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ABSOLUTE CONFIGURATION OF β,γ-CAROTENE AND BIOSYNTHETIC IMPLICATIONS*

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Abstract—Racemic γ-ionone, partly resolved via its menthydrazone, was used for total synthesis of β,γ-carotene enriched in the 6′*R* and 6′*S* enantiomers. By CD correlation with natural β,γ-carotene isolated from *Caloscypha fulgens* 6′*S*-chirality is demonstrated for the natural carotene. Biosynthetic implications regarding the cyclization reaction are discussed.

INTRODUCTION

Naturally occurring C_{40}-carotenoids with γ end-group (**B**) [1] include β,γ-carotene (**1**) from the fungus *Caloscypha fulgens* [2] and the green variety of the aphid *Microsiphium liriodendri* [3] and γ,γ-carotene (**2**) from the latter source [3].

The chirality of this end-group has hitherto been unsolved, and we now report assignment of absolute configuration of β,γ-carotene (**1d**) by a synthetic approach.

RESULTS AND DISCUSSION

Previously we have published [4] the total synthesis of racemic β,γ-carotene (**1b**) and racemic γ,γ-carotene (**2b**) from racemic γ-ionone (**3**).

We now report the partial resolution of γ-ionone (**3**) via its menthydrazone (**4**) by the procedure of Sobotka *et al.* [5–7] and total synthesis of β,γ-carotene enriched in the 6′*R*-enantiomer (**1c**) and the 6′*S*-enantiomer (**1d**).

γ-Ionone (**3**) was purified by column chromatography and converted to the menthydrazone (**4**) by the published method [5]. Repeated crystallization of **4** from ethanol and then diisopropyl ether [8] gave an enrichment of the (*d*)-γ-ionone-(*l*)-menthydrazone (**4c**) in the crystals and of the (*l*)-γ-ionone-(*l*)menthydrazone (**4d**) in the first mother liquor. The menthydrazones enriched in **4c** and **4d** were cleaved separately by steam distillation in the presence of acetic anhydride to provide (+)-γ-ionone enriched in the 6′*R*-enantiomer (**3c**) and (−)-γ-ionone enriched in the 6′*S*-enantiomer (**3d**) [6]. (+)-(*R*)-γ-Ionone (**3c**) exhibited a weak, reproducible, positive Cotton effect at 250–280 nm (EPA solution), consistent with the results obtained by Ohloff *et al.* [9, 10]. The appropriate γ-ionones (**3c** and **3d**) were converted by Grignard reactions with vinyl magnesium bromide, which caused no racemization at C-6′ [11], into the vinyl-γ-ionols (**5c** and **5d**). These were reacted with triphenylphosphine hydrobromide to give the phosphonium salts **6c** and **6d** by standard procedure [12]. The corresponding ylids, generated with butenoxide [13], gave with β-apo-12′-carotenal (**7**) β,γ-carotene enriched in the 6′*R*-enantiomer (**1c**) and the 6′S-enantiomer (**1d**). The Δε-values of **1c** obtained relative to those of natural β,γ-carotene (**1**), suggest *ca* 7% enrichment of the 6′*R*-enantiomer (**1c**) in the synthetic sample; the optical purity of **1c** being lower as expected.

The CD-spectrum of **1c** was opposite to that of natural β,γ-carotene (**1**) isolated from *Caloscypha fulgens* (Fig. 1) thus proving 6′*S*-chirality (**1d**) for the natural β,γ-carotene. A weak, but reproducibly negative CD at 330 and 235 nm for synthetic (6′*S*)-β,γ-carotene (**1d**) is consistent with this conclusion.

Thus, the configuration of natural β,γ-carotene (**1d**) at C-6′ is opposite to that of all C_{40}-carotenoids with an ε-end group (**F**) [14], and the same as recently established for the C_{50}-carotenoid sarcinaxanthin (end group **G**) [15].

