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Cleavage Products of Lycopene Produced by in Vitro Oxidations: Characterization and Mechanisms of Formation

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The aim of this study was to produce in vitro oxidation products of lycopene, which could be possible in vivo metabolites. An oxidation of lycopene with potassium permanganate gave a range of lycopene degradation compounds resulting from the oxidative cleavage of one or two carbon−carbon double bonds. Eleven apo-lycopenals/ones and six apo-carotendials were obtained and tentatively characterized by HPLC-DAD-MS. Apo-11-lycopenal and apo-8,6′-carotendial were isolated and characterized by ^{1}H NMR for the first time. Lycopene was submitted to an oxidation by atmospheric oxygen catalyzed by a metalloporphyrin, a model system of the active center of cytochrome P450 enzymes. *(Z)*-Isomers, monoxides, and cleavage compounds of *(E)*-lycopene were formed. We propose a mechanism of oxidation of lycopene by this system.

KEYWORDS: Lycopene; oxidation; apo-lycopenal/one; apo-carotendial

INTRODUCTION

Carotenoids are plant secondary metabolites which present a potential beneficial effect on human health (*1*). Except for the well-known provitamin A activity of some carotenoids, they could also be involved in protective effects against degenerative diseases such as cancers or cardiovascular diseases. The mechanism by which they act in vivo is not fully understood. Hypotheses are: antioxidant actions (*2*), influence on the immune system (*3*), and interaction with gap junctional intercellular communication (GJIC) (*4*), but metabolites, and particularly oxidative metabolites of carotenoids could contribute in vivo to effects attributed to the native molecule (*5*−*7*). However, only a few metabolites have been detected in vivo, one reason being their low concentration and the difficulty of detection in biological fluids.

Lycopene, the red pigment of tomato, is one of the most interesting carotenoids considering beneficial effects on health (*8*, *9*). It has been suggested that lycopene could be involved in protection against chronic diseases (*10*, *11*) such as prostate cancer (*12*). It is frequently consumed in the Western world through fresh or processed tomatoes. Even if lycopene has been proven to be the most effective carotenoid as singlet oxygen quencher in vitro (*13*), no clear mechanisms of action in vivo have been found. Only one metabolite of lycopene was detected in vivo, namely, 2,6-cyclolycopene-1,5 diol (*14*). Recently, Kim et al. (*15*) have described the formation of cleavage compounds of lycopene under different conditions of autoxidation, but so far these compounds have not been found in vivo.

This investigation was designed to characterize oxidation products of lycopene obtained by two different systems of oxidation, with potassium permanganate and by atmospheric oxygen catalyzed by a metalloporphyrin.

MATERIALS AND METHODS

Chemicals. $KMnO_4$, cetyltrimethylammonium bromide, carbonyl-ruthenium(II)-tetraphenylporphyrin (Ru(CO)TPP), 3-chloroperoxybenzoic acid, $CaCl_2$, Na_2SO_4, basic Al_2O_3, NH_4OAc, were purchased from Sigma-Aldrich (St Quentin Fallavier, France). Methanol was from Merck (Darmstadt, Germany) and of HPLC grade. All other solvents were from Carlo Erba (Val de Reuil, France). Ethanol, toluene, and methylene chloride were of analytical grade. Acetone, *n*-hexane, *tert*-butyl methyl ether (MTBE) and tetrahydrofuran (THF) were of HPLC grade. Methylene chloride was poured through basic alumina before use to eliminate traces of acidity. Water was purified through a Millipore Q-Plus.

Spectrophotometric Apparatus. For purity controls, UV−vis spectra were recorded on a Cary-1E (Varian). Measurements were made at room temperature in quartz cells (length 1 cm).

Analytic HPLC-DAD-MS Apparatus and Conditions. HPLC analyses were carried out with a Hewlett-Packard (HP) model 1050 equipped with a quaternary pump solvent delivery and a diode array detector (DAD) switched in line with a Micromass Platform LCZ 4000. The column used was a 250 × 4.6 mm i.d., 5 μm, YMC Pack C30 (YMC Inc., Wilmington, NC) equipped with a 20 × 4.6 mm, 5 μm, C-30 precolumn. The column was kept at 27 °C. Absorption spectra were recorded between 220 and 600 nm. Acquisition of the mass data between *m*/*z* 100 and 700 was performed in the positive electrospray mode. The program used for data analyses was Masslynx version 3.4.

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Parameters, and especially cone voltage, were optimized in order to avoid fragmentation. The following gradient system was used with H_2O containing NH_4OAc 25 mM (solvent A), methanol containing NH_4OAc 25 mM (solvent B), and MTBE (solvent C): 0 to 2 min, %A−%B−%C, 40−60−0; 5 min, %A−%B−%C, 20−80−0; 10 min, %A−%B−%C, 4−81−15; 60 min, %A−%B−%C, 4−11−85; 80 min, %A−%B−%C, 4−11−85; 80.01 min, %A−%B−%C, 0−100−0. The flow was 1 mL/min.

Preparative HPLC Apparatus and Conditions. For apo-8,6′-carotendial, preparative HPLC was carried out with a Waters model 600 equipped with a quaternary pump solvent. The column used was a 250 × 20 mm i.d., 5 μm, YMC Pack C30 (YMC Inc., Wilmington, NC) equipped with a 21.2 × 30 mm i.d., 10 μm C-18 precolumn. The column was kept at 25 °C. The following gradient system was used with H_2O (solvent A), methanol (solvent B), THF (solvent C) and acetone (solvent D): 0 min, %A−%B−%C−%D, 40−60−0−0; 10 min, %A−%B−%C−%D, 30−65−5−0; 15 min, %A−%B−%C−%D, 25−65−10−0; 20 min, %A−%B−%C−%D, 20−65−15−0, 30 min, %A−%B−%C−%D, 15−55−15−15; 35 min, %A−%B−%C−%D, 10−45−10−35; 40 min, %A−%B−%C−%D, 5−30−5−60; 50 min, %A−%B−%C−%D, 0−0−0−100. The flow was 15 mL/min.

