Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 18, pp. 2343-2348, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00431-9

A THEORETICAL INVESTIGATION OF ENDOCYCLIC ALLYLIC CARBON-CENTERED RADICAL FORMATION IN RETINOIC ACID

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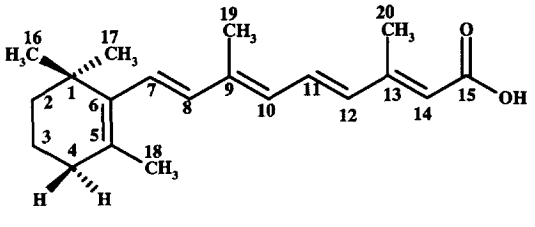
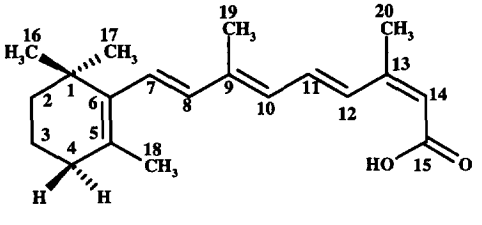
Abstract. We have carried out theoretical calculations that suggest that C4 carbon-centered radical formation in (*E*)- and (13*Z*)-retinoic acid, catalyzed by cytochromes P450 and PGH synthase, occur by a direct H-atom abstraction mechanism rather than an electron abstraction mechanism. In addition, our calculations indicate that C4 radical formation in the 13*Z*-isomer is thermochemically preferred compared with the all *trans*-isomer.

A combination of computer-aided theoretical techniques have been used to gain insight into the molecular mechanisms involved in cytochrome P450 (P450)- and prostaglandin H synthase (PHS)-catalyzed oxidation of retinoic acid geometric isomers that yield the corresponding C4-hydroxylated products. (*E*)-Retinoic acid (RA) and (13*Z*)-retinoic acid ((13*Z*)-RA) represent major in vivo metabolites of retinol (vitamin A) and retinyl acetate and oxidation of retinol to RA is necessary for its biological activity in epidermis as well as its teratogenic effects.[1] RA is the pharmacologically active ingredient of RetinA®, used topically in the treatment of acne[2] and photoaged skin[3] where it undergoes ~50% photochemical isomerization to the 13*Z* isomer.[4] The major phase I metabolism of RA and (13*Z*)-RA involves hydroxylation at the secondary endocyclic allylic position (C4) by P450[5,6] and the peroxidase activity of PHS,[7-10] yielding 4-hydroxy-RA and 4-hydroxy(13*Z*)-RA, respectively.

