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(*E*/*Z*)-Isomeric carotenes

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Abstract: Selected examples of new developments dealing with the preparation of (*Z*)-isomeric carotenes are presented. Some unpublished results on the synthesis and characterization of geometrical isomers of β,β-carotene, (*rac*)-β,ε-carotene and ψ,ψ-carotene (lycopene) are included. Thermal isomerization of (all-*E*)-carotenes and the significance of (*Z*)-isomers in carotene biosynthesis are addressed.

INTRODUCTION

Investigations on stereomutation of carotenes began in the mid-thirties (ref. 1). It was found that, in solution, (*E*/*Z*)-isomerization of carotenoids is promoted by heat, light, active surfaces and catalytic amounts of acids or iodine (cf. ref. 2). Stereoisomeric mixtures were prepared by refluxing in an inert solvent (e.g. petroleum ether, hexane, cyclohexane) or by traces of iodine. These (*E*/*Z*)-mixtures were partly separated by column chromatography. In 1962, Zechmeister summarized the pioneering experimental work of the preceding decades in a monograph which is still a standard reference (ref. 3). Progress in stereocontrolled synthesis of (*Z*)-polyene isoprenoids was periodically assessed in reviews (refs. 4-6).

Considering the relatively mild conditions required for (*E*/*Z*)-isomerization, special care is necessary to prevent the formation of (*Z*)-isomeric artefacts during extraction from natural sources. However, the occurrence of (*Z*)-isomers in various plants was unambiguously established. In 1942, prolycopene was isolated as a crystalline compound from Tangerine tomatoes (ref. 7). In 1979, its (7*Z*,9*Z*,7'*Z*,9'*Z*)-lycopene geometry was independently determined by Englert *et al.* (ref. 8) and Pattenden *et al.* (ref. 9) using ^{1}H- and ^{13}C-NMR spectroscopy. Phytoene, generally regarded as the initial C_{40}-hydrocarbon in the biosynthesis of carotenoids, is isolated as the (15*Z*)-isomer from most natural sources (refs. 10, 11). It is also the isomer formed in cell-free systems with phytoene-synthesizing ability (e.g. ref. 12). Further examples of (*Z*)-carotenes isolated from Nature are (9*Z*)-β,β-carotene from the alga *Dunaliella* (refs. 13, 14), (5 *Z*)-neurosporene from ripe hips of *Rosa pomifera* (ref. 15) and (5*Z*)-lycopene from tomatoes (ref. 16). Very recently, (15*Z*)-β,β-carotene was identified spectroscopically in the photosynthetic reaction center of spinach (ref. 17).

(*Z*)-Isomeric carotenes have frequently been involved in studies carried out in recent years. Content and the isomeric ratio of various carotene (*Z*)-isomers were investigated in fresh and processed food (refs. 18, 19) and also in human blood plasma (refs. 20-23). It was shown that the formation of lycopene (*Z*)-isomers increases during the processing of food (ref. 23). Studies on intestinal absorption, distribution and metabolism of (9*Z*)- and (13*Z*)-β,β-carotene in humans and various animals have been carried out (refs. 24-27).

SIGNIFICANCE OF (*Z*)-ISOMERS IN CAROTENE BIOSYNTHESIS

Purified chromoplast preparations from daffodil (*Narcissus pseudonarcissus* L.) were shown to synthesize β,β-carotene from radiolabelled isopentenyl diphosphate. Recently, it has been demonstrated that the membrane-bound carotenogenic reaction sequence in daffodil chromoplasts can be subdivided *in vitro* into three reaction segments (Fig. 1) by varying the incubation parameters. In the first segment, (15*Z*)-phytoene *1* is desaturated to (15*Z*)-ζ-carotene *3* in the dark. A photoisomerization of accumulated (15*Z*)-ζ-carotene *3* to (all-*E*)-ζ-carotene *4* is a prerequisite for the functioning of the second segment, i.e. the desaturation to prolycopene *6*. The third segment, the cyclization of prolycopene to β,β-carotene, proceeds only in the absence of O_2 and involves additional (*E*/*Z*) isomerizations (refs. 28, 29).

