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Conformational Analysis of Retinoic Acids: Effects of Steric Interactions on Nonplanar Conjugated Polyenes

Bryan D. Cox[†], Donald D. Muccio, and Tracy P. Hamilton^{*}

Department of Chemistry, University of Alabama at Birmingham, Birmingham, AL 35294

Abstract

Retinoic acids and other vitamin A analogs contain a trimethylcyclohexenyl ring in conjugation with a polyene chain joined at carbon-6 (C6) and carbon-7 (C7). A MP2-SCS/cc-pVDZ//B3LYP/6-31G(d) 2-D potential energy surface was computed for all-*trans* retinoic acid, which had 6 minima (3 enantiomeric pairs). The global minima were distorted *s-gauche* enantiomers ($\phi_{6-7} = 53^\circ$) with half-chair conformations of the ring. Distorted *s-gauche* enantiomers ($\phi_{6-7} = 55^\circ$) with inverted half-chair ring conformations were 1.7 kJ/mol above the global minima. The *s-trans* enantiomers ($\phi_{6-7} = 164^\circ$) were 11.3 kJ/mol above the global minima. Steric energies were computed by the method of Guo and Karplus to identify key structural elements in retinoic acids which determines their conformation. Small molecule crystal structures in the CCDC database with trimethylcyclohexenyl ring and exocyclic double bonds have ring-chain geometries near to one of the 6 energy minima of retinoic acids, except for retinaldehyde iminium cations.

Keywords

Retinoic acid; conformational analysis; steric energy; substituted cyclohexene ring inversion; β ionone; retinaldehyde iminium cations

Introduction

Well before the discovery of vitamin A by McCollum in 1907, many cultures recognized that dietary intake of liver/fish oils reversed night blindness[1]. Today it is well recognized that adequate dietary intake of vitamin A (retinol) from animal sources or provitamin A

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^{*}Corresponding Author: hamilton@uab.edu, CHEM 277, Department of Chemistry, UAB, Birmingham, AL 35294, Telephone 205-515-6935, Fax 205-934-2543.

[†]Present Address: Department of Chemistry, Emory University, Atlanta, GA 30322

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SUPPLEMENTARY INFORMATION

Supplementary Information Table 1 summarizes the τ_{6-7} and τ_{2-3} values obtained from CCDC crystal structures that contain a trimethylcyclohexenyl ring in conjugation with a polyene chain. Supplementary Information Table 2 provides the energy (in hartrees) of the low energy conformers obtained using the SCS-MP2/cc-pVDZ//B3LYP/6-31G(d) method. Supplementary Information Tables 3 and 4 report low vibrational frequencies that verify the largest molecule minima are truly minima. Supplementary Information Table 5 has Cartesian coordinates in angstrom for all minima.

from plants (carotenoids like β -carotene) is essential for normal nutrition[2]. In mammals, vitamin A dependent tissues first oxidize retinol to retinaldehyde (vitamin A aldehyde), which is the chromophore used in the visual system. Retinaldehyde is irreversibly oxidized to retinoic acid (vitamin A acid), which is essential for normal epithelial tissue growth and differentiation. Retinoic acid prevents epithelial tissues from developing cancer-like lesions first observed in vitamin A deficient animals[3], but it can not restore vision in vitamin A deficient animals. In 1987, the discovery of nuclear retinoic acid receptors (RARs) independently by the Chambon and Evans groups highlighted the role of retinoic acid as a hormone whose signaling controls epithelial cell growth, differentiation and development[4,5]. Three retinoic acid isomers (all-*trans*-retinoic acid or ATRA; 13-*cis*-retinoic acid or 13cRA; 9-*cis*-retinoic acid or 9cRA – Figure 1) are potent agonists for RARs and are currently used clinically to treat skin diseases or cancers[6].

Naturally occurring vitamin A analogs (retinol, retinaldehyde, retinoic acid), carotenoids (α -carotene, β -carotene, γ -carotene), and some terpenes (e.g., β -ionone) contain a common structural feature: one or two trimethylcyclohexenyl rings in conjugation with a polyene chain (Figure 1A). In 1963 Stam and MacGillavry[7] determined the first crystal structure of ATRA in a triclinic crystal. The C5-C6 double bond of the trimethylcyclohexenyl ring of ATRA was in a non-planar *cis*-like conformation relative to the conjugated polyene chain (C6-C7 torsion angle of $\sim 35^\circ$). Since this first observation 50 years ago, non-planar polyene structures about the trimethylcyclohexenyl ring have also been observed in x-ray crystal structures of 13-*cis*-retinoic acid[8], retinaldehydes[9–11], and β -carotenes[12–14]. Ten years after the first observation of a distortion in the polyene chain of ATRA, Stam solved the crystal structure of ATRA in a monoclinic crystal[15]. In contrast to their previous structure, the C5-C6 double bond of the trimethylcyclohexenyl ring of ATRA was nearly planar and more conjugated with the polyene chain (C6-C7 torsion angle of 193°). Interestingly, the monoclinic crystal was meta-stable and irreversibly converted to the triclinic form upon heating to 80°C . Other vitamin A analogs crystallize with a nearly planar *s-trans* conformation between the polyene chain and the trimethylcyclohexenyl ring. These structures are fewer in number and include retinaldehyde iminium salts[16,17].

