Retinoic Acid Oxidation at High Oxygen Pressures: Evidence for Spin-Forbidden Direct Addition of Triplet Molecular Oxygen[1]

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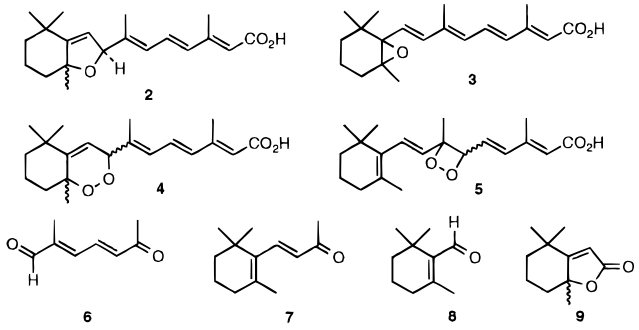
Introduction

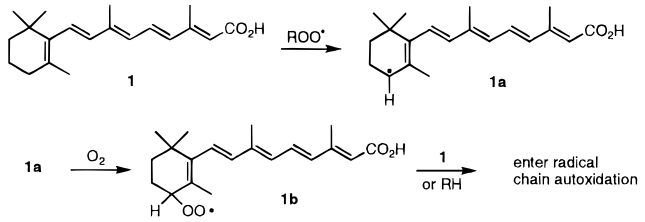
Retinoic acid (**1**), a biologically active polyene vital for mammalian development with activity against a number of cancers and dermatological diseases in humans,[2] is known to react rapidly with molecular oxygen. Retinoic acid has been shown to undergo initiated autoxidation in solution by a mechanism involving free radical chain reactions.[3] A thorough kinetic analysis was carried out for this process; however, the mechanism of retinoid autoxidation put forward was never clarified since a thorough product analysis was not carried out.[3] With few exceptions the resulting complex reaction mixtures from other studies have not been well characterized, and this has led to the assumption that abstraction of an activated secondary H atom from the substituted cyclohexenyl ring by a peroxyl radical is responsible for the observed product distribution (Scheme 1).

A comprehensive study has been reported for the oxidation of *all-trans*-retinoic acid (**1**) in 90% ethanol in which the major "dark" products were characterized.[4] Among the products common to retinoid oxidation, e.g., the furan **2** and the epoxide **3**, were a number of products that had not previously been identified. These included minor quantities of the cyclic peroxides **4** and **5** and high yields of the olefinic fragmentation compounds **6**−**9**. It was suggested that the cyclic peroxide **4** and **5** and the fragmentation products **6**−**9** were produced by a radical-cation chain mechanism rather than by autoxidation.[4]



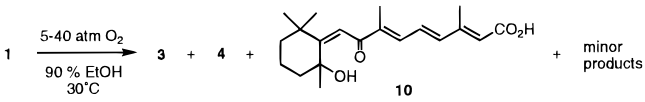
We have recently been investigating the *initial* stages of the reaction of *all-trans*-retinoic acid with molecular oxygen with a view of clarifying the mechanism(s) of oxygenation. Herein,

Scheme 1



we report our preliminary results concerning the mechanism for the formation of the endoperoxide **4**.

Preliminary oxygen uptake studies showed that reaction of **1** with O_2 is self-initiated and autocatalytic under ambient conditions in solvents such as benzene. Despite this, **1** initially reacts with O_2 rather slowly under similar conditions in 90% ethanol, the solvent system of this study. Reactions were, therefore, carried out at higher oxygen concentrations by increasing the oxygen pressure.[5] It is our contention that the results of previous retinoic acid oxidation studies were obscured by prolonged oxidation resulting in mixtures dominated by numerous secondary products.[4] In the present study, reaction mixtures were analyzed[6] at low (<5%) conversions of starting material. Surprisingly simplified product distributions (eq 1)