Apo-11-lycopenal was isolated after HPLC using an Agilent 1100 analytical system. Column and eluant were the same as those described above for analytical HPLC-DAD-MS.

NMR Apparatus and Conditions. NMR analysis were performed on a Bruker Avance DRX-500 apparatus equipped with a cryoprobe. ^{1}H NMR of apo-8,6′-carotendial was performed in $CDCl_3$. ^{1}H NMR of apo-11-lycopenal was performed in $CDCl_3$ and CD_3OD.

Preparation of Lycopene. Lycopene was extracted from tomato oleoresin kindly supplied by Naturex (Avignon, France). Tomato oleoresin (20.56 g) was mixed with *n*-hexane (100 mL), the insoluble fraction was filtered and washed twice with *n*-hexane (15 mL) and twice with ethanol (10 mL). The solid was dried overnight under reduced pressure in a desiccator containing $CaCl_2$. Crude lycopene (1.2 g) was obtained. Purity was checked by UV−vis spectrophotometry and HPLC-DAD-MS. No other compounds were detected. Lycopene was kept at −20 °C under an atmosphere of argon.

Oxidation of Lycopene by $KMnO_4$. Lycopene (39.8 mg) and cetyltrimethylammonium bromide (8.1 mg) were dissolved in methylene chloride/toluene (20 mL/20 mL). An aqueous solution of $KMnO_4$ (12 mL, 15 mg $KMnO_4$/mL water) was added to the organic solution. The reaction mixture was stirred on a magnetic stirrer at room temperature for 1 h and filtered through an Acrodisc, 0.45 μm, 32 mm PVDF filter. The organic phase was washed with water (30 mL), filtered through a PVDF filter and dried on Na_2SO_4. After evaporation under reduced pressure, the residue was dissolved in 2.2 mL CH_3OH/MTBE (80/20, v/v) for HPLC-DAD-MS analysis.

Oxidation of Lycopene by Atmospheric Oxygen Catalyzed by Ru(CO)TPP in the Presence of 3-Chloroperoxybenzoic Acid. Ru(CO)TPP (4.1 mg) was dissolved in methylene chloride (5 mL), 3-chloroperoxybenzoic acid (1.9 mg) was added, and the reaction mixture was stirred for 5 min. Then, lycopene (13 mg) was added. Aliquots of 0.5 mL were taken from the reaction mixture after 1, 5, 24, 48, and 96 h, filtered through PVDF filters, and diluted with 0.5 mL of CH_3OH/MTBE (80/20, v/v) for HPLC-DAD-MS analysis.

RESULTS AND DISCUSSION

Oxidation of Lycopene with Potassium Permanganate. The oxidation of lycopene by potassium permanganate was performed in a biphasic system using cetyltrimethylammonium bromide as phase transfer catalyst. After 1 h of reaction at room temperature, an HPLC-DAD-MS analysis showed that all lycopene was consumed, and a range of products was formed. The absorbance and total ion chromatograms (TIC) obtained by UV−vis detection and by mass detection respectively of the extracted reaction mixture are presented in **Figure 1**. The majority of the products were tentatively identified by detecting the corresponding molecular weight for all of them, and for most of them, the UV−vis spectral data, which were compared with literature values when available. For two compounds, we were able to confirm their structures, because it was possible to purify them by preparative HPLC and subsequently analyze them by ^{1}H NMR.

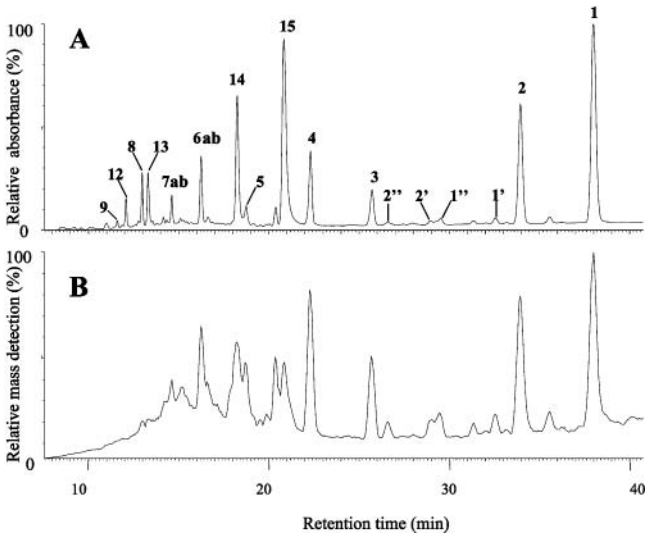

Figure 1. HPLC chromatograms obtained by (A) UV–vis detection and (B) mass spectrometric detection of the compounds resulting from the oxidation of lycopene by potassium permanganate. Numbers correspond to compounds listed in **Figure 2** and **3**. **1′** and **1′′** are *(Z)*-isomers of compound **1**. **2′** and **2′′** are *(Z)*-isomers of compound **2**.

