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Animal Carotenoids

8.* Synthesis of β,γ-Carotene and γ,γ-Carotene

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The detailed synthesis of racemic β,γ-carotene (*12*) and optically inactive γ,γ-carotene (*10*) is reported. Both carotenes were synthesized by routes which, in principle, have been used for other carotenoids. Physical properties of intermediates and products and comparative studies with naturally occurring compounds are reported.

In an earlier communication[1] we briefly outlined the approach to the synthesis of β,γ-carotene (*12*, 5,18-didehydro-5,6-dihydro-β,β-carotene by the proposed IUPAC nomenclature[2,3]) and γ,γ-carotene (*10*, 5,18,5′,18′-tetradehydro-5,6,5′,6′-tetrahydro-β,β-carotene). The full details are now reported.

The synthesis was prompted by the finding of β,γ-carotene (*12*) by Arpin *et al.*[4] in the fungus *Caloscypha fulgens* (Pers.) Boud. and of both β,γ-carotene (*12*) and α,α-carotene (*10*) in the aphid *Macrosiphum liriodendri* Monell.[5,6] These are to date the only sources of carotenes with an exocyclic terminal methylene group.

RESULTS AND DISCUSSION

The synthesis of both carotenes (*10* and *12*) followed the $C_{15}+C_{10}+C_{15}$ approach used by a number of workers for the preparation of cyclic and acyclic carotenoids[7,8] (Scheme 1). Construction of the phosphonium salts containing the γ- and β-end groups (*8* and *4*) was followed by selective condensation of the γ-C_{15} phosphoran of *8* with C_{10}-dial (*9*) to yield γ,γ-carotene (*10*) and the intermediate γ-C_{25}-al (*11*). The β-C_{15} phosphoran of *4* was then reacted with the γ-C_{25}-al (*11*) to give β,γ-carotene (*12*).

β-Ionone (*1*) was condensed in a Horner reaction with ethyl diethylphosphonoacetate[9] in the presence of sodium methoxide to give methyl β-

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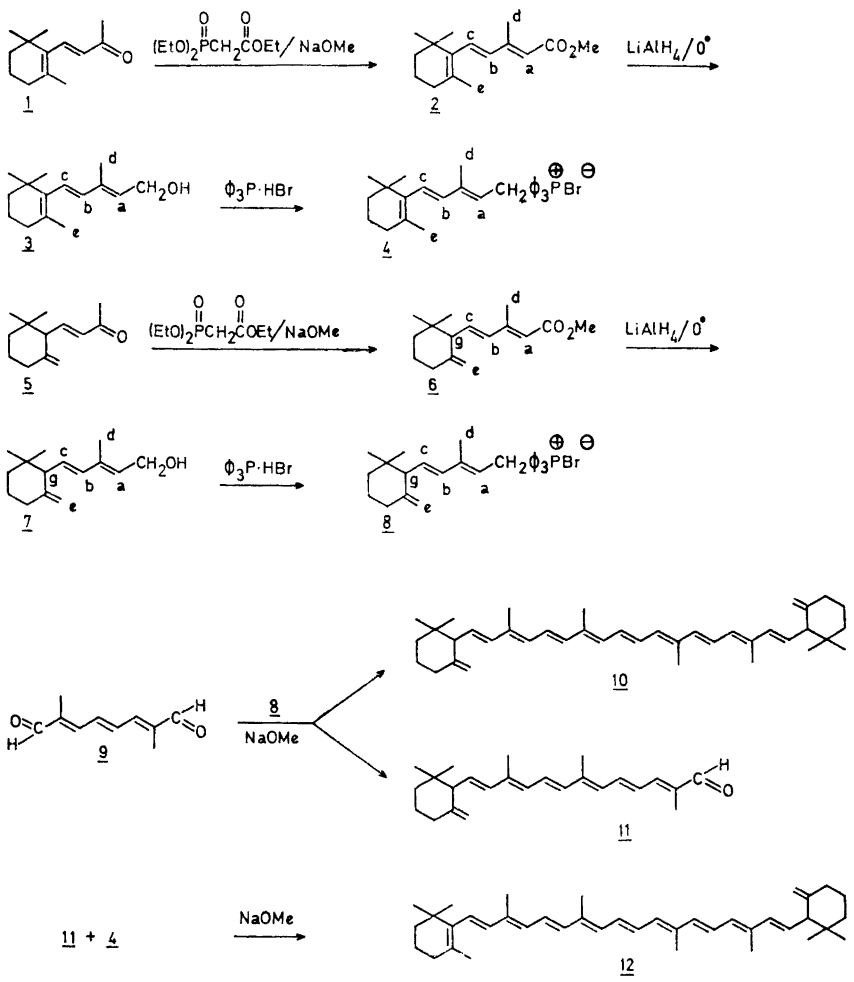

