Proposed Mechanisms for the Formation of Synthetic and Naturally Occurring Metabolites of Lycopene in Tomato Products and Human Serum

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Oxidation of lycopene (**I**) with *m*-chloroperbenzoic acid has been previously shown to yield lycopene 1,2-epoxide (**II**) and lycopene 5,6-epoxide (**III**) as major products and lycopene 1,2;5,6-diepoxide (**IV**), lycopene 1,2;5′,6′-diepoxide (**V**), lycopene 5,6;5′,6′-diepoxide (**VI**), and lycopene 1,2;1′,2′-diepoxide (**VII**) as minor products. Similarly, the acid-catalyzed rearrangement products of these epoxides have been identified as 2,6-cyclolycopene-1,5-epoxide A (**VIII**) and B (**IX**), 2,6-cyclolycopene-1,5-diol A (**X**) and B (**XI**), 1,16-didehydro-2,6-cyclolycopen-5-ol (**XII**), and lycopene 1,6;2,5-diepoxide (**XIII**). On the basis of the stereochemistry of the observed products (**VIII**−**XI**), here, it is proposed that the acid-catalyzed rearrangement of lycopene 5,6-epoxide may be proceeded by S_N1- and/or S_N2-type mechanisms, whereas an S_N2-type mechanism is preferred for the rearrangement of lycopene 1,2-epoxide. Details of the acid-catalyzed rearrangement of lycopene epoxides by S_N1- and S_N2-type reactions are presented. Although **II** and **VII**−**XI** are present in tomato products at low concentrations, only **X** and **XI** are found in human serum. Possible pathways leading to the formation of the synthetic compounds and the oxidative metabolites of lycopene in tomato products and human serum are discussed.

Keywords: *1,16-Didehydro-2,6-cyclolycopen-5-ol; 2,6-cyclolycopene-1,5-diol; 2,6-cyclolycopene-1,5-epoxide; lycopene 1,6;2,5-diepoxide; lycopene 1,2-epoxide; lycopene 5,6-epoxide; lycopene oxidation products; new serum carotenoids; carotenoid metabolites*

INTRODUCTION

The nutritional significance of lycopene in the prevention of cancer has recently been demonstrated in an epidemiological study of prostate cancer (Giovannucci et al., 1995) and in an in vivo study of colon cancer involving rodents (Narisawa et al., 1996). We recently reported on the identification of two oxidative metabolites of lycopene, with a novel five-membered ring end-group, in human serum and milk (Khachik et al., 1997). These metabolites were identified as a pair of diastereomeric 2,6-cyclolycopene-1,5-diols A (**X**) and B (**XI**). Because only the relative but not the absolute configuration of the asymmetric centers at C(2), C(5), and C(6) for these metabolites were established, each diastereomer may exist as a pair of enantiomers. To establish the structure of the lycopene metabolites, we recently reported a convenient method for the partial synthesis of these compounds from lycopene (Khachik et al., 1998). This involved oxidation of lycopene with *m*-chloroperbenzoic acid (MCPBA) followed by acid-catalyzed rearrangement of the resulting lycopene epoxides. The major products of the reaction of lycopene (**I**) with MCPBA were lycopene 1,2-epoxide (**II**) and lycopene 5,6-epoxide (**III**). However, a number of diepoxides of lycopene were also formed as minor products; these were lycopene 1,2;5,6-diepoxide (**IV**), lycopene 1,2;5′,6′-diepoxide (**V**), lycopene 5,6;5′,6′-diepoxide (**VI**), and lycopene 1,2;1′,2′-diepoxide (**VII**). All compounds with a 5,6- or 5′,6′-epoxy group were only tentatively assigned and could not be isolated due to their instability. The acid-catalyzed rearrangement products of these epoxides were identified by ^1H and ^{13}C NMR spectroscopy as 2,6-cyclolycopene-1,5-epoxide A (**VIII**) and B (**IX**), 2,6-cyclolycopene-1,5-diol A (**X**) and B (**XI**), 1,16-didehydro-2,6-cyclolycopen-5-ol (**XII**), and lycopene 1,6;2,5-diepoxide (**XIII**). Although detailed identification of the lycopene derivatives obtained by partial synthesis was provided in our previous publication, the reaction pathways leading to the formation of these compounds were not described (Khachik et al., 1998).

In this paper, we discuss the possible mechanisms of acid-catalyzed rearrangement of synthetic lycopene epoxides based on the stereochemistry of the observed products from these reactions. At present, it is not clear if lycopene metabolites (**X** and **XI**) in human serum are due to the in vivo oxidation of lycopene or if these compounds may be of dietary origin. Therefore, here, we describe some of the possible pathways that may explain the presence of the oxidative metabolites of lycopene in tomato products and human serum.

