Stereochemical Assignment of Prolycopene and other Poly-*Z*-Isomeric Carotenoids in Fruits of the Tangerine Tomato *Lycopersicon esculentum* var. ' Tangella '

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The acyclic carotenoid pigments phytoene, phytofluene, ζ-carotene, neurosporene, and lycopene found in fruits of the Tangerine tomato *Lycopersicon esculentum* var. ' Tangella ', were separated by chromatography and characterised. Spectroscopic data and comparison with synthetic model compounds established that the pigments have the stereochemistries 15*Z*- (1), 15*Z*, 9'*Z*- (2), 9*Z*, 9'*Z*- (3), 9*Z*, 7'*Z*, 9'*Z*- (4), and 7*Z*, 9*Z*, 7'*Z*, 9'*Z*- (5) respectively; the remaining double bonds in the pigments have the *E*-geometry.

The data suggest that phytoene (1), rather than the widely accepted ζ-carotene (3), is the clear branch point for poly-*Z*-carotene formation in the Tangerine tomato. In addition, the data established that: (*a*) during the desaturation of phytoene (1) to phytofluene (2) in the Tangerine tomato, at the same time as the 11'*E*-double bond is introduced, the 9'-double bond undergoes specific *E*-to-*Z* isomerisation, (*b*) as the *E*-disubstituted double bond at C-11 is introduced during the formation of ζ-carotene (3) from phytofluene (2), the double bonds at C-15 and C-9 in the phytofluene undergo *Z*-to-*E* and *E*-to-*Z* isomerisation, respectively, and (*c*) during the biosynthesis of prolycopene (5) from ζ-carotene (3) *via* proneurosporene (4) in the Tangerine tomato fruit, the C-7 and C-7' double bonds are each introduced specifically with *Z*-geometry.

' Prolycopene ' is a poly-*Z*-isomer of the carotenoid lycopene. It was first reported in fruits of the Tangerine tomato *Lycopersicon esculentum* var. ' Tangella ' by Zechmeister in 1941,[1] but has since been found in a number of berries and flowers.[2] Along with its congeners, prolycopene is responsible for the striking tangerine colour of Tangella fruits compared to the normal red colour of the common, commercial tomato.

Although the stereochemistry of prolycopene was investigated extensively by Zechmeister and his co-workers during the period 1941—1961,[2b] its exact stereostructure has remained a mystery. With the recent advances in the application of n.m.r. spectroscopy as a stereochemical probe amongst isomeric polyenes (see immediately preceding paper,[3] and refs. cited therein) and the development of methodology for the controlled synthesis of poly-*Z*-polyene isoprenoids,[4] we have been able to examine the detailed stereochemistry of this intriguing carotenoid and its congeners from Tangella. In this paper we summarise the outcome of this investigation.[5]

Freeze-dried segments of ripe fruits of the Tangerine tomato were extracted with hexane, and prolycopene, along with its C_{40}-biosynthetic precursors phytoene, phytofluene, ζ-carotene, and neurosporene, were separated by chromatography. Unlike some previous investigations, only single isomers of each carotenoid were isolated in this study.[6]

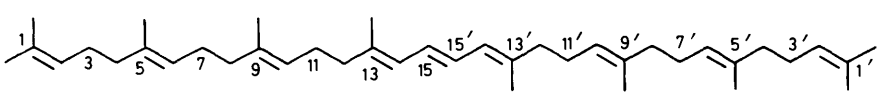
The phytoene isolated, like that from common red-coloured tomato fruits,[7] was found to have the 13*E*, 15*Z*, 13'*E*-stereochemistry [*viz.* (1)] † about the triene chromophore, and the unconjugated trisubstituted double bonds (at C-5, C-9, C-9', and C-5') were each shown to have an *E*-configuration. Thus, the nine absorptions in the sp^2-region of the noise-decoupled ^{13}C n.m.r. spectrum of the phytoene immediately demonstrated that the sample was isomerically pure, and furthermore that it was a symmetrical isomer. The 1 : 3 ratio of ' in-chain ' [δ 1.69, 1(1')*E*-Me] to ' out-of-chain ' [δ 1.62, 1(1')*Z*-Me, 5(5')-Me, 9(9')-Me] methyl resonances in the ^1H n.m.r. spectrum established that each of the four non-conjugated double bonds capable of isomerisation in phytoene has the *E*-configuration,[8] and this fact was confirmed by inspection of the vinyl methyl [δ 16.0, C-5(5'), C-9(9')] and vinyl methylene [δ 39.8, C-4(4'), C-8(8')] resonances in the ^{13}C n.m.r. spectrum.

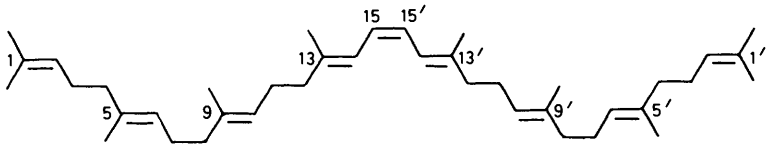
The carbon shifts of C-12(12') (δ 40.5) and 13(13')-Me (δ 16.55) immediately defined the *E*-geometry of the double bonds at C-13(13'), and the presence of a strong i.r. maximum at 765 cm^{-1}, with no corresponding absorption at *ca.* 960 cm^{-1}, provided compelling support for a *Z*-configuration about the central disubstituted double bond in the phytoene. Finally, comparison of the spectroscopic features outlined above for the natural product with those of the synthetic C_{20}-analogue (7)[9] fully supported the stereochemical assignment shown in structure (1) for phytoene; the comparative ^{13}C n.m.r. data are collected on formulae (6) and (7).

The i.r. spectrum of phytofluene (2) from the Tangerine tomato fruit showed that the polyene contains both *Z*-($\nu_{max.}$ 775 cm^{-1}) and *E*-($\nu_{max.}$ 960 cm^{-1}) disubstituted double bonds, and the ^{13}C n.m.r. spectrum established that the stereochemistry of one of the terminal trisubstituted double bonds in the conjugated pentaene chromophore is *Z*-(δ 24.1, 9'-Me), whilst the other, at C-13, has an *E*-configuration (δ 16.6, 13-Me). The ratio (2 : 5) of ' in-chain ' [δ 1.70, 1(1')*E*-Me] to ' out-of-chain ' [δ 1.63, 1(1')*Z*-Me, 5(5')-Me, 9-Me] methyl resonances in the ^1H n.m.r. spectrum demonstrated that the stereochemistries of the non-conjugated double bonds at C-5, C-9, and C-5' in the phytofluene each have the *E*-configuration; this was confirmed by inspection of relevant chemical shift data [δ 16.0, 5(5')-Me, 9-Me; δ 39.7, C-4(4'), C-8] in the ^{13}C n.m.r. spectrum.