Scheme 1.

ionylideneacetate (*2*) in 90 % yield. During the reaction ester interchange occurred. The product consisted of 65 % *trans* and 35 % *cis* isomers (around the new double bond). Reduction with lithium aluminium hydride gave *β*-ionylidene-ethanol (*3*) which when reacted with triphenylphosphine hydrobromide gave the corresponding phosphonium salt (*4*).

Synthetic, racemic *γ*-ionone (*5*) when reacted with ethyl diethylphosphonoacetate gave *γ*-ionylideneacetate (*6*) in 90 % yield (*cis* and *trans* as in *2* above). During the reaction *ca.* 10 % isomerization [10] to the *β*-analogue (*2*) was observed. Lithium aluminium hydride reduction followed by reaction with triphenylphosphine hydrobromide gave the phosphonium salt *8* in 70 % yield.

C_{10}-dial (*9*) was prepared by selective hydrogenation of the acetylenic analogue with central triple bond.[11] Reaction of the γ-C_{15}-phosphoran of *8* with C_{10}-dial (*9*) gave γ,γ-carotene (*10*; 20 % yield) as the double condensation product and the γ-C_{25}-al (*11*; 50 % yield) as the monocondensation product. The latter was purified and used in a second Wittig reaction with the phosphoran of *4* to give β,γ-carotene (*12*; 15 % yield).

In the above Wittig reactions the phosphorans were generated externally with sodium methoxide and added to a methanolic solution of the appropriate aldehyde (*11* or *9*). The reactions were monitored by thin layer chromatography and in each case excess amounts of phosphorans were required.

The chromatographic properties of the synthetic (*10* and *12*) and natural carotenes were identical on direct comparison. R_F values are given in Table 1.

Sufficient β,γ-carotene of natural origin was available for mixed melting point determination with the synthetic compound (*12*). No depression was observed.

The electronic absorption data of synthetic β,γ-carotene (*12*) and γ,γ-carotene (*10*; spectrum not published elsewhere) are given in Fig. 1 and in Experimental. These are identical in every respect with those of the corresponding carotenes from natural sources.

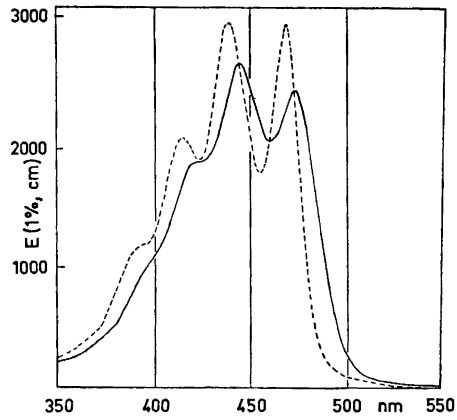

Fig. 1. Absorption spectrum in visible light of synthetic, *trans* β,γ-carotene (*12*, ——) and synthetic, *trans* γ,γ-carotene (*10*, – –) in petroleum ether.

Infrared spectra (KBr) of natural [4] and synthetic β,γ-carotene (*12*, Fig. 2) showed good agreement. Fig. 2 also includes the IR spectrum of γ,γ-carotene (*10*) not reported elsewhere; the 889 cm^{-2} absorption ascribed to $R_1R_2C=CH_2$ being of diagnostic value.