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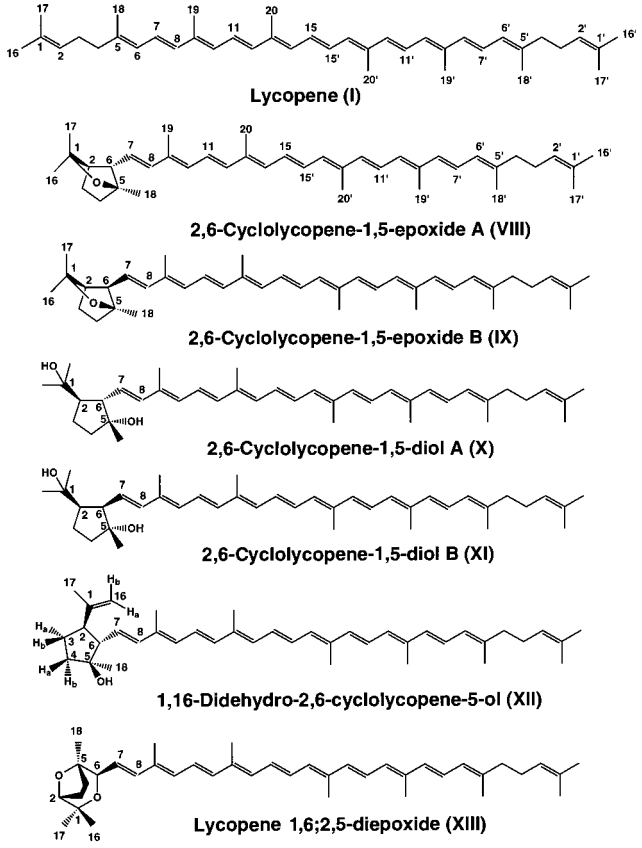

Figure 1. Chemical structures of lycopene and the acid-catalyzed rearrangement products of lycopene epoxides prepared by partial synthesis. Only the relative but not the absolute configurations of the asymmetric centers at C(2), C(5), and C(6) for lycopene derivatives with novel five-membered ring end-group are known.

EXPERIMENTAL PROCEDURES

The details of the reactions of lycopene with MCPBA and the acid-catalyzed reactions of lycopene oxides have been described previously (Khachik et al., 1998). Therefore, only a brief summary of the reaction conditions that promote the rearrangement of lycopene epoxides is described here.

Flash Chromatography on n-Silica Gel. A crude mixture of lycopene 1,2-epoxide and lycopene 5,6-epoxide that contained minor quantities of several diepoxides of lycopene was passed through a flash column containing n-silica gel (60−200 mesh, J. T. Baker, Phillipsburg, NJ) using petroleum ether/acetone in the range of 90:10 to 80:20 as eluent.

Dilute Sulfuric Acid. A crude mixture of lycopene epoxides was treated with 1% sulfuric acid in acetone for 15 min or with aqueous sulfuric acid (0.1%) for 24 h at room temperature under an atmosphere of nitrogen.

RESULTS AND DISCUSSION

The major products of the reaction of lycopene with MCPBA were identified as lycopene 1,2-epoxide and lycopene 5,6-epoxide. In addition, a number of lycopene diepoxides were formed as minor products. Purification of a crude mixture of these epoxides by flash chromatography on n-silica gel resulted in the formation of a number of rearrangement products (**VIII**−**XII**) with novel five-membered ring end-groups, which are shown in Figure 1. These lycopene derivatives are presumably formed due to the acidic nature of the hydroxyl groups on specific sites of n-silica gel that may promote these reactions. The acid-catalyzed reactions of carotenoid epoxides are not unprecedented, and the most prominent example is the rearrangement of 5,6-epoxides to the corresponding 5,8-epoxides (Karrer and Jucker, 1946). We found lycopene 1,2-epoxide to be fairly stable during flash chromatography on n-silica gel, and it only partially rearranged to compounds **VIII** and **IX**. However, numerous attempts to isolate and purify lycopene 5,6-epoxide by flash chromatography and preparative HPLC on various adsorbents were unsuccessful. On the basis of these observations, we concluded in our earlier publication that the observed 2,6-cyclolycopene-1,5-epoxide A (**VIII**) and B (**IX**) and their respective diols (**X**) and (**XI**), for the most part, result from the rearrangement of lycopene 5,6-epoxide (Khachik et al., 1998).

