

tion products on Sephadex LH-20.⁸ In the present study a few experiments with lignin model compounds in the system Sephadex LH-20/dimethylformamide were performed. The results (I, $K_d' = 0.72$; II, $K_d' = 0.54$; IX, $K_d' = 0.46$) indicate that the gel filtration properties of this system in certain respects resemble those of the systems polystyrene gel/tetrahydrofuran and Sephadex G-25/dimethyl sulfoxide.

Experimental. A Waters Gel Permeation Chromatograph Model 200 (column set B1: $3 \times 10^3 \text{ \AA}$, $5 \times 10^2 \text{ \AA}$, 10^2 \AA , and 60 \AA) was used for gel filtration on polystyrene gel with tetrahydrofuran as an eluent. For the experiments with Sephadex gels the following columns and solvents were used:

Sephadex G-25 (fine)/water. Amount of gel, 27 g; bed volume, 133 ml; column length, 35 cm. *Sephadex G-25 (fine)/dioxane-water (1:1).* Amount of gel, 47 g; bed volume, 199 ml; column length, 57 cm. *Sephadex G-25 (fine)/dimethyl sulfoxide.* Amount of gel, 32 g; bed volume, 198 ml; column length, 56 cm. *Sephadex LH-20/dioxane-water (9:1).* Amount of gel, 8 g; bed volume, 32 ml; column length, 18 cm. *Sephadex LH-20/dimethylformamide.* Amount of gel, 8 g; bed volume, 36 ml; column length, 20 cm.

Gel filtration. The samples (1–3 mg) were dissolved in 1–2 ml of the eluent and applied to the columns. The UV absorbance at 280 nm of the effluent from the columns was continuously recorded by means of a Beckman DB spectrophotometer equipped with a 1 mm flow cell and a recorder. In most experiments the flow rate was 5–10 ml/h.

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Fungal Carotenoids

VII.* Synthesis of β,γ - and γ,γ -Carotene with Terminal Methylene Groups

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Recently Arpin *et al.*¹ reported the isolation and identification of β,γ -carotene (10) from the discomycete *Caloschypha fulgens*. The new IUPAC nomenclature rules for carotenoids² are used here. The γ -terminus features an exocyclic methylene group common to terpenes, but previously unreported in carotenes. We now report the synthesis of β,γ -carotene (10) according to the route outlined in Scheme 1 and confirm the assigned structure. Symmetrical γ,γ -carotene (8) was also synthesized according to the same basic route.

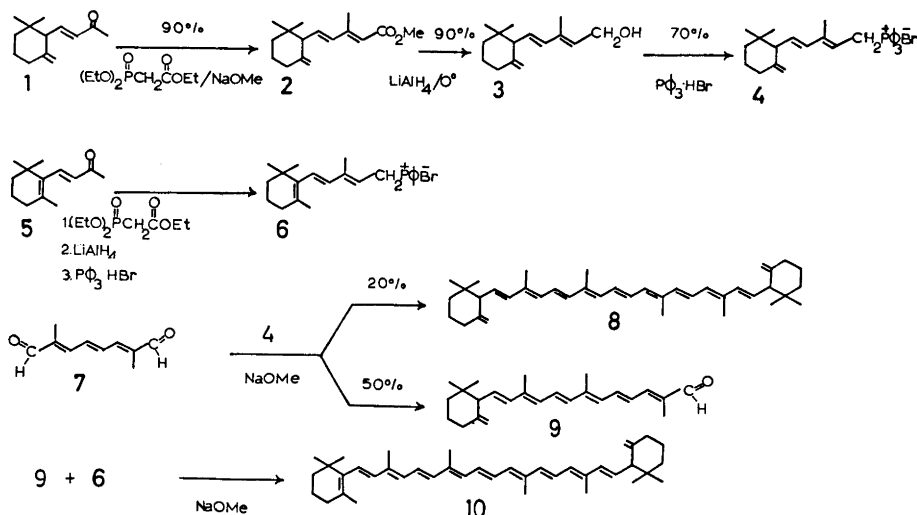
Synthetic β,γ -carotene (10), purified by column chromatography on deactivated alumina and silica gel plates and crystallized from benzene-methanol solution, melted at 174°C, undepressed on admixture with a natural specimen.

