# Reaction of Carotenoids and Ferric Chloride: Equilibria, Isomerization, and Products

Yunlong Gao and Lowell D. Kispert\*

Department of Chemistry, University of Alabama, Box 870336, Tuscaloosa, Alabama 35487-0336 Received: January 10, 2003; In Final Form: March 28, 2003

In the oxidation of carotenoids, ethyl all-*trans*-8'-apo- $\beta$ -caroten-8'-oate and all-*trans*- $\beta$ -carotene, with ferric chloride, several equilibria occur between Fe<sup>3+</sup>, Fe<sup>2+</sup>, Cl<sup>-</sup>, the neutral carotenoid, and its radical cation and dication. The radical cation and dication were found to abstract an electron from Fe<sup>2+</sup>. Isomerization of carotenoids occurs during the oxidation. In the presence of air, a stable product is formed in high yield during the oxidation. <sup>1</sup>H NMR, LC-MS, and optical studies show that this product is the 5,8-peroxide of the starting material. A mechanism for the formation of this compound is proposed.

#### Introduction

It is known that carotenoids serve as light harvesting agents in photosystems,<sup>1,2</sup> act as "triplet valves" to harmlessly dissipate the energy of chlorophyll (Chl) triplets, and are scavengers of singlet oxygen which can destroy Chl.<sup>3</sup> In the absence of antioxidants, the carotenoids are unstable to heat, light, and air/ oxygen, degrading to compounds with smaller molecular weights.<sup>4</sup> Oxidation of carotenoids under various conditions and in different systems<sup>5–8</sup> has been used to generate products. Most of the products identified to date are carbonyl compounds of the apo- $\beta$ -carotenoid or apo- $\beta$ -carotenone types. In the oxidation of all-trans carotenoids with ferric chloride (FeCl<sub>3</sub>) in anhydrous dichloromethane,<sup>9</sup> carotenoid radical cations (Car<sup>++</sup>) and cis isomers are formed in the reaction mixture.

Car<sup>•+</sup> is also formed in photosystem II reaction centers.<sup>10–12</sup> The formation and accumulation of the radical cations were found to be induced by the photoaccumulated primary electron acceptor radical P680<sup>•+</sup> (Car donates an electron to P680<sup>•+</sup>). Car<sup>•+</sup> can be reduced by electron donation from a monomeric Chl and also by cytochrome b<sub>559</sub>.<sup>13</sup> To understand the roles of Car<sup>•+</sup> in photosystem II reaction centers, the physiochemical properties of Car<sup>•+</sup> need to be studied. It has been shown<sup>14</sup> that the use of FeCl<sub>3</sub> to form Car<sup>•+</sup> also allows for the convenient optical study of the carotenoid dication (Car<sup>2+</sup>) formed from Car++ at room temperature. Formation of Car2+ electrochemically requires low temperature (-70 °C) procedures and dealing with the resulting difficulties with moisture. However, it has been shown that high yields of cis isomers are obtained by the electrochemical method due in part to the better control of the reaction conditions. Nonetheless, degradation of the carotenoids by electrochemical and FeCl<sub>3</sub> oxidation methods is smaller than by thermal methods.<sup>9</sup> It has also been shown<sup>15</sup> when dichloromethane solutions of carotenoids are treated with small amounts of FeCl<sub>3</sub> that extensive photodegradation occurs upon subsequent irradiation with near UV to visible light. Further questions arise as to why there are differences between electrochemical and FeCl<sub>3</sub> preparation of Car<sup>•+</sup> as well as what products are formed upon reaction of O<sub>2</sub> with Car<sup>•+</sup>. To address these questions, we choose two natural carotenoids ethyl all*trans*-8'-apo- $\beta$ -caroten-8'-oate (**I**) and all-*trans*- $\beta$ -carotene (**II**) (see Scheme 1).





All-trans-β-carotene (II)

It was found that several equilibria exist among  $Fe^{3+}$ ,  $Fe^{2+}$ , the neutral carotenoid, and its radical cation and dication. The radical cation and dication can also abstract an electron from  $Fe^{2+}$ . A stable oxidation product was formed in the presence of O<sub>2</sub>. LC-MS, <sup>1</sup>H NMR, and optical studies show that this product is the 5,8-peroxide of Car. A reaction mechanism for the formation of this compound is proposed.