Table 1. ^{1}H NMR of Apo-11-lycopenal (**8**) in CD_3OH at 500 MHz[a]

proton	δ in ppm (in CD_3OD) (multiplicity, coupling constant in Hz)
CH_3–C(1) *trans*	1.68 (s)
CH_3–C(1) *cis*	1.62 (s)
H–C(2)	5.09 (m) (in $CDCl_3$)
H–C(3) and H–C(4)	2.17 (m)
CH_3–C(5)	1.90 (d, $J = 1.0$)
H–C(6)	6.05 (d, $J = 11.0$)
H–C(7)	7.14 (dd, $J_1 = 11.0$, $J_2 = 15.2$)
H–C(8)	6.34 (d, $J = 15.2$)
CH_3–C(9)	2.34 (d, $J = 1.0$)
H–C(10)	5.93 (d, $J = 8.4$)
H–C(11)	10.05 (d, $J = 8.4$)

[a] s = singlet, d = doublet, dd = doublet of doublet, m = multiplet.

Analysis of the extracted reaction mixture showed the presence of two types of oxidation compounds, those resulting from a single oxidative cleavage, and others derived from a double oxidative cleavage of lycopene. The first type of compounds contain on one end an aldehyde or a ketone group and on the other end of the molecule the ψ-end group of lycopene. These were called apo-lycopenal and apo-lycopenone, respectively. The second category of products obtained contain two aldehyde end groups and were called apo-carotendials, because their structure could have been obtained from any carotenoid and not only from lycopene. The chemical structures of the identified apo-lycopenals/ones and apo-carotendials are shown in **Figure 2** and **Figure 3**. ^{1}H NMR of apo-11-lycopenal (**8**) and apo-8,6′-carotendial (**14**) are presented for the first time in **Tables 1** and **2**.

When modern analytical techniques were not yet available, potassium permanganate was used for the oxidative fission of carotenoids to elucidate their constitution. Large degradation fragments identified as apo-carotenals were obtained (*17*). In

Table 2. Maximum Wavelengths of Absorption of Apo-carotendials Obtained from the Oxidation of Lycopene by Potassium Permanganate

product no.	product name	λ_{max} (nm) experimental	λ_{max} (nm) literature	no. of carbon–carbon conjugated double bonds
12	apo-8,12′-carotendial	396, 414		5
13	apo-6,12′-carotendial or apo-10,8′-carotendial	422, 440		6
6b	apo-8,8′-carotendial = crocetinedial	442, 466	410, 433, 459 (EtOH) (*23*)	7
7b	apo-6,10′-carotendial	444, 464		7
14	apo-8,6′-carotendial	464, 485 (shoulder)	453, 484, 517 (carbon disulfide) 452, 480 (petroleum ether) (*24*)	8
15	apo-6,6′-carotendial	484, 505 (shoulder)		9

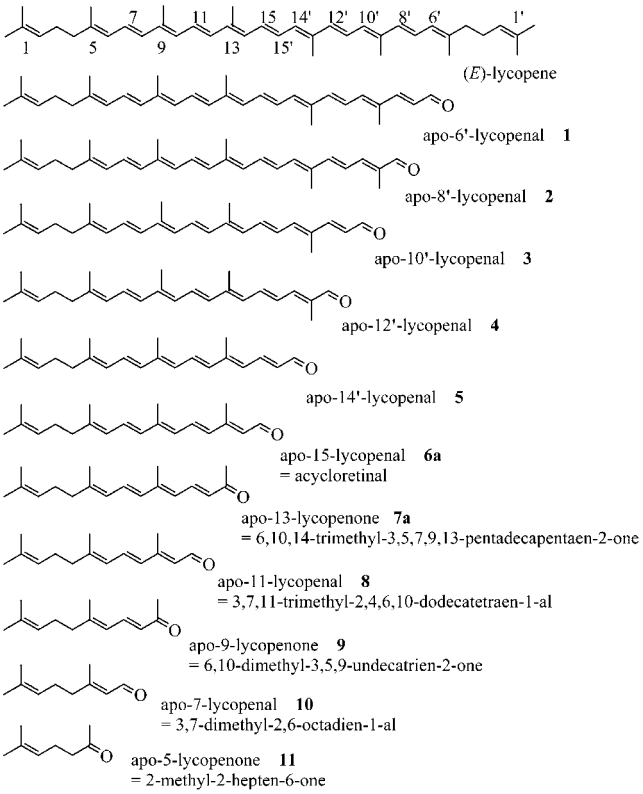

Figure 2. Structures of apo-lycopenals and apo-lycopenones detected by HPLC-DAD-MS obtained from the oxidation of lycopene by potassium permanganate.

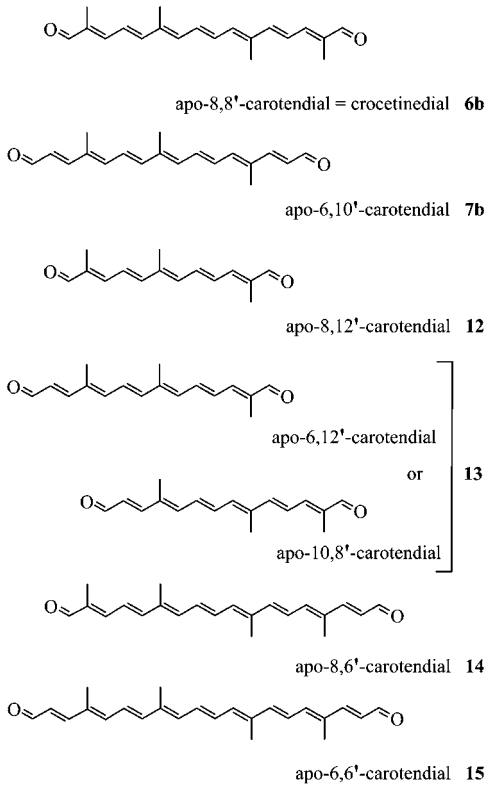

Figure 3. Structures of apo-carotendials detected by HPLC-DAD-MS obtained from the oxidation of lycopene by potassium permanganate.

