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CAROTENOID DIFFERENCES IN ISOGENIC LINES OF TOMATO FRUIT COLOUR MUTANTS

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Key Word Index—*Lycopersicon esculentum*; Solanaceae; carotenoids; *cis*- and *trans*-isomers.

Abstract—The main pigment present in fruits of tomato lines isogenic with the cultivar 'Ailsa-Craig', but with different fruit colours, is all-*trans*-β-carotene. Most of the tomato lines also contain *cis*-phytoene, all-*trans*-phytofluene, all-*trans*-ζ-carotene, all-*trans*-neurosporene, all-*trans*-lycopene and all-*trans*-α-carotene. Delta-del fruits accumulate all-*trans*-δ-carotene as the major pigment, and Tangerine coloured fruits contain massive amounts of the intriguing di-*cis*-ζ-carotene, tri-*cis*-neurosporene and tetra-*cis*-lycopene (also known as 'prolycopene'); smaller amounts of *cis*-phytoene and di-*cis*-phytofluene are also found in Tangerine tomato fruits.

INTRODUCTION

Without doubt, one of the most intriguing unsolved problems in carotenoid biosynthesis is the mechanism and stereochemistry of the sequential 'desaturation' steps whereby phytoene (7,8,11,12,7′,8′,11′,12′-octahydrolycopene, **1**) is converted into lycopene (**5**) via phytofluene (7,8,11,12,7′,8′-hexahydrolycopene, **2**), ζ-carotene (7,8,7′,8′-tetrahydrolycopene, **3**) and neurosporene (7,8-dihydrolycopene, **4**). Although the carotenoids **2–5** are most frequently found in nature with the all-*trans* configurations about their polyene chromophores [1], a recent study has shown that the same pigments present in Tangerine ('Tangella') tomato fruits, contain two or more *cis*-double bonds in their conjugated polyene segments, i.e. di-*cis*-phytofluene (**10**), di-*cis*-ζ-carotene (**11**), tri-*cis*-neurosporene (**12**) and tetra-*cis*-lycopene (**13**) [2, 3]. This study not only provides an insight into the fascinating genetic steering in Tangella tomato fruits, compared to the common red-coloured variety, but the results could have a bearing on current theories of the biosynthesis of the acyclic carotenoids **2–5** [4]. In particular, the results strongly suggest that *cis*-carotenoids may be more widely involved in general carotenoid biosynthesis than hitherto imagined.

As a prelude to some studies of the biosynthetic mechanisms involved in the desaturation of phytoene (**1**) to lycopene (**5**) in nature, we have carried out a comprehensive examination of the extent of carotenoid *cis–trans* isomerisation in tomato lines with different fruit colours [5–7]. The outcome of this investigation is presented here.

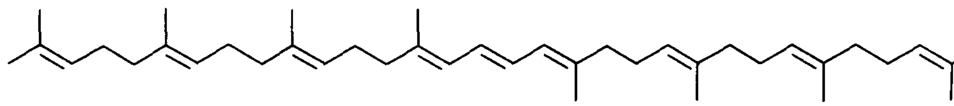
RESULTS AND DISCUSSION

Tomato seed, produced from tomato lines isogenic with the cultivar 'Ailsa Craig' but with different fruit colours were obtained from L. A. Darby, who has described the breeding of these tomato lines in considerable detail [8, 9]. The carotenoid pigments produced by six of the varieties, displaying the widest colour variation in the fruit (i.e. yellow, tangerine, apricot, orange, orange–red and red), were then separated from each other after extraction of the freeze-dried fruits and repeated chromatography. The structures and the stereochemistries of each separated carotenoid were then ascertained by a combination of spectroscopic measurements (i.e. UV/visible, IR, ^{1}H NMR, ^{13}C NMR; plus mass spectroscopy and comparison of data with those obtained from our earlier work [2, 3] and from the work of others [1, 10]). A summary of the carotenoid compositions of the six tomato lines investigated is shown in Table 1.

Table 1 shows that the main pigment present in most of the fruits is all-*trans*-β-carotene (β,β-carotene, **6**). Most of the tomato lines were found to contain varying amounts of the acyclic precursors, i.e. *cis*-phytoene (**9**), *trans*-phytofluene (**2**), all-*trans*-ζ-carotene (**3**), all-*trans*-neurosporene (**4**) and all-*trans*-lycopene (**5**), to β-carotene, and all the lines contained small amounts of all-*trans*-α-carotene [(6′*R*)-β,ε-carotene, **7**]. Significantly, orange–red coloured fruits of the 'Ailsa Craig' (i.e. Delta del) were found to accumulate all-*trans*-δ-carotene [(6*R*)ε,ψ-carotene, **8**] as the major pigment (cf. ref. [7]).