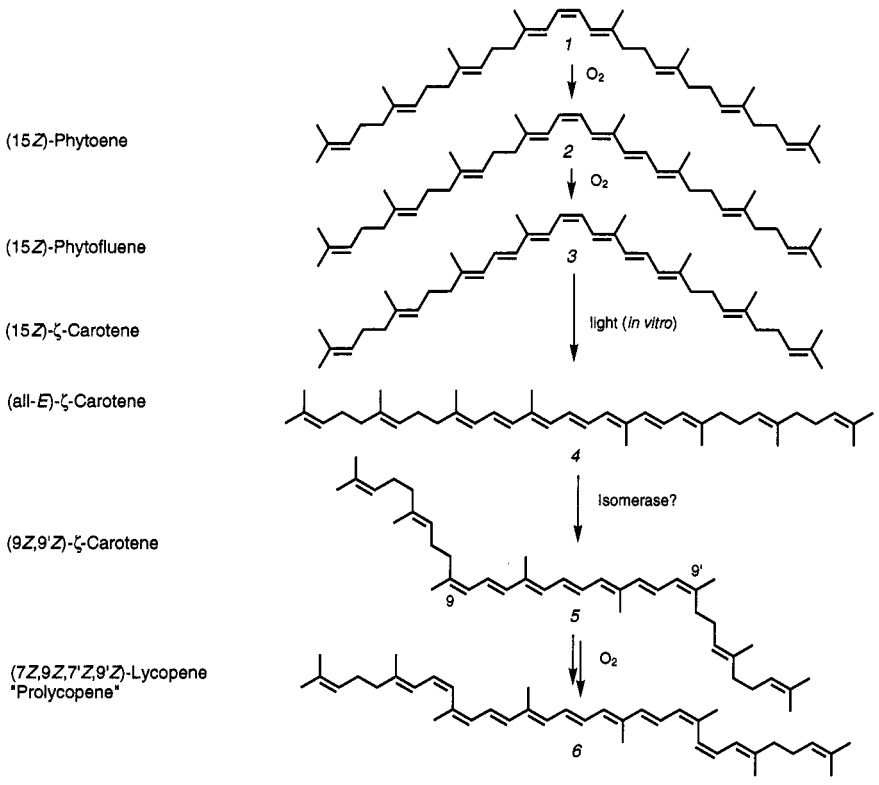


Fig. 1 Desaturation reactions *in vitro* in chromoplast membranes from *Narcissus pseudonarcissus* (refs. 28, 29)

THERMAL ISOMERIZATION PROCESS

The thermal (*E/Z*)-isomerization of a given carbon-carbon double bond of the acyclic polyene chain can be described as a 180° rotation about this bond. An illustrative example is the conversion of (all-*E*)-lycopene to its (15*Z*)-isomer. The energy profile of this process was calculated by a refined version of our in-house MAB-program (ref. 30). A 90° rotation about the central 15-double bond (Δ^{15}) leads to the 90°-twisted lycopene with two orthogonal polyenyl radicals. Its activation enthalpy (E_a: 25 kcal/mol) reflects the barrier that separates the (*E*)- from the (*Z*)-isomer. An additional turn of 90° then produces (15*Z*)-lycopene (ΔE: 2 kcal/mol). Calculations of the steric energies of the other (mono-*Z*)-isomers relative to (all-*E*)-lycopene show that for the trisubstituted double bonds Δ^5, Δ^9 and Δ^{13}, the adoption of the (*Z*)-configuration involves no or little additional steric strain (ΔE<1 kcal/mol). In contrast, for the disubstituted double bonds Δ^7 and Δ^{11}, the (*Z*)-configuration leads to serious steric interaction between a methyl group and a hydrogen atom, resulting in a non-planar twisted conformation of the polyene chain (ΔE: 3-4 kcal/mol). It is worth noting that this structural distinction between the two types of double bonds in carotenoids and retinoids was first proposed in 1939 (ref. 31).

The rate of (*E/Z*)-isomerization and the thermodynamic equilibrium between the respective isomers are of special interest to chemists. A recent study (ref. 32) has reported kinetic and thermodynamic data on the thermal interconversion between (15*Z*)-, (13*Z*)- and (all-*E*)-β-carotene. Specific rate constants for each of the four isomerization pathways were determined between 37 and 69°C by NMR-analysis, and derived Arrhenius parameters were given. In addition, relative enthalpies of activation were estimated for the isomerization of (all-*E*)-β,β-carotene to the (mono-*Z*)-isomers on the basis of radical stabilization energies. The data explain the qualitative observation that isomerization rates decrease progressively as the rearranging double bond moves from the center of the polyene chain towards the periphery. Thus, it is not surprising that thermal isomerization of pure (9*Z*)-β-carotene (hexane, 80°C) does not predominantly afford the (all-*E*)-isomer but produces the following (di-*Z*)-isomers, which are listed in order of decreasing isomerization rate: (9Z,13'Z) > (9Z,15Z) > (all-*E*) > (9Z,13Z) (ref. 33).

The isolation of (9*Z*)- and (13*Z*)-lycopene (Fig. 2) is a recent example of the preparation of (*Z*)-isomers by stereomutation (ref. 34). In this case, the two desired isomers were produced by stirring (all-*E*)-lycopene in acetone at 100°C for 70 minutes in an autoclave, followed by preparative scale HPLC separations of the components of interest from the filtered reaction mixture and repeated crystallizations. This method was chosen because the synthesis of these isomers was difficult due to a high tendency of the intermediates to spontaneous isomerization, and the resulting difficulty of obtaining pure compounds.