The proton magnetic resonance spectrum of synthetic β,γ-carotene (*12*) was fully consistent with that reported for natural β,γ-carotene.[4] That of synthetic γ,γ-carotene (*10*; spectrum not available elsewhere) is reproduced in Fig. 3 including signal assignments.

The upper mass region of the mass spectra of β,γ-carotene (*12*) and γ,γ-carotene (*10*) are given in Fig. 4. Both carotenes showed the common M – 92, M – 106, and M – 158 losses on electron impact.[12,13] The M – 92/M – 106 in-

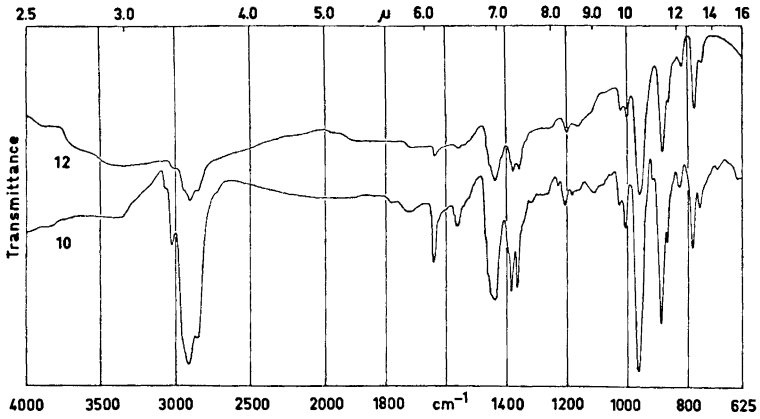

Fig. 2. Infrared spectra (KBr) of synthetic β,γ-carotene (*12*) and synthetic γ,γ-carotene (*10*).

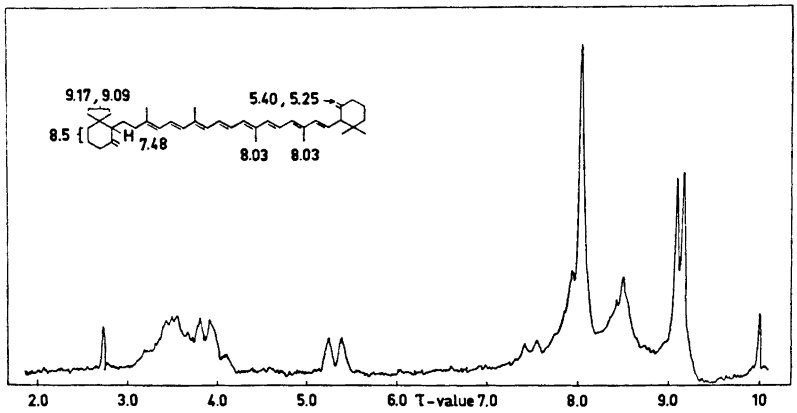

Fig. 3. Proton magnetic resonance spectrum (in $CDCl_3$) of synthetic γ,γ-carotene (*10*).

tensity ratio (1.62 for *12* and 1.57 for *10*) was within the expected range for bicyclic carotenoids with nine conjugated double bonds in the central acyclic chain.[14] Fragment ions of low intensity consistent with in-chain cleavages, some of which require hydrogen transfer, are indicated in Fig. 4. M − 123 ions compatible with cleavage of the C(6) − C(7) single bond of the γ-end were observed for both *10* and *12* and may be of diagnostic value. This same ion is observed in cleavages of the C(6) − C(7) bond of an α-end group but in this latter case an M − 56 ion which arises from a formal *retro*-Diels-Alder reaction is also noted.[12,15] An M − 137 ion, previously connected with carotenoids containing unsubstituted β-rings and ascribed to cleavage of the C(7) − C(8) double bond with hydrogen transfer to the smaller fragment,[12] was observed for β,γ-carotene (*12*) and not for γ,γ-carotene (*10*). Doubly charged molecular