To our surprise, apart from the Tangerine coloured fruits, none of the varieties were found to contain significant amounts of poly-*cis* isomers of the carotenoids already mentioned. By contrast, the Tangerine fruits contained massive amounts of the intriguing di-*cis*-ζ-carotene (**11**), tri-*cis*-neurosporene (**12**) and tetra-*cis*-lycopene (also known as 'prolycopene') (**13**); smaller amounts of mono-*cis*-phytoene (**9**) and di-*cis*-phytofluene (**10**) accompanied these carotenoids in the fruits.

It is clear from our investigation that the tomato colour mutants examined are of less value than we had anticipated for studying details of the biosynthesis of the carotenoids they accumulate. It is also clear however, that the Tangerine tomato fruits offer an exciting possibility of examining in detail the biosynthesis of the interesting poly-*cis*-carotenoids (**10–13**) and this possibility is now being examined.



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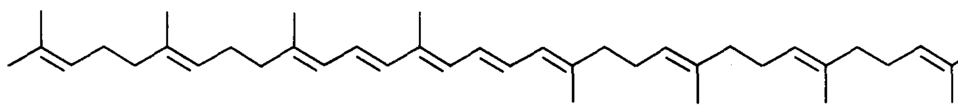

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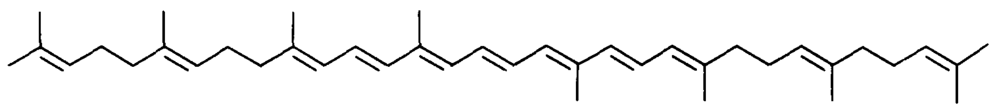

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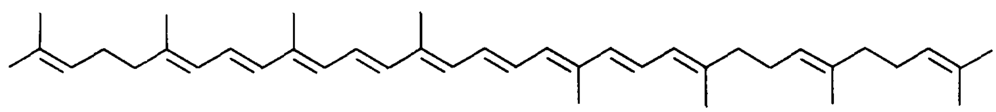

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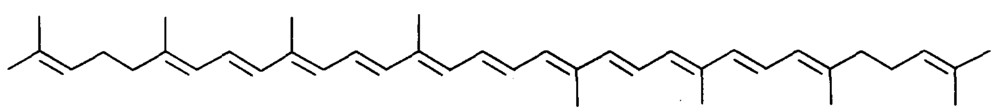

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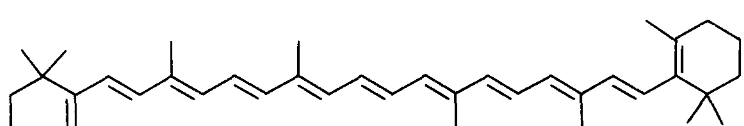

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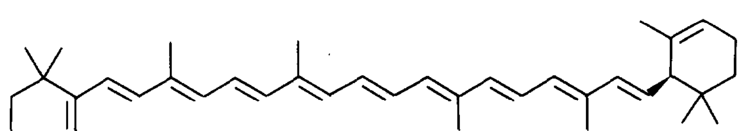

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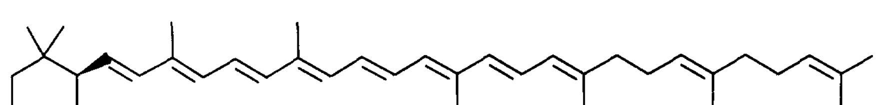

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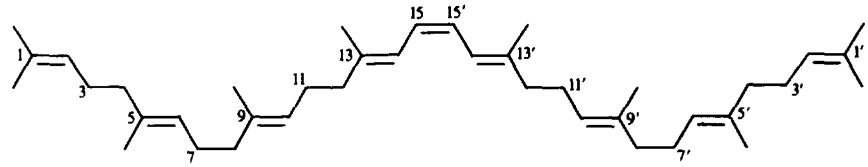
EXPERIMENTAL

Tomato seeds, produced from a breeding project of tomato lines isogenic with the cultivar 'Ailsa Craig', but with different fruit colours, were obtained from the Glasshouse Crops Research Institute, Littlehampton, W. Sussex, England. Fruits from the following genotypes were grown: yellow flesh r, tangerine t, apricot at, orange, delta del, and high pigment hp. The growth and properties of these genotypes has been described by L. A. Darby [8, 9].

Due to the sensitivity of carotenoid pigments to isomerisation in light or on heating, and to aerial oxidation, strict methods of handling were adhered to throughout [11].

All solvents were redistilled before use, and solns were dried over $MgSO_4$ and concd under red. pres.