## **Experimental Section**

Ethyl all-*trans*-8'-apo- $\beta$ -caroten-8'-oate (**I**) was a gift from Hoffmann-LaRoche, Basel; all-*trans*- $\beta$ -carotene (**II**) and ferric chloride (FeCl<sub>3</sub>) were purchased from Sigma; and benzyltriethylammonium chloride (BTAC), silver tetrafluoroborate (Ag-BF<sub>4</sub>), and anhydrous dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) were purchased from Aldrich. Acetonitrile of HPLC grade, obtained from Fisher, was treated with potassium carbonate before use. All-*trans*- $\beta$ carotene was purified by extraction with benzene and precipitation with methanol. The carotenoids were stored in the dark at -14 °C in a desiccator containing CaSO<sub>4</sub> and were allowed to warm to room-temperature just before use. Tetrabutylammonium hexafluorophosphate (TBAHFP) was obtained from Fluka.

The optical spectra were recorded with a double beam Shimadzu UV–visible 1601 spectrophotometer (190–1100 nm) using 1 cm quartz cells. A Vydac 201 TP54 polymeric C<sub>18</sub> column (250 × 4.6 mm i.d.) packed with 5  $\mu$ m particles (Hesperia, CA) and a Shimadzu LC-600 pump with a SPD-M10AVP PDA detector were used for the HPLC separation and detection. LC-MS experiments were performed by directing the column effluent to an APCI (atmospheric pressure chemical ionization) MS. Acetonitrile was the mobile phase. A funnel

<sup>\*</sup> To whom correspondence should be addressed.



**Figure 1.** UV/vis/NIR spectra (a) of pure I in  $CH_2Cl_2$ , (b) after adding 1 equiv FeCl<sub>3</sub>, (c) 5 min later, (d) 10 min later.



**Figure 2.** UV/vis/NIR spectra (a) pure **II** in  $CH_2Cl_2$ , (b) after adding 0.2 equiv FeCl<sub>3</sub>, (c) 5 min later, (d) 10 min later.

containing silica gel was used for rapid filtration of the solution to remove inorganic species and ions before injection.

Osteryoung square-wave voltammetry (OSWV) was performed with a BAS-100 B/W electrochemical analyzer in a conventional three-electrode system at room temperature. TBAHFP was the supporting electrolyte. A platinum disk electrode was selected as working electrode, a platinum wire was selected as the counter electrode, and a saturated calomel electrode (SCE) was selected as the reference electrode.

The bulk electrolysis experiment was performed by using a platinum gauze as the working electrode, a platinum wire as the counter electrode, and a silver wire as the pseudo reference electrode. The applied electrolysis potential was 1 V.

Separation of products was performed on a silica gel column; the eluent was  $CH_2Cl_2$ . <sup>1</sup>H NMR spectra of the products in  $CDCl_3$  were determined with a Bruker AM 360 (<sup>1</sup>H, 360.13 MHz) spectrometer.

AM1<sup>16</sup> and ZINDO/S<sup>17,18</sup> semiempirical molecular orbital calculations were carried out using Hyperchem 6.03 software on a Dell computer (Pentium III).

# **Results and Discussion**

Figures 1 and 2 show the UV/vis spectra of the oxidation of I and II with one equiv of FeCl<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> in the presence of air. The absorption near 440 nm is due to the neutral starting material. The absorption peak in the near infrared region is due to the  $D_0 \rightarrow D_2$  transition of the carotenoid radical cation (Car<sup>++</sup>).<sup>14</sup> The intensity of this peak decreases with time and a new absorption peak about 30 nm blue-shifted compared to the starting material increases with time, indicating that Car<sup>++</sup> reacts with other species to form the product. Identification of the



Figure 3. HPLC showing product formation in the reaction of I (a) and II (b) with FeCl<sub>3</sub>. The solid line is that of the product, and the dashed line is that of the starting material.