The carbon chemical shift of δ 12.4 for 13'-Me in the phytofluene clearly demonstrated that the double bond at C-13' has an *E*-configuration (predicted shift *ca.* δ 20 for corresponding *Z*-double bond). Once this feature was estab-

† The system of numbering carotenoids recommended by I.U.P.A.C. is used throughout this paper,[10] *viz.*:





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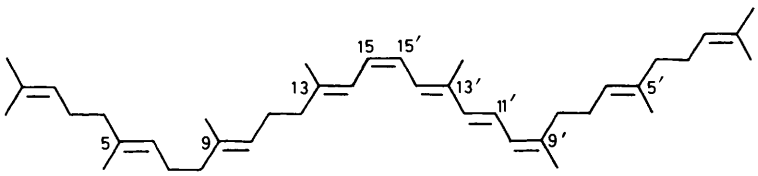

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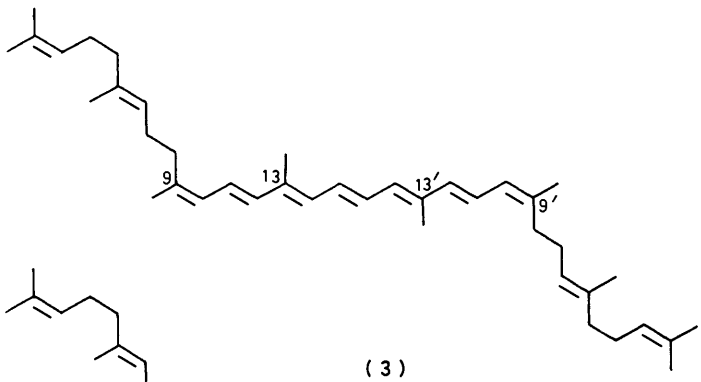

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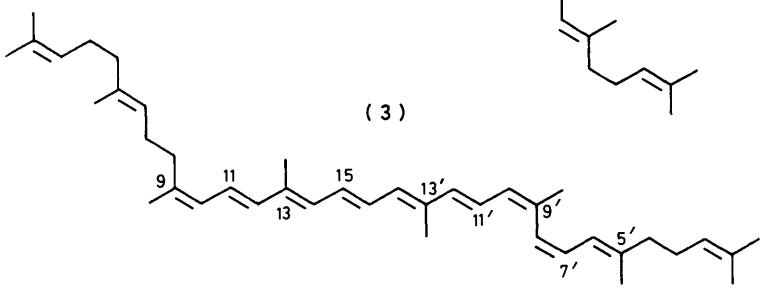

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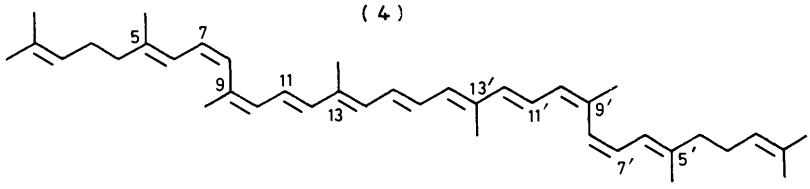


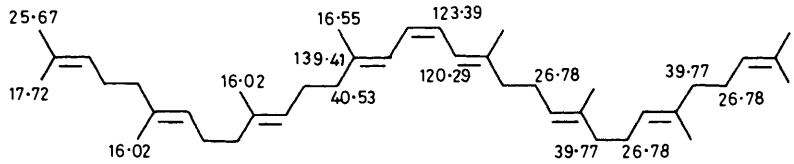
(5)

lished, comparative vinyl methyl shift data in the ^{1}H n.m.r. spectrum of the carotenoid were supportive of a 15*Z*, 11′*E*-[*i.e.* (8)] rather than an 15*E*, 11′*Z*-[*i.e.* (9)] configuration for the molecule. Thus, application of the shift parameters discussed earlier [3] predicted a chemical shift of δ 1.91 for the C-13′-methyl protons when the neighbouring C-15-double bond is *Z*-, but δ 2.03 when the corresponding C-11′-double bond has a *Z*-configuration; the observed chemical shift for the C-13′-methyl resonance in the phytofluene is δ 1.91.

Analysis of chemical-shift data for vinyl methyl and vinyl methylene carbons in synthetic model compounds [3] then permitted us to predict the chemical shifts of the vinyl methyl and vinyl methylene carbon atoms associated with the pentaene chromophores in the four geometrical isomers of phytofluene which remained possibilities. These data, summarised on formulae (11), (12), (13), and (14), were calculated from data estimated from synthetic model compounds for the all-*E*-chromophore (10) in phytofluene, and from the shift parameters summarised in the preceding paper.[3] Comparing these data with those obtained for the natural product: δ 12.4, 16.6, 24.1, 32.75, and 40.5, clearly demonstrated that natural phytofluene from the Tangerine fruits has the 13*E*, 15*Z*, 13′*E*, 11′*E*, 9′*Z*-stereochemistry [*i.e.* (11)] about its pentaene chromophore, and hence stereostructure (2). This stereochemical assignment was confirmed by synthesis of the authentic di-*Z*-C_{30}-octaene (15),[11] and comparison of i.r. and n.m.r. spectral data; the comparative ^{1}H n.m.r. and ^{13}C n.m.r. spectral data are collected on the formulae in Figure 1.

The stereochemical assignments determined for phytoene and phytofluene from Tangerine tomato fruits suggest, for the first time, that phytoene (1) rather than the widely accepted ζ-carotene (3),[12] is the clear branch point for poly-*Z*-carotene formation in this variety of tomato. In addition, the data demonstrate that during the desaturation of phytoene to phytofluene (2) in the Tangerine tomato, at the same time as the 11′*E*-double bond is introduced, the 9′-double bond undergoes an interesting and specific *E*-to-*Z* isomerisation.