1973, Ben-Aziz et al. (*18*) mentioned that apo-6′- and apo-8′-lycopenal were obtained by oxidation of lycopene with potassium permanganate. Ukai et al. (*19*) described a protocol for the reaction which gave apo-6′-lycopenal as the main product after 44 h, whereas around 50% of the lycopene was left. We optimized experimental conditions to completely oxidize lycopene and to obtain mono oxidative cleavage compounds as major products. A mixture of methylene chloride/toluene (60/40, v/v) to solubilize lycopene and the use of cetyltrimethylammonium bromide as phase transfer catalyst were found to be the best compromise. After the complete disappearance of lycopene, we detected eight apo-lycopenals, three apo-lycopenones (**Figure 2**), and six apo-carotendials (**Figure 3**). The former compounds result from the oxidative cleavage of one carbon−carbon double bond of lycopene, thus giving a range of products from the longest apo-6′-lycopenal (**1**) to the shortest one detected, apo-5-lycopenone (**11**). Tentative structure assignments were possible with the detection of the molecular weight and the maximum wavelength of absorption compared to those found in the literature (*15*). Mono cleavage compounds of lycopene have been tentatively identified by Kim et al. (*15*) using HPLC analysis with UV−vis detection. Our results were comparable to what they obtained for the series of compounds containing 11 (apo-6′-lycopenal (**1**)) to 3 conjugated double bonds (3,7,11-trimethyl-2,4,6,10-dodecatetraen = apo-11-lycopenal (**8**)). We tentatively identified three more compounds containing lower numbers of conjugated double bonds: 6,10-

Table 3. ^{1}H NMR of Apo-8,6′-carotendial (**14**) in $CDCl_3$ at 500 MHz[a]

proton	δ in ppm (multiplicity, coupling constant in Hz)
H–C(6′)	9.58 (d, $J = 7.8$)
H–C(7′)	6.19 (dd, $J_1 = 15.2$, $J_2 = 7.8$)
H–C(8′)	7.16 (d, $J = 15.2$)
CH_3–C(9′)	1.97 (s)
H–C(10′) H–C(11′) H–C(12′)	6.67 (m)
CH_3–C(13′)	2.00 (s)
H–C(14′)	6.38 (d, $J = 11.0$)
H–C(15′) H–C(15)	6.67 (m)
H–C(14)	6.44 (d, $J = 10.8$)
CH_3–C(13)	2.00 (s)
H–C(11) H–C(12)	6.67 (m)
H–C(10)	6.93 (d, $J = 9.5$)
CH_3–C(9)	1.89 (s)
H–C(8)	9.45 (s)

[a] s = singlet, d = doublet, dd = doublet of doublet, m = multiplet.

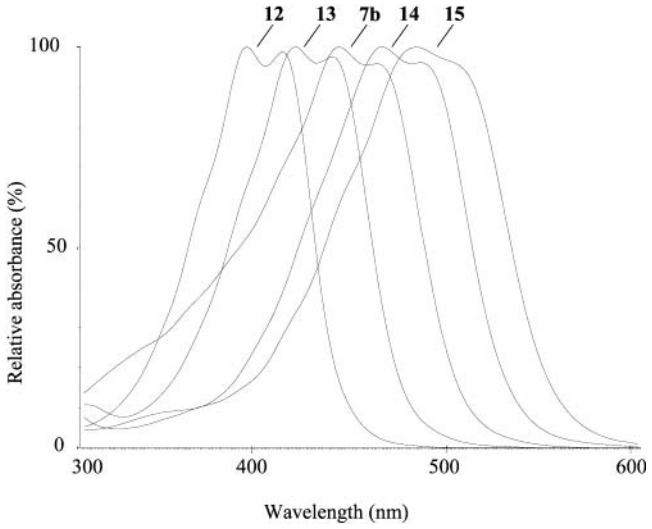

Figure 4. UV–vis spectra of apo-carotendials from the oxidation of lycopene by potassium permanganate. The number indicates the peak number given in **Figure 3**.

dimethyl-3,5,9-undecatriene-2-one (apo-9-lycopenone, **9**); 3,7-dimethyl-2,6-octaene-1-al (apo-7-lycopenal, **10**); and 2-methyl-2-heptene-6-one (apo-5-lycopenone, **11**). Compounds **10** and **11** were detected by their mass, but their UV−vis spectra was not possible to find, probably because these compounds are present in very small amounts and have a low molecular extinction coefficient; as a consequence, they are not visible in **Figure 1**. However, confirmation of the structure assignment can be obtained only when products are characterized by NMR. We thus confirmed the assignment of apo-11-lycopenal (**8**) by ^{1}H NMR (**Table 1**). We did not purify the other mono-cleavage compounds, as NMR data have been published for apo-6′-lycopenal (**1**) (*19*), apo-8′-lycopenal (**2**) (*20*, *21*), apo-10′-lycopenal (**3**) (*20*), apo-12′-lycopenal (**4**), and apo-15-lycopenal (**6a**) (*22*).