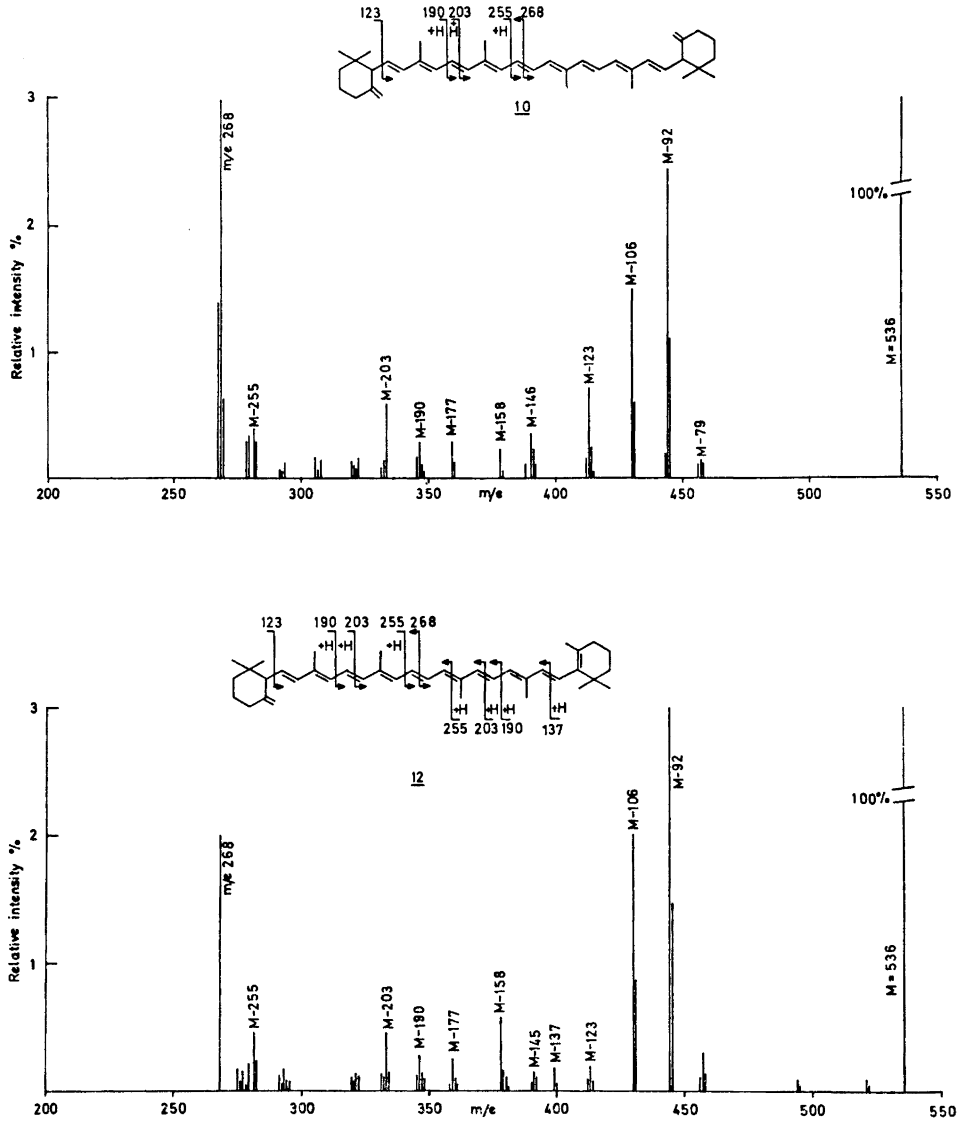

Fig. 4. Mass spectra of synthetic β,γ-carotene (*12*) and of synthetic γ,γ-carotene (*10*).

ions or ions due to cleavage of the central double bond (*m/e* 268) were abundant in both cases. M − 190, M − 203, and M − 255 peaks attributed to in-chain cleavages were also noted for both *10* and *12*.

Certain minor differences in the M − 92/M − 106 ratio and the low-intensity fragment ions of the previously published [4] mass spectrum of natural β,γ-carotene (*12*) and that of synthetic *12* given in Fig. 4 are ascribed to different

recording conditions. Thus natural *12* gave a mass spectrum essentially identical with the one presented for synthetic *12*, when recorded under similar conditions. The differences observed are accounted for by some variation in the hydrogen transfer pattern of certain in-chain cleavages; the present M − 123 contra the previously discussed [4] M − 135 ion may be explained by alternative processes of electron impact and thermal character.