In 1971, Honig *et al.* [18] used semi-empirical methods to establish that the distorted *s-cis* geometries (*s-gauche*) of β -ionone and retinaldehydes were lower in energy than the planar *s-trans* geometries by about 20 kJ/mol. Their NMR measurements (NOE and J-coupling analyses) supported significant populations of the non-planar conformers of β -ionone and retinaldehydes. The Poirier group executed a series of Hartree-Fock investigations of retinal analogues, including rotation about the 12-*s* bond [19] and rotation about the 6-*s* bond [20]. The analog that Poirier and Yadav used in the latter study is almost identical to our compound **11** (*vide infra*). In 2008, 6 low-energy conformers of β -ionone were determined using the B3LYP/6-31G(d,p) level of theory, but the *s-gauche* conformers were similar in energy to the *s-trans* conformers (only 2 kJ/mol higher in energy than *s-gauche*)[21]. Using a variety of semi-empirical or *ab initio* computational approaches, the low-energy conformers of carotenes (including β -carotene) have also been reported in the literature. For β -carotene, the low energy conformers had a distorted *s-gauche* orientation of the trimethylcyclohexenyl ring relative to the polyene chain, but the stability of the *s-gauche*

conformer relative to the *s-trans* conformer ranged considerably (4–30 kJ/mol)[22–25]. Over the past twenty years, many high-level computational studies were performed on retinaldehyde and corresponding Schiff bases to better understand their spectroscopic and chiro-optical properties, or to examine their role in photochemistry as chromophores for rhodopsin and bacteriorhodopsin[26–33]. The conformational analysis of retinoic acids were studied to a lesser extent. Recently, an important study by Merz and coworkers thoroughly searched the polyene chain conformations of retinoic acids at a high level of theory. They showed significant errors in the conformations of the polyene chain in crystal structures of retinoic acid bound to proteins[34,35].

In this study we address how the trimethylcyclohexenyl ring inversion influences the potential energy about the C6-C7 torsion in retinoic acids. Experimental[36] and computational methods[37] have established that cyclohexenes interconvert rapidly between two half-chair conformations with C2-C3 torsion angles (vitamin A numbering) of about 60°. In 2002, Shishkina *et al.* [38] demonstrated that cyclohexene inverts through a twisted-boat intermediate, with an activation energy of 23 kJ/mol at the MP2 level of theory. The trimethylcyclohexenyl rings of vitamin A analogs or β -carotene are expected to invert between similar half-chair conformations with the C16 and C17 methyl groups at C1 (*gem*-dimethyl groups) flipping between *pseudo*-axial and *pseudo*-equatorial positions (Figure 1B). Guo and Karplus evaluated steric energies in simple methyl-substituted 1,3-butadienes[39], and we use a similar approach here to reveal the steric interactions/energies present in retinoic acid conformers. We next survey all crystal structures with a trimethylcyclohexenyl ring system with an exocyclic bond or polyene chain and evaluate if they populate the low-energy minima determined for retinoic acids. To study why retinaldehyde alkyl iminium salts adopt planar trimethylcyclohexenyl ring/polyene chain geometries in crystal structures, we calculate the energies of the *s-gauche* and *s-trans* conformers of vitamin A analogs with different terminal functional groups.

Computational Methods

Geometry optimizations and energy calculations were performed using the Parallel Quantum Solutions (PQS) version 3.3 software package[40] on local clusters. The results presented were obtained from SCS-MP2[41] single-point energy calculations with Dunning's cc-pVDZ basis set[42] using B3LYP/6-31G(d)[43,44] optimized structures. The C5-C6-C7-C8 torsional (τ_{6-7}) energy potentials of molecules **1–11** (Figure 2) were calculated every 10° with constrained optimization starting from $\tau_{6-7} = 180^\circ$. The SCS-MP2/cc-pVDZ//B3LYP/6-31G(d) potential energy curves were scanned by 1° increments in the vicinity of minima in order to obtain best predictions for τ_{6-7} . To reveal artifacts, energies were calculated by changing the torsional angle by 10° in each direction. Bond orders were computed using the Mulliken population analysis at the B3LYP/6-31G(d) level. The ring puckering coordinates were determined as described by Cremer and Pople[45] using the RING program based on the published algorithm[46].

Torsional steric energies (δE) were calculated from the torsional energies using the approach described by Guo and Karplus[39]. The steric energy about the single bond, $\delta E(\tau_{6-7})$, was determined as:

$$\delta E(\tau_{6-7}) = [E(B, \tau_{6-7}) - E(A, \tau_{6-7})] - [E(B, \tau_{0,6-7}) - E(A, \tau_{0,6-7})]$$

where $E(B, \tau_{6-7})$ and $E(A, \tau_{6-7})$ are the torsional potentials for the substituted molecule (B) and the reference molecule (A), respectively. $E(\tau_{0,6-7})$ is the torsion angle at which $[E(B, \tau_{6-7}) - E(A, \tau_{6-7})]$ is the global minimum. The reference molecule was 1,3-butadiene for molecules **2–11**, (2E,4E,6E,8E)-undeca-2,4,6,8,10-pentaenoic acid for ATRA, (2Z,4E,6E,8E)-undeca-2,4,6,8,10-pentaenoic acid for 13cRA, and (2E,4E,6Z,8E)-undeca-2,4,6,8,10-pentaenoic acid for 9cRA.

The 2D potential energy surface of ATRA was generated by varying τ_{6-7} every 15° and the C1-C2-C3-C4 torsional angle (τ_{2-3}) every 10° . The τ_{6-7} was constrained, and τ_{2-3} was varied between -80° and $+80^\circ$ by 10° and a constrained optimization was performed. The torsional energy plot was generated with Origin 8.0, and the energies were presented relative to the global minimum.

