Superoxidation

DOI: 10.1002/ange.200501346

# Superoxidation of Bisretinoids[**]

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In addition to their role in vision, retinoids, for example, **1a**–**k** and their derivatives, have been found to affect fetal development, cell differentiation, metabolism, and organ and cell regulation.[1] They also provide lead structures for various anticancer, anti-inflammatory, and antibacterial drugs. In plants and food, they act as hormones, pigments, flavors, aromas, and as defense substances.[1]

The polyenes **1a**–**k** react readily with singlet oxygen.[2,3] Reactions with oxygen can be initiated by light, in the presence or absence of a photosensitizer, or by autoxidation.[3] The oxidized products often show biological activity and are responsible for various aromas and tastes of food.[2,3] Identification of the oxygenated products is complicated by the fact that the initial species are reactive dioxetanes, peroxides,

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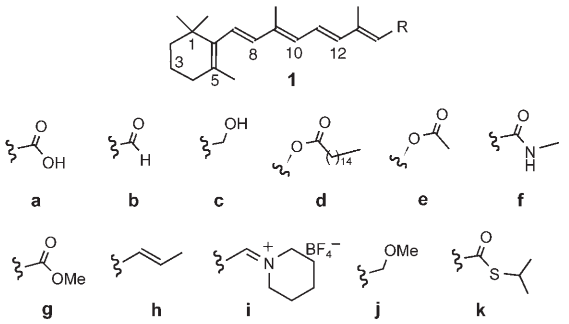
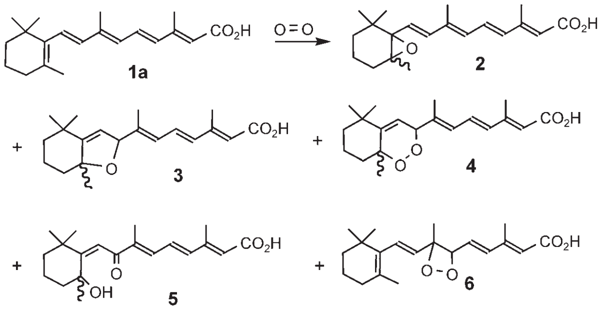
[**] We thank the National Institutes of Health (GM 036564 to K.N.) and the National Science Foundation (CHE 04—10655, N.J.T.) for financial support.



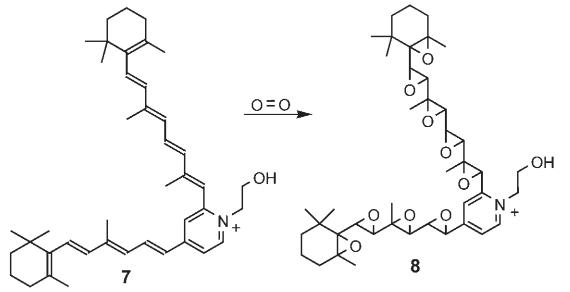
epoxides, and endoperoxides that can undergo secondary reactions and rearrangements upon chromatographic separation and identification.[2,3] Furthermore, various *cis/trans*, stereo-, and regioisomers are often obtained.

To date, nine different products have been reported for the reaction of retinoic acid (**1a**) with singlet oxygen (Scheme 1).[3f,g] These products include epoxide **2**, furan **3**



***Scheme 1.*** The reaction of retinoic acid (**1a**) with singlet oxygen.

(through rearrangement of **2**), endoperoxide **4**, hydroxyketone **5**, dioxetane **6**, and four degradation products (products with molecular weights lower than that of the parent retinoic acid). Similar oxidation products have also been reported for the oxidation of retinal (**1b**),[3d,e] retinol (**1c**),[3a,c] and retinol palmitate (**1d**).[3a] In most cases, the oxidation is reported to stop upon the addition of two oxygen atoms, with either the major or sole initial product proposed to be 5,8-endoperoxide (similar to **4**).[3] In contrast, A2E (**7**), a bisretinoid isolated from human lipofuscin, reacts with singlet oxygen to form the polyepoxide species **8** (Scheme 2).[4a] This is in contrast to retinoids **1a–d**, which add two oxygen atoms. In the case of **7**, a maximum of nine oxygen atoms can add to the polyene chain. The epoxides of **7** have been shown to be biologically active and are speculated to be involved in age-related macular degeneration.[4b] Polyepoxide **8** was not isolated, and its structure was proposed based on mass-spectrometric measurements.[4a]



***Scheme 2.*** The reaction of A2E (**7**) with singlet oxygen to form the polyepoxide species **8**.

To explain the difference between the reactivity of **7** and that of retinoids **1a–d** towards singlet oxygen and to explore the reactivity of singlet oxygen towards polyenes, several bisretinoids **10–12** (Schemes 5–7) as well as several monoretinoids **1a–c** and **1e–k** (Scheme 1) were treated with singlet oxygen. Through MS analysis of the reactant mixtures, it was concluded that the polyene chains, when held in proximity to one another, react readily with oxygen to give superoxygenated species. On the other hand, the oxidation of spatially isolated polyenes stops upon the addition of two oxygen atoms.