β,γ-Carotene (*12*) has an asymmetric carbon atom at C − 6′ and the natural compound is, as expected, optically active.[16] It is not yet known whether the absolute configuration is 6′*R* (as in natural β,ε-carotene [3]) or 6′*S*.

Provided optically active γ-ionone was available the synthesis of natural β,γ-carotene or its enantiomer by the scheme outlined here should be feasible, assuming no extensive racemization of the conjugated ketone *5* occurred during the Horner reaction. However, conversion of γ-ionone (*5*) to γ-ionol followed by condensation of the γ-ionol-derived phosphonium salt with the appropriate β-C_{27}-aldehyde may be a preferred route to the optically active carotene *12*.

EXPERIMENTAL

General. Solvents were of reagent grade and were purified by distillation before use.

Preparative chromatography was carried out on silica gel G plates and thin layer chromatography on silica gel HF_{254} plates. Mixtures of petroleum ether (b.p. 40 − 65°) and acetone were used for development. Woelm Al_2O_3 (deactivated with 2 % water) was used for column chromatography. For comparative chromatography of synthetic and natural carotenes, Schleicher & Schüll paper No. 288 (Al_2O_3) was used after heat activation at 90° for 2 h. Mixtures of petroleum ether and diethyl ether were used to develop the paper chromatograms.

Melting points were determined on an Electrothermal melting point apparatus in sealed, evacuated tubes and are uncorrected.

Electronic absorption spectra were recorded on a Coleman Hitachi 124 spectrometer and IR spectra on a Perkin-Elmer 257 spectrometer using potassium bromide disks or liquid films. PMR spectra were obtained on a Varian A-60 A instrument using deuteriochloroform solutions with tetramethylsilane as internal standard. An AEI MS902 instrument was used for recording the mass spectra; the electron bombardment energy was 70 eV and the accelerating voltage 8 kV. Carotenes were recorded at 200°.

Gas chromatography involved the use of a Perkin-Elmer F11 Flame Ionization Instrument equipped with a 180 × 0.6 cm column packed with 10 % Silicon OV-17 (methyl-50 % phenylsilicon, Serva, Heidelberg) on Chromosorb W.

Methyl β-ionylideneacetate (2). Sodium methoxide in methanol was added dropwise to a stirred solution of β-ionone (*1*, 15 g) and ethyl diethylphosphonoacetate (18 g) in dry benzene (40 ml). The mixture was stirred at 40° for 10 h; the reaction being monitored by TLC on silica HF_{254} plates. The reaction mixture was poured over ice, extracted with ether and the organic layer washed with water until the aqueous extract was neutral to phenolphthalein. The ether solution was dried over sodium chloride and the solvent removed under vacuum. The residue consisted of 17.3 g (90 %) of *2*. Analyses by GLC showed 65 % *trans*- and 35 % *cis*-isomers. *2* had b.p. 118 − 120°/0.3 torr; λ_{max} (methanol) 260, 295 nm; ν_{max} (liq.) 3020 − 2820 (CH), 1715 (C=O), 1610 (conj. C=C), 1235 (C−O), 1135 (C−O) cm^{-1}; τ ($CDCl_3$; *trans*-isomer, signal assignments with reference to *2*, Scheme 1) 8.98 (6H, *gem.* dimethyl), 8.30 (3H, CH_3-*e*), 7.65 (CH_3-*d*), 6.28 (3H, OCH_3), 4.23 (1H, H-*a*), 3.92 d (1H, J = 16 Hz, H-*b*), 3.48 d (1H, J = 16 Hz, H-*c*); *m/e* 248 (M), 233 (M − 15), 217 (M − 31) and 189 (M − 59).