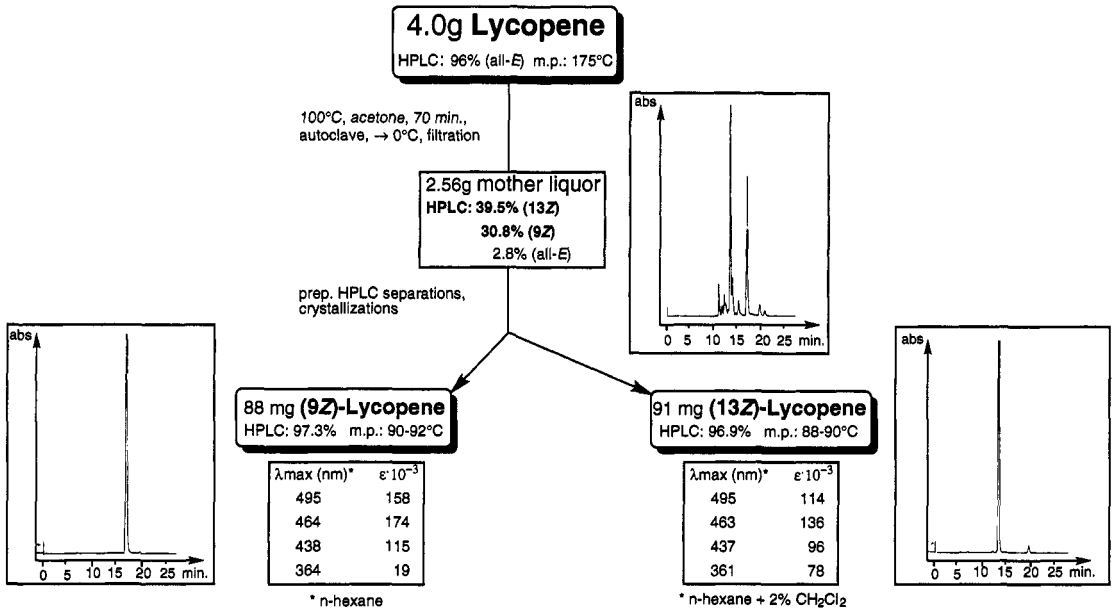


Fig. 2 Preparation of (9*Z*)- and (13*Z*)-lycopene (Conditions prep. HPLC: stationary phase: Kromasil 10 µm, mobile phase: hexane + 0.1‰ N-ethyldiisopropylamine, detection: 350nm; conditions analytical HPLC: stationary phase: Nucleosil 300-5, mobile phase: hexane + 0.5‰ N-ethyldiisopropylamine, detection: 469nm)

SPECTROSCOPY AND ANALYSIS

It has been demonstrated conclusively that the elucidation of the structure of geometrical isomers can be performed very efficiently by ^{1}H-NMR spectroscopy at high magnetic fields. The use of ^{13}C-NMR is frequently hampered by the need for larger quantities than are available from stereomutation experiments or biological samples. An excellent review is given by Englert (ref. 35), based on two decades of experience and on co-operation with leading carotenoid research groups.

HPLC separation of β,β-carotene isomers in combination with ^{1}H-NMR analysis was first reported in 1981 (refs. 36, 37). Subsequently, other HPLC techniques suitable for the separation of the geometrical isomers of lycopene (ref. 38), β,β-carotene (ref. 39) and β,ε-carotene (ref. 40) were developed. The chromatographic analysis of (*E*/*Z*) carotenoid isomers was recently reviewed (ref. 41).

TOTAL SYNTHESIS

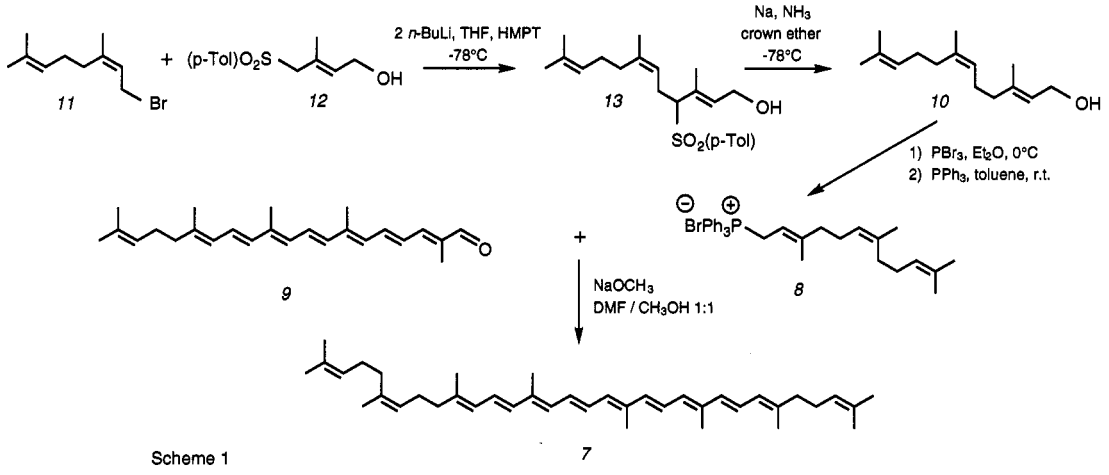
The availability of well characterized (*Z*)-isomeric carotenes is indispensable for the unambiguous identification of samples isolated from natural sources, and for the determination of biological activities of individual isomers.