The photoreactions were carried out for 1–3 h at 25 °C in $CH_2Cl_2$ in the presence of tetraphenylporphyrin (TPP) as a photosensitizer. The irradiation was effected by a 300-W halogen lamp that was equipped with a 400-nm cut-off filter. The exposure of solutions of the reagents in acetone (in a borosilicate glass vile with a UV cut-off of 340 nm) to sunlight for 3–6 h in the absence of added sensitizer gave similar results. All samples were analyzed directly by MS without any further manipulation. Figure 1 shows the fast-atom-bombardment (FYB) mass spectrum of the products of the amide **1f** after 3 h of irradiation (Scheme 3). The peak at $m/z$ 314 corresponds to **1f** $[M+1]$. The spectrum has a strong signal at $m/z$ 346, which corresponds to the mass of **1f** plus two oxygen atoms $[M+1]$. The smaller peak at $m/z$ 328 represents the mono-oxygen adduct of **1f** $[M-1]$. No peaks were found above $m/z$ 346 which suggests that oxidation of **1f** stops cleanly after the addition of two oxygen atoms.

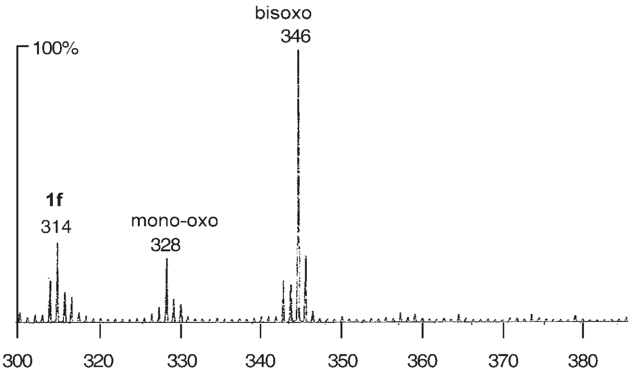
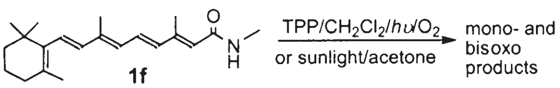


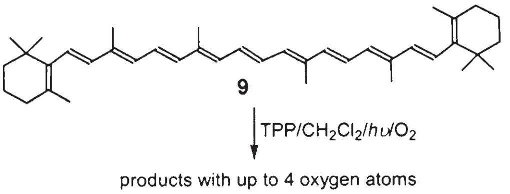
***Figure 1.*** FAB MS of the products of **1f**, 3 h after irradiation (300 W) at 25 °C in $CH_2Cl_2$ in the presence of tetraphenylporphyrin as a photosensitizer. The maximum increase by $m/z$ 32 (two oxygen atoms)



***Scheme 3.*** The irradiation of amide **1f**. Oxidation was shown to cease after the addition of two oxygen atoms.

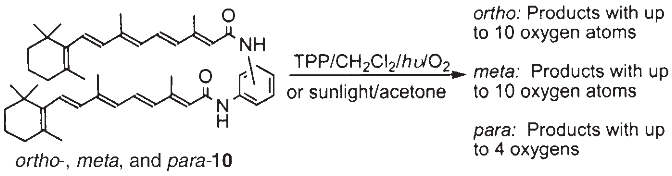
Similarly, retinoids **1a**–**c** and **1e**–**k** were prepared, irradiated, and the reactant mixture analyzed by MS. All the measured spectra were similar to that shown in Figure 1, with a maximum peak at +32 Da, which corresponds to the parent ion of the retinoid plus two oxygen atoms, and a smaller peak at +16 Da, which corresponds to the addition of one oxygen atom to the parent ion. The peaks located at 16 and 32 Da are consistent with literature reports for the formation of the 5,6-epoxide and the 5,8-endoperoxide, respectively, which are two well-characterized photo-oxidation products of retinoic acid (**1a**), retinal (**1b**), retinol (**1c**), and retinol palmitate (**1d**).[3]