Small-molecule crystal structures that contained the trimethylcyclohexenyl ring with an exocyclic propylene fragment were collected from the CCDC small-molecule crystal structure database using the Conquest software package. The search yielded 27 total crystal structures. Five of the x-ray structures were β -carotenoids that contained two trimethylcyclohexenyl rings. The list of τ_{6-7} , τ_{2-3} values for the published crystal structures are provided in Supplementary Information.

Results and Discussion

Methyl and t-Butyl Substituted 1,3-Butadiene Analogs of Retinoic Acid

Initially, we evaluated several levels of theory to find a method which determined energies and geometries of conjugated systems with sufficient accuracy yet was computationally efficient for molecules of the size of vitamin A. The geometries predicted by the B3LYP/6-31G(d) level of theory are closer to experimental results than the geometries predicted by many other levels of theory with similar computational efficiency[47]. Computations performed at the SCS-MP2 level with large basis sets[48] resulted in energies near experimental and CCSD(T) results[49]. Furthermore, a systematic evaluation of different levels of theory showed that the MP2/cc-pVDZ energies were comparable to MP2/CBS[50]. Here we used single-point calculations performed at the SCS-MP2/cc-pVDZ level of theory using the B3LYP/6-31G(d) optimized structures.

The potential energy curve for of 1,3-butadiene (**1**) was computed as a function of the torsional angle about the carbon-carbon single bond (Figure 3A). The *s-trans* conformer at $\tau = 180^\circ$ was the global minimum. Two local minima at $\tau = \pm 37^\circ$ (*s-gauche*) were found which were 12.1 kJ/mol greater in energy than the *s-trans* conformer (Table 1). Three maxima were found at $\tau = \pm 101^\circ$ and at $\tau = 0^\circ$ (*s-cis*) that were 25.1 and 15.1 kJ/mol greater in energy than the *s-trans* conformer, respectively. All minima and maxima of the 1,3-butadiene torsional potential from SCS-MP2/cc-pVDZ// B3LYP/6-31G(d) were within 0.8 kJ/mol of CCSD(T) calculations with large basis sets[49]. Furthermore, the energy of the *s-gauche* conformer relative to the *s-trans* conformer was very near to the experimentally-derived enthalpy change for *s-trans* to *s-gauche* inversion (12.1 kJ/mol)[50].

To determine the low-energy conformers of retinoic acids, the steric interactions between the trimethylcyclohexenyl ring and the polyene chain that govern them, torsional energy potentials were calculated for 1,3-butadiene analogs with alkyl substitutions that systematically mimicked portions of the trimethylcyclohexenyl ring or polyene chain of retinoic acids. [Note the use of the vitamin A numbering scheme so that a direct comparison can be made between the position of an alkyl substituent on butadiene and the corresponding group in vitamin A acids.] Four butadiene analogs (**2–5**) with methyl substitutions on the butadiene frame were examined to estimate the steric effects from C1, C4, C9, and C18 of retinoic acids. For butadiene analogs **2–5**, the global energetic minima occurred at $\tau_{6-7} = \pm 180^\circ$ (Figure 3A). Steric interactions for methyl-substituted 1,3-butadiene analogs **2–5** were largest in the planar *s-cis* and *s-trans* conformations and were minimal at $\tau_{6-7} = \pm 90^\circ$ (Figure 3B). The torsional energy potential of butadiene-analog **2** contained methyl groups to mimic C4 and C9 of retinoic acids. As expected, the energies were within 0.4 kJ/mol of those found for 1,3-butadiene over all torsional angles, and these methyl groups introduced essentially no steric effects onto the energy torsional potential for the C6-C7 torsional angle.

When methyl groups were introduced to mimic either C1 (**3**) or C18 (**4**) of retinoic acid, the energy of the *s-gauche* conformers decreased relative to the global minimum at *s-trans* (Figure 3; Table 1). When the steric energy curve was examined, the more informative perspective was that the steric energy of the *s-trans* conformer increased relative to *s-gauche* conformer. The steric interactions in the *s-trans* structures occurred between the C18 methyl group and H7 in the case of analog **3** (5.4 kJ/mol) or between the C1 methyl group and H8 in the case of analog **4** (3.8 kJ/mol). Butadiene analog **5** included methyl group substitutions that mimicked both C1 and C18. The steric energy of the *s-trans* conformer of butadiene analog **5** increased by 14.2 kJ/mol, which was nearly the sum of the steric interactions from **3** and **4**. Guo *et al.* [39] previously performed calculations on these butadiene analogs at the HF/6-31G(d) level of theory, and they also demonstrated approximately additive steric energies. The τ_{6-7} of the *s-gauche* conformer of butadiene analog **5** was twisted 17° more than butadiene analog **2** (Table 1). The *s-gauche* conformer of butadiene analog **5** was more distorted than **3** and **4** in order to minimize steric interactions of C18 methyl with H8 and C1 methyl with H7, which were greatest at the *s-cis* barrier ($\tau_{6-7} = 0^\circ$).

Introducing methyl substitutions into the 1,3-butadiene frame lowered the relative energy of the *s-gauche* conformers and increased the distortion of this conformer (especially for analog **5**), but methyl substitutions alone were not sufficient to destabilize the *s-trans* conformer to the extent required in order to make the *s-gauche* conformers the global minima. This suggests that the *gem*-dimethyl groups (C16 and C17) at C1 in the trimethylcyclohexenyl ring of retinoic acids are important groups for controlling the relative energies of the low-energy conformers. To investigate the steric effects of the C16/C17 *gem*-dimethyl groups on the C6-C7 torsional potential, torsional energy profiles were generated for analogs **6** and **7** which included a *t-butyl* group at the C6 position on the 1,3-butadiene frame (Figure 2). Butadiene analog **7** also included a methyl group to mimic C18 of retinoic acid. For these two butadiene analogs, the *s-gauche* conformers were the global minima and were significantly more stable than the *s-trans* conformer (Figure 3A). For analog **6**, the *s-trans* conformer was in a shallow well that was 14.2 kJ/mol above the *s-gauche* conformers.