Acid-catalyzed reactions of the crude mixture of lycopene mono- and diepoxides in dilute sulfuric acid solutions resulted in the formation of similar rearrangement products. However, lycopene 1,2-epoxide was shown to be much more reactive in acidic solutions and, along with lycopene 5,6-epoxide, was completely converted to compounds **VIII**−**XII**. Another product of these reactions was lycopene 1,6;2,5-diepoxide (**XIII**), which was formed at low yields in dilute sulfuric acid solutions. This compound is believed to result from the acid-catalyzed rearrangement of the tentatively identified lycopene 1,2;5,6-diepoxide, which was one of the minor products from the reaction of lycopene with MCPBA.

In an another experiment, a purified sample of 2,6-cyclolycopene-1,5-epoxide A (**VIII**) was shown to convert to 2,6-cyclolycopene-1,5-diol A (**X**) in dilute sulfuric acid (0.1%) almost quantitatively; similarly, epoxide **IX** yielded diol **XI**. Therefore, when a crude mixture of lycopene 1,2-epoxide (**II**) and lycopene 5,6-epoxide (**III**) is subjected to acidic media, these compounds would be expected to first undergo rearrangement to epoxides **VIII** and **IX**, which are then converted to diols **X** and **XI**, respectively. However, as will be discussed later, the direct formation of the diols **X** and **XI** from epoxides **II** and **III** without the involvement of epoxides **VIII** and **IX** cannot be ruled out.

The relative stereochemistry of the diols **X** and **XI** indicates that the hydroxylated isopropyl group at C(2) and the hydroxyl group at C(5) are always in a *trans* geometry. Although the relative stereochemistries of these epoxides and diols at C(2), C(5), and C(6) have been determined by NMR, the absolute configurations of these compounds are not known at present (Khachik et al., 1997b). Among all possible configurational isomers of the diols **X** and **XI**, only four stereoisomers (structures e−h, Figure 2) result in this stereospecific arrangement. These isomers can be formed from acid-catalyzed ring opening of two pairs of diastereomeric epoxides **VIII** and **IX** as shown in Figure 2. Each of these diastereomeric epoxides consisted of a pair of enantiomers (structures a/b and c/d and e/f and g/h, respectively, Figure 2). We have been able to separate and characterize only two diastereomeric epoxides and two diastereomeric diols. Therefore, each epoxide and diol probably consists of a pair of its corresponding enantiomer that could not be separated by HPLC.

Acid-Catalyzed Rearrangement of Lycopene Monoepoxides. The mechanism of the acid-catalyzed rearrangement of lycopene 5,6-epoxide (**III**) and lycopene 1,2-epoxide (**II**), which are the major products of oxidation of lycopene, is shown in Figure 3. The protonation of lycopene 5,6-epoxide (**III**) may be followed

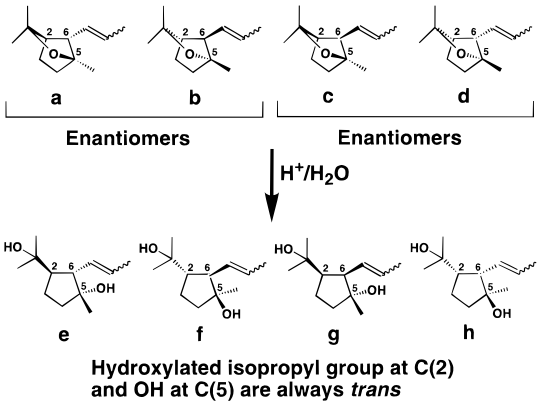

Figure 2. Stereochemistry of all possible 2,6-cyclolycopene-1,5-epoxides and their corresponding diols in which the hydroxylated isopropyl group at C(2) and the hydroxyl group at C(5) are in a *trans* geometry.

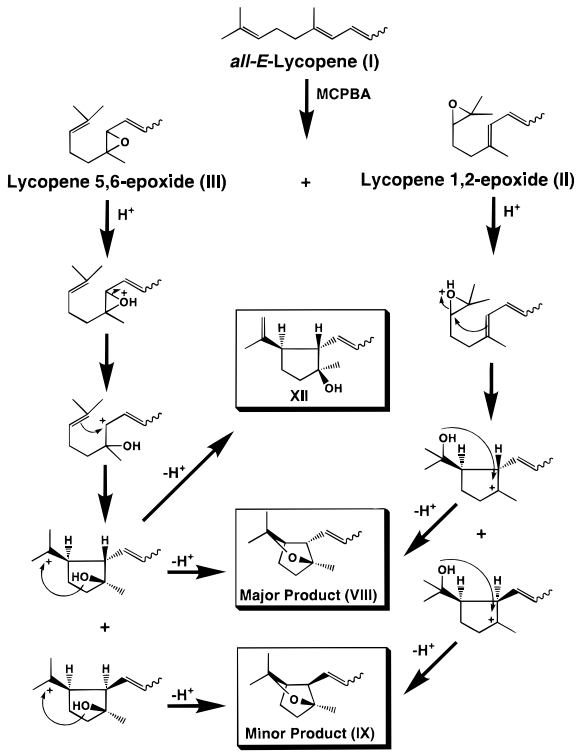

Figure 3. Proposed pathways for acid-catalyzed rearrangement of lycopene 5,6-epoxide and lycopene 1,2-epoxide to diastereomeric 2,6-cyclolycopene-1,5-epoxides **VIII** and **IX** with the inversion of configuration at C(6). Reaction conditions are described in the text.