In comparison with phytofluene, the establishment of the



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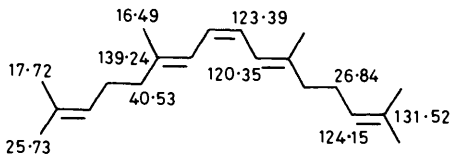

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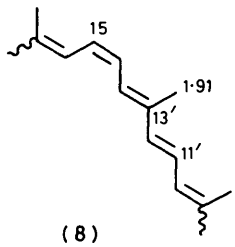

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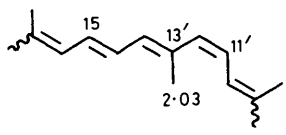

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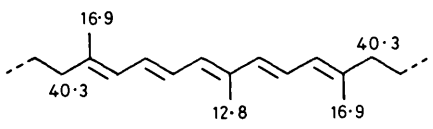

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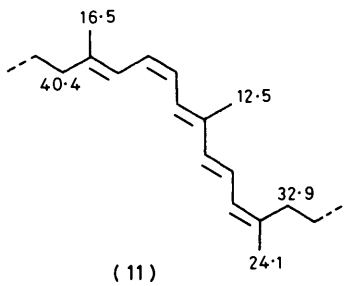

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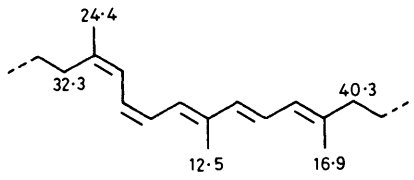

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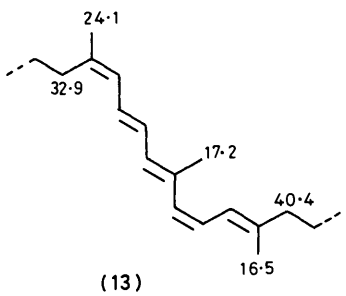

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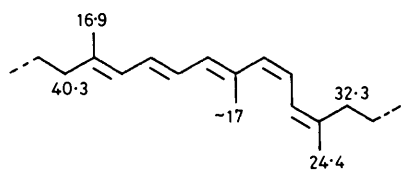

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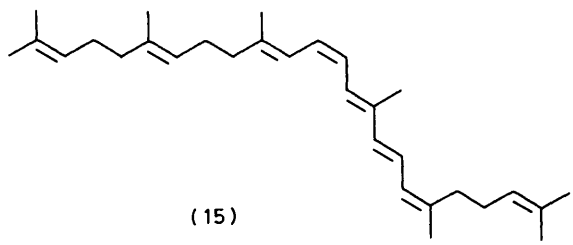


(15)

stereostructure for ζ-carotene (3) found in Tangerine tomato fruits was a simple task. Firstly, the pigment displayed only eleven sp^2-carbon resonances in its noise-decoupled ^{13}C n.m.r. spectrum, indicating that it is symmetrical. The ^{13}C n.m.r. spectrum also established straightaway that the terminal trisubstituted double bonds, at C-9 and C-9′, of the heptaene chromophore each have the *Z*-configuration [δ 32.8, C-8(8′); δ 24.1, 9(9′)-Me]. The absence of absorption at 760 cm^{-1} in the i.r. spectrum suggested that the stereochemistries of the three disubstituted double bonds are *E*-, and the relative strengths (1 : 2) of the vinyl methyl resonances at δ 1.70 [1(1′)*E*-Me] and δ 1.63 [1(1′)*Z*-Me, 5(5′)-Me] in the ^1H n.m.r. spectrum established that both of the non-conjugated double bonds capable of isomerisation also have the *E*-configuration.[8] In addition, the chemical shift (δ 12.75) for the 13(13′)-Me in the ^{13}C n.m.r. spectrum, firmly established an *E*-configuration for each of the remaining trisubstituted double bonds at C-13 and C-13′ in the ζ-carotene.

Further, more refined application of the n.m.r. shift parameters described in the preceding paper[3] fully supported the *Z*9, *Z*9-stereochemistry (3) for ζ-carotene, and this was confirmed by comparison of data (for n.m.r. data see Figure 2) with those of the authentic synthetic C_{30}-analogue (16).[11] Interestingly therefore, as the *E*-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

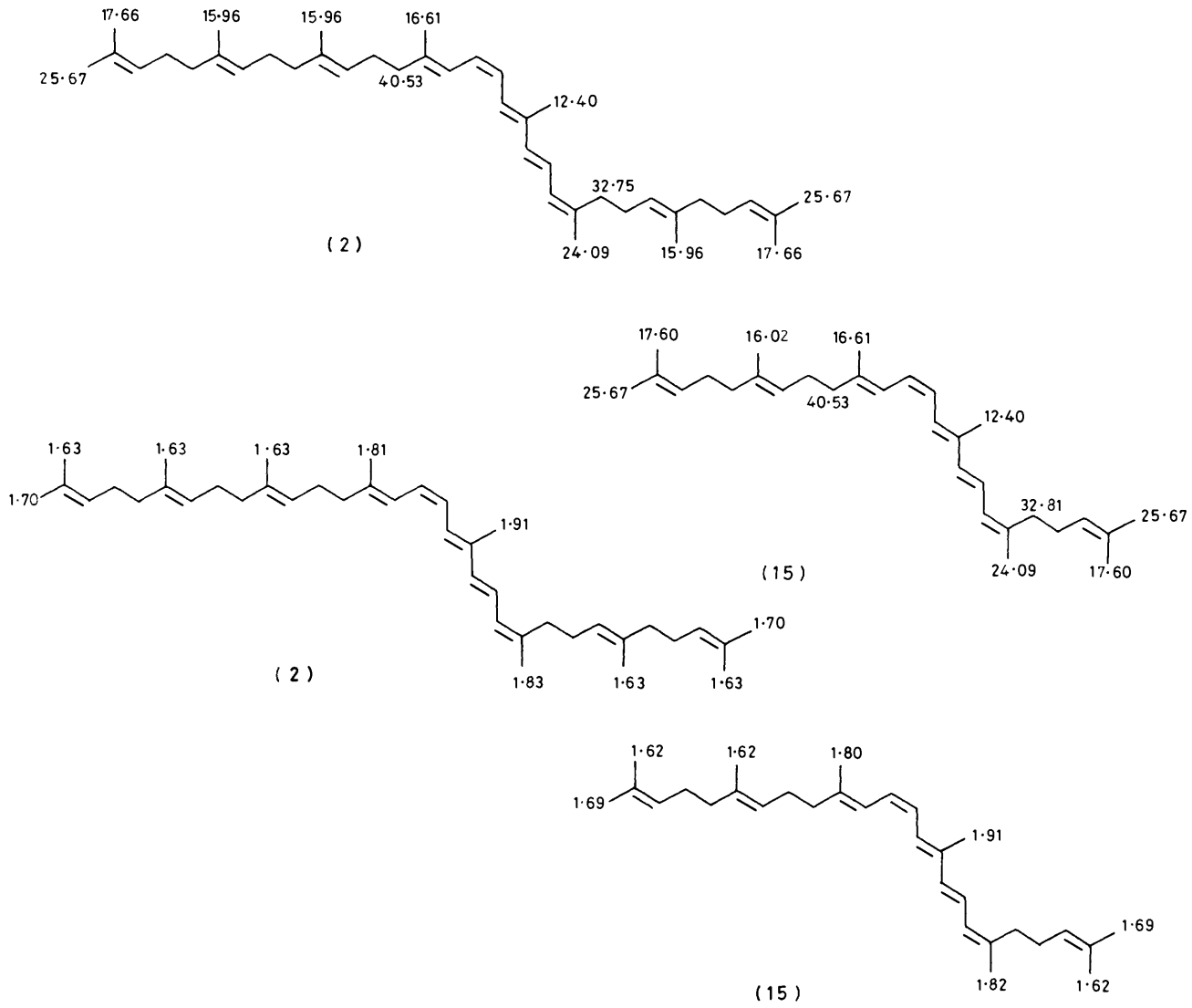
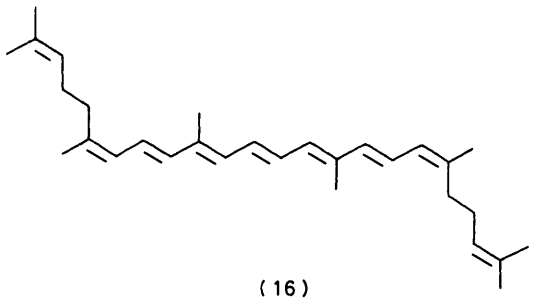