Analogue **7** was effectively unstable at the *s-trans* conformer, and this analogue possessed a high-energy barrier (30.1 kJ/mol) at $\tau_{6-7} = 180^\circ$. When the steric energies were examined (Figure 3B), the *s-trans* conformer of butadiene analogue **6** contained 30.1 kJ/mol of steric interactions when planar (Table 1) which was mainly attributed to the steric clash between the *t-butyl* group and H8. This steric energy was much larger than the steric energies for the *s-gauche* conformers (<2.1 kJ/mol for **1–6**). For analogue **7**, this effect was enhanced by a factor of two; the *s-trans* conformer had 50.6 kJ/mol of steric energy compared to 5.0 kJ/mol for the *s-gauche* conformer. As a consequence of these steric interactions, the optimized *s-gauche* conformers were significantly distorted about τ_{6-7} (Table 1). The low-energy *s-gauche* conformers for butadiene analogue **7** adopted a conformation in which the *t-butyl* was staggered with respect to the H7 (Figure 4A). This conformation of the *t-butyl* group is unlikely to occur in retinoic acids since the orientation of the C16/C17 *gem*-dimethyl group is restricted by the cyclohexenyl ring (Figure 4B).

Methyl-Substituted Cyclohexene Analogs of Retinoic Acid

To better understand how the trimethylcyclohexenyl ring influences the steric interactions about the C6-C7 torsion of retinoic acids, the torsional potentials of alkyl-substituted cyclohexene analogs with an exocyclic propylene group were computed (**8** – **11**). Previous studies demonstrated that cyclohexene exists predominantly in two low-energy half-chair conformations[38], and the conformation of the ring is defined by the C2-C3 torsional angle (τ_{2-3}). For the $\tau_{2-3} \sim +60^\circ$ ring conformation, the C17 *gem*-methyl group is *pseudo*-axial, and the C17 methyl group is *pseudo*-equatorial in the $\tau_{2-3} \sim -60^\circ$ ring conformation (Figure 1B). The general ring puckering coordinates (Q , ϕ , and θ)[45] for these two ring conformations are provided in Figure 1B, and the optimized ring structures of cyclohexene analogs **8** – **11** did not significantly deviate from these values.

The torsional potentials for the cyclohexene analogs **8**–**11** are shown only for one ring conformation ($\tau_{2-3} \sim +60^\circ$) (Figure 5A). The opposite ring conformation ($\tau_{2-3} \sim -60^\circ$) displayed a mirror image potential curve (data not shown). The τ_{6-7} torsional profile and steric energy profile for cyclohexene analog **8** was very similar to the methyl-substituted butadiene analog **4** (Figure 5A and 5B). For cyclohexene analog **8**, the global energetic minimum was *s-trans*, and the *s-gauche* conformers were 9.6 and 10.9 kJ/mol higher in energy. Analog **9** had a methyl group to mimic the C18 group of vitamin A, and the energy of the *s-gauche* conformer decreased relative to the global minimum at *s-trans*. Congruent to the results from the butadiene analogs **6**–**7**, the *s-gauche* conformers became the global minima for the cyclohexene analogs **10**–**11**, which included the C16/C17 *gem*-dimethyl substitutions on the cyclohexenyl ring. The *s-trans* conformer for **10** was a local minimum, whereas it was a shoulder on the potential curve for **11**. This was in contrast to the shallow *s-trans* minimum of butadiene analogs **6** and **7**. For butadiene analogs **6** and **7**, the *t-butyl* group was flexible and optimized in a staggered conformation relative to H7. The presence of the cyclohexene ring in analogs **10** and **11** constrained the C16/C17 to eclipse H7, raising the energy of the *s-gauche* conformers (Figure 4). Therefore, the *s-gauche*/*s-trans* energy difference was smaller for **10** and **11** than for **6** and **7**. The results by Poirier and Yadav [20] for a compound very similar to our **11** gave good agreement with our curve, considering the limitations in 1989 of a small basis, Hartree-Fock wavefunction and absence of high

accuracy benchmarks. They concluded that τ_{6-7} angles were very close to +60 and -60 for the global minima, *s-trans* is not a minimum, and that the barriers between the *s-gauche* conformations were 18.9 and 21.7 kJ/mol.

The τ_{6-7} torsional energy potentials of butadiene analogs **1–7** were symmetric about 180°. The torsional energy potentials of cyclohexene analogs **8–11** were asymmetric due to the presence of a single conformation of the cyclohexenyl ring ($\tau_{2-3} \sim +60^\circ$; Figure 1B) with the C17 methyl group *pseudo*-equatorial (**17Eq**). For this **17Eq** half-chair ring conformation, the distorted *s-gauche* conformers of **10–11** with **positive** τ_{6-7} were the global minima (Conformer **P17Eq**). The second distorted *s-gauche* conformer with **negative** τ_{6-7} (Conformer **N17Eq**) was slightly higher in energy for **10** and **11**. In each *s-gauche* conformer, the C18 methyl tilted out of the C5 = C6 double bond plane (C7-C6-C5-C18 = +8°) disrupting the symmetry of the steric interactions between C18 methyl and H8. For cyclohexene analog **10**, the *s-trans* conformer with τ_{6-7} near to 180° was much higher in energy (Conformer **T17Eq**) due to 21.3 kJ/mol steric energy primarily from C16/C17 interactions with H8. For cyclohexene analog **11**, the **T17Eq** conformer was further destabilized due to significant steric interactions between the C16/C17 and H8 (present in cyclohexene analog **10**) and between C18 and H7, summing to 33.4 kJ/mol of steric interactions (Figure 5B). Thus for the cyclohexene analog **11**, which most closely mimicked retinoic acids, the two distorted *s-gauche* conformers convert to a high-energy *s-trans* conformer (meta-stable) with activation energies ranging from 12.5 to 16.3 kJ/mol:

