic analysis. Due to the potential diradical character of some of the structures considered in this work, the

(24) Isotope effects (k_H/k_D of about 6) have been measured in the [1,7]-H sigmatropic shift of the previtamin D system: (a) Okamura, W. H.; Hoeger, C. A.; Miller, K. J.; Reischl, W. *J. Am. Chem. Soc.* **1988**, *110*, 973–974. (b) For simple trienes, the values are lower; see: Okamura, W. H.; Elnagar, H. Y.; Ruther, M.; Dobreff, S. *J. Org. Chem.* **1993**, *58*, 600–610.

(25) *all-trans*-Retinal-14,19,19-d_3 is formed by direct rotation about the C11−C12 double bond in 11-*cis*-retinal-14,19,19-d_3 which, in turn, is formed via [1,7]-H sigmatropic shift driven H/D scrambling from the parent 11-*cis*-retinal-19,19,19-d_3 (see Figure 5).

(26) Sreeruttun, R. K.; Ramasami, P.; Wannere, C. S.; Paul, A.; von R. Schleyer, P.; Schaeffer, H. F., III. *J. Org. Chem.* **2005**, *70*, 8676–8686.

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(28) (a) Becke, A. D. *Phys. Rev. A* **1988**, *38*, 3098–3100. (b) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648–5652.

(29) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B.* **1988**, *37*, 785–789.

(30) Foresman, J. B. Frisch, *Æ. Exploring Chemistry with Electronic Structure Methods*, 2nd ed.; Gaussian, Inc.: Pittsburgh, PA, 1996.

internal and external stability of the wave functions was computed via the Hermitian stability matrices **A** and **B** in all cases.[31] For all structures exhibiting unstable restricted wave functions, the spin-symmetry constraint of the wave function was released (i.e., expanding the SCF calculation to an unrestricted space, UB3LYP), leading to stable unrestricted wave functions.

The methodology here described yielded very accurate activation barriers for the 11-*cis*-retinal to *all-trans*-retinal isomerization ($\Delta G^{\#}$= 27.3 kcal/mol at the B3LYP level) as confirmed through comparison with experimental data ($\Delta G^{\ddagger} = 26.2$ kcal/mol).[7]

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Supporting Information Available: Cartesian coordinates, SCF energies, and the number of imaginary frequencies for each structure are available. A two-dimensional potential energy surface of the direct double bond isomerization and procedures for the preparation of 11-*cis*-retinal-19,19,19-d_3, protocols followed for the kinetic experiments and NMR spectra of the isomerization producs are also provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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(31) Bauernschmitt, R.; Ahlrichs, R. *J. Chem. Phys.* **1996**, *104*, 9047–9052.