Figure 1. Comparative ^{13}C n.m.r. and ^{1}H n.m.r. data ($\delta_{TMS} = 0$) for natural 15*Z*, 9′*Z*-phytofluene (2) from 'Tangella' and the synthetic C_{30}-octaene (15)



(16)

phytofluene undergo *Z*-to-*E* and *E*-to-*Z* isomerization respectively.

Iodine-catalysed isomerisation of the neurosporene ($\lambda_{max.}$ 410, 433, 459 nm) isolated from Tangella fruits resulted in a significant increase in the strength and the wavelength (*i.e.* to 415, 438, 466 nm) of the visible absorption bands, suggesting that the molecule has at least one (and probably more) non-terminal *Z*-double bond in its chromophore. Inspection of the relative strengths of the olefinic C–H out-of-plane bands at 765 and 965 cm^{-1} in the i.r. spectrum suggested that the pigment contains three *E*- and one *Z*-disubstituted double bonds.

The ^{13}C n.m.r. spectrum of neurosporene showed quite clearly that the geometry of one of the terminal trisubstituted double bonds of the nonaene chromophore is *Z*- (δ 32.8, C-8) whilst the other has an *E*-configuration (δ 40.35, C-4′). Furthermore, the ^{13}C n.m.r. spectrum established that the nonaene chromophore contains one other *Z*- (δ 24.7) and two other *E*-trisubstituted (δ 12.6, 12.75) double bonds (9′-Me, 13′-Me, 13-Me). Working from the estimated chemical shifts for the sp^3-carbon nuclei on the nonaene chromophore of all-*E*-neurosporene (17), and the shift parameters described earlier,[3] we were able to predict the carbon chemical-shift data collected on formulae (18)—(23) for the various isomeric polyene fragments possible in the natural product. In this manner, and taking all features together, either of the stereostructures (24) or (25) could be assigned to neurosporene from Tangella (observed shifts: δ 12.6, 12.75, 16.6, 24.15, 24.7, 32.8, 40.35). We were disappointed to find that our shift parameter data were insufficiently sensitive to distinguish between these two possibilities. In view of the stereostructure