Calculations on Retinoic Acids: ATRA, 9cRA, 13cRA

To explore the impact of the increased conjugation of the retinoic acid polyene chain and *E/Z* isomerization, the τ_{6-7} torsional potentials of ATRA, 9cRA, and 13cRA were compared to cyclohexene analog **11** for a single trimethylcyclohexenyl ring conformation (**17Eq** or $\tau_{2-3} \sim +60^\circ$) (Figure 6A). The torsional potentials for ATRA, 9cRA, and 13cRA were surprisingly close, within 0.8 kJ/mol of each other for all τ_{6-7} angles. This demonstrated that the steric factors affecting torsion around the C6-C7 bond were primarily local in nature. The torsional energy potentials for the **17Eq** ($\tau_{2-3} \sim +60^\circ$) ring conformation contained three conformers: the low-energy conformer at $\tau_{6-7} \sim 60^\circ$ (**P17Eq**), followed by a second distorted *s-gauche* conformer at $\tau_{6-7} \sim 300^\circ$ (**N17Eq**), and a high energy conformer at $\tau_{6-7} \sim 170^\circ$ (**T17Eq**). Exact values for τ_{6-7} for each retinoic acid are in Table 1. The torsion potential curve of cyclohexene analog **11** was very similar to those of retinoic acids. The largest difference was that the *s-trans* conformer (**T17Eq**) is a minimum for ATRA/9cRA/13cRA, because the relative energy of *s-trans* has been lowered by 2.1 kJ/mol compared to cyclohexene analog **11** (Figure 6A; Table 1). The C6-C7 bond order of **T17Eq** was greater for ATRA/9cRA/13cRA than cyclohexene analog **11** (1.128 for ATRA or 9cRA versus 1.102 for analog **11**) due to increased conjugation from the extended polyene chain of ATRA/9cRA/13cRA relative to cyclohexene analog **11**. The steric energy potentials of ATRA/9cRA/13cRA were very similar to cyclohexene analog **11** for all τ_{6-7} torsional angles (Figure 6B), indicating that this cyclohexene analog mimicked steric interactions extremely well.

The effects of the trimethylcyclohexenyl ring inversion on the C6-C7 torsional potential were examined by calculating the 2-D potential energy surface of ATRA with respect to both τ_{6-7} and τ_{2-3} torsional angles (Figure 7). The surface revealed 6 low-energy conformers for ATRA, which are three pairs of enantiomers. Global minima occurred at the *s-gauche* enantiomers (**P17Eq** and **N17Ax**), another pair of *s-gauche* enantiomers which were 1.7 kJ/mol higher in energy (**N17Eq** and **P17Ax**), and two *s-trans* enantiomers (**T17Eq** and **T17Ax**) where were 11.3 kJ/mol greater in energy than the global minima. The structures of the 6 low-energy conformers are provided in Figure 8 and their important structural features are summarized in Table 2.

The ring inversions between *s-gauche* conformers (**P17Eq** $\rightleftharpoons$ **P17Ax** or **N17Ax** $\rightleftharpoons$ **N17Eq**) are connected by a saddle point with an activation energy of 25.5 kJ/mol above the global minimum (black X's in Figure 7). The conformation of the ring at the transition state was a twist-boat (ring puckering coordinates of $Q = 0.58$; $\phi = 0, 180^\circ$; and $\theta = 90^\circ$), which was the same type of transition state reported for cyclohexene [38]. The ring interconversion for *s-gauche* conformers occurred without significant change in τ_{6-7} . For *s-trans* conformers (τ_{6-7} at 164°), high-energy intermediates (67 kJ/mol) prevented ring inversion without changes in τ_{6-7} . To determine the low-energy pathways of ring inversion for *s-trans* conformers (without proceeding first through *s-gauche* minima), τ_{2-3} was varied from -60° to $+60^\circ$ starting from the *s-trans* conformers while τ_{6-7} was allowed to optimize. The ring inversion between *s-trans* conformers are connected by a transition states at τ_{6-7} of 138° (black + 's in Figure 7) with twist-boat ring conformations (ring puckering coordinates of $Q = 0.58$; $\phi = 0, 180^\circ$; and $\theta = 90^\circ$). These transition states were 26.4 kJ/mol above the *s-trans* conformers, and 37.2 kJ/mol higher than the global minima. This pathway followed along energetic ridges which are more visible when the 2D potential energy surface is viewed edge-on (Supplementary Information Figure 1). The pathway resembles a road on a mountainside, where a slight deviation to the side away from the peaks will result in a trajectory down towards the *s-gauche* minima. Limited twisting about τ_{6-7} provided a ring inversion pathway between the *s-trans* conformers that avoided the high-energy planar intermediate without passing through the *s-gauche* conformation.

