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CAROTENE EPOXIDES OF *LYCOPERSICON ESCULENTUM*

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato; carotenoids; epoxides; apo-lycopenals.

Abstract—A series of oxygenated carotenoids has been isolated from tomatoes. Two of these compounds have been identified, by comparison of their chromatographic and spectroscopic properties with those of semisynthetic samples, as epoxides of lycopene (1,2-epoxy-1,2-dihydro-ψ,ψ-carotene and 5,6-epoxy-5,6-dihydro-ψ,ψ-carotene). The other related compounds have been identified by their chromatographic, spectroscopic and chemical properties as mutatochrome (5,8-epoxy-5,8-dihydro-β,β-carotene) and epoxides of phytoene (1,2-epoxy-1,2,7,8,11,12,7′,8′,11′,12′-decahydro-ψ,ψ-carotene), phytofluene (1,2-epoxy-1,2,7,8,11,12,7′,8′-octahydro-ψ,ψ-carotene and 1,2-epoxy-1,2,7,8,7′,8′,11′,12′-octahydro-ψ,ψ-carotene) and ζ-carotene (1,2-epoxy-1,2,7,8,7′,8′-hexahydro-ψ,ψ-carotene). The presence in tomatoes of apo-6′-lycopenal (6′-apo-ψ-caroten-6′-al), 8′-apo-lycopenal (8′-apo-ψ-caroten-8′-al) and lycoxanthin (ψ,ψ-caroten-16-ol) has been confirmed by comparison with authentic samples.

INTRODUCTION

THE CAROTENOID hydrocarbons of several strains of tomato have been well characterized,[1-3] but no recent systematic work on the xanthophylls of tomatoes has been reported.[4] Kuhn and Grundmann[5] reported that the xanthophyll fraction of normal tomatoes comprised mainly lutein (β,ϵ-carotene-3,3′-diol) and zeaxanthin (β,β-carotene-3,3′-diol) and Curl[6] used counter-current distribution to demonstrate the presence of a large number of xanthophylls, including the normal chloroplast xanthophylls. Curl[6] also detected lycoxanthin (ψ,ψ-caroten-16-ol, X) in the monol fraction in agreement with the earlier work of Zechmeister and von Cholnoky.[7] Zechmeister and Pinckard[8] isolated a compound that was more polar than phytofluene but had a similar absorption spectrum. They concluded that this was a monohydroxyphytofluene which they named phytofluenol.

Recently we reported[9] the characterization of a compound that was identified from its chromatographic and spectroscopic properties as phytoene epoxide (1,2-epoxy-1,2,7,8,11,12,7′,8′,11′,12′-decahydro-ψ,ψ-carotene, I), and noted the presence in tomatoes of other related compounds with similar chromatographic polarity. We now report the characterization of several of these compounds.

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RESULTS

Chromatographic examination of the unsaponifiable material from an acetone extract of tomatoes showed the presence of a large number of oxygenated carotenoids. A group of pigments with polarity intermediate between the hydrocarbons and the monohydroxy-carotenoids could, however, be isolated, and several of the individual compounds in this group have been purified and characterized.

Carotene Epoxides

Included in this fraction was a series of compounds with polarity similar to that of squalene-2,3-epoxide and mutatochrome (5,8-epoxy-5,8-dihydro-β,β-carotene, VII). These compounds were separated by successive TLC in three systems to give a series similar to the series of hydrocarbons present in the extract. The compounds could not be saponified, did not form acetates or trimethylsilyl ethers, and their absorption spectra were not changed by $NaBH_4$ or dilute acid. This shows the absence of ester, hydroxyl, carbonyl and cyclic 5,6-epoxide groups. The pigments were characterized by their chromatographic properties, absorption spectrum and MS. Insufficient material was available (*ca.* 100 μg of each pigment) for confirmation of the proposed epoxide structures by NMR spectroscopy, but two of the compounds were shown to be identical (chromatographic properties, absorption and mass spectra) with two epoxides of lycopene produced by treatment of lycopene with *m*-chloroperbenzoic acid.