by ring opening and cyclization by an S_N1-type mechanism to form the two five-membered ring intermediate carbocations with epimerization at C(6). Cyclization of the resulting carbocations yields the corresponding epoxides **VIII** (major product) and **IX** (minor product). The rearrangement of lycopene 5,6-epoxide according to this mechanism may also be accompanied by epimerization at C(2). Therefore, all of the resulting protonated five-membered ring carbocations in which the protonated isopropyl group at C(2) and the hydroxyl group at C(5) are in a *trans* geometry and cannot cyclize to epoxides **VIII** and **IX** can react with water to yield diols **X** and **XI** as well as their enantiomers. Proton elimination from the five-membered-ring carbocations with the correct relative stereochemistry can also account for the formation of another observed product,

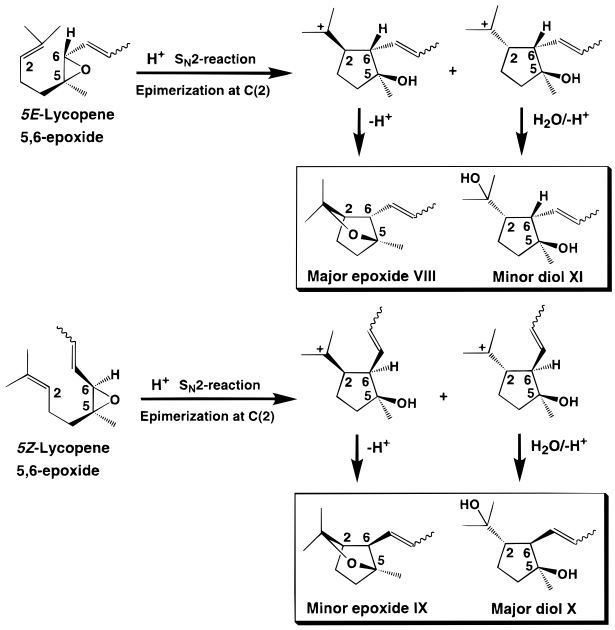

Figure 4. Rearrangement of *5E*- and *5Z*-lycopene 5,6-epoxides by an S_N2-type mechanism. Only the major epoxide **VIII** and the minor diol **XI** can be formed from *5E*-lycopene 5,6-epoxide, whereas the minor epoxide **IX** and the major diol **X** would be expected from *5Z*-lycopene 5,6-epoxide.

XII. Whereas only relative but not absolute chirality at C(2), C(5), and C(6) is known for **XII**, its diastereomer with the inversion of configuration at C(6) was not among the observed products.

On the contrary, the formation of epoxides **VIII** and **IX** from lycopene 1,2-epoxide (**II**) must proceed by an S_N2-type mechanism. This is because the ring opening of the protonated lycopene 1,2-epoxide by an S_N1-type mechanism would be expected to form a tertiary rather than a secondary carbocation. In such a case, the cyclization of the resulting intermediate cannot produce the observed epoxides **VIII** and **IX**. Similarly, the fact that purified lycopene 1,2-epoxide (**II**) in dilute sulfuric acid (1%) can be partially converted to **XII** suggests that this compound results from the ring opening of the epoxide **VIII**, followed by proton elimination.

It is imperative to point out that Lu et al. (1995) and Yokota et al. (1997) have also studied the oxidation products of lycopene. However, these investigators have reported on the isolation and characterization of only epoxide **VIII** and diol **X**, whereas the corresponding diastereomers of these compounds (epoxide **IX** and diol **XI**) as well as compounds **XII** and **XIII** were not observed. The rearrangement of lycopene 5,6-epoxide to the observed products described above is similar to the one proposed by Yokota et al. (1997). In addition, these investigators suggested that cyclization of lycopene 5,6-epoxide to epoxide **VIII** can proceed by either S_N1- or S_N2-type reaction.