Most of the syntheses reported for (*Z*)-isomeric carotenes use Wittig or Horner condensations in the final step. However, these reactions frequently lead to mixtures of (*Z*)- and (*E*)-geometrical isomers formed about the newly introduced double bond. All attempts to synthesize trisubstituted double bonds in polyenes by the Wittig process lead to mixtures of geometrical isomers. Scope and limitations of Wittig and Horner condensations have been reviewed in 1981 at the Liverpool meeting (ref. 5).

In this section, selected examples of stereoselective syntheses are briefly discussed.

(5*Z*)-7,8-Dihydro-ψ,ψ-carotene (*7*) [(5*Z*)-Neurosporene]

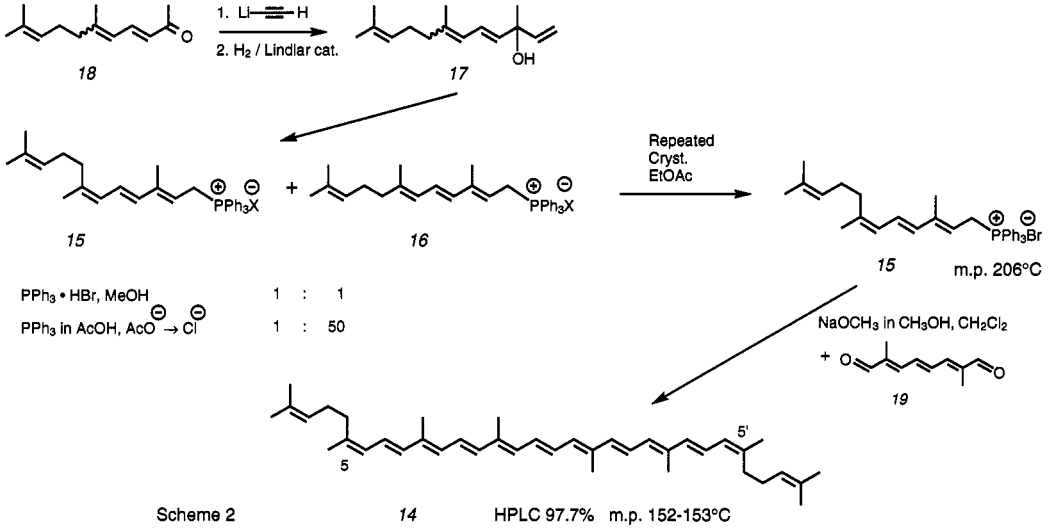
(5*Z*)-Neurosporene *7* was synthesized by using C_{15}-(mono-*Z*)-farnesylphosphonium salt *8* and C_{25}-aldehyde *9* in the final Wittig condensation (Scheme 1). (2*E*,6*Z*)-Farnesol *10* was prepared stereoselectively by alkylation of nerylbromide *11* with the dilithium derivative of the hydroxysulphone *12*, followed by reductive removal of the sulphone group in the coupling product *13* (ref. 42).



Scheme 1

(5*Z*,5'*Z*)-ψ,ψ-Carotene (*14*) [(5*Z*,5'*Z*)-Lycopene]

Recently, an efficient synthesis of (5*Z*,5'*Z*)-lycopene *14* based on a new interesting route to the (mono-*Z*)-phosphonium salt *15* was developed (refs. 38, 44). A stereoisomeric mixture of vinyl-ψ-ionol *17*, prepared from commercial (5*E*/*Z*)-ψ-ionone *18* was added to a suspension of triphenylphosphonium hydrobromide in methanol. The mixture was stirred at room temperature and then evaporated. One precipitation from ethyl acetate followed by four crystallizations from ethanol/ethyl acetate afforded 93%-pure *15* in 8.5% yield. The Wittig reaction of *15* with C_{10}-dialdehyde *19* afforded (5*Z*,5'*Z*)-lycopene *14* which retained the (*Z*)-configuration of the terminal conjugated double bonds.