Isolation of carotenoids from tomato fruits. Freeze-dried segments of the ripe tomato fruits (0.4 kg, equivalent to *ca* 6.6 kg fresh fruit) were chopped rapidly in a Waring blender with Me_2CO–petrol (bp 40–60°) (1 : 1). The extraction was carried out batchwise using 80 g of tomato fruits and 200 ml of solvent; each time the pulp was filtered off and the filter-cake was then re-extracted with fresh solvent (3 × 200 ml). The combined solvent extracts were poured into water (150 ml), and the brightly coloured petrol layer was separated and then evaporated to 50 ml at room temp. *in vacuo*. The residue was filtered through a short column (25 × 2.5 cm) of alumina, using Me_2CO–hexane (1 : 10) as eluant (*ca* 1000 ml), to remove chlorophylls and other polar material. Evaporation of the solvents *in vacuo* then left a mixture of carotenoids (1.1–2.7 g) as a dark-red coloured oily solid. The individual carotenoids were separated from the mixture by



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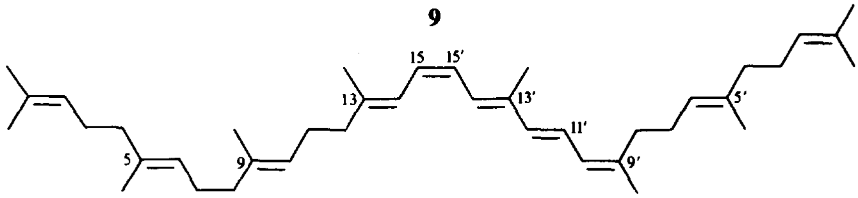

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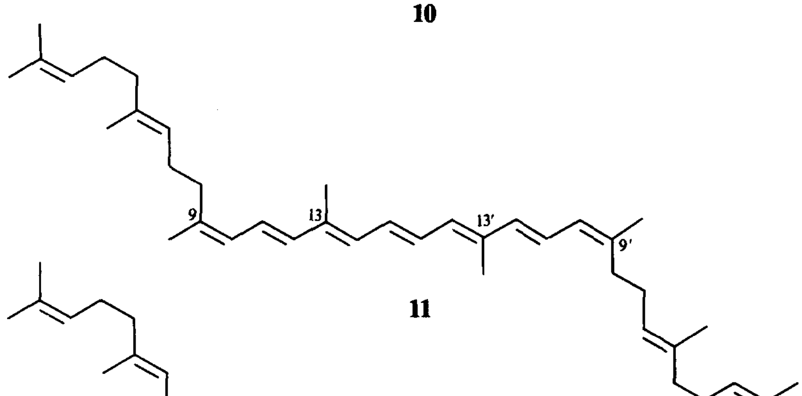

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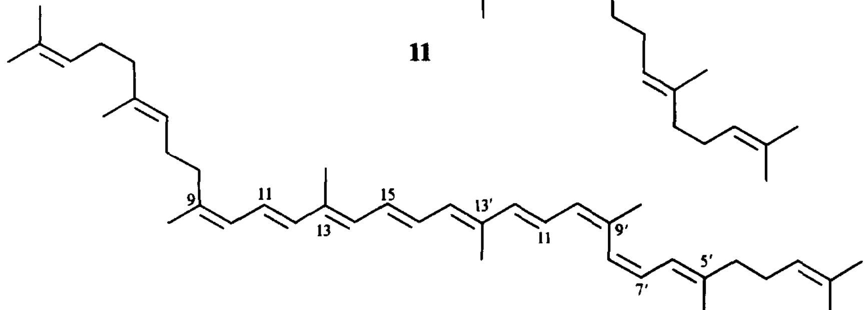

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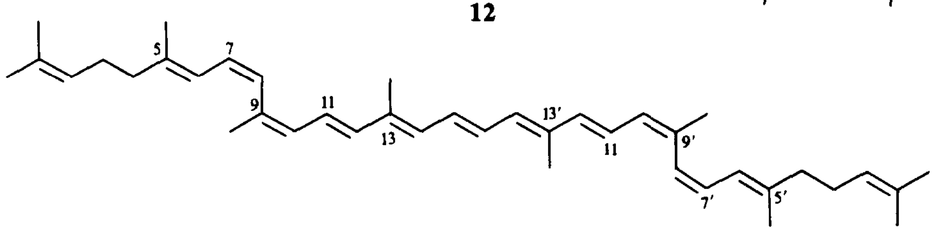

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Table 1. Carotenoid differences in isogenic lines of tomato fruit colour mutants

	Yellow	Tangerine	Apricot	Orange	Orange–red	High pigment
Phytoene (**9**)		*		+	+	**
Phytofluene (**2**)	+		+	*	*	**
cis-Phytofluene (**10**)		+				
ζ-Carotene (**3**)			+	+	*	**
cis-ζ-Carotene (**11**)		**				
Neurosporene (**4**)	*		+	+		+
cis-Neurosporene (**12**)		**				+
Lycopene (**5**)				+		**
cis-Lycopene (**13**)		**	+			+
α-Carotene (**7**)	+	+	+	+	+	+
β-Carotene (**6**)	**	+	**	**	*	**
δ-Carotene (**8**)					**	

+, Carotenoid present; *, carotenoid present in substantial amounts; **, major carotenoid present.

careful chromatography on a column of magnesium oxide (30 × 4.5 cm) using increasing proportions of Me_2CO in *n*-hexane as eluant. Fractions from the column were analysed by electronic absorption spectroscopy which showed that the carotenoids were eluted in the following order: phytoene, phytofluene, α-carotene, β-carotene, δ-carotene, proneurosporene, neurosporene, prolycopene, lycopene.