The rearrangement of *5E*- and *5Z*-lycopene 5,6-epoxide to the observed products by an S_N2-type mechanism would be expected to yield products with different stereochemistry as shown in Figure 4. Cyclization of the protonated *5E*-lycopene 5,6-epoxide with epimerization at C(2) results in the formation of two intermediates that can afford the major epoxide **VIII** and the minor diol **XI**. On the other hand, the rearrangement of the protonated *5Z*-lycopene 5,6-epoxide with epimerization at C(2) can yield the minor epoxide **IX** and the

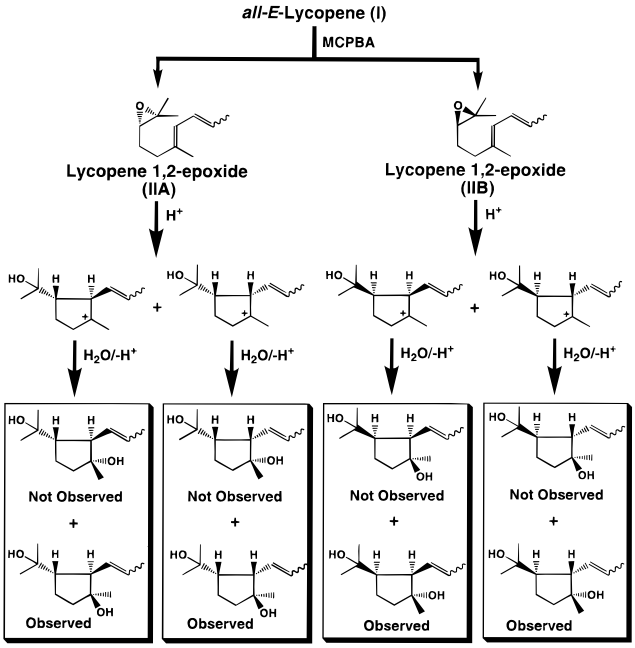

Figure 5. Rearrangement of lycopene 1,2-epoxides **IIA** and **IIB** by an S_N2-type mechanism. All of the observed lycopene epoxides (**VIII** and **IX**) and diols (**X** and **XI**) can be obtained by this mechanism.

major diol **X**. Only one of the enantiomers of *5E*- and *5Z*-lycopene 5,6-epoxide is shown in the mechanism depicted in Figure 4. The other enantiomers of these compounds can rearrange by a similar mechanism to give the enantiomers of epoxides **VIII** and **IX** as well as diols **X** and **XI**. The purity of lycopene employed in these reactions was shown to be 96% by spectrophotometric, NMR, and HPLC-UV−vis/MS as described previously (Khachik et al., 1998). NMR analysis of the crystalline sample of lycopene did not show the presence of any significant amount of geometrical isomers. However, HPLC analysis of this sample according to the method of Hengartner et al. (1992), which simultaneously separates 14 geometrical isomers of lycopene, revealed the presence of 95% *all-E*- and 5% *5Z*-lycopene. Therefore, even though the lycopene employed as the starting material for the partial synthesis of the epoxides predominantly consisted of the *all-E* isomer, the *5E/5Z* isomerization of this compound during the epoxidation reaction cannot be ruled out. Consequently, due to this complication, it is difficult to determine the extent to which the rearrangement of lycopene 5,6-epoxide to the observed products may proceed by S_N1- and/or S_N2-type reaction.

As pointed out earlier, the rearrangement of lycopene 1,2-epoxide would be expected to proceed by an S_N2-type reaction. This accounts for the formation of the observed epoxides **VIII** and **IX** (Figure 3) as well as diols **X** and **XI** as shown in Figure 5. The rearrangement of lycopene 1,2-epoxides **IIA** and **IIB** to the four possible five-membered ring carbocation intermediates by an S_N2-type reaction, followed by the addition of water and deprotonation, results in the formation of eight diols. The relative stereochemistry at C(2), C(5), and C(6) in four of these diols is identical with that of the observed diols **X** and **XI**.

Acid-Catalyzed Ring Opening of 2,6-Cyclolycopene-1,5-epoxide. The possible mechanisms of acid-catalyzed ring opening of epoxide **VIII** are shown in Figure 6. Following protonation, epoxide **VIII** can be converted to diol **X** by an S_N1-type reaction. Therefore, in a stepwise mechanism, the protonated epoxide may undergo ring opening by pathway A or B as shown in Figure 6. Pathway A gives a tertiary carbocation, but addition of water to this intermediate results in the formation of a product in which the hydroxylated isopropyl group at C(2) and the hydroxyl group at C(5) are in a *cis* geometry. This is opposite to the stereochemical arrangement in the observed diol (**X**). However, proton elimination from the carbocation formed by pathway A is the most likely route to compound **XII**, which is one the major products of the acid-catalyzed reactions of lycopene epoxides.