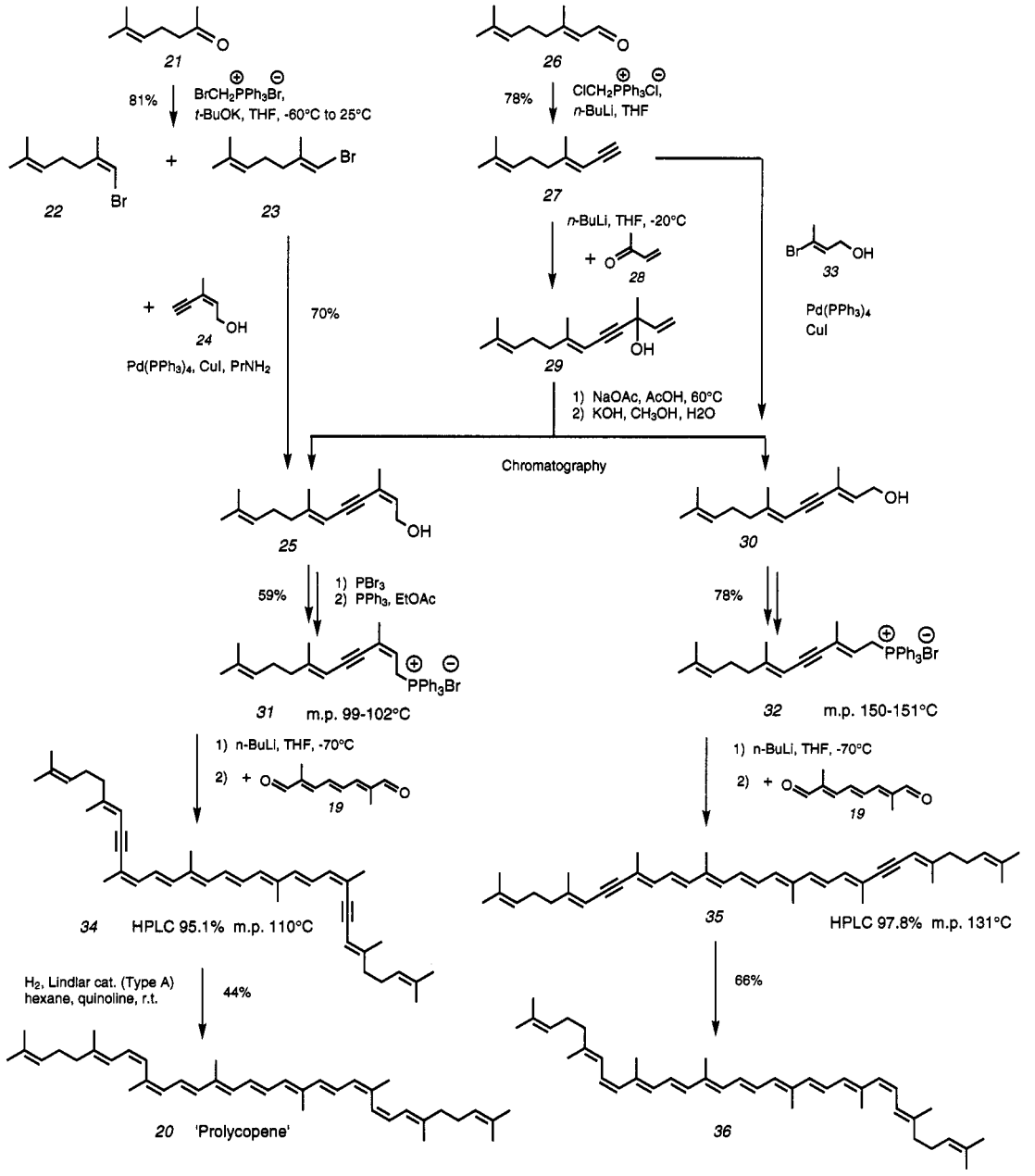
Scheme 2 *14* HPLC 97.7% m.p. 152-153°C

(7*Z*,9*Z*,7'*Z*,9'*Z*)-ψ,ψ-Carotene (*20*) [Prolycopene]

In 1987, the total synthesis of prolycopene *20* was reported by two groups. The Pattenden and Robson synthesis (ref. 43) started from methylheptenone *21*. A Wittig condensation between *21* and bromomethyl-triphenylphosphonium bromide led to a 3:1 mixture of the isomers *22* and *23*, from which the (*E*)-isomer *23* was separated by gas chromatography. A coupling reaction between *23* and the methylpentynol *24*, which is the C_6-building block in Isler's vitamin A synthesis, gave *25*.