Small Molecule X-Ray Structures with β -ionone Rings

The CCDC small molecule crystal structure database was searched for structures that contained a trimethylcyclohexenyl ring (' β -ionone' ring) in conjugation with an exocyclic propylene fragment similar to retinoic acids and other vitamin A analogs. The search yielded 27 unique structures, five of which were β -carotenoids that contained two ring-polyene chain fragments (Supplementary Information). The τ_{6-7} , and τ_{2-3} torsional angles obtained from the crystal structures were placed onto the calculated 2-D potential energy surface of ATRA (shown as dots in Figure 9). Only one crystal structure had a conformation which was not contained within the low-energy wells of the 2-D potential energy surface of retinoic acids (Figure 9). There were 18 crystal structures of the ringpolyene chain with conformations within the global *s-gauche* minima wells (**P17Eq** or **N17Ax**). There were 5 crystal structures with conformations in the slightly higher-energy *s-gauche* conformer wells (**N17Eq** and **P17Ax**). Only 8 crystal structures were positioned near to the *s-trans* minima (**T17Eq** and **T17Ax**). Two of the three reported crystal structures of ATRA had

conformations that were near the global *s-gauche* minima ($[\tau_{6-7}, \tau_{2-3}] = [42^\circ, 57^\circ]$) [15,51]. The third ATRA crystal structure had a conformation near the *s-trans* minimum ($[\tau_{6-7}, \tau_{2-3}] = [194^\circ, -63^\circ]$) [15], but as mentioned previously this structure was meta-stable and interconverted to the distorted *s-gauche* conformer when heated. To date, there are no published crystal structures of 9cRA. The 2D potential energy surface of retinoic acids provided a surface for understanding the ring-polyene chain conformers of not only retinoic acids but also many other vitamin A structures and related analogs.

Nearly all of the small molecule crystal structures displayed a half-chair conformation of the trimethylcyclohexenyl ring that fell within 10° of the predicted minima of $\tau_{2-3} = \pm 60^\circ$. However, the structure of 2,6-di-*cis*-4-hydroxyretinoic acid γ -lactone [52] had a distorted *s-gauche* conformation with a nearly planar trimethylcyclohexenyl ring ($[\tau_{6-7}, \tau_{2-3}] = [48^\circ, 5^\circ]$). In this structure the methylene carbons (C2, C3) and the C16,C17 *gem*-dimethyl group of the trimethylcyclohexenyl ring had high B values. Thackeray and Gafner [52] proposed that the trimethylcyclohexenyl ring of the γ -lactone was a 1:1 mixture of the two low-energy half-chair conformations and not a transition state. Our calculations are consistent with the rapid interconversion between the *s-gauche* conformations through a ~ 25 kJ/mol transition state of the trimethylcyclohexenyl ring of this γ -lactone.

Like the γ -lactone structure, the crystal structure of 13cRA had a disordered trimethylcyclohexenyl ring [8]. Unlike the γ -lactone structure, the C6-C7 torsional angle in the crystal structure of 13cRA was nearly *s-trans* ($\tau_{6-7} = 180^\circ$). In this structure, the crystal was solved using two equally populated half-chair conformations of the trimethylcyclohexenyl ring ($\tau_{2-3} = \pm 66^\circ$). Malpezzi *et al.* [8] proposed that the disorder could be either static or dynamic. Based on the 2D potential energy surface for ATRA/9cRA/13cRA, inversion of the trimethylcyclohexenyl ring of 13cRA restricted to $\tau_{6-7} = 180^\circ$ would have a large energy barrier due to a planar cyclohexene transition state. Because of the high energy inversion barrier and the inability of 13cRA to distort about τ_{6-7} without cracking the crystal, dynamic disorder in the trimethylcyclohexenyl ring was unlikely to be present in the 13cRA crystal.

End Group Effects on the *s-gauche*/*s-trans* Energy Difference of Vitamin A Analogs

The room temperature Boltzmann populations of the 6 low-energy conformers favor the 2 pairs of *s-gauche* enantiomers over the *s-trans* enantiomers by a large ratio (99:1). Eight small molecules crystal structures were found in the planar *s-trans* conformers (**T17Eq** or **T17Ax**). Crystal packing forces certainly play an important role in stabilizing high energy conformers. However, three of the crystal structures that occupy *s-trans* conformational space (either **T17Eq** or **T17Ax**) were retinaldehyde iminium cations (see Supplemental Information). To evaluate the effects of the vitamin A end group on C6-C7 conformer energies, the energies of *s-gauche* and *s-trans* conformers of retinoids with different end groups were calculated using the **17Eq** ring conformation (Table 3). End groups ranged in polarity and were selected to reflect the oxidation state of the vitamin A (alcohol) and related vitamin A analogs (aldehyde; carboxylic acid; methyl ester; methyl imine; methyl iminium cation). A methyl group was used to mimic less polar structures like carotenenes. The *s-gauche* conformer for vitamin A and analogs with polar groups were generally 11.3 – 11.7

kJ/mol lower in energy than the *s-trans* conformer for all polar end groups except for the retinaldehyde methyl iminium cation. For this polar and charged end group, the *s-trans* conformer was only 5.0 kJ/mol greater in energy than the *s-gauche* conformers. Terstegen and Buß [28] observed this previously in their calculations on retinaldehyde iminium cations using DFT calculations.