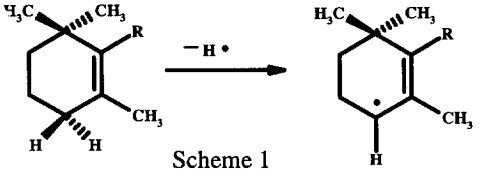


RA

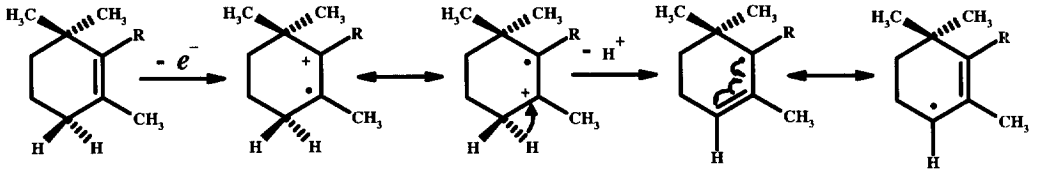


(13Z)-RA

Both P450s and PHS contain heme as a prosthetic group, and appear to generate similar catalytic intermediates involving the oxidation of the resting ferric state by two oxidizing equivalents yielding a ferryl (Fe(IV)=O) intermediate and porphyrin π cation radical or protein radical (reviewed in references 11 and 12). Both enzymatic systems catalyze the C4 retinoid hydroxylation where the rate limiting step consists of the formation of the corresponding C4 endocyclic secondary allylic carbon-centered radical.[9,11] However, the tertiary structure of the proteins impose important mechanistic differences that affect the substrate access to the heme-derived higher oxidation states and the regiospecificity of the reaction (reviewed in reference 13). P450s catalyze 2-electron aliphatic hydroxylations involving oxene insertion mechanisms as well as electron abstraction from carbon-carbon double bonds and even single bonds.[11] In contrast, heme peroxidases have been shown to hydroxylate aromatic systems via sequential one-electron abstraction mechanisms.[14] It is unclear whether the rate determinant step, retinoid carbon-centered radical formation, occurs via a direct H-atom abstraction or electron-abstraction mechanism. The present study is important because the enzymatic metabolic mechanisms are relevant to the toxicity and pharmacological activity of retinoids, and the mechanisms and energetics characterizing C4 carbon centered radical formation have not been investigated. Therefore, to compare the energetics characterizing electron- versus H-atom abstractions in *conjugated* allylic functions in non-aqueous environment and gain insight into the molecular mechanisms of RA oxidation, we have carried out molecular mechanical calculations (MM), using Tripos force field parameters[15] and molecular orbital calculations (MO), using the semiempirical quantum mechanical program AM1.[14] We report calculated heats of formation (ΔH_f) as well as E_{HOMO} (energy of the highest occupied molecular orbital) and E_{LUMO} (energy of the lowest unoccupied molecular orbital) values for the parent retinoids and corresponding free radicals.



Scheme 1



Scheme 2

Table 1 lists the ΔH_f, E_{HOMO}, and E_{LUMO} values characterizing the parent retinoic acid isomers as well as their corresponding C4 radicals and C5,C6 radical-cations for RA or (13*Z*)-RA to the to the corresponding C4 radical, via direct H-atom abstraction mechanism (Scheme 1), or electron abstraction from the 5,6-double bond yielding a π-cation-radical intermediate which deprotonates (Scheme 2). The calculated heats of formation in Table 1, indicate similar values for both RA and (13*Z*)-RA based on RHF or UHF calculations. The results in Tables 2 indicate that the calculated $\Delta\Delta H_f$ values characterizing C4 carbon-centered radical formation are 13.11 and 11.70 kcal·mol^{-1} for the *E*- versus 13*Z*-geometric isomers of retinoic acid from RHF or UHF calculations. On the other hand, the enthalpy differences for the formation of the C5,C6 radical-cations from the parent retinoids are 171.91 and 170.77 kcal·mol^{-1} respectively for RA and (13*Z*)-RA. This enormous difference in calculated heats of formation suggest that retinoid oxidation by P450 or PHS, proceeds by a mechanisms involving H-atom abstraction (at C4) rather than electron abstraction from the C5-C6 double bond yielding a radical-cation intermediate.

We have previously reported that the rate of PHS-catalyzed oxidation of the 13*Z*-isomer is ~3-fold higher compared with RA as evidenced by dioxygen uptake studies, RA-C4 peroxyl radical-dependent epoxidation of the peroxyl radical probe (+)-benzo[*a*]pyrene-7*S*,8*S*-dihydrodiol, and EPR experiments using 2-methyl-2-nitrosopropane as a spin trap under anaerobic condition.[9,10] These rate differences may reflect differences in steric interactions of the geometric isomers 13*Z*- and RA within the PHS active site. Assuming that transition state and free radical intermediate energies are proportional, the -3.37 or -1.41 kcal·mol^{-1} enthalpy difference characterizing C4 carbon radical formation in the 13*Z* versus *E*-isomer (Table 3) (the former probably being more accurate due to the absence of spin restrictions), cannot exclude electronic contributions in these kinetic results. Additionally, the slightly higher HOMO energy in the *cis* isomer would facilitate the C4-H homolytic cleavage. Further insights into the oxidative mechanism are expected from current experiments using C4-deuterated analogues and investigation of the kinetic isotope effects, as well as solvation dependent MO calculations.