In the case of apo-carotendials issued from the cleavage of two carbon−carbon double bonds, only the double bonds 5−6 (or 5′−6′), 7−8 (or 7′−8′), 9−10 (or 9′−10′), and 11−12 (or 11′−12′) were affected by the cleavage. We did not detect apo-carotendials/ones resulting from cleavages of double bonds closer to the center of the molecule (15−15′ carbon−carbon double bond). No cleavage was detected on the double bond which is the farthest from the center of the molecule, i.e., the 1−2 double bond, which is not conjugated and is trisubstituted; this could explain its non reactivity to the oxidation with potassium permanganate. Four of the six apo-carotendials tentatively identified, apo-6,10′-carotendial (**7b**), apo-8,12′-carotendial (**12**), apo-6,12′- or -10,8′-carotendial (**13**), and apo-6,6′-carotenendial (**15**) (**Figure 3**) were not mentioned in the literature. The structure of the apo-carotendials were assigned by detecting their molecular weight and their maximum absorption in UV−vis spectra, which were consistent either with their number of conjugated double bonds or with data found in the literature when available (**Table 3**) (*23*, *24*). These data were available only for the apo-8,8′-carotenedial (also called crocetinedial) (**6b**) and for apo-8,6′-carotendial (**14**). The differences in value of the maximum wavelength can be accounted for by the different solvents used. It is interesting to

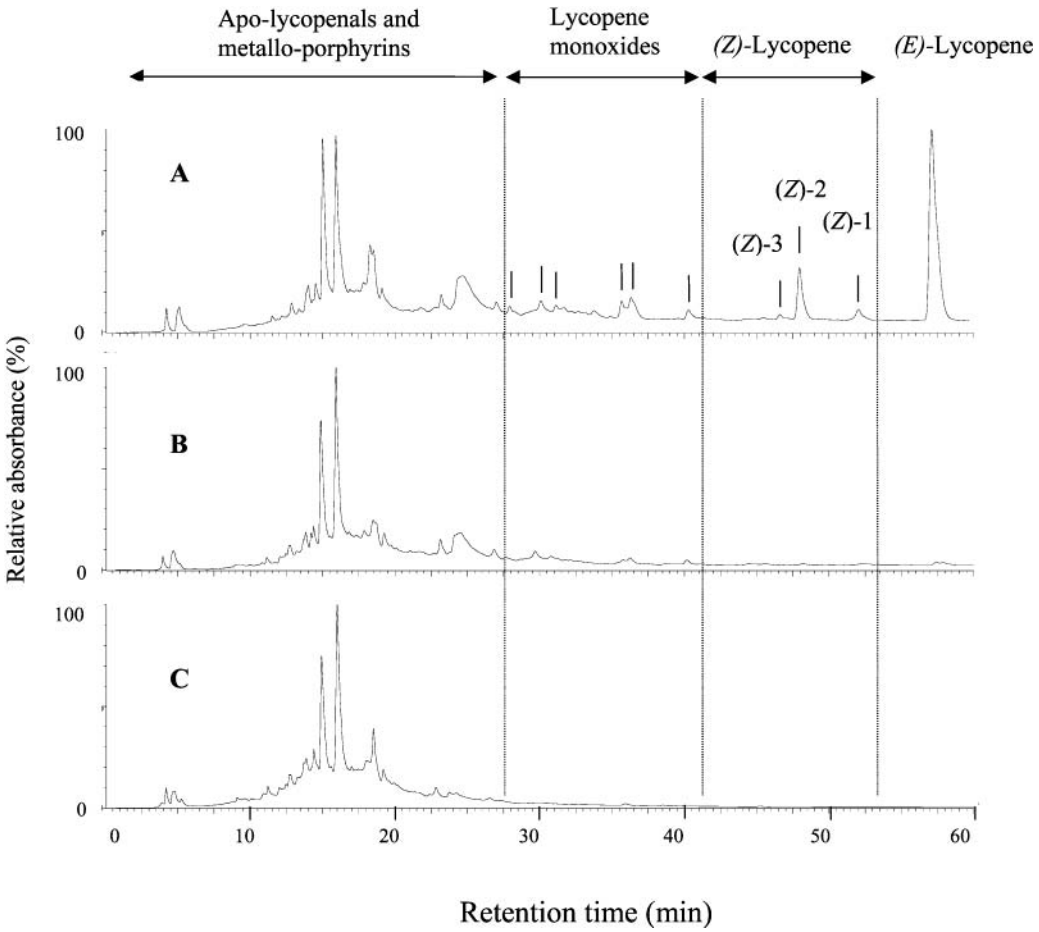

Figure 5. HPLC chromatograms obtained by UV–vis detection of the compounds issued from the oxidation of *(E)*-lycopene by oxygen, catalyzed by Ru(CO)TPP in the presence of 3-chloroperoxybenzoic acid after (A) 1 h, (B) 24 h, and (C) 96 h.