In the synthesis designed by Hengartner, Bernhard, Englert and Glinz (refs. 38, 44), the starting material was citral 26, which was converted to the acetylenic hydrocarbon *27*. Addition of *27* to methylvinyl ketone *28* in the presence of lithium bromide afforded the tertiary alcohol *29*. Allylic rearrangement of *29* in acetic acid/sodium acetate and subsequent saponification gave a mixture of the isomeric alcohols *25* and *30* in a 2:1 ratio. The two alcohols were separated by chromatography, converted with phosphorus tribromide to the corresponding bromides and reacted with triphenylphosphine to yield, without observable isomerization, the two isomeric Wittig salts *31* and *32*.
In addition, a more efficient method for the preparation of the (all-*E*)-alcohol *30* was developed *via* the coupling reaction of *27* with (*E*)-bromobutenol *33*. Wittig reaction of the (2*Z*,6*E*)-phosphonium salt *31* with the C_{10}-dialdehyde *19*, and butyllithium as a base, followed by thermal isomerization, gave the (9*Z*,9'*Z*)-diacetylene *34*. In

the final step, the two triple bonds of *34* were hydrogenated over Lindlar catalyst. Prolycopene, m.p. 109°C was obtained after chromatography and two crystallizations, first from hexane, and then from dichloromethane/methanol 1:10. The (all-*E*)-phosphonium salt *32* was used for the synthesis of (7*Z*,7'*Z*)-lycopene *36*, following an analogous sequence (ref. 38).

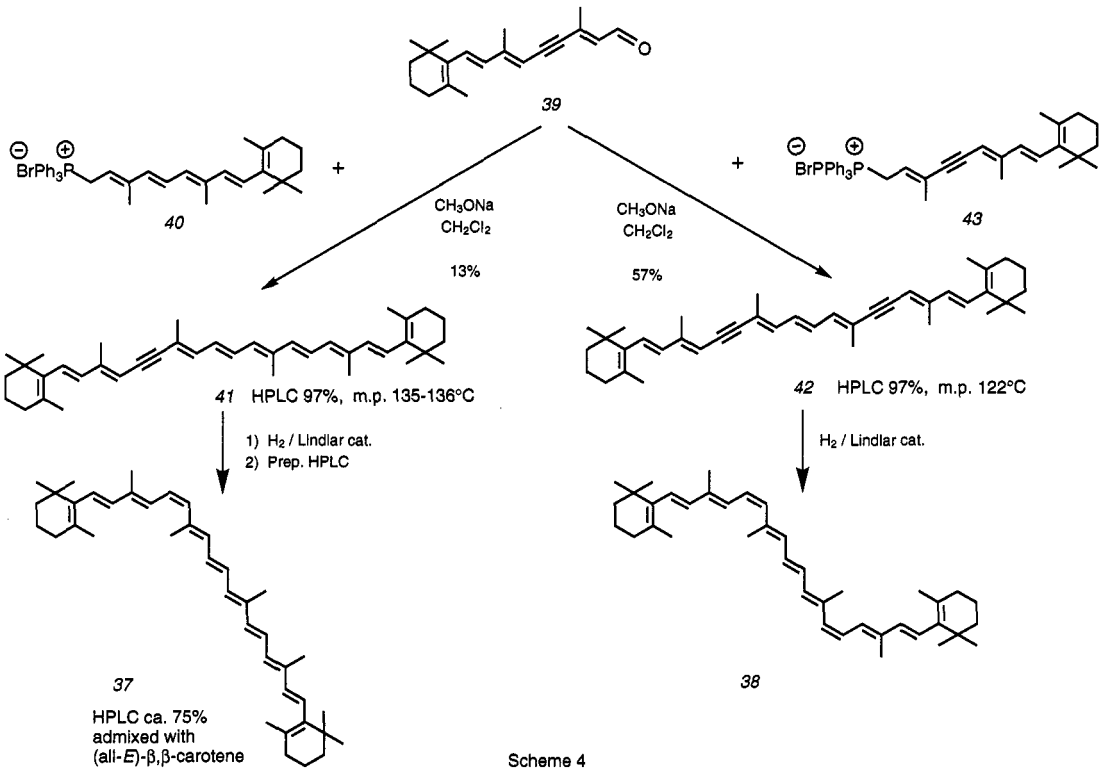


Scheme 3

(11*Z*)-β,β-Carotene (*37*) and (11*Z*,11'*Z*)-β,β-Carotene (*38*)

(11*Z*)-β,β-Carotene *37* and (11*Z*,11'*Z*)-β,β-carotene *38* were synthesized by using 11,12-didehydroretinal *39* (ref. 45) as a common intermediate (Scheme 4). Wittig condensation of aldehyde *39* with retinyl-triphenylphosphonium salt *40* (prepared from vitamin A acetate by analogy to ref. 46) provided 11,12-didehydro-β,β-carotene *41* as a deep red solid. Catalytic semihydrogenation of intermediate *41* over Lindlar catalyst gave a 1:3 mixture of (all-*E*)-β,β-carotene, and its thermally unstable (11*Z*)-isomer *37*.