Table 1. Energies for ground state RA, (13*Z*)-RA radical intermediates, and 4-hydroxylated products.

Compound	E_{LUMO} (eV)	E_{HOMO} (eV)	ΔH_f (kcal·mol^{-1}) RHF	ΔH_f (kcal·mol^{-1}) UHF
RA	-0.981	-8.451	-68.57	-75.30
4-radical-RA	-4.140	-8.689	-39.50	-62.19
5-radical-RA	-9.234	-12.515	108.32	96.61
4-hydroxy-RA	-0.930	-8.501	-108.35	
(13*Z*)-RA	-0.912	-8.362	-67.32	-74.30
4-radical-(13*Z*)-RA	-4.107	-8.507	-41.62	-62.60
5-radical-(13*Z*)-RA	-9.178	-12.498	107.91	96.47
4-hydroxy-(13*Z*)-RA	-0.831	-8.425	-109.29	

Table 2. Enthalpy differences ($\Delta\Delta H_f$) between intermediate and parent retinoid (kcal·mol^{-1}).

(Intermediate) - (reactant)	RHF	UHF
(4-radical-RA) - (RA)	29.07	13.11
(5-radical-RA) - (RA)	176.89	171.91
(4-hydroxy-RA) - (RA)	-39.78	-
(4-radical-(13Z)-RA) - (13Z)-RA	25.70	11.70
(5-radical-(13Z)-RA) - (13Z)-RA	175.23	170.77
(4-hydroxy-(13Z)-RA) - (13Z)-RA	-41.97	-

Table 3. Enthalpy differences ($\Delta\Delta\Delta H_f$) between 4-radical-RA and 4-radical-(13Z)-RA (kcal·mol^{-1}).

Method	ΔH_f
RHF	+3.37
UHF	+1.41

Acknowledgments

This study was partially supported by grants from the NIH (529 ES06765-03), DoD Office of Naval Research (ONR-4114502), Murphy Oil Corporation Research Award, American Cancer Society, and The National Sciences Foundation (EHR-9108762).

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15. Molecular models for the ground states were built and optimized using SYBYL 6.2 force field parameters (Tripos, Inc., St. Louis Missouri). The resulting geometries were subjected to full energy optimization using Restricted Hartee-Fock (RHF) SCF MO as implemented in the AM1[16] method from the MOPAC 6.0 package (QCPE), Department of Chemistry, Indiana University, Bloomington, Indiana). A fixed valence geometry conformational search at 5° increments about the dihedral angle defined by atoms 1-6-7-8 was made using electronic and steric energy terms. The lowest energy conformer was selected for a second full geometry optimization and calculation of heats of formation, orbital energies, electron densities and partial atomic charges with AM1. The geometries of the C5 and C6 radicals were optimized using Unrestricted Hartree-Fock (UHF) calculations and RHF using the half-electron method. UHF calculations were also performed in the ground state for comparative purposes.[17] All computations were performed with the PRECISE key word. Additionally, the geometry and energetics of the X-ray crystallographic structure of RA (VITAAC10) from the Cambridge Structural Data base-CDS-Unity-Version 5.12, was compared against

the theoretically built molecular models. The minimized structure of RA was nearly superimposable with the published X-ray crystallographic structure. This was reflected by comparison of our calculated-minimized coordinates with the X-ray crystallographic coordinates which yielded a root-mean-square (rms) deviations of heavy atom overlays in ring and side chain of less than 0.7 Å for RA. The major difference was the dihedral angle formed by atoms C1-C6-C7-C8 wich is +126.8° in the computer generated model and -139.4° in the crystal structure of RA.

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(*Received in USA* 4 *April* 1997; *accepted* 8 *August* 1997)