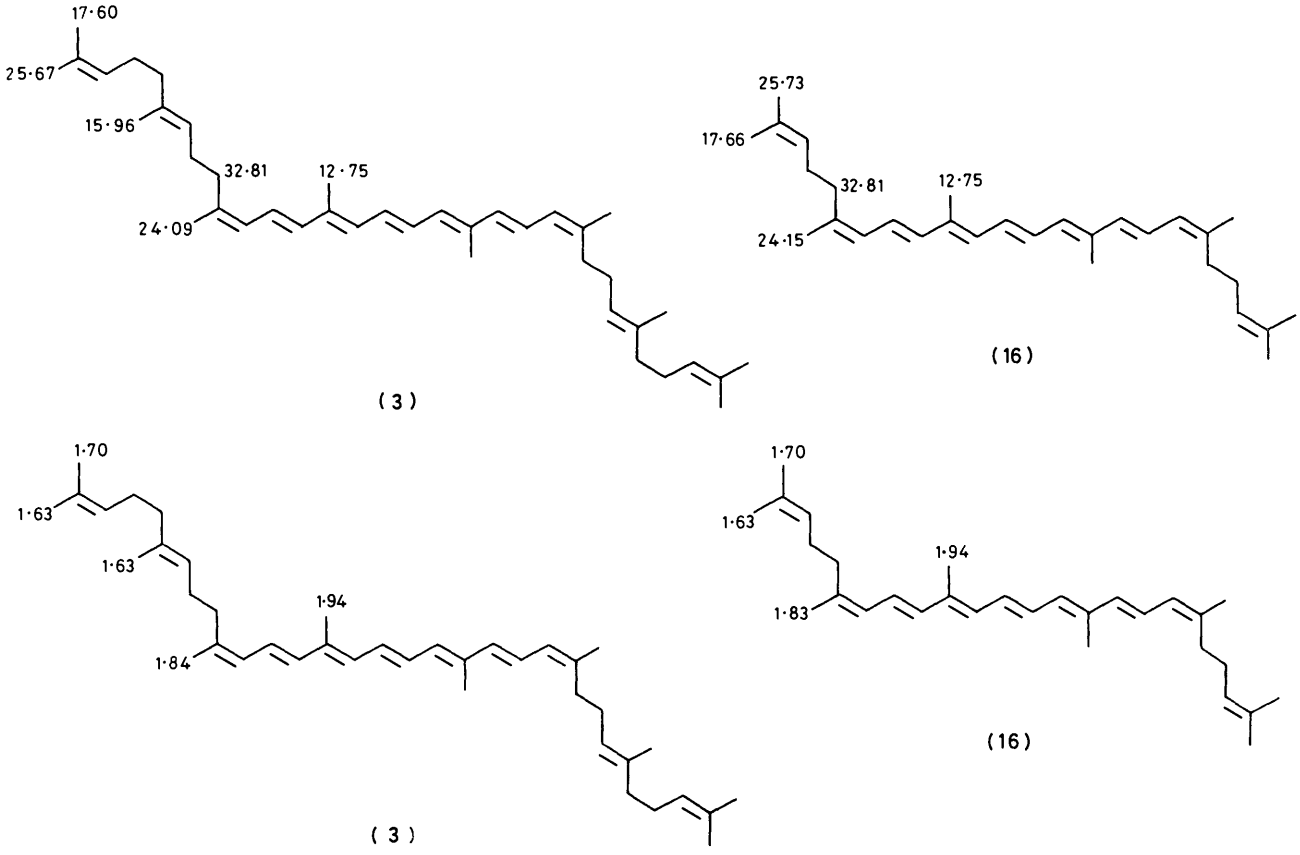


Figure 2. Comparative ^{13}C n.m.r. and ^{1}H n.m.r. data ($\delta_{TMS} = 0$) for natural 9*Z*, 9′*Z*-ζ-carotene (3) from 'Tangella' and the synthetic C_{30}-nonaene (16)

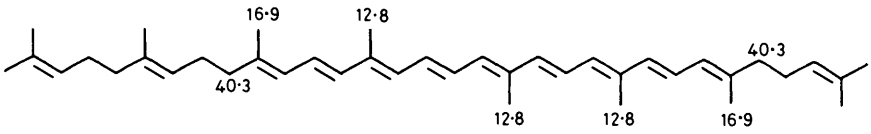
of ζ-carotene (3), the biosynthetic precursor of neurosporene in Tangella, it is inconceivable that neurosporene would have the stereostructure (25) since the *in vivo* conversion [(3) ⟶ (25)] would involve no fewer than three *E*-to-*Z*, in addition to two *Z*-to-*E*, double bond inversions. The *in vivo* conversion of 9*Z*, 9′*Z*-ζ-carotene (3) into stereostructure (24), by contrast, would simply involve the introduction of one double bond with *Z*-geometry. Consequently, we assigned the stereochemistry of neurosporene from Tangella as 9*Z*, 7′*Z*, 9′*Z*-[*viz.* (24) ≡ (4)].

The symmetrical 7*Z*, 9*Z*, 7′*Z*, 9′*Z*-stereochemistry (5) ≡ (26) for 'prolycopene' found in Tangerine tomato fruits followed logically from comparison of its spectral data with those accumulated for its congeners and synthetic model compounds. Thus, the seven signals in the sp^3-region of the noise-decoupled ^{13}C n.m.r. spectrum of prolycopene established that the molecule contains a symmetrical chromophore, and i.r. data showed that the pigment contains both *Z*-($\nu_{max.}$ 780 cm^{-1}) and *E*-($\nu_{max.}$ 960 cm^{-1}) disubstituted double bonds. Furthermore, the relative strengths of these i.r. maxima strongly suggested that prolycopene contains two *Z*- and three *E*-disubstituted double bonds.

Inspection of the sp^3-carbon absorptions in the ^{13}C n.m.r. spectrum of prolycopene [see formula (26) for details] illustrated that they fell into exactly the same pattern as those observed from one end of the chromophore in proneurosporene (4), and in consequence they were interpreted in the same way. The chemical shifts of 5(5′)-Me (δ 16.55) and C-4(4′) (δ 40.3) showed clearly that the double bonds at the ends of the chromophore in prolycopene each have an *E*-configuration. A comparison with the data for all-*E*-lycopene (27) (^{13}C n.m.r. data are included on the diagram), and using the shift parameter data summarised in the previous paper,[3] showed that the precise position of 5(5′)-Me in prolycopene further required that the double bonds at C-7(7′) have a *Z*-configuration.

The carbon chemical shifts (*i.e.* δ 12.6, 24.7) of the methyl groups positioned 'deep' on the chromophore [*i.e.* 9(9′)-Me, 13(13′)-Me] in prolycopene demonstrated that one must be attached to a *Z*-double bond while the other is attached to an *E*-double bond. Furthermore, as with proneurosporene (4), the highly deshielded signal (δ 24.7) could only be explained by a polyene segment of the type (28). On the basis of shift parameter data, the more shielded methyl carbon signal at δ 12.6, which is necessarily associated with an *E*-double bond, could be part of either the all-*E*-segment (29) or the mono-*Z*-segment (30); the alternative segment (31) was certainly excluded. With all these data, it was clear that prolycopene has the symmetrical tetra-*Z*-structure (5). In contemporaneous studies, the research groups led by Englert and by Weedon have independently established the 7*Z*, 9*Z*, 7′*Z*, 9′*Z*-stereochemistry (5) for prolycopene using largely high frequency ^{1}H n.m.r. spectroscopy and the application of spin-spin decoupling and inter-proton nuclear Overhauser experiments.[13]

In many respects the stereostructure now established for prolycopene fulfils Zechmeister's requirements of over twenty years standing.[1] On the basis of the detailed features of the electronic absorption spectrum, and its change on equilibration, Zechmeister predicted the overall straight molecular form of prolycopene, and the approximate number of *Z*-double bonds we now know it contains. At the same time,



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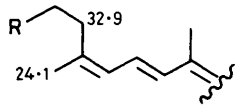

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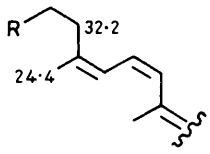

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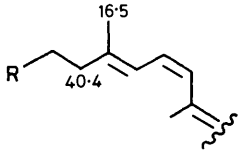

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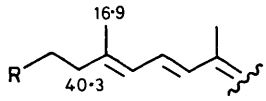

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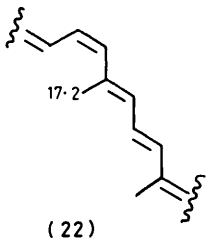

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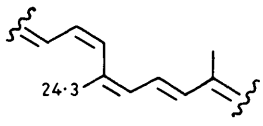