Phytoene Epoxide (1,2-*epoxy*-1,2,7,8,11,12,7′,8′,11′,12′-*decahydro*-ψ,ψ-*carotene*, I)

The characterization of this compound has been reported previously.[9]

Phytofluene Epoxides (1,2-*epoxy*-1,2,7,8,7′,8′,11′,12′-*octahydro*-ψ,ψ-*carotene*, II, *and* 1,2-*epoxy*-1,2,7,8,11,12,7′,8′-*octahydro*-ψ,ψ-*carotene*, III)

Closely associated with the phytoene epoxide, but separable from it by TLC on MgO-Kieselgur was a fraction whose bright greenish-white fluorescence under UV light, and UV absorption spectrum (λ_{max} in petrol. at 331, 348, 367 nm) indicated it to be a phytofluene derivative. The mass spectrum showed the parent ion, M^+ to be at *m/e* 558 (50%, $C_{40}H_{62}O$), but no fragment ion was present at *m/e* 540 ($M-H_2O$), thus confirming the absence of hydroxyl groups. [Fragment ions due to loss of water are observed in the mass spectra of hydroxyphytofluenes from *Rhodospirillum rubrum*[10] (1,2,7,8,11,12,7′,8′-octahydro-ψ,ψ-caroten-1-ol) and berries of *Solanum dulcamara*[11] (probably 7,8,11,12,7′,8′-hexahydro-ψ,ψ-caroten-16-ol).] Major fragment ions were observed at *m/e* 353 (16%, M-205, metastable at *m/e* 223; $353^2/558 = 223{\cdot}3$) and 405 (5%, M-153, metastable at *m/e* 294; $405^2/558 = 294{\cdot}0$) due to cleavage of the 'bis-allylic' C-11′,12′ and C-7,8 bonds respectively.[12] This is consistent with structure II, 1,2-epoxy-1,2,7,8,7′,8′,11′,12′-octahydro-ψ,ψ-carotene. The MS also contained strong fragment ions at *m/e* 421 (2·5%, M-137, metastable at *m/e* 318; $421^2/558 = 317{\cdot}6$) and 337 (8%, M-221, metastable at *m/e* 203·5; $337^2/558 = 203{\cdot}5$) as expected for cleavage of the C-7′,8′ and C-11,12 bonds of the isomeric phytofluene epoxide, 1,2-epoxy-1,2,7,8,11,12,7′,8′-octahydro-ψ,ψ-carotene (III). The ratios of the intensities of the 353 and 405 to the 337 and 421 ions indicated that II and III were present in a ratio of *ca.* 2:1.

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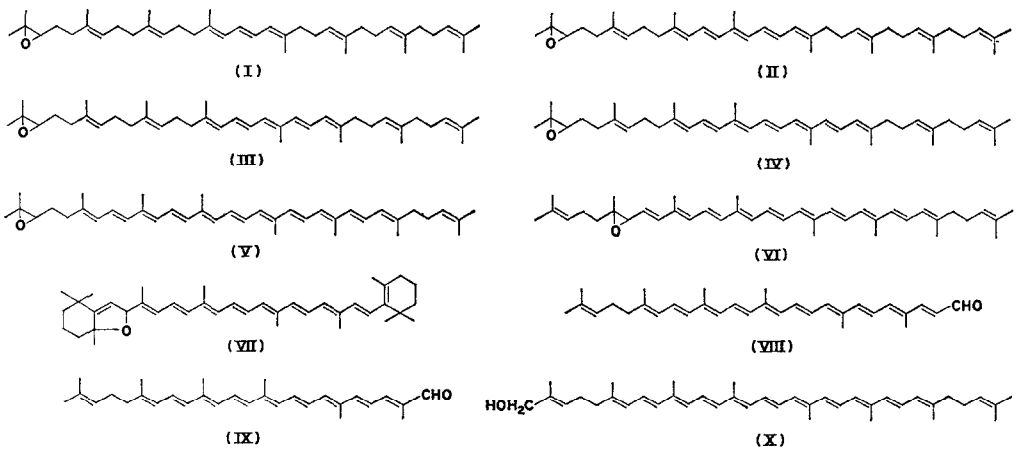
ζ-Carotene Epoxide (1,2-*epoxy*-1,2,7,8,7′,8′-*hexahydro*-ψ,ψ-*carotene*, IV)