Prior to detailed spectroscopic investigation each carotenoid was further purified using either medium pressure chromatography on silica (20–44 microns) or TLC on silica gel plates (Fluka Kieselgel HF_{254}).

Stectroscopic characterisation of carotenoids. Full details of the spectroscopic characterisation of *cis-* and *trans*-phytoene (**1** and **9**), di-*cis*-phytofluene (**10**), di-*cis*-ζ-carotene (**11**), tris-*cis*-neurosporene (**12**) and tetra-*cis*-lycopene (**13**) have recently been published [2, 3] whereas most spectroscopic data on all-*trans* carotenoids are in the earlier literature and in reviews [11]. In this study, for the first time, the stereochemistries of the major pigments present in the tomato fruits were determined unambiguously using proton and carbon magnetic resonance spectroscopy. Whenever insufficient material was available for NMR analysis, an indication of the *cis*-geometry of a pigment was ascertained by inspection of ultraviolent-visible absorption data before and after iodine-catalysed equilibration [12].

Carotenoids in yellow flesh fruits. By the general procedure chromatography separated all-*trans*-β-carotene (**6**) as the main pigment, whose structure and stereochemistry followed from MS, UV/visible, IR and ^{1}H NMR analysis. The presence of α-carotene (**7**) and *trans*-phytofluene (**2**) was demonstrated by UV/visible, IR and MS analysis, and *trans*-neurosporene (**4**) was detected by UV/visible spectroscopy. No evidence for the presence of any other carotenoid was obtained.

Carotenoids in tangerine flesh fruits. Chromatography separated di-*cis*-ζ-carotene (**11**), tri-*cis*-neurosporene (**12**) and tetra-*cis*-lycopene (**13**) as the major pigments; their spectral data (particularly their ^{13}C NMR data) were identical with those reported earlier [2, 3]. *cis*-Phytoene (**9**) was also separated, and its stereochemistry followed from the ^{13}C NMR spectrum. Smaller amounts of *cis*-phytofluene (**10**) (UV/visible, MS) and of α- and β-carotenes (**7** and **6**) (UV/visible only) were also present in the fruits.

Carotenoids in apricot flesh fruits. Chromatography separated all-*trans*-β-carotene (**6**) as the major pigment (UV/visible, IR, ^{1}H NMR, ^{13}C NMR, MS). The presence of phytofluene (**2**), ζ-carotene (**3**), neurosporene (**4**), tetra-*cis*-lycopene (**13**) and α-carotene (**7**) were established by UV/visible spectroscopy.

Carotenoids in orange flesh fruits. Chromatography separated all-*trans*-β-carotene (**6**) as the major pigment (UV/visible, IR, ^{1}H NMR, ^{13}C NMR, MS) and phytofluene (**2**) as the minor pigment. The presence of *cis*-phytoene (**9**), ζ-carotene (**3**) neurosporene (**4**), lycopene (**5**) and α-carotene (**7**) were established by UV/visible spectroscopy.

Carotenoids in delta del (orange–red flesh) fruits. Chromatography separated all-*trans*-δ-carotene (**8**) as the major pigment (UV/visible, IR, ^{1}H NMR, MS), and *trans*-phytofluene (**2**) (UV/visible, IR, ^{1}H NMR, MS), ζ-carotene (**3**) (UV/visible, ^{1}H NMR, MS) and β-carotene (**6**) (both *cis* and *trans*-isomers present) as minor pigments. The presence of *cis*-phytoene (**9**) (MS, ^{1}H NMR), lycopene (**5**) (UV/visible, MS) and α-carotene (**7**) (UV/visible, IR, ^{1}H NMR) was also confirmed.

Carotenoids in high pigment tomatoes. Chromatography separated *cis*-phytoene (**9**) (UV/visible, ^{13}C NMR), *cis*-15-phytofluene (UV/visible), ζ-carotene (**3**) (UV/visible), lycopene (**5**) (UV/visible, ^{13}C NMR) and β-carotene (**6**) (^{1}H NMR, ^{13}C NMR) as major pigments. Smaller amounts of neurosporene (**4**), *cis*-neurosporene (**12**), *cis*-lycopene (**13**) and α-carotene (**7**) were detected by UV/visible spectroscopy.

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