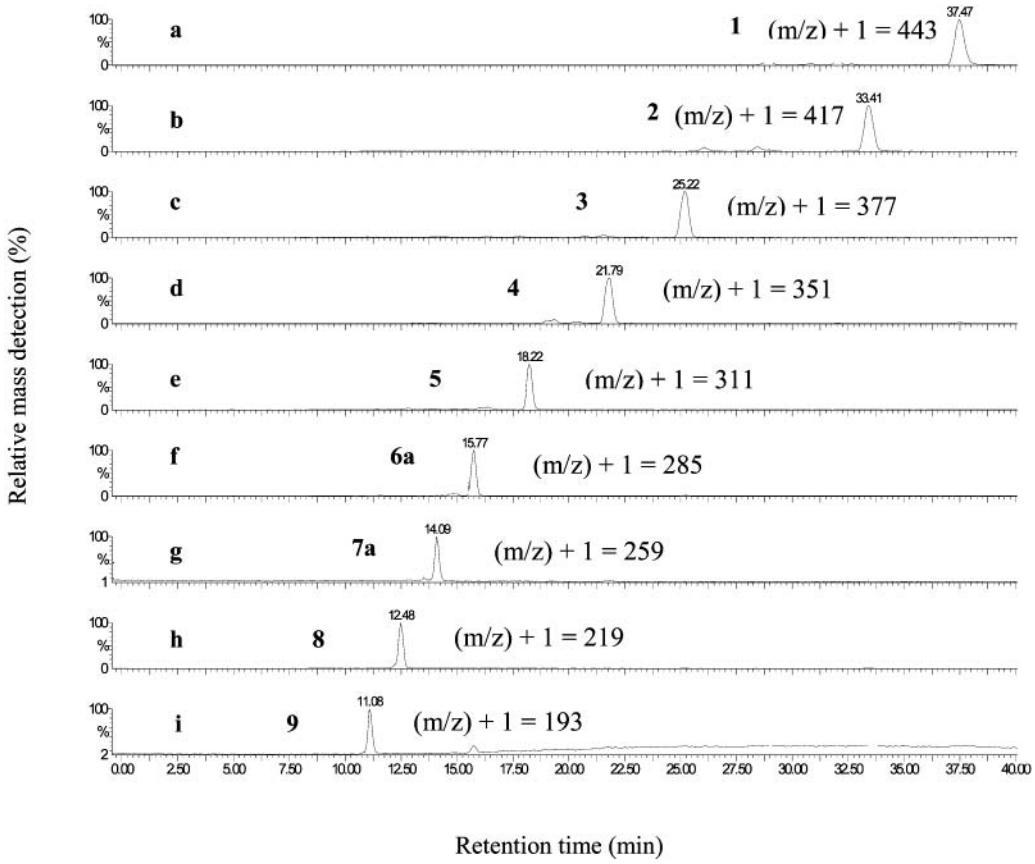

Figure 6. HPLC chromatograms obtained by mass detection of the apo-lycopenals at (a) (*m*/*z*) + 1 = 443, **1**; (b) (*m*/*z*) + 1 = 417, **2**; (c) (*m*/*z*) + 1 = 377, **3**; (d) (*m*/*z*) + 1 = 351, **4**; (e) (*m*/*z*) + 1 = 311, **5**; (f) (*m*/*z*) + 1 = 285, **6a**; (g) (*m*/*z*) + 1 = 259, **7a**; (h) (*m*/*z*) + 1 = 219, **8**; (i) (*m*/*z*) + 1 = 193; **9**. Number of products correspond to compounds listed in **Figure 2**.

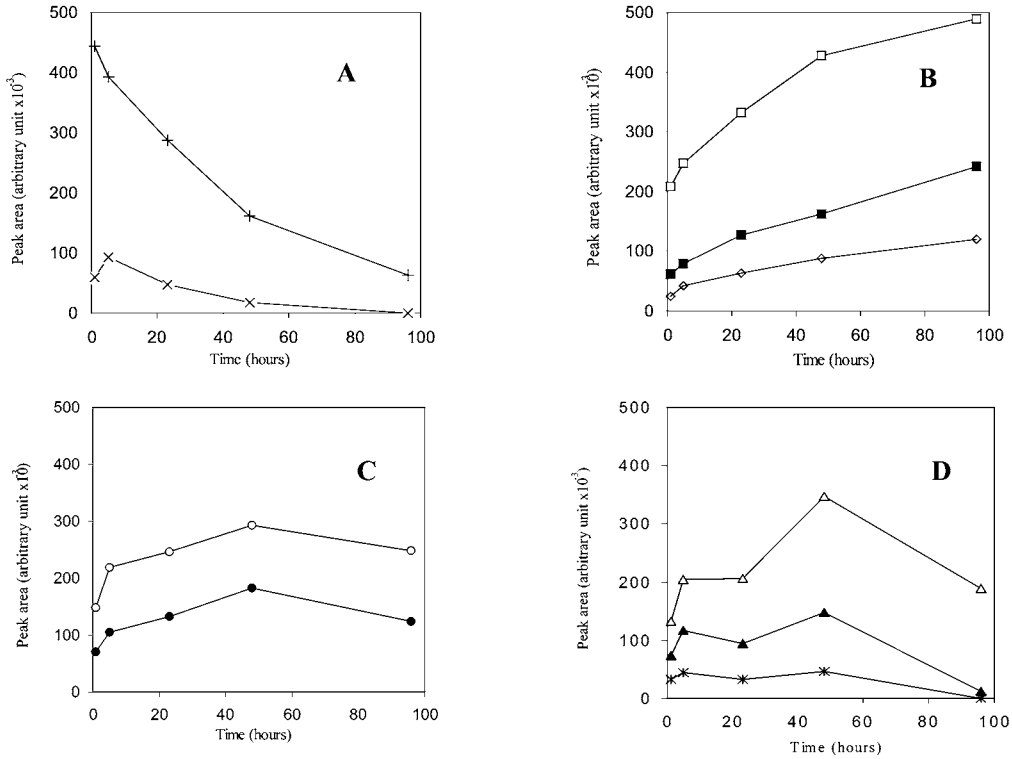

Figure 7. Evolution with time of the mass peak area of (A) lycopene monoxides + and apo-6′-lycopenal ×; (B) apo-13-lycopenone □, apo-11-lycopenone ■, and apo-9-lycopenal ◇; (C) apo-15-lycopenal ● and apo-14′-lycopenal ○; and (D) apo-12′-lycopenal △, apo10′-lycopenal ▲, and apo-8′-lycopenal ∗.