This compound, closely related to I, II and III, had λ_{max} (petrol.) at 377, 400, 425 nm indicative of the conjugated heptaene chromophore of a ζ-carotene derivative. The mass spectrum had the parent ion M^+ at *m/e* 556 (74%, $C_{40}H_{60}O$) and major fragment ions at *m/e* 419 (4%, M-137, metastable at *m/e* 316; $419^2/556 = 315{\cdot}8$) and 403 (4·5%, M-153, metastable at *m/e* 292; $403^2/556 = 292{\cdot}1$) due to cleavage of the bis-allylic C-7′,8′ and C-7,8 bonds respectively. No fragmentations involving the loss of water were detected. The experimental data are consistent with structure IV, 1,2-epoxy-1,2,7,8,7′,8′-hexahydro-ψ,ψ-carotene.



Lycopene-1,2-*epoxide* (1,2-*epoxy*-1,2-*dihydro*-ψ,ψ-*carotene*, V)

A further compound was obtained which had an absorption spectrum similar to that of lycopene, with λ_{max} (petrol.) at 443, 469, 500 nm. The MS showed the molecular ion M^+ at *m/e* 552 (100%, $C_{40}H_{56}O$) and had a major fragment ion at *m/e* 483 (2%, M-69, metastable at *m/e* 423; $483^2/552 = 422{\cdot}6$) and a less intense ion at *m/e* 467 (1%, M-85) due to cleavage of the C-3′,4′ and C-3,4 bonds respectively. Fragment ions were also observed at *m/e* 473 (1%, M-79), 460 (6%, M-92, metastable at *m/e* 383, $460^2/552 = 383{\cdot}3$), 446 (28%, M-106) and 394 (2%, M-158) due to fragmentation of the polyene chain,[12,13] and multiple losses of 69 or 85 m.u. from these fragments were also observed, especially at *m/e* 377 (2%, M-106-69, metastable at *m/e* 319; $377^2/446 = 318{\cdot}7$). No fragmentations involving loss of water were detected. This compound was therefore assigned structure V, lycopene-1,2-epoxide (1,2-epoxy-1,2-dihydro-ψ,ψ-carotene). The absorption spectrum and MS were identical to those of a sample of lycopene-1,2-epoxide obtained by reaction of lycopene with *m*-chloroperbenzoic acid, and the tomato compound and the semi-synthetic lycopene epoxide were not separated by TLC on silica gel G with 10% ether in petrol., or on MgO-Kieselgur G (1:1) with acetone–benzene–petrol. (2:2:1) as developing solvent.

Lycopene-5,6-*epoxide* (5,6-*epoxy*-5,6-*dihydro*-ψ,ψ-*carotene*, VI)

Isomeric with the above (V) (MW552, $C_{40}H_{56}O$) but separable from it by TLC on MgO-Kieselgur G was a compound with absorption spectrum (λ_{max} in petrol. at 431, 454,

[13] VETTER, W., ENGLERT, G., RIGASSI, N. and SCHWIETER, U. (1971) in *Carotenoids* (ISLER, O., ed.), p. 189, Birkhäuser, Basel.