β-Ionylidene-ethanol (3). A solution of *2* (10 g) in ethyl ether (40 ml) was added to a suspension of lithium aluminium hydride (1.7 g) in ether (30 ml) at 0 − 5° over a period of 30 min. Stirring was continued for 1 h during which time the reaction was followed by TLC (disappearance of the starting material). The complex was decomposed by the

addition of wet methanol followed by a 10 % aqueous solution of ammonium chloride. The ether solution was washed with water and the solvent evaporated under vacuum to yield 8.5 g (90 %) of *3*; b.p. 140–145°/0.3 torr; λ_{max} (methanol) 237, 265 nm; ν_{max} (liq.) 3320 (OH), 3020–2820 (CH), 1010 (CH_2OH), 970 (*trans* CH=CH) cm^{-1}; τ ($CDCl_3$; with reference to *3*, Scheme 1) 8.98 (6H, *gem.* dimethyl), 8.30 (3H, CH_3-*e*), 8.14 (3H, CH_3-*d*), 5.71 d (2H, J=7 Hz, CH_2OH), 4.27 t (1H, J=7 Hz, H-*a*), 3.91 (2H, olefinic H-*b*,*c*); *m/e* 220 (M).

β-Ionylidene-ethyltriphenylphosphonium bromide (4). A solution of *3* (5 g) and triphenylphosphonium bromide (9 g) in methanol-chloroform (40 ml, 1:1) was stirred at 20° for 48 h. The solution was washed with water, the organic layer dried with sodium sulfate and the solvents removed under vacuum. The residue was triturated with ethyl acetate to give 9.2 g (75 %) of *4*. After recrystallization from methylene chloride-ethyl acetate *4* melted at 123°; τ ($CDCl_3$; see Scheme 1) 9.03 (6H, *gem.* dimethyl), 8.53 (3H, CH_3-*d*), 8.37 (3H, CH_3-*e*), 5.25 dd (2H, J_{H-P}=15. Hz, J_{H-H}=8 Hz, CH_2P), 4.70 (1H, complex coupling, H-*a*), 4.00 (2H, olefinic H-*b*,*c*) and 2.4–1.9 (aromatic H).

Methyl γ-ionylideneacetate (6). Sodium methoxide in methanol was added to a solution of γ-ionone (*5*, 2 g) and ethyl diethylphosphonoacetate (2.4 g) in a manner analogous to that described for the preparation of *2*. After work-up, GLC analysis showed a mixture of 52 % *trans-6*, 38 % *cis-6*, and 10 % *2*. The oily product was purified by column chromatography on Al_2O_3 to give 2.32 g (90 %) *6*; λ_{max} (methanol) 260 nm; ν_{max} (liq.) 3080–2840 (CH), 1718 (C=O), 1610 (conj. C=C), 892 ($R_1R_2C=CH_2$) cm^{-1}; τ ($CDCl_3$; see Scheme 1) 9.17, 9.08 (6H, *gem.* dimethyl), 7.68 (3H, CH_3-*d*), 7.45 d (1H, J=7 Hz, H-*g*), 6.28 (3H, OCH_3), 5.28, 5.40 (2H, $=CH_2$-*e*), 5.76 (1H, H-*a*), 4.03–3.70 (2H, olefinic H-*b*,*c*); *m/e* 248 (M), 233 (M–15), 217 (M–31), 205 (M–43), 201 (M–47), 192 (M–56), 189 (M–59) and 177 (M–71).

γ-Ionylidene-ethanol (7). *6* (2.2 g) was reduced with lithium aluminium hydride (0.4 g) as described for the preparation of *3*. After work-up, *7* (2 g, 90 %) was obtained; λ_{max} (methanol) 238 nm; ν_{max} (liq.) 3350 (OH), 3020–2860 (CH), 1640 (C=C), 1035 (CH_2OH), 893 ($R_1R_2C=CH_2$) cm^{-1}; τ ($CDCl_3$; see Scheme 1) 9.18, 9.10 (6H, *gem.* dimethyl), 8.25 (CH_3-*d*), 7.80 d (1H, J=6 Hz, H-*g*), 5.72 d (2H, J=6 Hz, CH_2OH), 5.30, 5.40 (2H, $=CH_2$-*e*), 4.5–3.7 (3H, olefinic H-*a*,*b*,*c*); *m/e* 220 (M), 205 (M–15), 194 (M–26), 179 (M–41), 176 (M–44), 161 (M–59) and 136 (M–84).