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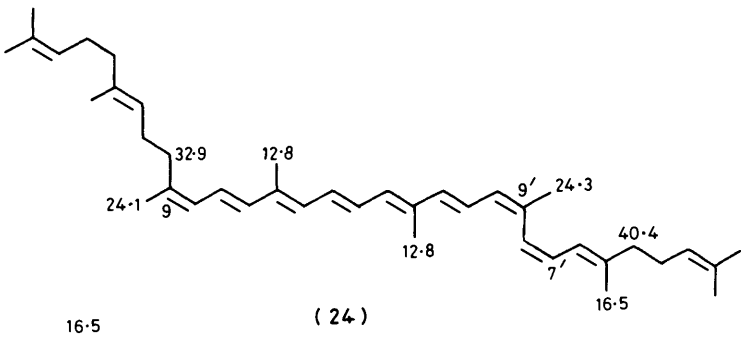
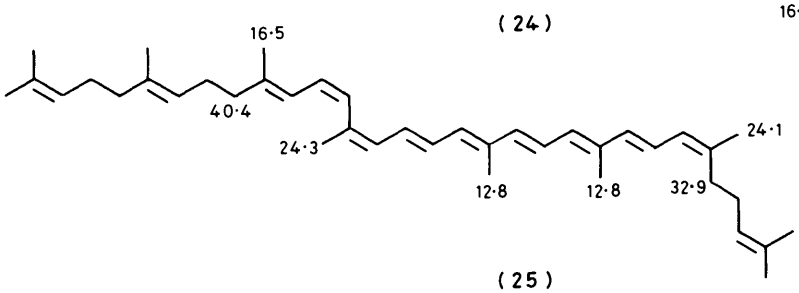


(δ_{TMS} = 0)

(24)



(25)

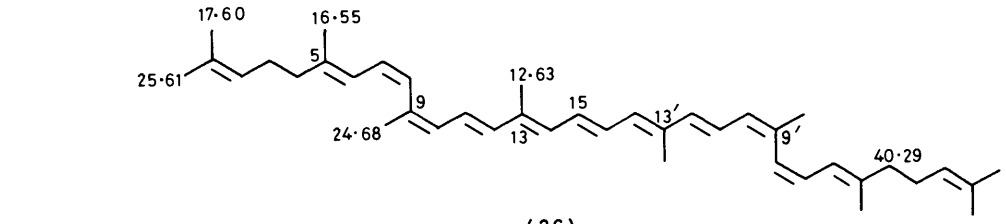
however, he would be surprised to learn that the pigment contains two sterically hindered *Z*-double bonds!

The establishment of the stereostructures of the acyclic carotenoid pigments in the Tangerine tomato fruit provides an insight into the fascinating genetic steering found in this variety compared to the common red tomato fruits. Since, however, little is known of the stereochemistry of hydrogen loss during the desaturation of phytoene (1) to prolycopene (5), it is not possible at this time to provide a mechanistic rationale of the interesting enzymic reactions involved in the dehydrogenation-isomerisation steps between these carotenoids; this must await further research.

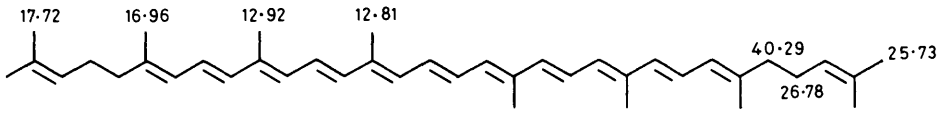
Experimental

For general experimental details see ref. 3. Due to the sensitivity of carotenoids to geometrical isomerisation and to aerial oxidation, strict methods of handling were adhered to throughout. At no time was a carotenoid left out of solution and in contact with the air. Furthermore, carotenoids were left in solution only for a minimum length of time and the solutions were never heated above room temperature. All solutions containing carotenoids were handled in subdued light or in darkness, and all columns used in chromatographic separations were wrapped in metal foil. Carotenoids were left adsorbed on active surfaces for the minimum time possible. The addition and removal of carotenoid samples from p.l.c. plates was performed rapidly and with the plate under a blanket of nitrogen. When carotenoid samples were stored for short periods, for example before final purification and spectroscopic analyses, they were stored at 0 °C in the dark, and under vacuum.

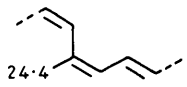
The entire extraction and purification procedure and accumulation of data for the five carotenoids isolated in this



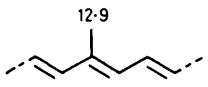
(δ_{TMS} = 0) (26)



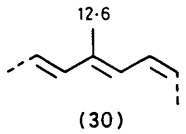
(27)



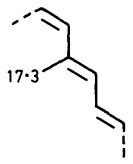
(28)



(29)



(30)



(31)

study was carried out within four weeks, and every stage of purification was monitored for possible changes in isomeric composition by electronic absorption spectroscopy.

Isolation of Acyclic Carotenoid Pigments from Tangerine Tomato Fruits.—Freeze-dried segments of the ripe Tangerine tomato fruits (600 g: equivalent to *ca.* 10 kg of fresh fruit) were chopped rapidly in a blender with acetone–n-hexane (1 : 1). The pulp was filtered off and re-extracted with fresh solvent until it was almost colourless. The combined organic extracts were poured into water, and the clear, brightly coloured hexane layer was then separated. The hexane extracts were partially concentrated, and the residue was then passed through a short column of alumina with 10% acetone in n-hexane as eluant to remove chlorophylls and other highly polar metabolites, and also any remaining fibres. The hexane solution was evaporated under reduced pressure to leave a dark red solid (2.26 g) which was chromatographed on magnesium oxide with increasing proportions of acetone in n-hexane as eluant. Fractions eluted from the column were analysed by electronic absorption spectroscopy which showed that the five carotenes of interest had been largely separated from each other. The appropriate fractions were combined and then evaporated under reduced pressure to leave the crude carotenoids which were purified further as described below:

(i) 15*Z*-*Phytoene* (1). The combined fractions containing phytoene from the preliminary column (which contained traces of phytofluene) were purified by chromatography on a single 40 × 40 cm p.l.c. plate (Fluka Kieselgel HF_{254}), developed with n-hexane. The zone which was more weakly adsorbed than the highly fluorescent phytofluene band was removed from the plate to give 15*Z*-*phytoene* (80 mg) as a colourless oil, $\lambda_{max.}$ (n-hexane) 265infl. (18 400), 276infl. (30 700), 286 (37 500), and 296infl. (27 200) nm (with a slight increase in intensity, though no significant change in wavelength, on iodine-catalysed equilibration), $\nu_{max.}$ (film) 1 635, 835, and 765 cm^{-1}; δ_H 6.38—6.06 (m, 4 H, olefinic protons on chromophore), 5.13 (br, 6 H, olefinic protons on isolated double bonds), 2.15—2.02 (m, 24 H, CH_2CH_2), 1.78 [6 H, 13(13′)-Me], 1.69 [6 H, 1(1′)*E*-Me], and 1.62 [18 H, 1(1′)*Z*-Me, 5(5′)-Me, 9(9′)-Me]; δ_C 16.02 [5(5′)-Me, 9(9′)-Me], 16.55 [13(13′)-Me], 17.72 [1(1′)*Z*-Me], 25.67 [1(1′)*E*-Me], 26.78 [C-3(3′), C-7(7′), C-11(11′)], 39.77 [C-4(4′), C-8(8′)], 40.53 [C-12(12′)], 120.29 [C-14(14′)], 123.39 [C-15(15′)], 123.97, 124.27, 124.44 [C-2(2′), C-6(6′), C-10(10′)], 131.17 [C-1(1′)], 134.91, 135.32 [C-5(5′), C-9(9′)], and 139.41 [C-13(13′)] p.p.m. [*m/z* 544 (94%, *M*), 476 (4%), 450 (8%), 410 (6%), 339 (100%, $M - C_{15}H_{25}$) (Found: M^+, 544.4964. $C_{40}H_{64}$ requires *M*, 544.5008)].