To further explore this change in relative energies, the torsion energy potentials of the all-*trans*retinaldehyde methyl imines and methyl iminium cations were calculated in a single ring conformation (Figure 10). The torsion energy potential for retinaldehyde methyl imine had a global minimum at $\tau_{6-7} \sim 60^\circ$ (**P17Eq**), a local minimum at $\tau_{6-7} \sim 300^\circ$ (**N17Eq**) that was 1.7 kJ/mol greater in energy, and a high-energy local minimum at $\tau_{6-7} \sim 160^\circ$ (**T17Eq**) that is 10.9 kJ/mol greater in energy than the global minimum (similar to retinoic acids, compare Figure 10 to Figure 6). For the retinaldehyde methyl iminium cation, the **P17Eq** *s-gauche* minimum was less twisted by about 10° ($\tau_{6-7} = 45^\circ$) compared to other vitamin A analogs (Table 3). The relative energy of the *s-trans* conformer (**T17Eq**) was lowered to only 5.0 kJ/mol above the global minima. The C6-C7 bond orders were compared for distorted *s-gauche* conformers of the retinaldehyde methyl iminium cation to its unprotonated form. The retinaldehyde methyl iminium cation had larger bond orders for the rotatable bonds (1.134) than those in distorted conformers of other vitamin A analogs with uncharged end groups (1.035 ± 0.003) (consistent with more conjugation and hence less twisted τ_{6-7}). Increased conjugation also occurred in the *s-trans* conformers of the retinaldehyde methyl iminium cation (1.192 for the retinaldehyde methyl iminium cation compared to 1.126 ± 0.002 for other vitamin A analogs), which offset steric interactions and lowered the energy of these conformers to energies comparable to those of the *s-gauche* conformers.

Conclusions

This study evaluates the structure and energetics of low-energy conformations of retinoic acids. We provide computational evidence that retinoic acids (and other molecules with the β -ionone ring) have six low-energy conformers with respect to the trimethylcyclohexene ring - polyene chain orientation. For retinoic acids, the relative energies of these conformers are independent of *E/Z*-isomerization about either C9-C10 (9cRA) or C13-C14 (13cRA). We determine the *s-gauche* conformers (2 sets of enantiomeric pairs) are more stable than *s-trans* conformers primarily due to a 21.3 kJ/mol steric interaction between the C16/C17 gem-dimethyl groups and H8. We provide evidence that ring inversion of retinoic acids (and other molecules which contain the β -ionone ring) occur between *s-gauche* or *s-trans* conformers with activation energies slightly greater than that of cyclohexene. In solution, these molecules should rapidly interconvert among conformers which would be difficult to trap and study individually. We examined the x-ray structures of similar molecules with β -ionone rings and found that each is located within one of the low-energy potential wells of retinoic acid conformers. While it is known that crystal packing forces can stabilize low-energy conformations which are not global minima, these forces are rarely sufficient to confine molecules in the crystal to a high energy conformation near transition states or even far outside of local minima. Indeed, we establish that most of the crystal structures had geometries near to the low-energy conformers found for retinoic acids (**P17Eq**, **N17Ax**,

P17Ax, N17Eq). Some structures, however, contain β -ionone rings in the planar conformational space. In Stam's structure on ATRA [15], its β -ionone ring was nearly planar in the monoclinic crystal. Since this crystal is metastable, crystal packing forces are probably responsible for trapping ATRA in a higher energy local minimum. In other structures with planar β -ionone ring conformations, such as the retinaldehyde iminium salts, the *s-trans* geometry will have increased conjugation between C6-C7. The positive charge of the protonated imine delocalizes along the polyene chain, increases conjugation between C6 and C7, and therefore stabilizes the *s-trans* geometry sufficiently so that crystal packing forces favor that conformation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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- Steric energies in substituted butadienes are computed.
- Minimum energy conformations of retinoids are explained in steric and electronic terms.
- The steric energy is unaffected by length or E/Z geometry of the polyene chain.
- Crystal structures of retinoids fall within minima on a single 2-D potential surface.

Figure 1.

A) Chemical structures of 9cRA and ATRA. The structure used for an electronic energy reference in the steric energy calculations are highlighted in red. The retinoic acids are shown in the *s-cis* conformer about the C6-C7 torsion angle. B) Two low-energy ring conformations of the trimethylcyclohexenyl ring in both 9cRA and ATRA. Also provided are the C2-C3 torsion angles and the ring puckering coordinates (Q , ϕ , and θ) of the two ring conformations.

Figure 2.

Butadiene analogs **1–7** and cyclohexene analogs **8 – 11** used in this study are displayed in the *s-cis* conformer about the C6-C7 torsion angle. The numbering scheme used for **1 – 11** are related to ATRA so that comparison can be made between the diene systems and corresponding alkyl substitutions.

Figure 3.

A) Torsion energy potential for butadiene analogs **1–6**: **1** and **2** (Black line), **3** (Red circles), **4** (Green squares), **5** (Blue triangles), **6** (Orange diamonds), and **7** (Purple circles) calculated using the SCS-MP2/cc-pVDZ//B3LYP/6-31G(d) level of theory. B) Steric energy potential for butadiene analogs **1–6**: **3** (Red circles), **4** (Green squares), **5** (Blue triangles), **6** (Orange diamonds), and **7** (Purple circles) relative to 1,3-butadiene.

Figure 4. Optimized *s-gauche* conformations of the (A) *t*-butyl substituted 1,3-butadiene analog **7** and the (B) C16/C17-substituted cyclohexene analog **11**.

Figure 5.

A) Torsion energy potential of cyclohexene analogs **8** – **11**: **8** (Green squares), **9** (Blue triangles), **10** (Orange diamonds), and **11** (Purple circles) calculated at the SCS-MP2/cc-pVDZ// B3LYP/6-31G(d) level of theory. The cyclohexene analogs were calculated in the $\tau_{2-3} \sim +60^\circ$ ring conformation (17EQ) described in Figure 1. B) Steric energy potential of cyclohexene analogs **8**–**11** relative to 1,3-butadiene: **8** (Green squares), **9** (Blue triangles), **10** (Orange diamonds), **11** (Purple circles).