γ-Ionylidene-ethyltriphenylphosphonium bromide (8). *8* was prepared from *7* (2 g) and triphenylphosphonium bromide (3.7 g) in a manner identical to that described for the preparation of *4*. The phosphonium salt could not be crystallized and was dried to an amorphous powder under vacuum and used without further purification; τ ($CDCl_3$; see Scheme 1) 9.24, 9.31 (6H, *gem.* dimethyl), 8.60 (3H, CH_3-*d*), 7.60 d (1H, J=8 Hz, H-*g*), 5.53, 5.30 (2H, $=CH_2$-*e*), 5.25 dd (2H, J_{H-P}=16 Hz, J_{H-H}=8 Hz, CH_2-P), 4.70 (complex coupling, 1H, H-*a*), and 4.3–3.7 (2H, olefinic H-*b*,*c*).

γ,γ-Carotene (10) and *γ-apo-12′-carotenal (11).* Sodium methoxide was slowly added to a stirred solution of *8* (250 mg) in dry methanol (5 ml) until the phosphonium salt was completely converted to the deep red phosphoran. A solution of 2,7-dimethyl-2,4,6-trienedial (*9*, 75 mg, prepared from the acetylenic analogue [11]) was added and the mixture stirred. The progress of the reaction was monitored by TLC and additional phosphoran, generated externally, was added until analysis showed no starting dial (*9*). Water was added and the crude product extracted with ether, the ether extract dried over sodium sulfate and the solvent removed under vacuum. Preparative thick layer chromatography afforded *10* (49 mg, 20 % based on starting dial, mixture of *cis-* and *trans*-isomers) and *11* (80 mg, 50 % based on starting dial, mixture of *cis-* and *trans*-isomers). Both *10* and *11* were purified by re-chromatography. After repeated crystallizations from benzene-methanol all *trans-10* was isolated, m.p. 183°; λ_{max} (petroleum ether) 414, 438 E(1 %, 1 cm)=2960, 468 nm, (acetone) 416, 440 E(1 %, 1 cm)=2870, 469 nm, (ether) 415, 439, 469 nm, see Fig. 1; ν_{max} (KBr) 889 ($R_1R_2C=CH_2$), 963 cm^{-1} (*trans*-disubstituted double bonds), see Fig. 2; τ ($CDCl_3$) see Fig. 3 with assignments; *m/e* 536 (M), 457 (M–79), 444 (M–92), 430 (M–106), 413 (M–123), 378 (M–158), 346 (M–190), 333 (M–203), and 268, see Fig. 4. For *11*, λ_{max} (petroleum ether) 386, 405, 428 nm, ($CHCl_3$) 425 nm, (acetone) 413 nm; τ ($CDCl_3$) 9.16, 9.08 (6H, *gem.* dimethyl), 8.12 (3H, CH_3-*a*), 8.02, 7.97 (6H, CH_3-*b*,*c*), 7.49 d (1H, J=7 Hz, H-*d*), 5.43, 5.25 (2H, $=CH_2$-*e*), 4.1–3.0 (9H, olefinic), 0.53 (1H, H-*f*); *m/e* 350 (M), 335 (M–15), 322 (M–28), 307 (M–28–15), 227 (M–123), and 201 (M–149).

Co-chromatography of synthetic *10* and *10 ex Macrosiphum liriodendri* gave no separation, see Table 1.

Table 1. Chromatographic properties of natural and synthetic γ,γ-carotene (*10*) and β,γ-carotene (*12*).

Compound	R_F value S & S 288 paper		Al_2O_3 plates
10 (natural)	0.16[a]	0.28[b]	0.23[c]
10 (synthetic)	0.16[a]	0.28[b]	0.23[c]
12 (natural)	0.20[a]	0.40[b]	0.33[c]
12 (synthetic)	0.20[a]	0.40[b]	0.33[c]

[a] Petroleum ether. [b] 1 % ether in petroleum ether. [c] 5 % ether in petroleum ether.