Beta-carotene (**9**) was also examined (Scheme 4). The mass spectrum showed a maximum mass that corresponds to an increase of 64 Da, which is double that observed for the compounds **1a**–**c** and **1e**–**k**. Furthermore, peaks that correspond to the addition of one, two, and three oxygen atoms were also observed. The mass spectrum is consistent with the previously characterized products of singlet-oxygen-mediated oxidation products of beta-carotene, which are mixtures of bis and mono 5,6-epoxides and 5,8-endoperoxides.[3h]



***Scheme 4.*** The irradiation of beta-carotene (**9**), which results in the addition of up to four oxygen atoms.

Figure 2 shows the electrospray ionization mass spectrum obtained after 1 h of irradiation of a solution of bisretinoid *ortho*-**10** (Scheme 5). The peak at *m*/*z* 672 corresponds to the parent mass of *ortho*-**10**. Subsequent peaks (labeled with an ×) were found at increments of 16 Da, which corresponds to the addition of one oxygen atom. The peaks labeled with an * are sodium adducts (+23 Da). The spectrum shows the maximum *m*/*z* at 834 or 856 (834+Na) which corresponds to the addition of ten oxygen atoms. The spectrum is similar to that observed after the irradiation of **7,** which was reported to yield the polyepoxide species **8**. Superoxidation was also observed by MS for the isomer *meta*-**10**. When the isomer *para*-**10** was irradiated, the mass spectrum showed a maximum peak that corresponds to the addition of only four oxygens, and was similar to that observed for beta-carotene (**9**).



***Scheme 5.*** The irradiation of a solution of bisretinoid *ortho*-, *meta*-, and *para*-**10**, which results in the addition of ten, ten, and four oxygen atoms, respectively.

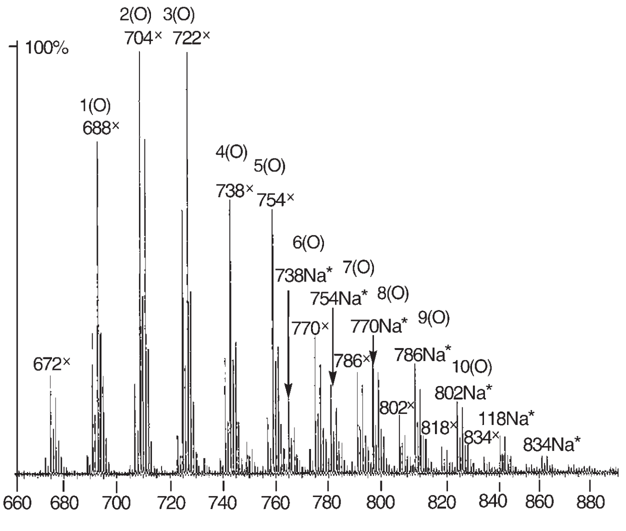
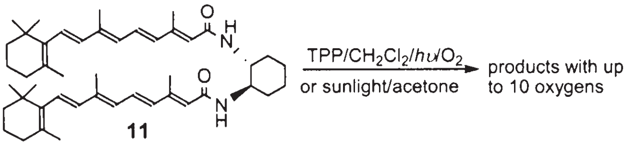


***Figure 2.*** ESI MS of the reaction mixture of *ortho*-**10** after irradiation (300 W) for 1 h at 25 °C in CH$_2$Cl$_2$ in the presence of tetraphenylporphyrin as a photosensitizer. Labels show the consecutive increase by 16 Da and the corresponding sodium adducts, × represents the addition of an oxygen atom, * are sodium adducts (+23 Da).

For the *trans*-substituted cyclohexane derivative **11**, there is no conjugation between the two polyene chains. However, superoxidation is still observed by MS (Scheme 6). When the two polyene chains are held together by a flexible linker as in **12**, oxidation was found mostly to stop upon the addition of one to four oxygen atoms (Figure 3 and Scheme 7). After the complete conversion of **12** (*m*/*z* 652) as shown by MS, only small peaks located at *m*/*z* 733 and 749, which correspond to



***Scheme 6.*** Irradiation of the *trans*-substituted cyclohexane derivative **11**, despite the absence of conjugation between the two polyene chains, resulted in superoxidation.