A priori a common C-5 carbonium ion, differing only in the position of proton loss, could be envisaged for the

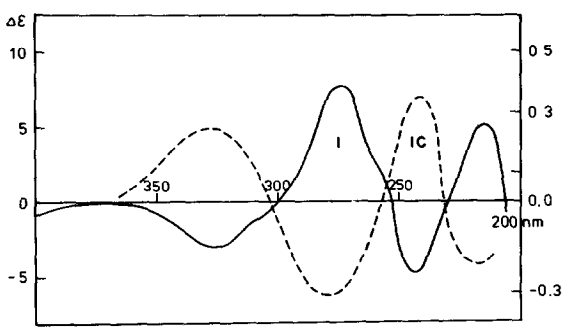

Fig. 1. CD spectra (EPA) of natural β,γ-carotene (**1**, left scale) and synthetic (6′*R*)-β,γ-carotene (**1c**, right scale).

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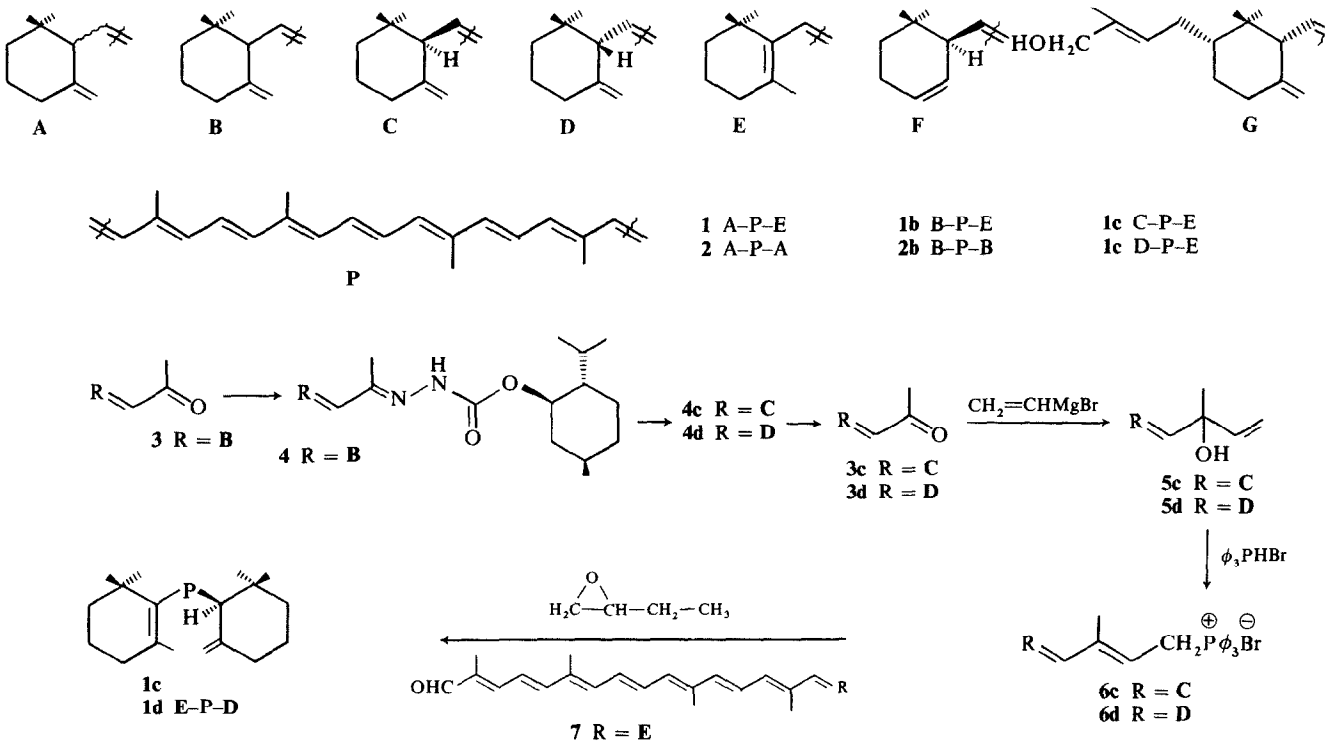