were observed and consisted of three major and several minor products. Two of the major products were identified[7] as 5,6-epoxy-5,6-dihydroretinoic acid (**3**) and 5,8-epidioxy-5,8-dihydroretinoic acid **(4)**. The third major product was identified[8] as 5-hydroxy-8-oxo-6,7-dihydroretinoic acid (**10**), which to the best of our knowledge has not previously been reported as an oxidation product of **1**. In addition to other unidentified compounds, *trans*- and *cis*-5,8-epoxy-5,8-dihydroretinoic acid (**2a** and **2b**, respectively), 2-methyl-6-oxo-2,4-heptadienal (**6**), β-ionone (**7**), cyclocitral (**8**), and dihydroactinidiolide were identified in minor amounts. All compounds, with the exception of **10**, have previously been identified and fully characterized by Oyler et al. as products of *all-trans*-retinoic acid oxidation but in quite different relative concentrations. Thus, Oyler et al. reported that **2** and **6**−**9** comprised the bulk of the product mixture while **3** and **4** were minor components,[4] whereas **2** and **6**−**9** are all minor products and **3** and **4** are major components (along with **10**) at the low substrate conversions used in the present work.

To minimize the importance of autoxidation, reaction of **1** with O_2 was carried out under conditions identical to those described above but in the presence of 1−10× molar excess of 2,6-di-*tert*-butyl-4-methylphenol (BMP), an efficient peroxyl radical scavenger. Under these conditions, yields of **4** and **10**

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(5) Oxidations employed 3 mM solutions of **1** (0.1% by weight) in 90% ethanol. For inhibited runs, the reaction mixtures generally contained 1−10× molar equivalents of antioxidant. Handling of photolabile retinoic acid oxidation solutions was carried out under Gold light to prevent photoisomerization and photoinitiation.

(6) Analytical HPLC was performed on a Hewlett-Packard Series II 1090 Liquid Chromotograph equipped with diode array UV−vis detector. Separations were achieved by using a 4.6 mm × 250 mm Spherisorb ODS2 5 m column operated with a tertiary gradient solvent system.

(7) On the basis of elution times, co-injection and comparison of UV specta of authentic, independently synthesized compounds.

(8) On the basis of its spectral and molecular weight data after isolation of the pure compound from oxidized **1**. UV−vis λ_{max} = 327 nm (acetonitrile); ^{1}H NMR ($CDCl_3$, 500 MHz) vinyl protons: 5.80 (s br, 1H, H-14), 6.12 (s br, 1H, H-7), ca. 6.15 (d, 1H, $J_{10,11}$ = 11.3 Hz, H-10, doublet obscured by H-7 (6.12)), 6.28 (d, 1H, $J_{11,12}$ = 15.3 Hz, H-12), 6.97 (dd, 1H, $J_{11,12}$ = 15.3, $J_{10,11}$ = 11.3 Hz, H-11); MS (ES) 332.1 ($M^{+\bullet}$).

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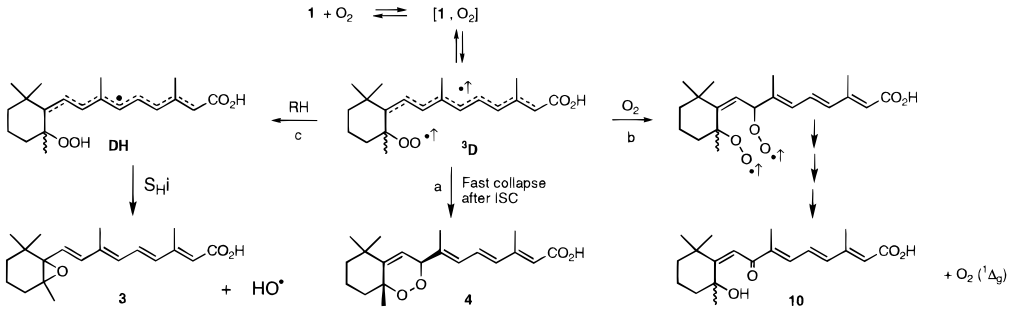
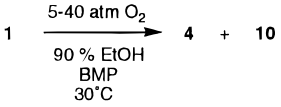
Scheme 2



were unaffected while formation of **3** was almost completely inhibited. There was also neglible formation of **2a**,**b**, **6**−**9**, and some other minor unidentified products even at >25% conversion of **1**. This suggests that **4** and **10** are not formed by autoxidation. However, it should be noted that similar concentrations of more efficient antioxidants, such as α-tocopherol[9] resulted in lower yields of both **4** and **10**.