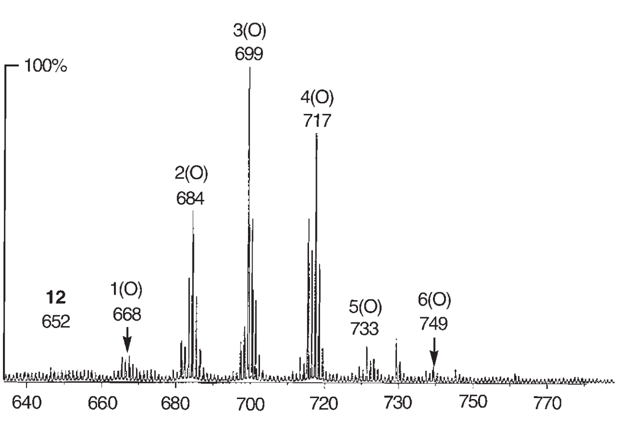
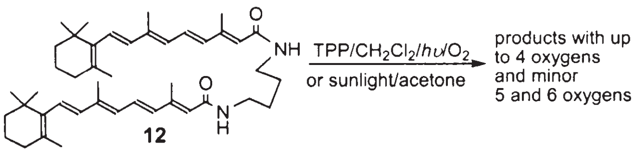


***Figure 3.*** FAB MS of the reaction mixture of **12** after irradiation (300 W) for 3.5 h at 25 °C in CH$_2$Cl$_2$, in the presence of tetraphenylporphyrin as a photosensitizer.



*Scheme 7.* The irradiation of **12**, in which the two polyene chains are held together by a flexible linker. No further oxidation occurred after the addition of one to four oxygen atoms.

the addition of five and six oxygen atoms, respectively, were found.

The $\lambda_{max}$ of **11** in ethanol is blue shifted by 5 nm relative to the similar retinoids **12** and **1 f**. Retinoid **11** has a fluorescence quantum yield in ethanol of 0.08 at 25 °C relative to 9,10-diphenylanthracene ($\Phi_F = 0.95$).[5] The fluorescence quantum yield of **12** and **1 f** were measured to be 0.07 and 0.05, respectively. This is in accord with the reported fluorescence quantum yields for retinal, retinol, and retinal acetate, which are between 0.03 and 0.01 at 25 °C.[6] Retinoids do not stack in apolar or polar solutions.[7] A slight increase in fluorescence is expected for **11**, as the proximity of the polyene chains restricts their movement, thus a greater amount of excited energy is emitted rather then dissipated by isomerization or vibrational motion. The blue shift in the absorption spectrum and the greater fluorescence of **11** relative to **12** and **1 f** suggest that the polyene chains in **11** are stacked. Similar observations have been reported for retinoids.[7]

The rate constants for the quenching of singlet oxygen by **11** and **1 f** were determined through pseudo-first-order treatment of the singlet oxygen phosphorescence decays by using TPP as a sensitizer and a pulsed Nd–YAG laser (532 nm) to generate singlet oxygen.[8] Rate constants of $(2.0 \pm 0.1) \times 10^6\,\mathrm{M}^{-1}\mathrm{s}^{-1}$ and $(1.9 \pm 0.1) \times 10^6\,\mathrm{M}^{-1}\mathrm{s}^{-1}$ were determined for the quenching of singlet oxygen by **11** and **1 f**, respectively. The determined values are in accord with the reported rate constants for the quenching of singlet oxygen by *trans*-retinal ($3.6 \times 10^6\,\mathrm{M}^{-1}\mathrm{s}^{-1}$) and retinol acetate ($7 \times 10^5\,\mathrm{M}^{-1}\mathrm{s}^{-1}$).[9] As the number of double bonds in bisretinoid **11** is doubled, an increased rate constant for quenching would have been expected. The observed value suggests that some of the double bonds are less accessible to singlet oxygen in the bisretinoid **11** when both retinal arms are in proximity.

The similarity between the rate constants of the quenching of singlet oxygen by **1 f** and **11** and the fact that different products are obtained from the reactions of monoretinoids and bisretinoids with oxygen suggest that they are oxidized by two species: singlet oxygen and oxygen radicals or oxygen radical anions. The endoperoxide products, typically observed after light-mediated oxidation of retinoids, are the products of the [2+4] addition of singlet oxygen. The higher energy oxygen radicals or oxygen radical anions are expected to give a more complex mixture of products,[10] as seen for the oxidation of the bisretinoids. Upon irradiation, retinoids **1 a**–**c** have been shown to produce both singlet oxygen and radical species.[11] It has also been reported that singlet oxygen plays a minor role in the oxidation of **7**.[12] Singlet oxygen chemistry often competes with or produces oxygen radicals or oxygen radical anions. In the case of the bisretinoids, the latter species may be formed to a greater extent and play a larger role in oxidation.

These data suggest that when two polyenes chains are able to communicate through space they react with oxygen upon exposure to light to give superoxidized species. Oxidation is limited, however, in the absence of such communication. The enhanced oxidative damage of bispolyenes versus monopolyenes may have implications with regard to the mechanisms of action of naturally occurring bispolyene compounds.[13]

Received: April 18, 2005
Revised: August 1, 2005
Published online: October 12, 2005

**Keywords:** alkenes · bisretinoids · carotenoids · photochemistry · singlet oxygen

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