product will be described later. Peak x which appears during the oxidation could be due to the dication of the starting material. Because, according to previous ZINDO/S calculations and optical studies,<sup>14</sup> the absorption peak of Car<sup>2+</sup> should appear between those of Car and Car<sup>•+</sup>. Peak x may also be due to the radical cation of the product. Figures 1 and 2 exhibit two welldefined isosbestic points, that is, wavelengths at which the absorbance does not change with time during the reaction. It is generally accepted that occurrence of an isosbestic point in a closed system requires that the changes in the concentrations of the various components be linearly related.<sup>19</sup> Because the dication was not produced during the initial oxidation (assume peak x is due to the dication) and formation of Car<sup>2+</sup> from Car<sup>•+</sup> is known to be fast,<sup>9</sup> the possibility that the product is from the dication can be ruled out. This was also demonstrated by the fact that the yield of the product is very low when the concentration of FeCl<sub>3</sub> is high (the dication of the starting material was produced at the initial oxidation). Whether the neutral starting material is a direct precursor of the product is unclear.

Parts a and b of Figure 3 show the HPLC of the products for I and II after 10 min reaction with 1 equiv of FeCl<sub>3</sub>, respectively. The small peaks close to the product are probably due to isomers of the product because the UV/vis absorption spectra of these peaks, which are obtained by the PDA detector, are similar to that of the product. The starting material disappears after 10 min reaction.

To ascertain whether the product is due to the reaction of Car<sup>•+</sup> with Cl<sup>-</sup>, the concentration of Cl<sup>-</sup> was increased by adding BTAC to the reaction mixture of **I** and FeCl<sub>3</sub> to observe whether the formation of the product increases. Surprisingly, the peak of Car<sup>•+</sup> disappeared at once, and the intensity of the starting material increased significantly (see Figure 4), indicating that Car<sup>•+</sup> is reduced to Car. Where does the electron come from? It is known<sup>20–22</sup> that Fe<sup>3+</sup> can react with Cl<sup>-</sup> to form FeCl<sub>4</sub><sup>-</sup> in H<sub>2</sub>O. Does this reaction also occur in CH<sub>2</sub>Cl<sub>2</sub>? Figure 5 shows the UV/vis spectrum of the reaction of FeCl<sub>3</sub> with 2 equiv of BTAC in CH<sub>2</sub>Cl<sub>2</sub>. The two peaks with absorption at 314 and 360 nm are typical for FeCl<sub>4</sub><sup>-</sup>.<sup>21</sup> The two peaks disappear after addition of Ag<sup>+</sup> to the solution due to the reaction

$$\operatorname{FeCl}_4^- + \operatorname{Ag}^+ \rightarrow \operatorname{FeCl}_3 + \operatorname{AgCl}$$

The reduction of  $Car^{\bullet+}$  is due to the decrease of  $Fe^{3+}$ 



**Figure 4.** UV/vis/NIR spectra showing the effect of increasing the concentration of  $Cl^-$  in the reaction of I and FeCl<sub>3</sub>. (a) Pure I in CH<sub>2</sub>-Cl<sub>2</sub>, (b) 1 min after adding 1 equiv FeCl<sub>3</sub> to I, (c) after adding 1 drop of saturated BTAC CH<sub>2</sub>Cl<sub>2</sub> solution.



**Figure 5.** UV/vis/NIR spectra showing the formation of FeCl<sub>4</sub><sup>-</sup> after 20  $\mu$ M FeCl<sub>3</sub> reacts with 100  $\mu$ M BTAC (solid line) and the dissociation of FeCl<sub>4</sub><sup>-</sup> after addition of 200  $\mu$ M AgBF<sub>4</sub> (dashed line).

concentration when FeCl<sub>4</sub><sup>-</sup> forms. The equilibrium is

$$Car + Fe^{3+} \rightleftharpoons Car^{\bullet+} + Fe^{2+}$$

According to the initial concentrations of Car and Fe<sup>3+</sup> and Figures 1a,b and 2a,b, the calculated equilibrium constants are about 1 and 2.5 for the redox reactions of **I** and **II**, respectively. The difference is probably due to the fact that Car<sup>•+</sup>(**I**) contains an electron-withdrawing group and favors the back reaction. The above experiment implies that Car<sup>•+</sup> can abstract an electron from Fe<sup>2+</sup>. Another experiment shows that decreasing the concentration of Cl<sup>-</sup> by adding AgBF<sub>4</sub> to the reaction mixture, which results in the formation of AgCl, can increase the concentration of the radical cation (see Figure 6).