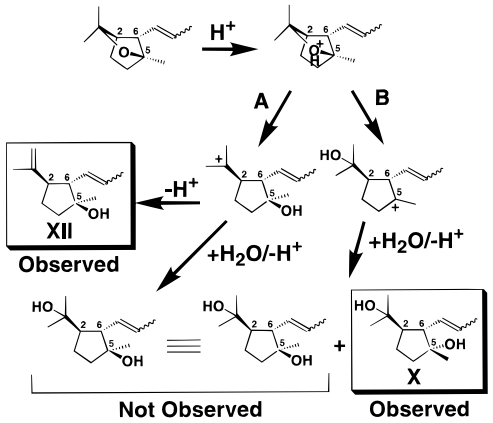

Figure 6. Acid-catalyzed ring opening of 2,6-cyclolycopene-1,5-epoxide A (**VIII**) to the observed products **X** and **XII** by an S_N1-type mechanism. Epoxide **IX** can form diol **XI** by a similar mechanism. Reaction conditions are described in the text.

Pathway B results in a flat tertiary carbocation, but in contrast to pathway A, upon addition of water, a new asymmetric carbon is generated at C(5). Therefore, according to pathway B, the inversion of configuration at C(5), in addition to the observed product **X**, would be expected to yield a diol, which has not been observed. Nevertheless, pathway B is the most likely route from epoxide **VIII** to diol **X**; diol **XI** can be formed by a similar mechanism from epoxide **IX**.

Acid-Catalyzed Rearrangement of Lycopene 1,2;5,6-Diepoxide. The acid-catalyzed ring opening of lycopene 1,2;5,6-diepoxide (**IV**), one of the minor products of the reaction of lycopene with MCPBA, is the most likely source for the presence of compound **XIII** among the observed products. As shown in Figure 7, the 5,6-epoxy ring of **XIII** would be expected to undergo hydrolysis first due to its much greater reactivity than the 1,2-epoxy ring. This has been clearly established from the stability of a mixture of synthetic lycopene 1,2-epoxide and lycopene 5,6-epoxide; whereas the former was readily isolated and purified, the latter underwent rearrangement during purification and could not be isolated. The next steps of this rearrangement are protonation of the 1,2-epoxy ring followed by ring formation between the C(5) hydroxyl group and C(2). After deprotonation, the allylic hydroxyl group of the resulting substituted oxolane at C(6) undergoes protonation followed by dehydration to yield an allylic carbocation. Finally, the formation of an oxygen bridge between the C(1) hydroxyl group and C(6) and loss of a proton can yield the observed product, **XIII**. It is imperative to note that the presence of three asymmetric centers in compound **XIII**, for which only the relative but not the absolute configuration is known,

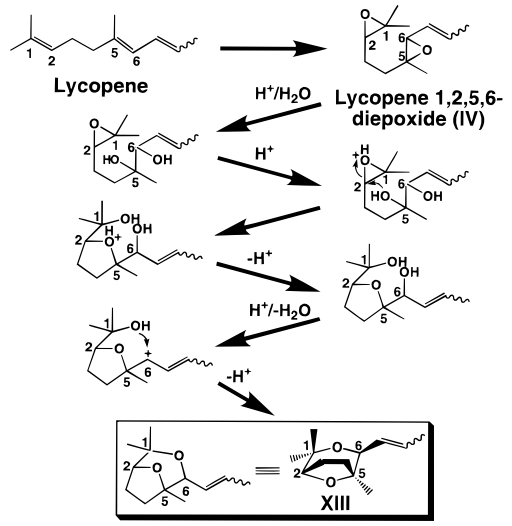

Figure 7. Acid-catalyzed ring opening of lycopene 1,2;5,6-diepoxide to the observed product **XIII**. Reaction conditions are described in the text.

offers many possibilities for the mechanism of cyclization. Therefore, in the absence of stereochemical considerations, the true mechanism leading to the formation of compound **XIII**, remains uncertain.