483 nm) indicative of an acyclic conjugated decaene chromophore. The mass spectrum, which had the parent ion M^+ at *m/e* 552 (80%, $C_{40}H_{56}O$) again showed no losses of water, but did contain fragment ions at *m/e* 473 (1%, metastable at *m/e* 405; $473^2/552 = 405{\cdot}3$), 460 (11%, metastable at *m/e* 383; $460^2/552 = 383{\cdot}3$), 446 (36%) and 394 (2%) due to losses of 79, 92, 106 and 158 m.u. respectively by rearrangement and cleavage of the polyene chain, and at *m/e* 483 (2·5%, M–69, metastable at *m/e* 423; $483^2/552 = 422{\cdot}6$), 391 (1%, M-92-69, metastable at *m/e* 332; $391^2/460 = 332{\cdot}4$) and 377 (3%, M-106-69, metastable at *m/e* 319; $377^2/446 = 318{\cdot}7$) due to cleavage of the C-3,4 and C-3',4' bonds. No losses of 85 m.u. were observed. From the available data, this compound was tentatively identified as lycopene-5,6-epoxide (5,6-epoxy-5,6-dihydro-ψ,ψ-carotene, VI) and this was confirmed by comparison with lycopene-5,6-epoxide obtained as a major product of the reaction of lycopene with *m*-chloroperbenzoic acid. The absorption spectrum and MS of the two samples were identical, and the two samples were not separated by TLC on silica gel G with 10% ether in petrol. or MgO–Kieselgur G (1:1) with acetone–benzene–petrol. (2:2:1) as solvent.

Mutatochrome (5,8-*epoxy*-5,8-*dihydro*-β,β-*carotene*, VII)

A compound was also isolated which had an absorption spectrum (λ_{max} in petrol. at 404, 427, 452 nm) and chromatographic properties identical to those of mutatochrome (5,8-epoxy-5,8-dihydro-β,β-carotene). The MS was very similar to that shown by Budzikiewicz *et al.*,[14] and had the parent ion, M^+ at *m/e* 552 (68%, $C_{40}H_{56}O$) and fragment ions at *m/e* 460 (1%, M-92, metastable at *m/e* 383; $460^2/552 = 383{\cdot}3$) and 446 (1%, M-106) and especially at *m/e* 472 (36%, M-80, metastable at *m/e* 404; $472^2/552 = 403{\cdot}6$), 205 (36%) and 165 (35%) indicative of the furanoid oxide structure.[12,15] This compound was therefore identified as mutatochrome, 5,8-epoxy-5,8-dihydro-β,β-carotene (VII).

Other Carotenoids

Three other acyclic carotenoids were also isolated from this chromatographic fraction. Two of these, which were similar in polarity to the epoxide series and had absorption spectra lacking in fine structure, typical of carotenoids containing a carbonyl group in conjugation with the polyene chromophore, were identified as apo-lycopenals. The third compound, which was more polar, was identified as lycoxanthin.

Apo-6'-lycopenal (6'-*apo*-ψ-*caroten*-6'-*al*, VIII)

The absorption spectrum of this compound (λ_{max} in ethanol at *ca.* 475 nm) indicated the presence of a carbonyl group in conjugation with a decaene system, and this was confirmed by a shift in the absorption maxima to 430, 455, 485 nm on reduction with $NaBH_4$. The spectrum of the reduced product indicated a decaene chromophore and showed fine structure typical of an acyclic carotenoid. The MS of the natural carbonyl compound had the parent ion, M^+ at *m/e* 442 (100%, $C_{32}H_{42}O$) and a strong fragment ion at *m/e* 373 (7·5%, M-69, metastable at *m/e* 315; $373^2/442 = 314{\cdot}8$) due to cleavage of the 'bis-allylic' C-3,4 bond. The spectroscopic data were consistent with the formulation of this compound as apo-6'-lycopenal (6'-apo-ψ-caroten-6'-al, VIII). The absorption spectrum and MS were identical to those of a sample of apo-6'-lycopenal obtained by $KMnO_4$ oxidation of lycopene, and

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the identity was confirmed by co-chromatography; the two samples could not be separated by TLC on silica gel G with 10% ether in petrol. or on MgO–Kieselgur G (1:1) with acetone–benzene–petrol. (2:2:1) as solvent. The presence of apo-6′-lycopenal in extracts of tomatoes has previously been reported by Winterstein *et al.*[16]