(ii) 15*Z*, 9′*Z*-*Phytofluene* (2). The combined fractions containing phytofluene from the preliminary column (containing traces of phytoene and β-carotene) were purified on a column of magnesium oxide using n-hexane as eluant to give a sample of phytofluene free from other carotenoids. Solvent residues remaining in the sample were removed by p.l.c. on a single 20 × 20 cm plate (silica gel G), developed with 1% acetone in n-hexane, to give 15*Z*, 9′*Z*-*phytofluene* (30 mg) as a yellow oil with intense blue-green fluorescence in solution when irradiated with u.v. light (λ = 366 nm), $\lambda_{max.}$ (n-hexane) 249, 257, 304infl., 318infl., 331, 348, 367 nm [with a distinct increase in intensity, though only a slight increase in wavelength, of absorption on the main band (304, 318, 331, 348, 367 nm), and a decrease in intensity of absorption in the *cis* peak region (249, 257 nm), on iodine-catalysed equilibration], $\nu_{max.}$ (film) 1 630, 960, 885, 835, 775 cm^{-1}; δ 6.69—5.91 (m, 7 H, olefinic protons on chromophore), 5.14 (br, 5 H, olefinic protons on isolated double bonds), 2.21—2.04 (m, 20 H, CH_2–CH_2), 1.91 (3 H, 13′-Me), 1.83 (3 H, 9′-Me), 1.81 (3 H, 13-Me), 1.70 [6 H, 1(1′)*E*-Me], 1.63 [15 H, 1(1′)*Z*-Me, 5(5′)-Me, 9-Me]; δ_C 12.40 (13′-Me), 15.96 [5(5′)-Me, 9-Me], 16.61 (13-Me), 17.66 [1(1′)*Z*-Me], 24.09 (9′-Me), 25.67 [1(1′)*E*-Me], 26.67 [C-3(3′), C-7(7′), C-11], 32.75 (C-8′), 39.71 [C-4(4′), C-8], 40.53 (C-12), 120.52, 123.27, 123.80, 124.21, 124.44, 125.26, 125.79, 126.55, 130.99, 131.11, 134.79, 135.26, 135.38, 135.73, 139.06, and 140.35 p.p.m. [*m/z* 542 (56%, *M*), 405 (50%,

$M - C_{10}H_{17}$), 337 (100%, $M - C_{15}H_{25}$) (Found: M^+, 542.4804. $C_{40}H_{62}$ requires M, 542.4851)].

(iii) 9Z, 9'Z-ζ-*Carotene* (3).—The combined fractions containing ζ-carotene from the preliminary column (containing traces of β-carotene and proneurosporene), were purified by chromatography on magnesium oxide using increasing proportions of acetone (1 to 6%) in n-hexane as eluant to give ζ-carotene free from other carotenoids. Solvent residues were removed by p.l.c. on a single 20 × 20 cm plate (silica gel G) developed with 4% acetone in n-hexane, to give 9Z, 9'Z-ζ-*carotene* (30 mg) as a very viscous orange oil, $\lambda_{max.}$ (n-hexane) 284, 294, 325infl., 341infl., 361, 379, 401, and 427 nm [with a small decrease in both wavelength and intensity of absorption in the main band (379, 401, 427 nm), and an increase in intensity of absorption in the *cis* peak region (284, 295 nm), on iodine-catalysed equilibration], $\nu_{max.}$ (film) 1 635, 1 595, 960, and 830 cm^{-1}; δ_H 6.65—5.89 (m, 10 H, olefinic protons on chromophore), 5.09 (br, 4 H, olefinic protons on isolated double bonds), 2.22—2.03 (m, 16 H, CH_2CH_2), 1.94 [6 H, 13(13')-Me], 1.84 [6 H, 9(9')-Me], 1.70 [6 H, 1(1')*E*-Me], 1.63 [12 H, 1(1')*Z*-Me, 5(5')-Me]; δ_C 12.75 [13(13')-Me], 15.96 [5(5')-Me], 17.60 [1(1')*Z*-Me], 24.09 [9(9')-Me], 25.67 [1(1')*E*-Me], 26.72 [C-3(3'), C-7(7')], 32.81 [C-8(8')], 39.76 [C-4(4')], 123.74, 124.38, 124.56, 126.72, 129.47, 131.05, 131.46, 135.14, 135.38, 135.84, and 139.24 p.p.m. [*m/z* 540 (100%, *M*), 403 (14%, $M - C_{10}H_{17}$) (Found: M^+, 540.4480. $C_{40}H_{60}$ requires *M*, 540.4695)].