Pathways Leading to the Formation of Lycopene Metabolites in Tomato Products and Human Serum. The origin of the lycopene metabolites **X** and **XI** in human serum is not very well understood at present. This is primarily due to the instability of lycopene, which makes this compound readily susceptible to oxidation. A systematic outline of some of the possible scenarios that may contribute to this complication is depicted in Figure 8. The comparative profile of the synthetic derivatives of lycopene with the distribution of the lycopene metabolites in tomato paste, tomato juice, and human serum is summarized in Table 1. This can help to provide some insight into various processes that may be ultimately responsible for the presence of the lycopene metabolites in human serum. Lycopene 1,2-epoxide and lycopene 5,6-epoxide are undoubtedly the precursors of the lycopene metabolites in human serum. Lycopene 1,2-epoxide is found in tomato products (Khachik et al., 1992a; Tonucci et al., 1995), whereas only epoxides **VIII** and **IX**, the rearranged forms of lycopene 5,6-epoxide and lycopene 1,2-epoxide, are found in tomato paste and tomato juice (Khachik et al., 1998). The rearrangement of lycopene 5,6-epoxide may be part of the natural metabolism in tomatoes and due to the presence of acids in this fruit. Another likely source for the formation of lycopene epoxides is the preparation of tomato products by processing tomatoes at relatively high temperatures, which can effect the oxidation of lycopene to these compounds. Finally, lycopene may also undergo in vivo oxidation in humans as part of its normal metabolism to form these lycopene epoxides. Conversion of lycopene 1,2-epoxide (**II**) and lycopene 5,6-epoxide (**III**) to epoxides **VIII** and **IX** followed by hydrolysis to diols **X** and **XI** may also take place in the human stomach in the presence of acids. The presence of low concentrations of some of the lycopene metabolites in tomatoes and human serum may also be related to the physiological function of lycopene as a radical scavenger. Unfortunately, the fact that diols **X** and **XI** are also found in tomato products at low concentrations makes it difficult to differentiate

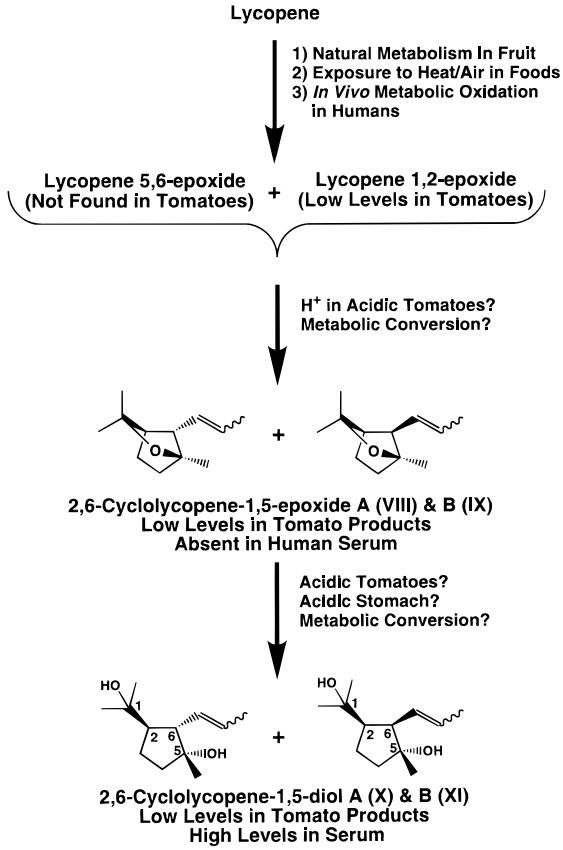

Figure 8. Possible pathways for the formation of the oxidation and acid-catalyzed rearrangement products of lycopene in tomato products and human serum.

Table 1. Comparative Profile of the Synthetic Derivatives of Lycopene with the Distribution of the Lycopene Metabolites in Tomato Paste, Tomato Juice, and Human Serum

compound[a]	tomato paste	tomato juice	human serum
lycopene (**I**)	+++[b]	+++	+++
lycopene 1,2-epoxide (**II**)	+[c]	+	−[d]
lycopene 5,6-epoxide (**III**)	−	−	−
lycopene 1,2;5,6-diepoxide (**IV**)	−	−	−
lycopene 1,2;5′,6′-diepoxide (**V**)	−	−	−
lycopene 5,6;5′,6′-diepoxide (**VI**)	−	−	−
lycopene 1,2;1′,2′-diepoxide (**VII**)	+	+	−
2,6-cyclolycopene-1,5-epoxide A (**VIII**)	+	+	−
2,6-cyclolycopene-1,5-epoxide B (**IX**)	+	+	−
2,6-cyclolycopene-1,5-diol A (**X**)	+	+	+
2,6-cyclolycopene-1,5-diol B (**XI**)	+	+	+
1,16-didehydro-2,6-cyclolycopen-5-ol (**XII**)	−	−	−
lycopene 1,6;2,5-diepoxide (**XIII**)	−	−	−

[a] With the exception of lycopene, which was isolated from tomatoes, all other compounds have been prepared by partial synthesis from lycopene. [b] Indicates presence at high concentrations. [c] Indicates presence at low concentrations. [d] Indicates absence.

among the various processes that may ultimately be responsible for the presence of these compounds in human serum. If the in vivo oxidation of lycopene in humans is indeed taking place, one would not expect to find the resulting epoxides **II**, **III**, **VIII**, and **IX** in the serum. This is because, to date, our extensive analysis of the serum of human volunteers who consume large quantities of fruits and vegetables rich in carotenoid epoxides has not revealed the presence of these group of carotenoids in humans (Khachik et al., 1992b, 1998).

