

## Naturally Occurring Poly-*cis* Carotenoids. Stereochemistry of Poly-*cis* Lycopene and its Congeners in 'Tangerine' Tomato Fruits

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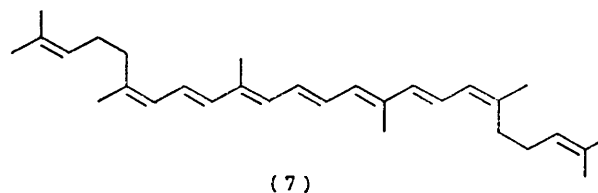
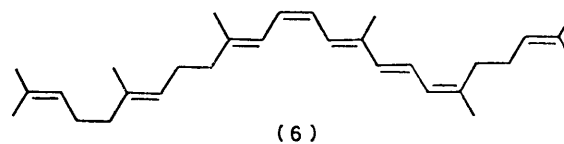
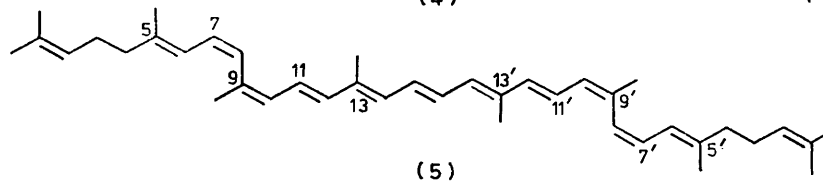
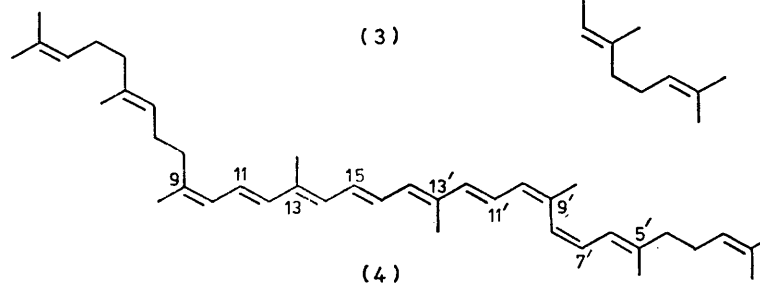
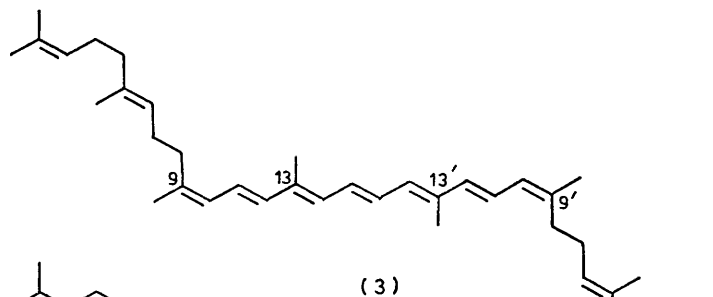
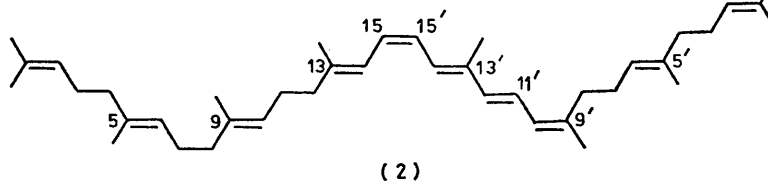
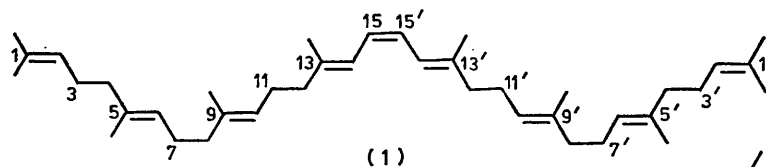
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**Summary** Spectroscopic data and comparison with synthetic model compounds establish that the carotenoid pigments phytoene, phytofluene,  $\zeta$ -carotene, neurosporene, and lycopene found in the Tangerine tomato fruit have the stereochemistries C-15-mono-*cis* (**1**), C-15, C-9'-di-*cis* (**2**), C-9, C-9'-di-*cis* (**3**), C-9, C-9', C-7'-tri-*cis* (**4**), and C-7, C-9, C-9', C-7'-tetra-*cis* (**5**), respectively; the remaining double bonds in the pigments have the *trans*-geometry.