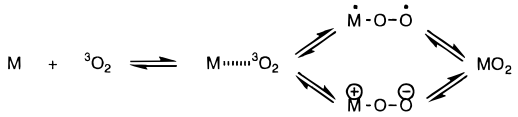


These results suggest that, at early stages of oxidation, the product mixture is relatively simple and that two concurrent processes are at play, one involving an inhibitable radical chain process (autoxidation) which leads to formation of **3**, and another where **1** apparently undergoes a direct reaction with triplet molecular oxygen to give **4** and **10**. Formation of **4** and **10** is, however, quickly overshadowed, as the oxidation proceeds by production of **2**, **3**, and **6**−**9** (among others) by autoxidation. Although BMP does not inhibit the formation of **4** and **10**, the ability of tocopherols to partially inhibit their formation suggests that oxygen-centered radical intermediates are involved in their formation. The experimental evidence collected here does not support the participation of radical-cation chains, at least in the formation of **4** and **10**, although work supporting such intermediates has been published for other unsaturated systems.[12] Kinetic data[13] would seem to preclude reaction of $1^{\bullet+}$ and O_2 in any solvent system, let alone one comprised of ethanol containing 10% water (where H_2O behaves as a weak nucleophilic radical-cation quencher with a bimolecular rate constant of 3.5×10^5 M^{-1} s^{-1}).[13] Our preliminary solvent dependence studies suggest that yields of **4** (as well as **10**) are actually higher in aqueous systems.

The possibility that **10** is derived from rearrangement of endoperoxide **4** was considered; however, control experiments, where 3 mM solutions of **4** were subjected to oxidation conditions (retinoic acid absent) similar to those for retinoic acid, did not result in any detectable formation of **10**.

We can only speculate as to the exact nature of the process that gives rise to direct addition of molecular oxygen and products **4** and **10**. Although considered rare, there have been a number of "direct" olefinic oxidations reported that do not appear to involve radical chains or singlet oxygen.[12,14−16]

At this point we prefer to think of the direct addition of O_2 in terms of a mechanism involving a retinoic acid−ground state oxygen encounter complex [**1**, $^3O_2$].[16] It is well-known that



oxygen-saturated organic solvents produce charge-transfer spectra, the intensity of which in many cases has been shown to be proportional to the oxygen concentration.[17] For most organic compounds (M), the spectrum is collisional in nature with no stable complex between O_2 and the substrate being formed. The importance of charge transfer may increase significantly for [**1**, $^3O_2$] complexes with the enhanced donor strength of retinoic acid, an electron-rich polyene with a low ionization potential.[18] Oxygenation may be initiated when electron donor−acceptor complexes collapse reversibly to form triplet biradicals (carbon−peroxyl biradical) ^{3}D and/or zwitterionic intermediates as depicted in Scheme 2. For simplicity, we have not included the possibility of zwitterionic intermediates in Scheme 2. Fast ring closure of biradical ^{3}D after obligatory intersystem crossing would result in endoperoxide **4** formation (path a), a process that must occur very rapidly to avoid hydrogenation via BMP. At high oxygen concentrations, ^{3}D may be intercepted by O_2, resulting in an unstable tetroxide which would be expected to undergo cleavage to **10** via a mechanism similar to that described by Russell for bimolecular reaction of primary and/or secondary peroxyls.[19] Formation of **10** would be expected to increase as the concentration of O_2 increases if this mechanism is correct. Experiments designed to follow the formation of **10** as a function of O_2 concentration are underway. Preliminary results reveal that formation of **10** may be attenuated at lower O_2 concentrations.

Inhibitable radical chain autoxidation is initiated when ^{3}D (Scheme 2) abstracts hydrogen from solvent or substrate, resulting in DH, a radical which in turn would undergo intramolecular homolytic substitution (S_Hi), yielding epoxide **3** and a chain carrying hydroxyl, HO• (path c, Scheme 2). Although according to this mechanism epoxide **3** is formed in the initiating steps, the bulk of **3** that is formed in the uninhibited autoxidation of **1** likely arises from a chain propagating step in which a peroxyl RO_2• adds to C5 of **1** followed by an S_Hi reaction to produce the epoxide.

Further work, aimed at examining the role of radical intermediates in the propagation process, is currently in progress.

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(9) α-Tocopherol (α-T) scavenges peroxyls ~200 times faster than BMP.[10] In aqueous media α-T is about 3 times more reactive than BMP.[11]

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