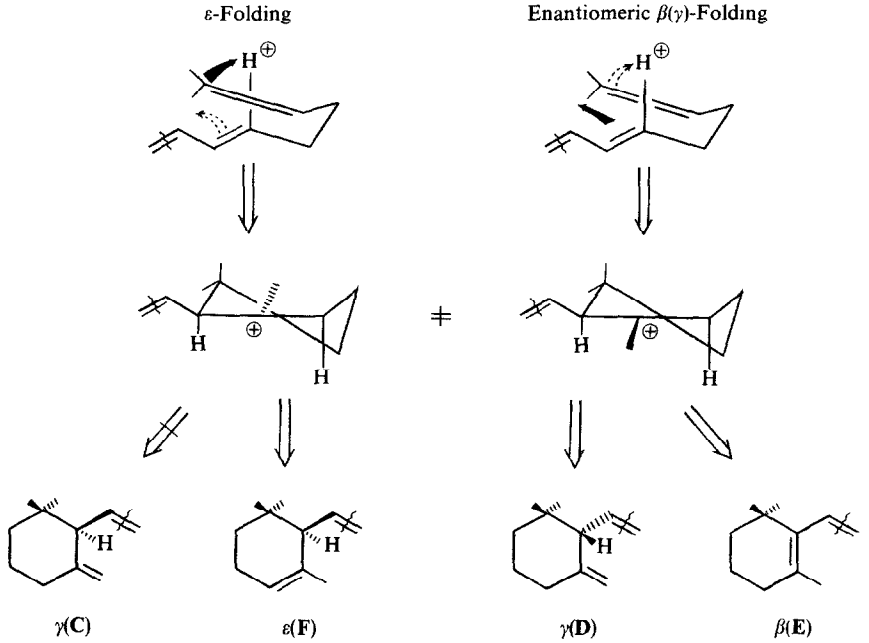
in vivo cyclization of the aliphatic C_9-unit to ε (**F**) and γ(**A**) end groups. However, this is not consistent with opposite stereochemistry at C-6 for these end groups. Opposite foldings of the presumed common aliphatic precursor are required to fit the stereochemical findings. This, in turn, implies that different enzymes must be responsible for cyclization to ε(**F**) and γ(**D**) end groups.

According to the work by Bu'Lock *et al.* [16] on trisporic acid biosynthesis, enantiomeric foldings are required for the *in vivo* cyclization to the ε (**F**) and β (**E**) end groups. The β-folding and γ-folding consequently are identical, but different enzymatic systems are still likely to be involved.

In *Caloscypha fulgens* [17] as well as in *Microsiphium liriodendri* [3] carotenoids with γ and β end-groups are reported, but ε end-groups are missing.

EXPERIMENTAL

Materials and methods. These were as commonly employed in our laboratory [4]. HPLC was carried out on a Du Pont 830 Liquid Chromatograph, CD spectra recorded with a Roussel Jouan Dicrographe and $[\alpha]_D$ values on a Carl Zeiss Polarimeter.



γ-Ionone (3). 3 (10 g), obtained from Firmenich & Cie, was chromatographed on a Si gel column (eluent 5–10% isopropyl ether in hexane) to effect separation from α-ionone (*ca* 1%) and β-ionone (*ca* 8%, estimated from GC, *cf* ref. [18]). 3 had UV λ_{max}^{EtOH} 234 nm: IR ν_{max} (liq.) and NMR δ ($CDCl_3$) in agreement with the published spectra [17]: MS *m/e* 192 (M), M-15, M-43, 121, 109 and 43.

(d,l)-γ-Ionone-(l)-menthydrazone (4). (±)-Ionone (3, 9.45 g) and (−)-menthydrazide (10.52 g, prepared from ethyl (−)-menthylcarbonate and hydrazine hydrate by published procedure [19]) in EtOH (75 ml, containing 2% NaOAc and 1% AcOH) was refluxed for 2.5 hr. Crystallization from EtOH gave **4**, 12.1 g (63%); $[\alpha]_D^{20} = -39 \pm 3°$. Attempts to separate **4** into its diastereomers by TLC based on kieselgel, polyamide, cellulose or alumina failed, nor was separation by GC or HPLC successful.