ALTHOUGH the majority of carotenoids found in nature show an all-*trans* configuration about the conjugated polyene chromophore, a few contain one or more *cis*-double bonds, and there is circumstantial evidence that this isomerism plays an important role in carotenoid biosynthesis, and perhaps in some of their biological functions.<sup>1,2</sup> The pigment 'prolycopene' (a poly-*cis* lycopene) found in the Tangerine tomato fruit, is without doubt the Cinderella

of the natural *cis*-carotenoids. First isolated in 1941, its stereochemistry was investigated extensively by Zechmeister and his co-workers during the ensuing two decades.<sup>2,3</sup>

synthesis of poly-*cis* polyene isoprenoids,<sup>5</sup> we have now been able to investigate the detailed stereochemistry of polycopene and its congeners. The results of this investi-



With the recent advances in the application of  $^{13}\text{C}$  n.m.r. spectroscopy as a stereochemical probe amongst polyenes,<sup>4,5</sup> and the development of methodology for the controlled

gation, summarised here, have a significant bearing on current theories of biogenetic transformations of acyclic carotenoids.<sup>6</sup>

The carotenoid pigments from freeze-dried segments of ripe fruits of the Tangerine tomato were extracted with hexane, and then separated by column chromatography on magnesium oxide using increasing amounts of acetone in hexane as eluant. The phytoene isolated, like that from standard red tomatoes,<sup>7</sup> was found to have the C-13-*trans*, C-15-*cis*, C-13'-*trans* stereochemistry (*viz.* 1) about the triene chromophore; this assignment followed unambiguously from comparison of spectra with authentic isomeric C<sub>20</sub>-triene models whose syntheses were reported earlier.<sup>5a</sup> In addition, the unconjugated trisubstituted double bonds (at C-5, C-9, C-9', and C-5') in the phytoene were each shown to have the *trans*-configuration by inspection of <sup>1</sup>H and <sup>13</sup>C n.m.r. data.

The i.r. spectrum of phytofluene from the Tangerine tomato fruit showed that the polyene contained both *cis* ( $\nu_{\max}$  775 cm<sup>-1</sup>) and *trans* ( $\nu_{\max}$  960 cm<sup>-1</sup>) disubstituted double bonds, and the <sup>13</sup>C n.m.r. spectrum established that the stereochemistry of one of the terminal trisubstituted double bonds in the pentaene chromophore was *cis* ( $\delta$  24.1 p.p.m., C-9'-Me), whilst the other was *trans* ( $\delta$  16.6 p.p.m., C-13-Me). The ratio (2:5) of 'in-chain' ( $\tau$  8.29) to 'out-of-chain' ( $\tau$  8.36) methyl resonances in the <sup>1</sup>H n.m.r. spectrum confirmed that the stereochemistries of the double bonds at C-5, C-9, and C-5' were each *trans*. Convincing evidence that the *cis*-disubstituted double bond was located at C-15 rather than at C-11' in the phytofluene came from comparison between the chemical shift of the C-13'-methyl resonance ( $\tau$  8.07) in the <sup>1</sup>H n.m.r. spectrum, and those of model systems (predicted  $\tau$  8.07 when C-15-*cis*, but  $\tau$  7.94 when C-11'-*cis*).<sup>8</sup> These data, together with additional comparison of <sup>13</sup>C n.m.r. data with model systems, permitted us to assign the C-13-*trans*, C-15-*cis*, C-13'-*trans*, C-11'-*trans*, and C-9'-*cis*-stereochemistry (*viz.* 2) to the phytofluene. This stereochemical assignment was confirmed by synthesis of the authentic di-*cis*-C<sub>30</sub>-octaene (6),<sup>8</sup> and comparison of i.r. and n.m.r. absorption spectra. Thus, during the desaturation of phytoene to phytofluene in the Tangerine tomato, at the same time as the C-11'-*trans* double bond is introduced, the C-9' double bond undergoes specific *trans*-to-*cis* isomerisation.

ζ-Carotene (3) from the Tangerine tomato fruit displayed only eleven *sp*<sup>2</sup> carbon resonances in its <sup>13</sup>C n.m.r. spectrum indicating that the molecule was symmetrical. The <sup>13</sup>C n.m.r. spectrum also established that the terminal trisubstituted double bonds (at C-9 and C-9') of the heptaene chromophore each had the *cis*-configuration ( $\delta$  32.8 p.p.m. C-8 and C-8'). The absence of absorption at 760 cm<sup>-1</sup> in the i.r. spectrum suggested that the stereochemistries of the three disubstituted double bonds were *trans*, and the chemical shift of the C13(13')-Me groups ( $\delta$  12.8 p.p.m.) in the <sup>13</sup>C n.m.r. supported a *trans* geometry for the C-13 and C-13' double bonds. Taken together the spectral data supported the C-9, C-9' di-*cis* stereochemistry (3) for ζ-carotene,

and this was confirmed by comparison of data with the authentic synthetic C<sub>30</sub>-analogue (7).<sup>5b</sup> Interestingly therefore, as the *trans*-disubstituted double bond at C-11 is introduced during the formation of ζ-carotene from phytofluene, the double bonds at C-15 and C-9 in the phytofluene undergo *cis*-to-*trans* and *trans*-to-*cis* isomerisation, respectively.†