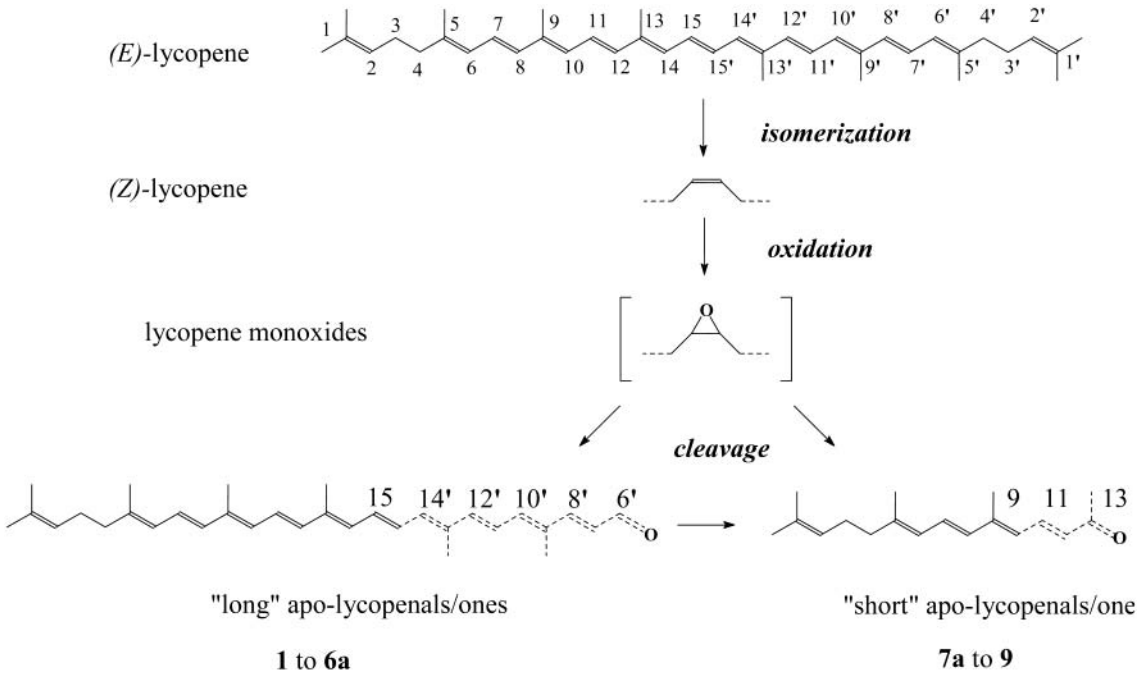

Figure 8. Possible mechanism of formation of apo-lycopenals and apo-lycopenones by oxidation of *(E)*-lycopene with oxygen from air catalyzed by Ru(CO)TPP.

note that the UV−vis spectra of apo-lycopenals were bell-shaped, whereas for the apo-carotendials, a fine structure present in the UV−vis spectra of lycopene was partly observed (**Figure 4**). Thus, two maximums of absorption were measured for apo-carotendials (**Table 2**). Only the UV−vis data were published for apo-8,6′-carotendial (**14**) (*24*), we purified and characterized it for the first time by ^{1}H NMR (**Table 3**). For one of the products detected and attributed to an apo-carotendial (**13**), the molecular weight and UV−vis spectra were not sufficient to distinguish between two structural possibilities (**Figure 3**). ^{1}H NMR would allow determination of the precise chemical structure of the compound; however, we did not succeed so far in purifying the compound.

Oxidation of Lycopene with a Metalloporphyrin. We studied the catalytic oxidation activity of a metalloporphyrin on lycopene. A similar system was tested before on β-carotene (*25*). These models mimic the active center of cytochrome P450 enzymes, which are involved in the in vivo transformation of xenobiotics. These enzymes are present in the liver, the organ in which carotenoids can be stored and eventually metabolized, thus it was of interest to examine the potential activity of such a model system on a carotenoid of nutritional importance like lycopene.

The oxidative catalytic species *trans*-dioxoruthenium(VI)-tetraphenylporphyrin $Ru(O)_2(TPP)$ is initially formed from carbonylruthenium(II)-tetraphenylporphyrin Ru(CO)(TPP) by oxidation with 3-chloroperoxybenzoic acid. After transferring oxygen to the substrate, $Ru(O)_2(TPP)$ is regenerated by atmospheric oxygen (*26*). The reaction of this catalytic system on lycopene was followed by HPLC-DAD-MS analysis of the extracted reaction mixture over a period of 96 h. Lycopene disappeared after 24 h (**Figure 5**), but the composition of the reaction mixture underwent evolution even after the complete disappearance of the starting material, indicating that the catalytic system was efficient not only on lycopene, but also on its degradation products initially formed. Three types of products were detected: *(Z)*-isomers, compounds with a molecular weight of $553 = M_{\text{lycopene}} + 16 + 1$, which we called lycopene monoxides, and cleavage compounds assigned to apo-lycopenals. No products resulting from a double oxidative cleavage (apo-carotendial) were detected over the 96 h period. Almost all the apo-lycopenals/ones that were obtained by oxidation of lycopene with potassium permanganate were detected, except the short-chain apo-5-lycopenone (**11**) and apo-7-lycopenal (**10**). These compounds are volatile, and even if they had been formed, they could have evaporated during the time of the experiment. The analysis of the reaction mixture at different times showed very complex chromatograms that allowed the degradation compounds to be distinguished mainly by their molecular weight, but for most of the compounds it was not possible to obtain their UV−vis spectra. In **Figure 6**, the detection of apo-lycopenals/ones by their molecular weight is shown. Their retention time is comparable to the same compounds obtained with oxidation by potassium permanganate (**Figure 1**).