(d)-γ-Ionone-(l)-menthydrazone (4c). **4**, after recrystallization twice from diisopropyl ether was enriched in **4c**, yield 2.72 g: mp 175–176°, $[\alpha]_D^{20} = -37 \pm 4°$ (EtOH); UV λ_{max}^{hexane}: 258 nm (ε = 3740); IR ν_{max}^{KBr} cm^{-1}: 3210 (NH), 1730 (O—C=O), 1705 (O=C—NH), 1640 (C=N), 890 (=CH_2); NMR ($CDCl_3$): δ 0.80 (3 H, *d*, *J* = 6 Hz), 0.865 and 0.90 *s* (6 H, *gem.* Me), 0.90 (6 H, *d*, *J* = 7 Hz), 0.65–2.32 (15 H), 1.95 (3 H, *s*), 4.55 and 4.73 (2 H, =CH_2), 7.67 (1 H, NH); MS *m/e*: 388 (M), M-15, M-28, M-138, 175, 139, 97, 95, 83, 69, 57, 55; CD (EPA) Δε (230 nm + 1.95, 260 nm 0).

(l)-γ-Ionone-(l)-menthydrazone (4d). The ethanolic mother liquor of **4** was enriched in **4d**.

(+)-*(R)-γ-Ionone* (3c). **4c** (2.6 g) and phtyhalic anhydride (6.2 g) in H_2O (48 ml) was steam distilled, the distillate being extracted with Et_2O and chromatographed on a Si gel column (2% Et_2O in hexane) to give **3c** (0.94 g, 73%) with properties as given for **3**: CD (EPA) 320 nm 280–250 nm (reproducibly +).

(−)-*(S)-γ-Ionone* (3d). The ethanolic mother liquor of **4** was taken to dryness and cleavage to **3d** effected as for **3c** above; yield 0.19 g (13%).

(R)-Vinyl-γ-ionol (5c). Preparation by standard procedure [11] from vinyl bromide (6.9 ml), Mg (1.7 g) and (+)-γ-ionone (**3c**, 0.90 g) in dry THF; yield **5c** (0.49 g, 48%); UV λ_{max}^{hexane}: 216 nm; IR ν_{max}^{liq} cm^{-1}: 3480 (OH), 1645 (C=C), 890 (=CH_2); NMR ($CDCl_3$): δ 0.80 and 0.89 (6 H, *s*, *gem.* Me), 1.37 (3 H, *s*), 1.69 (1 H, *s*), 1.17–1.27 (6 H), 4.53 and 4.72 (2 H, =CH_2); MS *m/e* 220 (M), M-15, M-18, M-58, 109, 97, 95, 93, 84, 81, 71, 69, 55, 43 (100%).

(S)-Vinyl-γ-ionol (5d. Preparation as **5c**; yield 0.095 g (83%), exhibited UV, IR, NMR and MS properties as **5c**.

(R)-γ-Ionyliden-ethyltriphenylphosphonium bromide (6c). Prepared from **5c** (535 mg) by standard procedure [11]; yield **5c** (588 mg, mp 121–123°).

(S)-γ-Ionyliden-ethyltriphenylphosphonium bromide (6d). Prepared as above from **5d** (95 mg); yield non-crystalline **6d** (109 mg).

(6'R)-β,γ-carotene (1c). The phosphonium salt (**6c**, 176 mg), β-apo-12'-carotenal (**7**, 134 mg) and butenoxide (1.5 ml) was heated under N_2 in a sealed tube at 90° for 1.25 hr. Column chromatography (Al_2O_3, C_6H_6–hexane 3:1) gave **1c** (52 mg) as a *cis–trans* mixture. Crystallization twice from C_6H_6–MeOH gave all-*trans* **1c**, mp 171–172°; UV λ_{max}^{hexane}: 419, 443.5 (ε = 148200) and 471 nm; IR, NMR and MS data as reported for racemic **2** [4]; CD (Fig. 1).

(6'S)-β,γ-carotene (1d). Prepared from **6d** (109 mg) by the above procedure; yield 36.5 mg as a *cis–trans* mixture. Crystallization twice from MeOH–C_6H_6 provided **1d**, *ca* 4 mg, physical properties as for **1c**, except CD (EPA) 330 nm Δε = *ca* −0.1, 234 nm Δε = *ca* −0.3.

Natural β,γ-carotene (1). Remained from a previous study [2]. The CD spectrum is given in Fig. 1.

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