To examine whether  $\operatorname{Car}^{2+}$  can also abstract an electron from  $\operatorname{Fe}^{2+}$ , excess  $\operatorname{FeCl}_3$  (about 4 equiv) was added to I and II  $\operatorname{CH}_2$ - $\operatorname{Cl}_2$  solutions. Parts a and b of Figure 7 show the UV/vis spectra for the reaction mixtures of I and II, respectively. The absorption peak in the near infrared region is much broader than those in Figures 1 and 2. Because the absorption peak of  $\operatorname{Car}^{2+}$  should appear between the maxima of Car and  $\operatorname{Car}^{*+}$ , the broad peak could be due to overlap of  $\operatorname{Car}^{*+}$  and  $\operatorname{Car}^{2+}$ . These peaks disappear at once after addition of excess of BTAC and the peaks of neutral starting material reappear (the intensity of the neutral starting material is lower than that before addition of FeCl<sub>3</sub> because of the dilution of the solution after addition of FeCl<sub>3</sub> and BTAC solution), indicating that both  $\operatorname{Car}^{*+}$  and  $\operatorname{Car}^{2+}$ can abstract an electron from Fe<sup>2+</sup>.

OSWV was also used to examine the formation of  $Car^{2+}(I)$  when I was treated with 4 equiv of FeCl<sub>3</sub>. OSWV was first



**Figure 6.** UV/vis/NIR spectra showing the effect of adding AgBF<sub>4</sub> to the reaction solution of **I** and FeCl<sub>3</sub>. (a) Pure **I** in CH<sub>2</sub>Cl<sub>2</sub>, (b) 5 min after adding 1 equiv FeCl<sub>3</sub>, (c) after adding 1 drop of saturated AgBF<sub>4</sub> CH<sub>2</sub>Cl<sub>2</sub> solution to (b).



**Figure 7.** UV/vis/NIR spectra showing that the dication of **I** (a) and **II** (b) can also abstract an electron from  $Fe^{2+}$ . (1) Starting material; (2) after the starting material was treated with 4 equiv FeCl<sub>3</sub>, (3) after subsequent addition of a drop of saturated AgBF<sub>4</sub> (about 2 equiv of FeCl<sub>3</sub>).

used to determine the reduction potentials of  $Car^{2+}$  and  $Car^{*+}$ : oxidation of I for 1 min at 1100 mV (vs SCE) to produce  $Car^{2+}$ was then followed by scanning the voltage from +1100 to -100 mV. The reduction potentials for  $Car^{2+}$  and  $Car^{*+}$  are 928 and 800 mV (vs SCE), respectively (solid line in Figure 8). When I was treated with 4 equiv of FeCl<sub>3</sub> and then scanned from +1100 to -100 mV, the same reduction potential peaks for  $Car^{2+}$  and  $Car^{*+}$  appeared (dashed line in Figure 8), indicating that  $Car^{2+}$  is produced when I is treated with 4 equiv of FeCl<sub>3</sub>. The reduction potential peaks appear at 288 and 56 mV are attributed to those of iron ions. For II, the reduction potentials



**Figure 8.** OSWV of  $Car^{2+}$  and  $Car^{+}$  of **I**. Solid line: Oxidation of **I** for 1 min at 1100 mV (vs SCE) to produce  $Car^{2+}$  was then followed by scanning the voltage from +1100 to -100 mV. Dashed line: **I** was treated with 4 equiv of FeCl<sub>3</sub> and then scanned from +1100 to -100 mV.



**Figure 9.** HPLC showing equilibria exist in the reaction of **I** with FeCl<sub>3</sub>. (a) Pure **I**, (b) 30 s after **I** was treated with excess FeCl<sub>3</sub>, (c) solution b treated with 1 drop of saturated BTAC CH<sub>2</sub>Cl<sub>2</sub> solution (wavelength: 441 nm, the strongest absorbance of **I**, intensity normalized to strongest peak).

for  $\mbox{Car}^{\bullet +}$  and  $\mbox{Car}^{2+}$  are almost the same and cannot be distinguished by OSWV.