Figure 6.

A) Torsion energy potential of cyclohexene analog **11** (purple circles), ATRA (Black circles), 9cRA (Red triangles), and 13cRA (Blue Squares) calculated at the SCS-MP2/cc-pVDZ// B3LYP/6-31G(d) level of theory in the $\tau_{2-3} \sim +60^\circ$ ring conformation (17EQ, Figure 1). B) Steric energy potential of cyclohexene analog **11** (purple circles) and ATRA/9cRA/13cRA (Black circles).

Figure 7. Potential energy surface of ATRA for rotations about the τ_{6-7} and τ_{2-3} torsional angles. The transition states of ring inversion between *s-gauche* conformers are marked as “x” symbols, and the ring inversion transition states between *s-trans* conformers are marked as “+” symbols.

Figure 8.
 Geometries and energy barriers for the six low-energy all-*trans*-retinoic acid conformers.

Figure 9.
Potential energy surface of ATRA for rotations about the τ_{6-7} and τ_{2-3} torsional angles overlaid with small-molecule crystal structure conformations are (Black Circles)

Figure 10.

Torsion energy potential about the C6-C7 torsional angle calculated at the SCS-MP2/cc-pVDZ// B3LYP/6-31G(d) level for the all-*trans* retinoid terminating with a deprotonated Schiff base ($-\text{CH}=\text{N}-\text{CH}_3$, black circles) or the protonated Schiff base ($-\text{CH}=\text{N}^+\text{H}-\text{CH}_3$, Red squares) in the $\tau_{2-3} \sim +60^\circ$ ring conformation (17EQ, Figure 1).

Table 1

Geometries, relative energies, and steric energies (energy in kJ/mol) of retinoic acid analogs.^a

Analog	<i>s-trans</i> conformer			<i>s-gauche</i> conformers			<i>s-cis</i> barrier ^b		
	E ^c	δE ^d	τ ₆₋₇	τ ₂₋₃	E ^c	δE ^d	τ ₂₋₃	E ^c	δE ^d
1	0.0	-	180°	-	12.1	-	±37°	-	15.0
2	0.0	<0.1	180°	-	12.5	<0.1	±37°	-	15.5
3	0.0	5.4	180°	-	12.1	5.9	±46°	-	19.6
4	0.0	3.8	180°	-	10.5	2.1	±43°	-	16.7
5	0.0	14.2	180°	-	5.9	7.1	±54°	-	20.1
6	14.2	30.1	±174°	-	0.0	2.1	±50°	-	15.5
7	30.1	50.6	180°	-	0.0	5.0	±64°	-	29.3
8	0.0	4.6	±176°	±61°	9.6	1.7	±42°	±61°	15.0
9	0.0	13.4	±177°	±61°	10.9	2.9	-/+ 41°	±60°	16.7
10	5.9	21.3	±169°	±62°	8.4	8.8	-/+ 56°	±60°	1.3
11	13.4	33.4	±160°	±62°	0.0	4.2	-/+ 46°	±61°	17.6
ATRA	11.3	34.7	±164°	±62°	2.1	11.7	-/+ 59°	±60°	14.6
9cRA	10.9	33.9	±165°	±62°	1.7	10.9	-/+ 54°	±60°	15.5
13cRA	11.3	34.7	±165°	±62°	1.7	10.9	-/+ 54°	±60°	15.5
					1.7	10.9	-/+ 56°	±60°	25.5

^a Determined at the SCS-MP2/cc-pVDZ//B3LYP/6-31G(d) level of theory.

^b At τ₆₋₇ = 0°.

^c Energy given relative to the global minimum in kJ/mol.

^d Steric energy given in kJ/mol.

Table 2
Relative energies (kJ/mol) and ring puckering coordinates for six conformers of all-*trans*retinoic acid.

Conformer	τ_{6-7}	τ_{2-3}	E kJ/mol	Q	ϕ	θ
P17Eq	53°	62°	0.0	0.49	94°	53°
P17Ax	55°	-60°	1.7	0.47	-91°	133°
T17Eq	164°	62°	11.3	0.50	90°	54°
T17Ax	196°	-62°	11.3	0.50	-90°	126°
N17Eq	305°	60°	1.7	0.47	89°	47°
N17Ax	307°	-62°	0.0	0.49	-86°	127°

Table 3
Energies (kJ/mol) of retinoids with different end groups in the distorted *s-gauche* and planar *s-trans* conformations.

R	Distorted <i>s-gauche</i>			<i>s-trans</i>		
	E ^a	τ_{6-7}	E _h	E ^a	τ_{6-7}	E _h
-COOH	0.0	53°	-926.4709677	11.3	164°	-926.466622
-CH ₂ OH	0.0	56°	-852.5805171	11.7	162°	-852.5760970
-CHO	0.0	56°	-851.3970133	11.3	165°	-851.3927006
-CH ₃	0.0	55°	-777.5558318	11.7	165°	-777.5513892
-COOCH ₃	0.0	55°	-965.6354572	11.3	166°	-965.6310889
-CH=N-CH ₃	0.0	55°	-851.3970136	11.3	164°	-851.3927023
-CH=N ⁺ H-CH ₃	0.0	45°	-871.1211734	5.0	172°	-871.1192586

^a E in kJ/mol.