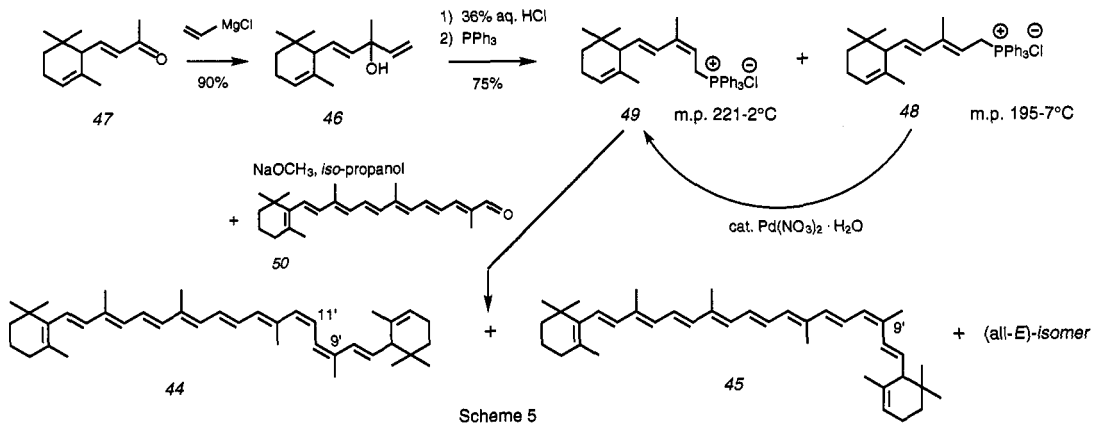
In an analogous approach, 11,12,11',12'-tetradehydro-β,β-carotene *42* was obtained by the Wittig coupling of aldehyde *39* with 11,12-didehydroretinyltriphenylphosphonium salt *43* (ref. 47). Catalytic semihydrogenation of precursor *42* over Lindlar catalyst gave the "relatively" stable crystalline (11*Z*,11'*Z*)-β,β-carotene *38*.



Scheme 4

(6'*RS*,9'*Z*,11'*Z*)-β,ε-Carotene (*44*) and (6'*RS*,9'*Z*)-β,ε-Carotene (*45*)

The vinylalcohol *46*, prepared from racemic α-ionone *47*, was converted to an (all-*E*/9*Z*)-mixture of phosphonium chlorides *48* and *49* (ca. 4:1). A Pd-catalysed isomerization in refluxing toluene provided the (9*Z*)-isomer *49* in ≥ 95% purity (m.p. 221-2°C). This is in contrast to the result obtained with the corresponding β-ring phosphonium chloride where only minor (*E*) → (*Z*)-isomerization occurred under the same conditions. A Wittig reaction with (9*Z*)-phosphonium chloride *49* and 12'-apo-β-caroten-12'-al *50* led to a mixture of (9'*Z*,11'*Z*)-β,ε-carotene *44*, (9'*Z*)-β,ε-carotene *45* and (all-*E*)-β,ε-carotene in a ratio of ca. 60:36:3. Direct crystallization of this (*E*/*Z*)-mixture (Et_2O/EtOH) furnished pure (9'*Z*,11'*Z*)-β,ε-carotene *44* (m.p. 122°C). From the evaporated mother liquor, pure (9'*Z*)-β,ε-carotene *45* (m.p. 130-1°C) was isolated by crystallization (Et_2O/EtOH).



Scheme 5

In the following table, literature references and selected analytical data for recently synthesized (*Z*)-isomeric carotenes are listed. In particular, additional results on β,β-carotene (*Z*)-isomers (cf. ref. 48) are provided.

TABLE. Selected analytical data of some recently synthesized (*Z*)-isomeric carotenes.