Apo-8′-lycopenal (*8′-apo-ψ-caroten-8′-al*, IX)

Closely associated with the above aldehyde (VIII) was a compound which had λ_{max} (ethanol) at *ca.* 465 nm and which, on $NaBH_4$ reduction gave a product with well-defined λ_{max} (ethanol) at 416, 441, 465 nm, indicating an acyclic nonaene chromophore, and showing that one carbonyl group had been reduced. The MS had the molecular ion, M^+ at *m/e* 416 (100%, $C_{30}H_{40}O$) and a fragment ion at *m/e* 347 (9%, M-69, metastable at *m/e* 289; $347^2/416 = 289{\cdot}4$) due to cleavage of the C-3,4 bond. From the available data, this compound was identified as apo-8′-lycopenal (8′-apo-ψ-caroten-8′-al, IX), the presence of which in extracts of tomatoes has also been reported previously.[16] The identification was confirmed by comparison of the absorption spectrum and MS with those of a sample of apo-8′-lycopenal obtained by $KMnO_4$ oxidation of lycopene, and by co-chromatography of the two samples, which were not separated by TLC on silica gel G with 10% ether in petrol. or on MgO–Kieselgur G (1:1) with acetone–benzene–petrol. (2:2:1) as solvent.

Lycoxanthin (*ψ,ψ-caroten-16-ol*, X)

Small amounts were isolated of a compound with an absorption spectrum (λ_{max} in ethanol at 445, 470, 502 nm) identical to that of lycopene but with the polarity of a monohydroxycarotenoid. The MS had the parent ion, M^+ at *m/e* 552 (100%, $C_{40}H_{56}O$), major fragment ions at *m/e* 534 (32%, $M-H_2O$, metastable at *m/e* 517; $534^2/552 = 516{\cdot}6$), 483 (10%, M-69, metastable at *m/e* 423; $483^2/552 = 422{\cdot}6$) and 467 (5%, M-85), and fragment ions due to multiple losses of the above fragments with toluene (92 m.u.) and xylene (106 m.u.). The details of the MS were very similar to those quoted by Markham and Liaaen-Jensen,[17] and illustrated by Enzell *et al.*[18] The absorption spectrum and MS were identical to those of lycoxanthin isolated from *Solanum dulcamara* fruits,[11] and the two samples were not separated by TLC on silica gel G with 40% ether in petrol. or on MgO–Kieselgur G with acetone–benzene–petrol. (2:2:1) as solvent. This compound was therefore lycoxanthin (ψ,ψ-caroten-16-ol, X) the presence of which in tomatoes has previously been reported.[6,7]

DISCUSSION

Tomato fruit contain a very large number of oxygenated carotenoids, most of which are present in very small amounts. Several of these compounds have been isolated and characterized by their chromatographic, spectroscopic and chemical properties as epoxides of the normal tomato acyclic carotenoid hydrocarbons phytoene, phytofluene, ζ-carotene and lycopene. The epoxide nature of these compounds was established by comparison of the lycopene derivatives with semi-synthetic epoxides prepared from lycopene. Although naturally occurring epoxides of cyclic carotenes are well known,[19,20] this is the first report of the occurrence of epoxides of acyclic carotenes in extracts of natural tissues. Members of

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this new series of carotenoid epoxides have also been detected in fruit of Woody Nightshade (*Solanum dulcamara*).[11]

The biological significance of the occurrence of these compounds is not known. They are present in very small quantities (*ca.* 100 μg isolated from 900 g fruit), but do not appear to be artefacts of the isolation and purification procedure.[21] It is possible that these epoxides of acyclic carotenoids may be formed by the fruit in response to injury, in a similar manner to the rapid formation of β-carotene epoxides in excised leaves.[22] It is also possible that these epoxides and the apo-lycopenals may be early products of the oxidative degradation of the carotenes in the senescing tissues,[23] since a preliminary investigation indicates that these compounds may be present in larger quantities in over-ripe tomatoes.[21]