β,γ-Carotene (12). Sodium methoxide was added dropwise to a stirred solution of *4* (100 mg) in dry methanol (5 ml). *11* (50 mg) in dry methanol was added to the red phosphoran solution. The reaction mixture was stirred at room temperature and the progress followed by TLC. Additional phosphoran was added until no starting aldehyde (*11*) remained. The solution was worked up as described for *10* and β,γ-carotene (*12*) isolated by preparative thick layer chromatography (11.5 mg, 15 % based on starting aldehyde *11*). After purification by plate chromatography and repeated crystallizations from benzene-methanol solution *trans*-β,γ-carotene (*12*) melted at 174°; λ_{max} (petroleum ether) 421, 444 E(1 %, 1 cm)=2600, 472 nm, (acetone) 425, 447.5, 475 nm, (ether) 422, 444, 473 nm, see Fig. 1; τ ($CDCl_3$, τ-values previously [1,4] reported were for CCl_4solution and not $CDCl_3$) 9.17, 9.09 (6H, *gem.*-dimethyl on γ-ring), 8.96 (6H, *gem.*-dimethyl on β-ring), 8.50 mult. (8H, non-allylic methylene), 8.28 (3H, end-of-chain methyl in β-ring), 8.03 (12H, in-chain methyl), 7.9 mult. (4H, allylic methylene), 7.49 d (1H, J=8 Hz, methine at C-6′), 5.25, 5.43 (2H, $=CH_2$), 4.1–3.1 (14H, olefinic); *m/e* 536 (M), 457 (M–79), 444 (M–92), 430 (M–106), 413 (M–123), 399 (M–137), 378 (M–158), 359 (M–177), 346 (M–190), 333 (M–203), and 268, see Fig. 4.

Mixed melting point determination with natural β,γ-carotene (m.p. 176°) gave no depression. Co-chromatography of synthetic *12* with natural material from two sources gave no separation, see Table 1. Comparison of the visible light absorption spectra of synthetic and natural β,γ-carotene showed compatibility in every detail.

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REFERENCES

1. Andrewes, A. G. and Liaaen-Jensen, S. *Acta Chem. Scand.* **25** (1971) 1922.
2. IUPAC. Tentative Rules of Carotenoid Nomenclature. *Biochemistry. In press.*
3. Straub, O. In Isler, O., Ed., *Carotenoids*, Appendix, Birkhäuser, Basel 1971.
4. Arpin, N., Fiasson, J.-L., Bouchez-Dangye-Caye, M. P., Francis, G. W. and Liaaen-Jensen, S. *Phytochemistry* **10** (1971) 1595.
5. Andrewes, A. G., Kjøsen, H., Liaaen-Jensen, S., Weisgraber, K. H., Lousberg, R. J. J. C. and Weiss, U. *Acta Chem. Scand.* **25** (1971) 3878.
6. Weisgraber, K. H., Lousberg, R. J. J. C. and Weiss, U. *Experientia* **27** (1971) 1017.
7. Mayer, H. and Isler, O. In Isler, O., Ed., *Carotenoids*, Birkhäuser, Basel 1971, Chapter 6.

8. Manchand, P. S., Rüegg, R., Schwieter, U., Siddons, P. T. and Weedon, B. C. L. *J. Chem. Soc.* **1965** 2019.
9. Wadsworth, W. S. and Emmons, W. D. *J. Am. Chem. Soc.* **83** (1961) 1733.
10. Willhalm, B., Steiner, U. and Schinz, H. *Helv. Chim. Acta* **41** (1958) 1359.
11. Surmatis, J. and Ofner, A. *J. Org. Chem.* **28** (1963) 2735.
12. Vetter, W., Englert, G., Rigassi, N. and Schwieter, U. In Isler, O., Ed., *Carotenoids*, Birkhäuser, Basel 1971, Chapter 4.
13. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **23** (1969) 727.
14. Enzell, C. R., Francis, G. W. and Liaaen-Jensen, S. *Acta Chem. Scand.* **22** (1968). 1054.
15. Svec, W. A., Harkness, A. L., Strain, H. H. and Katz, J. J. *Org. Mass Spectrom.* **6** (1972) 843.
16. Borch, G., Andrewes, A. G. and Liaaen-Jensen, S. *Unpublished results.*

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