HPLC was used to further corroborate the equilibria. Figure 9 shows that a stable product was produced 30 s after I was treated with excess FeCl<sub>3</sub> (about 4 equiv of I) and all of the starting material was consumed. The peaks of I and its cis isomers appeared after adding a drop of saturated BTAC CH<sub>2</sub>-Cl<sub>2</sub> solution to the reaction solution, indicating that one or more equilibria exist. Because the stable product was produced after all starting material was consumed, the neutral starting material is not a direact precursor of the product. Similar results were found for II. The identification of the cis isomers is according to previous results.<sup>9,23,24</sup> <sup>1</sup>H NMR spectroscopy and values of the Q ratio<sup>25</sup> (absorbance of the "cis peak"/absorbance of the most intense maximum) were used to identify these isomers. The mechanism proposed below can account for the formation of cis isomers

$$trans-\operatorname{Car}^{\bullet+} \rightleftharpoons cis-\operatorname{Car}^{\bullet+}$$
$$trans-\operatorname{Car}^{2+} \rightleftharpoons cis-\operatorname{Car}^{2+}$$
$$cis-\operatorname{Car}^{2+} + \operatorname{Fe}^{2+} \rightleftharpoons cis-\operatorname{Car}^{\bullet+} + \operatorname{Fe}^{3+}$$
$$cis-\operatorname{Car}^{\bullet+} + \operatorname{Fe}^{2+} \rightleftharpoons cis-\operatorname{Car} + \operatorname{Fe}^{3+}$$

According to a previous study,<sup>23</sup> isomerization of Car is mediated by  $Car^{\bullet+}$  and  $Car^{2+}$ .



SCHEME 3: Structure of the 5,8-Peroxide of Car



To determine whether the products are produced by participation of  $O_2$  present in air, the reactions were carried out in a cuvette in a  $N_2$  drybox. After 10 min, the cuvette was sealed and taken out of the drybox, and the spectra were then measured. The absorption peak of the product and peak x were absent, but they appeared after the reaction mixture was exposed to  $O_2$ . From Figure 1, we can see that if the reaction is carried out in the presence of air, a strong peak of a product appears 10 min later, indicating that  $O_2$  was involved in the formation of the product.

Based on the above results, the mechanism shown in Scheme 2 is proposed for the reaction of Car with  $FeCl_3$  in  $CH_2Cl_2$  in the presence of oxygen. According to this scheme, the concentration of the dication of the starting material should decrease as the concentrations of neutral and radical cation species decrease. Because, however, the intensity of peak x in Figures 1 and 2 increases with the decrease in concentration of these two species, peak x is not due to the dication of the starting material. Oxidation of the isolated product with  $FeCl_3$  in  $CH_2Cl_2$  shows that a peak with the same absorption as peak x appears, implying that it is due to the radical cation of the product.

APCI LC-MS can provide rapid identification and quantification of carotenoid oxidation products.<sup>26</sup> The  $[M + H]^+$  ion is usually detected by this method.<sup>26</sup> APCI LC-MS shows that the base peak has the molecular mass of the starting material plus 33 (493 and 569 for the two cases, respectively. The mass of **I** and that of **II** are 460 and 536, respectively), indicating that O<sub>2</sub> has added to Car. According to other studies,<sup>27–30</sup> the product was tentatively assigned to the 5,8-peroxide of Car (CarO<sub>2</sub>) (see Scheme 3).

<sup>1</sup>H NMR data for the 5,8-peroxides of Car have been reported only for short chain carotenoids such as the 5,8-peroxide of ethyl 15-apo- $\beta$ -caroten-15-oate.<sup>31</sup> To establish the structure of peroxides of Car with long chains such as **I** and **II**, NMR studies are needed. The <sup>1</sup>H NMR spectra (Figure 10) of **I** and its reaction product show that the chemical shifts of the protons of the product are almost the same as those of **I**, with the exception of H-7 and H-8. Similar results were found for **II**. The chemical shifts of H-7 and H-8 of the products are the same as those of the 5,8-peroxide of ethyl 15-apo- $\beta$ -caroten-15-oate reported by Pfoertner<sup>31</sup> (5.56 and 4.65, respectively).