EXPERIMENTAL

Materials. Tomatoes (900 g) were bought from a local shop. Lycopene-1,2-epoxide and lycopene-5,6-epoxide, prepared by treatment of lycopene with *m*-chloroperbenzoic acid, were kindly provided by Miss M. K. McLaurin. Apo-6′-lycopenal and apo-8′-lycopenal, obtained by $KMnO_4$ oxidation of lycopene were kindly provided by Mr. D. J. Brown. Lycoxanthin, isolated from *Solanum dulcamara* fruit, was the kind gift of Mr. S F. Dunkeyson. Petrol (light petrol, b p. 40–60°), C_6H_6 and Et_2O (diethyl ether) were dried over sodium-lead alloy, and acetone for chromatography was dried over K_2CO_3. All solvents were distilled before use, ether from reduced iron powder.

Extraction and purification methods. Lipid material was extracted with acetone and saponified by standard procedures.[24] The unsaponifiable material was chromatographed on a column of neutral alumina (100 g, activity grade III). Hydrocarbons were eluted with 20% C_6H_6 in petrol, and polar substances then removed with 5% EtOH in Et_2O. This polar fraction was rechromatographed on a second column of neutral alumina (40 g, activity grade III). Traces of lycopene were removed with 20% C_6H_6 in petrol, a second fraction was eluted with 10% Et_2O in petrol, and the remaining polar material was eluted with 5% EtOH in Et_2O.

The second (10% Et_2O in petrol) column fraction was chromatographed on thin layers of silica gel G in 10% ether in petrol. This gave several bands, three of which were removed and eluted. Band 1 (R_f 0·7) was pale yellow, and fluoresced greenish-white under UV light, whereas bands 2 (R_f 0 5) and 3 (R_f 0 35) were red Mutatochrome and the epoxides of phytoene, phytofluene and ζ-carotene were obtained from band 1 as previously described,[9] and were further purified by TLC on silica gel G with 5% Et_2O in petrol before determination of their MS

TLC of band 2 on MgO-Kieselgur G (1:1) in acetone–C_6H_6–petrol. (2:2:1) gave lycopene-1,2-epoxide (R_f 0 4) and lycopene-5,6-epoxide (R_f 0·6), and these were further purified by TLC on silica gel G with 8% Et_2O in petrol.

TLC of band 3 on MgO-Kieselgur G (1·1) with acetone–C_6H_6–petrol (2:2:1) gave two main products, apo-6′-lycopenal (R_f 0·3) and apo-8′-lycopenal (R_f 0·45), which were further purified by TLC on silica gel G with 10% Et_2O in petrol.

The third (5% EtOH in Et_2O) column fraction was chromatographed on thin layers of silica gel G with Et_2O–C_6H_6–petrol. (9:1:10). Many bands were observed, but a red band (R_f 0 7) was removed, eluted and rechromatographed in the same system. This red band was then chromatographed on a thin layer of MgO-Kieselgur G (1·1) with acetone–C_6H_6–petrol (2:2:1), to give lycoxanthin (R_f 0 3), which was further purified by TLC on silica gel G with 40% Et_2O in petrol.

Co-chromatography. Lycopene epoxides and apo-lycopenals were co-chromatographed with semisynthetic samples, and lycoxanthin with an authentic natural sample, on silica gel G and MgO-Kieselgur G (1:1) in the solvent systems described in the Results.

Chemical reactions. Attempted acetylation, silylation, acid isomerization and $NaBH_4$ reduction were performed by standard procedures.[24]

Spectra Absorption spectra of the epoxides were determined in petrol and of lycoxanthin and the apo-lycopenals in EtOH. MS were determined by Mrs. A M. Ball and Mr J. R. Ireland, in an A.E.I. MS12 instrument, with the direct insertion probe, and at an ion source temp. of 210–250°.

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