(Z)-isomers of lycopene were detected at 1 h and had almost disappeared after 24 h (**Figure 5**). The tentative attribution of the *(Z)*-isomers was made by detecting their mass, identical to that of *(E)*-lycopene, but also with their UV−vis spectrum. The *(Z)*-isomers detected are most likely mono-*(Z)*-isomers due to the hypsochromic shift of only 4−8 nm. For the isomer with a retention time close to 52 min (*(Z)*-1) (**Figure 5**), the absorbance in the UV region is low, indicating that the compound is decentrally isomerized but not completely otherwise the "*cis*-peak" would not be visible, it could correspond to the 9-*(Z)*-isomer. Peaks eluting at 48 (*(Z)*-2) and 46.5 min (*(Z)*-3) present both a "*cis*-peak" observed at 360 nm which have a high relative intensity ($\%A_B/A_{II}$ as defined in (*23*), 59 and 71%, respectively). These results suggest that the isomerized double bond is in both cases close to the center of the molecule, it is probably the 13-*(Z)*- and the 15-*(Z)*-isomers, respectively. Moreover, the order of the retention times and the values of $\%A_B/A_{II}$ found for the *(Z)*-isomers we detected are comparable to the results found by Yeum et al. (*25*), who used comparable HPLC conditions. Isolation and NMR studies would be necessary to confirm these tentative attributions. We have already

observed *(Z)*-isomerization of β-carotene in such an oxidative system (*26*). It is known that a *(Z)*-olefin is at least 10 times more reactive than the *(E)*-isomer in a competitive oxidation by a metalloporphyrin catalytic system similar to the one we used (*27*). However, the mechanism by which isomerization occurs is not known. It could be either metal- (*28*) or acid-catalyzed (*29*).

Compounds having a molecular weight of 553 = $M_{lycopene}$ + 16 + 1 were detected in a positive electrospray mode, suggesting the presence of epoxide groups on the chain of lycopene, but no other information on the structure could be obtained, because UV−vis spectra were not possible to obtain for these peaks. In a previous study with a similar such catalytic system, we had been able to detect β-carotene epoxides (*26*).

The evolution in time of these compounds was followed in a relative quantitative manner by using the total ion chromatograms (TIC) obtained by mass detection. As for UV−vis detection, we assumed that the area of the peaks of a mass chromatogram obtained at a precise mass (see for example **Figure 6**) is proportional to the quantity of the compound. Thus, by following the evolution in time of the peak areas at a precise molecular weight, we obtained information on the evolution of the relative quantity of the compound considered. When several peaks with close retention time were obtained at the same molecular weight, indicating the presence of *(Z)*-isomers, we added the areas of all peaks. Thus, for the lycopene monoxides, their global evolution in time is shown in **Figure 7A**. Their amount was at the maximum after 1 h of reaction and then decreased regularly until 96 h. For the longest-chain apo-lycopenal (i.e., apo-6′-lycopenal (**1**)), its amount increased until 5 h and then decreased continuously until complete disappearance at 96 h (**Figure 7A**). For the apo-13-lycopenone (**7a**), apo-11-lycopenal (**8**) and apo-9-lycopenone (**9**), their amount increased quickly during the first 5 h (**Figure 7B**) and then more slowly but continuously until 96 h. For apo-15-lycopenal (**6a**) and apo-14′-lycopenal (**5**), their evolution is comparable to the short-chain apo-lycopenals until 48 h, but then their amount decreased until 96 h (**Figure 7C**). For the longer-chain apo-12′-lycopenal (**4**), apo-10′-lycopenal (**3**), and apo-8′-lycopenal (**2**), their amount increased until 5 h, but then their evolution was not regular between 5 and 48 h (**Figure 7D**). It was stable and increased for the apo-12′-lycopenal (**4**), decreased and increased for the apo-10′-lycopenal (**3**) and apo-8′-lycopenal (**2**), but after 48 h, for all of them, their amount drastically decreased until 96 h to almost complete disappearance in the case of (**2**) and (**3**). To summarize the evolution of apo-lycopenals, we observed that the amount of the shortest ones (apo-9-lycopenone (**9**), apo-13-lycopenone (**7a**), and apo-11-lycopenal (**8**)) increased continuously until 96 h (**Figure 7B**), whereas the concentration of the longer ones (apo-6′-lycopenal (**1**), apo-8′-lycopenal (**2**), apo-10′-lycopenal (**3**), apo-12′- lycopenal (**4**), apo-14′- lycopenal (**5**), and apo-15-lycopenal (**6a**)) decreased after some time (5 or 48 h) (**Figure 7**, parts **A**, **C**, and **D**). The evolution of different products resulting from lycopene suggests a possible mechanism of formation of these compounds (**Figure 8**). *(Z)*-Isomers are first formed and then oxidized into epoxides forming the lycopene monoxides. The epoxide groups undergo an oxidative cleavage, giving rise to apo-lycopenals. Apo-lycopenals of all sizes are formed simultaneously from the beginning of the reaction, then the longer-chain apo-lycopenals (apo-15- to apo-6′-lycopenal, **6a** to **1**) are oxidatively cleaved and thus transformed into shorter apo-lycopenals/ones (apo-9- to apo-13-lycopenal/ones, **9** to **7a**).

Further studies are underway to detect oxidative cleavage compounds of lycopene formed by the action of extracts of cells containing cytochrome P450 enzymes and in vivo.

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