(iv) 9Z, 7'Z, 9'Z-*Neurosporene* ('*Proneurosporene*') (4). Solvent residues in the combined fractions from the preliminary column were removed by p.l.c. on a single 20 × 20 cm plate (silica gel G) developed with 10% acetone in n-hexane to give 9Z, 7'Z, 9'Z-*neurosporene* (30 mg) as a very dark red, very viscous oil, $\lambda_{max.}$ (n-hexane) 367infl., 390infl., 410, 433, and 459infl. nm [with a very large increase in both intensity and wavelength of absorption and the emergence of *cis* peaks on iodine-catalysed equilibration; equilibrium $\lambda_{max.}$ (n-hexane) 318, 330, 370infl., 394infl., 415, 438, and 466 nm], $\nu_{max.}$ (film) 1 635, 965, 885, 875, 830,a nd 765 cm^{-1}; δ_H 6.64—5.9 (m, 13 H, olefinic protons on chromophore), 5.06 (br, 3 H, olefinic protons on isolated double bonds), 2.19—1.99 (m, 12 H, CH_2CH_2), 2.05 (3 H, 9'-Me), 1.93 (3 H, 13-Me), 1.87 (3 H, 13'-Me), 1.84 (3 H, 9-Me), 1.82 (3 H, 5'-Me), 1.66 [6 H, 1(1')*E*-Me], 1.59 [9 H, 1(1')*Z*-Me, 5-Me]; δ_C 12.63 (13'-Me), 12.75 (13-Me), 16.02 (5-Me), 16.61 (5'-Me), 17.60 [1(1')*Z*-Me], 24.15 (9-Me), 24.68 (9'-Me), 25.67 [1(1')*E*-Me], 26.72 [C-3(3'), C-7], 32.81 (C-8), 39.77 (C-4), 40.35 (C-4'), 122.57, 123.80, 124.03, 124.32, 124.73, 126.14, 126.66, 129.47, 129.70, 129.88, 131.17, 131.52, 131.98, 135.20, 135.49, 136.08, 139.47, and 140.58 p.p.m. [*m/z* 538 (100%, *M*), 469 (2%, $M - C_5H_9$), 446 (17%), 401 (4%, $M - C_{10}H_{17}$), and 309 (8%), (Found: M^+, 538.4542. $C_{40}H_{58}$ requires *M*, 538.4538)].

(v) 7Z, 9Z, 7'Z, 9'Z-*Lycopene* ('*Prolycopene*') (5). The fractions containing prolycopene from the preliminary column contained significant quantities of 4-hydroxy-4-methylpentan-2-one (diacetone alcohol) formed from the acetone eluant under the catalytic influence of the magnesium oxide. The pentan-2-one was removed by washing an ether solution of the combined prolycopene fractions with water. The ether solution was then dried and concentrated under reduced pressure to leave the crude 7Z, 9Z, 7'Z, 9'Z-*lycopene* (160 mg) as a dark red solid. Crystallisation from n-hexane–ethanol gave rosettes of tiny red-orange flaky crystals, m.p. 112—113.5 °C (corrected) (lit.,[14] m.p. 111.5—112.5 °C), $\lambda_{max.}$ (n-hexane) 232 (26 500), 255 (21 900), 295 (18 700), 362infl. (18 100), 392infl. (45 000), 417infl. (81 800), 437 (100 700), 461infl. (74 000), and 487infl. (25 600) nm [with a tremendous increase in intensity (*ca.* 40% at the most intense band) and wavelength of absorption, as well as the appearance of *cis* peaks, on iodine-catalysed equilibration; equilibrium $\lambda_{max.}$ (n-hexane) 286, 296, 346, 361, 443, 468, and 500 nm], $\nu_{max.}$ (KBr) 1 625, 960, 880, 830, and 780 cm^{-1}; δ_H 6.65—5.98 (m, 16 H, olefinic protons on chromophore), 5.12 [br, 2 H, 2(2')-H], 2.09—2.07 (m, 8 H, CH_2CH_2), 2.01 [6 H, 9(9')-Me], 1.89 [6 H, 13(13')-Me], 1.82 [6 H, 5(5')-Me], 1.68 [6 H, 1(1')*E*-Me], and 1.59 [6 H, 1(1')*Z*-Me]; δ_C 12.63 [13(13')-Me], 16.55 [5(5')-Me], 17.60 [1(1')*Z*-Me], 24.68 [9(9')-Me], 25.61 [1(1')*E*-Me], 26.72 [C-3(3')], 40.29 [C-4(4')], 122.51, 123.91, 125.96, 126.14, 129.82, 131.52, 131.98, 135.32, 136.08, 136.20, and 140.64 p.p.m. [*m/z* 536 (93%, *M*), 467 (8%, $M - C_5H_9$), 445 (35%), 444 (100%) (Found: M^+, 536.4397. $C_{40}H_{56}$ requires *M*, 536.4382)].

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References

1 L. Zechmeister, A. L. Le Rosen, F. W. Went, and L. Pauling, *Proc. Natl. Acad. Sci. U.S.A.*, 1941, **27**, 468.
2 See: (*a*) L. Zechmeister and W. A. Schroeder, *J. Biol. Chem.*, 1942, **144**, 315 and (*b*) L. Zechmeister, '*cis–trans* Isomeric Carotenoids, Vitamins A, and Arylpolyenes,' Springer-Verlag, Vienna, 1962.
3 L. Carey, J. M. Clough, and G. Pattenden, *J. Chem. Soc., Perkin Trans. 1*, 1983, preceding paper.
4 For review see: G. Pattenden, 'Stereocontrolled Synthesis of Polyene Isoprenoids,' in *Carotenoid Chemistry and Biochemistry*, ed. G. Britton and T. W. Goodwin, Pergamon Press, 1982.
5 Preliminary communication: J. M. Clough and G. Pattenden, *J. Chem. Soc., Chem. Commun.*, 1979, 616.
6 *Cf.* R. J. H. Williams, G. Britton, J. M. Charlton, and T. W. Goodwin, *Biochem. J.*, 1967, **104**, 767; A. A. Qureshi, M. Kim, N. Qureshi, and J. W. Porter, *Arch. Biochem. Biophys.*, 1974, **162**, 108.
7 N. Khan, D. E. Loeber, T. P. Toube, and B. C. L. Weedon, *J. Chem. Soc., Perkin Trans. 1*, 1975, 1457.
8 J. B. Davis, L. M. Jackman, P. T. Siddons, and B. C. L. Weedon, *Proc. Chem. Soc.*, 1961, 261; *J. Chem. Soc. C*, 1966, 2154.
9 L. Barlow and G. Pattenden, *J. Chem. Soc., Perkin Trans. 1*, 1976, 1029.
10 See Appendix in 'Carotenoids,' ed. O. Isler, Birkhäuser Verlag, Basel, 1971.
11 J. M. Clough and G. Pattenden, *Tetrahedron Lett.*, 1979, 5043; *Tetrahedron*, 1981, **37**, 3911.
12 For recent summary see: B. H. Davies and R. F. Taylor, *Pure Appl. Chem.*, 1976, **47**, 211.
13 G. Englert, B. O. Brown, G. P. Moss, B. C. L. Weedon, G. Britton, T. W. Goodwin, K. L. Simpson, and R. J. H. Williams, *J. Chem. Soc., Chem. Commun.*, 1979, 545; G. Englert, *Helv. Chim. Acta*, 1979, **62**, 1497.
14 L. Zechmeister and J. H. Pinckard, *J. Am. Chem. Soc.*, 1947, **69**, 1930.

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