Therefore, the metabolic oxidation of lycopene in humans would be expected to involve enzymatic ring opening of epoxides **VIII** and **IX** to the observed diols **X** and **XI**. For example, the microsomal epoxide hydrolase has been reported to catalyze the *trans*-antiplanar addition of water to epoxides to give vicinal diols by involving an ester intermediate (Lacourciere and Armstrong 1993). Enzymes of this nature may also be responsible for the rearrangement of lycopene epoxides to 2,6-cyclolycopene-1,5-epoxides **VIII** and **IX** as well as their respective diols **X** and **XI**. However, if lycopene 1,2-epoxide and lycopene 5,6-epoxide are the initial products of in vivo oxidation of lycopene in humans, the enzymatic conversion of these epoxides to epoxides **VIII** and **IX** or their direct conversion to diols **X** and **XI** needs to be first established. In the absence of human metabolic studies with lycopene, labeled with a stable isotope, that is, ^{13}C, the in vivo oxidation of lycopene in humans would be a difficult task to accomplish.

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LITERATURE CITED

Giovannucci, E.; Ascherio, A.; Rimm, E. B.; Stampfer, M. J.; Colditz, G. A.; Willett, W. C. Intake of carotenoids and retinol in relation to risk of prostate cancer. *J. Natl. Cancer Inst.* **1995**, *87* (23), 1767−1776.

Hengartner, U.; Bernhard, K.; Meyer, K.; Englert, G.; Glinz, E. Synthesis, isolation, and NMR-spectroscopic characterization of fourteen (Z)-isomers of lycopene and of some acetylenic didehydro- and tetradehydrolycopenes. *Helv. Chim. Acta* **1992**, *75*, 1848−1865.

Karrer, P.; Jucker, E. Epoxides and furanoid oxides that derive from cryptoxanthin. *Helv. Chim. Acta* **1946**, *29*, 229.

Khachik, F.; Goli, M. B.; Beecher, G. R.; Holden, J.; Lusby, W. R.; Tenorio, M. D.; Barrera, M. R. The effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *J. Agric. Food Chem.* **1992a**, *40* (3), 390−398.

Khachik, F.; Beecher, G. R.; Goli, M. B.; Lusby, W. R.; Smith, J. C. Separation and identification of carotenoids and their oxidation products in extracts of human plasma. *Anal. Chem.* **1992b**, *64* (18), 2111−2122.

Khachik, F.; Spangler, C. J.; Smith Jr., J. C.; Canfield, L. M.; Pfander, H.; Steck, A. Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal. Chem.* **1997**, *69*, 1873−1881.

Khachik, F.; Steck, A.; Niggli, Urs; Pfander, H. Partial synthesis and structural elucidation of the oxidative metabolites of lycopene identified in tomato paste, tomato juice and human serum. *J. Agric. Food Chem.* **1998**, *46*, 4874−4884.

Lacourciere, G. M.; Armstrong, R. N. The Catalytic Mechanism of Microsomal Epoxide Hydrolase Involves an Ester Intermediate. *J. Am. Chem. Soc.* **1993**, *115*, 10466−10467.

Lu, Y.; Etoh, H.; Watanabe, N.; Ina, K.; Ukai, N.; Oshima, S.; Ojima, F.; Sakamoto, H.; Ishiguro, Y. A new carotenoid, hydrogen peroxide oxidation products from lycopene. *Biosci., Biotechnol., Biochem.* **1995**, *59* (11), 2153-2155.

Narisawa, T.; Fukaura, Y.; Hasebe, M.; Ito, M.; Aizawa, R.; Murakoshi, M.; Uemura, S.; Khachik, F.; Nishino, H. Inhibitory effects of natural carotenoids, α-carotene, β-carotene, lycopene and lutein on colonic aberrant crypt foci formation in rats. *Cancer Lett.* **1996**, *107*, 137−142.

Tonucci, L. H.; Holden, J. M.; Beecher, G. R.; Khachik, F.; Davis, C. S.; Mulokozi, G. Carotenoid content of thermally processed tomato-based food products. *J. Agric. Food Chem.* **1995**, *43*, 579−586.

Yokota, T.; Etoh, H.; Ukai, N.; Oshima, S.; Sakamoto, H.; Ishiguro, Y. 1,5-Dihydroxyiridanyl-lycopene in Tomato Puree. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 549−550.

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