Figure 10. <sup>1</sup>H NMR spectra in CDCl<sub>3</sub> of I (a) and the product (b) and (c).

TABLE 1:  $\lambda_{max}$  (nm) Calculated by ZINDO/S<sup>*a*</sup>

	$S_0 \rightarrow S_2$		$D_0 \rightarrow D_2$	
	Car	CarO <sub>2</sub>	Car•+	$CarO_2^{\bullet+}$
I II	415 (446) 434 (460)	400 (420) 398 (432)	814 (848) 872 (973)	720 (658) 843 (790)

 $^{\it a}$  The  $\lambda_{max}$  (nm) in  $CH_2Cl_2$  determined by experiment is in parentheses.

The optical absorption of CarO<sub>2</sub> is blue-shifted compared to that of Car because the conjugated chain of CarO<sub>2</sub> is shorter than that of Car. The maximum absorption wavelengths ( $\lambda_{max}$ ) of Car, CarO<sub>2</sub>, Caro+, and CarO<sub>2</sub>o+ by gas-phase ZINDO/S calculations are listed in Table 1. The maxima of these species in CH<sub>2</sub>Cl<sub>2</sub> determined by UV/vis are also included. The geometries of these species were optimized using the AM1 method. The restricted Hartree-Fock (RHF) formalism was used to optimize the geometries of the neutral species, and unrestricted Hartree-Fock (UHF) was used to optimize those of radical cations. The single-excitation configuration interaction was used to calculate the optical absorption spectrum. The weighting factors for  $\sigma - \sigma$  and  $\pi - \pi$  overlap are 1.267 and 0.585, respectively. The calculation shows that, although the absolute values of the calculated absorption wavelengths do not match the experimental value, the relative absorption wavelengths for the different species are in agreement with the experimental values.

The 5,8-peroxide of long-chain Car is a specific marker for singlet oxygen quenching by Car,<sup>30,32–34</sup> but it was also found in the thermal oxidation.<sup>28,35,36</sup> Formation of singlet O<sub>2</sub> is usually due to energy transfer from the excited state of a sensitizer to the triplet ground state of O<sub>2</sub>. In the present study, the reaction mixture was kept in the dark and singlet O<sub>2</sub> cannot be formed.

The formation of the 5,8-peroxide should be due to other mechanisms. It is known<sup>37</sup> that radical cations of organic compounds may react with  $O_2^{\bullet-}$  to form peroxides. A possible reaction mechanism for the formation of CarO<sub>2</sub> in the oxidation of Car with FeCl<sub>3</sub> in the presence of O<sub>2</sub> is shown below:

$$Car + Fe^{3+} \rightleftharpoons Car^{\bullet+} + Fe^{2+}$$
$$O_2 + Fe^{2+} \rightleftharpoons O_2^{\bullet-} + Fe^{3+}$$
$$Car^{\bullet+} + O_2^{\bullet-} \rightleftharpoons CarO_2$$
$$CarO_2 + Fe^{3+} \rightleftharpoons CarO_2^{\bullet+} + Fe^{2+}$$

However, according to this mechanism, the concentrations of Car<sup>•+</sup> and the radical cation of the product (CarO<sub>2</sub><sup>•+</sup>) cannot be linearly related, and there should be no isosbestic point between the peaks of Car<sup>•+</sup> and CarO<sub>2</sub><sup>•+</sup> in Figures 1 and 2. The same product was formed in the electrolysis of the neutral starting material in the presence of O<sub>2</sub>, indicating that the product is probably due to the reaction of Car<sup>•+</sup> and O<sub>2</sub> because O<sub>2</sub><sup>•-</sup> cannot be produced under our electrolysis conditions. We propose the following mechanism for the formation of CarO<sub>2</sub> in the oxidation of Car with FeCl<sub>3</sub> in the presence of O<sub>2</sub>:

$$Car + Fe^{3+} \rightleftharpoons Car^{\bullet +} + Fe^{2+}$$
$$Car^{\bullet +} + O_2 \rightarrow CarO_2^{\bullet +}$$
$$CarO_2^{\bullet +} + Fe^{2+} \rightleftharpoons CarO_2 + Fe^{3+}$$
$$CarO_2^{\bullet +} + Car \rightleftharpoons CarO_2 + Car^{\bullet +}$$