Isomer	HPLC	m.p. (°C)	Characterization	Ref.
β,β-Carotene (β-Carotene)				
(9*Z*)	99.4	134-5	UV/VIS[1]: 260(159), 340(127), 425(972), 445(1368), 473(1195). ^{1}H-NMR[2]: H(7,7') 6.18, H(8) 6.67, H(8') 6.13, H(10) 6.06, H(10') 6.15, H(11) 6.76, H(11') 6.64, H(12) 6.29, H(12') 6.35, H(14,14') 6.24, H(15,15') 6.63.	48
(13*Z*)	99.9	132-3	UV/VIS[1]: 274(156), 337(487), 423(833), 443(1123), 470(914). ^{1}H-NMR[2]: H(7,7') 6.2, H(8,8') 6.15, H(10) 6.20, H(10') 6.17, H(11) 6.64, H(11') 6.65, H(12) 6.89, H(12') 6.35, H(14) 6.10, H(14') 6.24, H(15) 6.80, H(15') 6.56.	48
(15*Z*)	99.3	149-51	UV/VIS[1]: 279(133), 336(561), 424(747), 447(978), 474(786). ^{1}H-NMR[2]: H(7) 6.15, H(8) 6.15, H(10) 6.15, H(11) 6.67, H(12) 6.41, H(14) 6.65, H(15) 6.39.	
(9*Z*,9'*Z*)	99.4	156-60	UV/VIS[1]: 268(157), 340(95), 420(943), 440(1339), 467(1153). ^{1}H-NMR[2]: H(7) 6.18, H(8) 6.67, H(10) 6.05, H(11) 6.75, H(12) 6.28, H(14) 6.23, H(15) 6.61.	48
(9*Z*,15*Z*)	98.8	137-8	UV/VIS[1]: 283(227), 336(302), 422(728), 442(975), 468(775). ^{1}H-NMR[2]: H(7,7') 6.19, H(8) 6.68, H(8') 6.14, H(10) 6.06, H(10') 6.16, H(11) 6.79, H(11') 6.68, H(12) 6.36, H(12') 6.42, H(14,14') 6.66, H(15,15') 6.39.	48
(11*Z*,11'*Z*)	90	114-6	UV/VIS[3]: 277(259), 337(310), 428(707), 447(814), 476(581). ^{1}H-NMR[2]: H(7) 6.22, H(8) 6.15, H(10) 6.66, H(11) 6.33, H(12) 5.97, H(14) 6.30, H(15) 6.60.	
(13*Z*,13'*Z*) (contains 11.8% of (13*Z*)-isomer)	85.8	161-3	UV/VIS[1]: 280(323), 340(153), 418(794), 436(1015), 461(791). ^{1}H-NMR[2]: H(7) 6.2, H(8) 6.14, H(10) 6.2, H(11) 6.65, H(12) 6.87, H(14) 6.09, H(15) 6.73.	
(13*Z*,15*Z*)	96.1	139-40	UV/VIS[1]: 284(324), 340(110), 417(898), 437(1155), 462(886). ^{1}H-NMR[2]: H(7,7') 6.19, H(8,8') 6.14, H(10,10') 6.16, H(11,11') 6.69, H(12) 6.89, H(12') 6.41, H(14) 6.51, H(14') 6.64, H(15) 6.56, H(15') 6.34.	48
(6'RS)-β,ε-Carotene (α-Carotene)				
(9'*Z*)	98.5	130-1	UV/VIS[1]: 266(179), 330(104), 418(958), 440(1424), 468(1285). ^{1}H-NMR[2]: H(6') 2.24, H(7) 6.18, H(7') 5.54, H(8) 6.12, H(8') 6.63, H(10) 6.15, H(10') 6.00, H(11) 6.64, H(11') 6.76, H(12) 6.35, H(12') 6.28, H(14,14') 6.24, H(15,15') 6.63.	
(9'*Z*,11'*Z*)	94.4	122	UV/VIS[1]: 268(232), 330(128), 419(893), 439(1127), 466(871). ^{1}H-NMR[2]: H(6') 2.22, H(7) 6.18, H(7') 5.59, H(8) 6.12, H(8') 6.65, H(10) 6.15, H(10') 5.92, H(11) 6.64, H(11') 6.45, H(12) 6.35, H(12') 6.49, H(14) 6.25, H(14') 6.29, H(15,15') 6.63.	
β,ψ-Carotene (γ-Carotene)				
(5'*Z*)	95	135-6	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	42
ε,ψ-Carotene (δ-Carotene)				
(5'*Z*)	85	134-5	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	42
ψ,ψ-Carotene (Lycopene)				
(5*Z*)	80	137-9	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	42
	95.6	143	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	38
(7*Z*)	93.4	126	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	38
(15*Z*)		105	UV/VIS	49
	93.8	ca. 100	UV/VIS, ^{1}H-NMR, ^{13}C-NMR, elemental analysis	38
(5*Z*,5'*Z*)	> 85	148-9	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	42
	97.7	152-3	UV/VIS, ^{1}H-NMR, ^{13}C-NMR, elemental analysis	38
(7*Z*,7'*Z*)	95.5	133	UV/VIS, ^{1}H-NMR, ^{13}C-NMR, elemental analysis	38
(7*Z*,9*Z*)	86.1	78-9	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	38
(9*Z*,9'*Z*)	93.3	135	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	38
(7*Z*,9*Z*,7'*Z*,9'*Z*)	95.1	109	UV/VIS, ^{1}H-NMR, ^{13}C-NMR, elemental analysis	38
7,8-Dihydro-ψ,ψ-carotene (Neurosporene)				
(5*Z*)	85	91-2	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	42
(5'*Z*)	80	93-5	UV/VIS, ^{1}H-NMR, ^{13}C-NMR	42

[1] λ_{max} in nm ($\varepsilon \cdot 10^{-2}$), in hexane/$CH_2Cl_2$ = 98:2. [2] Solvent $CDCl_3$. [3] λ_{max} in nm ($\varepsilon \cdot 10^{-2}$), in hexane/$CH_2Cl_2$ = 2:1.

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