Iodine-catalysed isomerisation of the isolated neurosporene (4) resulted in a significant increase in the strength and the wavelength of the visible absorption bands ( $\lambda_{\max}$  410, 433, and 459 nm → 415, 438, and 466 nm), and the relative strengths of the C-H out of plane bands at 765 and 965 cm<sup>-1</sup> in the i.r. spectrum suggested that the pigment had one *cis*-disubstituted double bond. The <sup>13</sup>C n.m.r. spectrum showed quite clearly that the geometry of one of the terminal trisubstituted double bonds of the nonaene chromophore was *cis* ( $\delta$  32.8 p.p.m., C-8) whilst the other was *trans* ( $\delta$  40.4 p.p.m., C-4'). Furthermore, the <sup>13</sup>C n.m.r. spectrum showed that the nonaene chromophore contained one other *cis*- and two other *trans*-trisubstituted double bonds. Application of shift parameter data, worked out for vinyl methyl carbon atoms from synthetic model polyenes,<sup>8</sup> made it possible to establish that the neurosporene had the C-9, C-9', C-7'-tri-*cis* stereochemistry shown, (4). The stereochemistry of polycopene (5) followed logically from comparison of its spectral data with those accumulated for its congeners and synthetic model compounds. In particular, the <sup>13</sup>C n.m.r. spectrum showed that the molecule was symmetrical, with the stereochemistries of the terminal trisubstituted double bonds at C-5 and C-5' both *trans* ( $\delta$  40.3 p.p.m., C-4, C-4'). The i.r. spectrum supported the presence of two *cis*-disubstituted double bonds, and the <sup>1</sup>H n.m.r. spectrum showed the non-equivalence of the vinyl methyl groups at C-9(9') ( $\tau$  7.99) and C-13(13') ( $\tau$  8.11). Finally, comparison between <sup>13</sup>C n.m.r. data obtained for all-*trans* lycopene and <sup>13</sup>C n.m.r. shift parameters derived from model compounds,<sup>8</sup> permitted the symmetrical (C-7, C-9, C-7', C-9') tetra-*cis* stereochemistry (*viz.* 5) to be assigned to polycopene [ $\delta$  12.6 (C-13-Me), 16.6 (C-5-Me), and 24.7 (C-9-Me) p.p.m., calculated shifts:  $\delta$  12.8, 16.5, and 24.3 p.p.m., respectively].‡ During the biosynthesis of polycopene from ζ-carotene *via* neurosporene in the Tangerine tomato fruit, the C-7 and C-7' double bonds are therefore each introduced specifically with *cis* geometry.

The present work not only establishes the stereochemistry of polycopene and its congeners in the Tangerine tomato fruit but also provides an insight into the fascinating genetic steering found in this variety compared to the common red tomato fruits. Significantly our work suggests for the first time that phytoene (1), rather than the widely accepted ζ-carotene (3), is the clear branch point for poly-*cis* carotene formation in the Tangerine tomato.<sup>6</sup> Since little is known of the stereochemistry of hydrogen loss during the desatura-

† Only a single isomer of ζ-carotene was isolated in these studies (*cf.* A. A. Qureshi, N. Qureshi, M. Kim, and J. W. Porter, *Arch. Biochim. Biophys.*, 1974, **162**, 117; L. C. Raymundo and K. L. Simpson, *Phytochemistry*, 1972, **11**, 397).

‡ Dr. G. P. Moss and Prof. B. C. L. Weedon have independently established the C-7, C-9, C-7', C-9'-tetra-*cis* stereochemistry (5) for polycopene (*Pure Appl. Chem.*, 1979, **51**, 507).

tion of phytoene to polyycopene, it is not possible at this time however to provide a mechanistic rationale of the enzymic reactions involved in the dehydrogenation–isomerisation steps between these carotenes.

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<sup>1</sup> See 'Carotenoids,' ed. O. Isler, Birkhauser, Basel, 1971.

<sup>2</sup> L. Zechmeister, 'cis-trans-Isomeric Carotenoids, Vitamins A and Arylpolyenes,' Springer-Verlag, 1962.

<sup>3</sup> L. Zechmeister, A. L. LeRosen, F. W. Went, and L. Pauling, *Proc. Nat. Acad. Sci. U.S.A.*, 1941, **27**, 468; A. L. LeRosen and L. Zechmeister, *J. Amer. Chem. Soc.*, 1942, **64**, 1075.

<sup>4</sup> G. Englert, *Helv. Chim. Acta*, 1975, **58**, 2367; G. P. Moss, *Pure Appl. Chem.*, 1976, **47**, 97; M. Baranyai, J. Szabolcs, G. Toth, and L. Radics, *Tetrahedron*, 1976, **32**, 867.

<sup>5</sup> See: (a) L. Barlow and G. Pattenden, *J.C.S. Perkin I*, 1976, 1029; (b) J. M. Clough and G. Pattenden, *Tetrahedron Letters*, 1978, 4159, and references cited therein.

<sup>6</sup> For recent summary, see: B. H. Davies and R. F. Taylor, *Pure Appl. Chem.*, 1976, **47**, 211.

<sup>7</sup> N. Khan, D. E. Loeber, T. P. Toube, and B. C. L. Weedon, *J.C.S. Perkin I*, 1975, 1457.

<sup>8</sup> J. M. Clough and G. Pattenden, unpublished data.