This mechanism is consistent with the facts that the concentrations of  $Car^{\bullet+}$ ,  $CarO_2^{\bullet+}$ , and  $CarO_2$  are linearly related and that the concentrations of both  $CarO_2^{\bullet+}$  and  $CarO_2$  increase with the decrease of  $Car^{\bullet+}$  and Car.

### Conclusions

In the oxidation of carotenoids with FeCl<sub>3</sub>, there exist equilibria among the carotenoid, the radical cation, dication, and various inorganic species. In addition, the radical cation and dication can abstract an electron from Fe<sup>2+</sup>. This phenomenon is more pronounced for Car containing an electronwithdrawing group. Isomerization via Car<sup>•+</sup> and Car<sup>2+</sup> occurs during the oxidation. The 5,8-peroxide of Car is formed when the reaction mixture is exposed to O<sub>2</sub>. The formation of this compound is attributed to the reaction of Car<sup>•+</sup> with O<sub>2</sub>. Whether such a reaction exists in the reaction center of photosystem II, and thus protects it, needs further study. Because Car<sup>•+</sup> is also formed by photoillumination, the previous studies<sup>28-30,32-34</sup> need to be reexamined: Is the formation of the 5,8-peroxide of Car due to the oxidation of Car by singlet oxygen, or is it due to the reaction of Car<sup>•+</sup> with O<sub>2</sub>, or both? This study gives both NMR and MS evidence for the formation of the 5,8-peroxide of carotenoids with long chains. Previous studies  $^{28-30,32-36}$  gave only LC-MS evidence for the formation of the 5,8-peroxide of II because of difficulties in the purifications.

Oxidation of Car by singlet oxygen<sup>28–30,32–34</sup> or by thermal oxidation<sup>28,35,36</sup> in other systems affords many products, and the yield of 5,8-peroxide is very low so that separation of this compound on a preparative scale is impossible. The strong selectivity and high yield (more than 90%) of this compound produced by the method described in this study should facilitate

the synthesis and purification of this group of carotenoids so that physiochemical properties of these compounds can be studied in the future. These compounds may be precursors of other degradation products, when carotenoids are oxidized in the presence of  $O_2$ . Study of these compounds may contribute to understanding the degradation pathway of carotenoids in biological systems.

**Acknowledgment.** We thank Dr. E. Hand for helpful discussions. This work was supported by the Division of Chemical Sciences, Office of Basic Energy Sciences, Office of Energy Research of the U.S. Department of Energy under Grant No. DE-FG02-86ER13465.

### **References and Notes**

- (1) Koyama, Y. J. Photochem. Photobiol. B 1991, 9, 265.
- (2) Mimuro, M.; Katoh, T. Pure Appl. Chem. 1991, 63, 123.
- (3) Witt, H. T. Biochim. Biophys. Acta 1979, 505, 355.
- (4) Mordi, R. C. Chem. Ind. 1993, 3, 79.
- (5) Tang, G.; Wang, X.-D.; Russell, R. M.; Krinsky, N. I. *Biochemistry* 1991, 30, 9829.
  - (6) Handelman, G. J. Free Rad. Biol. Med. 1991, 10, 427.
- (7) El-Tinay, A. H.; Chichester, C. O. J. Org. Chem. 1970, 35, 2290.
  (8) Mordi, R. C.; Walton, J. C.; Burton, G. W.; Hughes, L.; Ingold, K.
- U.; Lindsay, D. A.; Moffatt, D. J. *Tetrahedron* 1993, 49, 911.
  (9) Wei, C. C.; Gao, G.; Kispert, L. D. J. Chem. Soc., Perkin Trans.
- 2 **1997**, 783. (10) Schenck, C. C.; Diner, B.; Mathis, P.; Satoh, K. *Biochim. Biophys.*
- (10) Schenck, C. C.; Diner, B.; Matnis, P.; Saton, K. *Biochim. Biophys.* Acta **1982**, 680, 216.
- (11) Mathis, P.; Rutherford, A. W. Biochim. Biophys. Acta 1984, 767, 217.
- (12) De Las Rivas, J. O.; Telfer, A.; Barber, J. Biochim. Biophys. Acta 1993, 1142, 155.
- (13) Hanley, J.; Deligiannakis, Y.; Pascal, A.; Faller, P.; Rutherford, A. W. *Biochemistry* **1999**, *38*, 8195.
- (14) Jeevarajan, J. A.; Wei, C. C.; Jeevarajan, A. S.; Kispert, L. D. J. Phys. Chem. **1996**, 100, 5637.