The synthetic and natural pigments could not be separated chromatographically.

Synthetic β,γ -carotene (10) had λ_{\max} 421, 444 [$E(1\%, 1\text{ cm}) = 2600$] and 472 [$E(1\%, 1\text{ cm}) = 2370$] nm in petroleum ether and 425, 447.5, [$E(1\%, 1\text{ cm}) = 2520$] and 475 [$E(1\%, 1\text{ cm}) = 2300$] nm in acetone. The IR spectrum (KBr) resembled those of common carotenes except for a strong absorption at 889 cm^{-1} ascribed to $R_1R_2C=CH_2$. The NMR spectrum (60 Mc) in $CDCl_3$ solution exhibited singlets at τ 9.17 (3H) and 9.10 (3H) due to *gem*-dimethyl on the γ -ring, broad singlets at τ 5.31 (1H) and 5.46 (1H) attributed to a $R_1R_2C=CH_2$ grouping with magnetically non-equivalent protons and a doublet at τ 7.57 (1H, $J = 8$ cps) ascribed to the methine proton at C-6'. Signals at τ 8.98 (singlet, 6H, *gem*-dimethyl) and τ 8.30 (singlet, 3H, end-of-chain methyl) were caused by the β -end group. Four in-chain methyl groups gave a singlet at τ 8.03

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(12H); non-allylic and allylic methylene groups gave rise to multiplets centered at τ 8.5 (8H) and 7.9 (4H), respectively, and the olefinic protons of the polyene chain (14H) produced signals in the τ 3.4–4.2 region. The mass spectrum exhibited the molecular ion at m/e 536. Fragment ions were observed at $M-92$, $M-106$, and $M-158$.³ No fragmentation within the rings was observed. Ions corresponding to in-chain cleavages were recorded, the strongest ion arising from fragmentation of the 15,15' bond.

The physical properties of synthetic β,γ -carotene (10) correspond to those reported for the natural compound.¹

Symmetrical γ,γ -carotene (8) has not been reported to occur in nature, although it is suspected to be present in the aphid *Macrosiphum liriodendri*.⁴ Crystallized from benzene-methanol, synthetic γ,γ -carotene (8) melted at 183°C. The IR spectrum again exhibited strong absorption at 889 cm^{-1} attributed to the exocyclic methylene groups. The absorption spectrum of γ,γ -carotene (8) in petroleum ether showed λ_{max} 414, 438 [$E(1\%, 1\text{ cm}) = 2960$] and 468 nm; in acetone at 416, 440 [$E(1\%, 1\text{ cm}) = 2870$], and 469 nm. The NMR spectrum in CDCl_3 displayed the expected signals: singlets at τ 9.17 (6H) and 9.10 (6H) due to *gem*-dimethyl of the γ -ring, broad signals at τ 5.31 (2H) and 5.46 (2H) ascribed to terminal methylene, doublet at τ 7.57 (2H, $J = 8$ cps) from methine at C-6, C-6', singlet at t 8.03 (12H)

due to in-chain methyl groups, multiplets centered at τ 7.9 and 8.5 from allylic and non-allylic methylene groups, and signals at τ 3.4–4.2 (olefinic protons). The mass spectrum of γ,γ -carotene (8) showed the molecular ion at m/e 536 and the usual $M-92$, $M-106$, and $M-158$ peaks.³ Other ions noted were accommodated by fragmentation within the polyene chain. On comparative chromatography (alumina paper) the symmetrical γ,γ -carotene (8) was adsorbed between β,γ -carotene (10) and β,ψ -carotene.

Further details will be published.

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