- (15) Gao, G.; Deng, Y.; Kispert, L. D. J. Phys. Chem. B 1997, 101, 7844.
- (16) Dewar, M. J. S.; Zoebisch, E. E.; Healy, E. F.; Stewart, J. J. P. J. Am. Chem. Soc. **1985**, 107, 3902.
  - (17) Ridley, J.; Zerner, M. C. Theor. Chim. Acta 1973, 32, 111.
  - (18) Ridley, J.; Zerner, M. C. Theor. Chim. Acta 1976, 42, 223.
  - (19) Cohen, M. D.; Fischer, E. J. Chem. Soc. 1962, 3044.
- (20) Obermoller, H. R.; White, D. A.; Lagos, S. *Hydrometallurgy* **1991**, 27, 63.
- (21) Russo, M. V.; Polzonetti, G.; Furlani, A. Synth. Methods 1991, 39, 291.
- (22) Aronson, F. L.; Hwang, L. L.-Y.; Ronca, N.; Solomon, N. A.; Steigman, J. *Inorg. Chem.* **1985**, *24*, 541.
- (23) Gao, G.; Wei, C. C.; Jeevarajan, A. S.; Kispert, L. D. J. Phys. Chem. 1996, 100, 5362.
- (24) Gao, G.; Deng, Y.; Kispert, L. D. J. Phys. Chem. B 1998, 102, 3897.
- (25) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. J. Am. Chem. Soc. **1985**, 107, 3902.
  - (26) Liebler, D. C.; McClure, T. D. Chem. Res. Toxicol. 1996, 9, 8.
- (27) Oyler, A. R.; Motto, M. G.; Naldi, R. E.; Facchine, K. L.; Hamburg, P. F.; Burinsky, D. J.; Dunphy, R.; Cotter, M. L. *Tetrahedron* **1989**, *45*,
- 7679. (28) Henry, L. K.; Puspitasari-Nienaber, N. L.; Jarén-Galán, M.;
- Breemen, R. B. V.; Catignani, G. L.; Schwartz, S. J. J. Agric. Food Chem. 2000, 48, 5008.
- (29) Stratton, S. P.; Liebler, D. C. Biochemistry, 1997, 36, 12911.
- (30) Stratton, S. P.; Schaefer, W. H.; Lieber, D. C. Chem. Res. Toxicol. 1993, 6, 542.
- (31) Pfoertner, K.-H. Helv. Chim. Acta 1975, 58, 833.
- (32) Junji, T.; Noriko, B.; Hiroki, H.; Ryo, Y. Abstr. Pap.-Am. Chem. Soc. 2001, 221st, AGFD-093.
- (33) Ryo, Y.; Kakue, T.; Koji, K. Biosci., Biotechnol., Biochem. 1998, 62, 1301.
- (34) Stratton, S. P.; Schaefer, W. H.; Liebler, D. C. *Chem. Res. Toxicol.* **1993**, *6*, 542.
- (35) Marty, C.; Berset, C. J. Food Sci. 1988, 53, 1880.
- (36) Marty, C.; Berset, C. J. Agric. Food Chem. 1990, 38, 1063.
- (37) Schaap, A. P.; Lopez, L.; Gagnon, S. D. J. Am. Chem. Soc. 1983, 105, 663.