

column (145 g). With 5% EtOAc in hexane a fraction containing **2** eluted followed by a fraction containing **1**; cholesterol was eluted with 20% EtOAc in hexane and oleic and linoleic acids with 50% EtOAc in hexane. Crude *trans*-phytol was further purified using a Water Associates high pressure liquid chromatograph equipped with 244 × 0.94 cm Si gel column (Porasil A, 37–75  $\mu$ m). *cis*-Phytol was also purified by HPLC (a 700 × 0.95 cm plus a 460 × 0.64 cm column). The solvent system used was 3% EtOAc in hexane.

*trans*-Phytol (**1**). Pure **1** was isolated as a clear colorless oil (0.014% yield from dry wt of alga). IR (KBr) 3.00, 6.82, 7.23 and 9.9  $\mu$ m; NMR (CCl<sub>4</sub>,  $\delta$ ), 0.90 (12H, *d*, *J* 6 Hz), 1.2–1.6 (20H, *m*), 1.64 (3H, *s*), 1.95 (2H, *m*), 4.05 (2H, *d*, *J* 6.4 Hz), 5.33 (1H, *t*, *J* 6.4 Hz); MS molecular ion *m/e* 296 (corresponds to C<sub>20</sub>H<sub>40</sub>O).

*cis*-Phytol. Compound **2** was isolated as a clear colorless oil (0.0028% yield from dry wt of alga). IR (CCl<sub>4</sub>) 2.92, 6.86, 7.30, 10.4  $\mu$ m; NMR (CCl<sub>4</sub>,  $\delta$ ), 0.91 (12H, *d*, *J* 6 Hz), 1.2–1.6 (20H, *m*), 1.71 (3H, *s*), 2.0 (2H, *m*), 4.48 (2H, *d*, *J* 6.4 Hz), 5.33 (1H, *t*, *J* 6.4 Hz); MS molecular ion *m/e* 296 (corresponds to C<sub>20</sub>H<sub>40</sub>O).

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## THE ISOLATION OF $\gamma$ -CAROTENE AND A POLY-*CIS*- $\gamma$ -CAROTENE FROM THE TANGERINE TOMATO\*

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**Key Word Index**—*Lycopersicon esculentum*; Solanaceae; tangerine tomato; poly-*cis*- $\gamma$ -carotene;  $\gamma$ -carotene.

**Plant and source.** *Lycopersicon esculentum*; Tangerine tomato genotype, variety Golden Jubilee. Field-ripened Tangerine tomato fruits were used in this study. The seeds were a generous gift from Dr. M. L. Tomes of the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana, U.S.A.

**Present work.** Tangerine (Golden Jubilee) tomato fruits are characterized by high concentrations of the poly-*cis* carotenes, proneurosporene and prolycopene [1,2] and poly-*cis*- $\zeta$ -carotene [3]. In further investigations, a pink contaminant of proneurosporene was resolved into two bands: the upper from the MgO-Hyflo SuperCel column was identified as  $\gamma$ -carotene (1.8  $\mu$ g/g dry w), and the lower as a poly-*cis*- $\gamma$ -carotene (3.0  $\mu$ g/g dry w). Poly-*cis*

carotenes normally exhibit strong bathochromic shifts and an increase in extinction upon I<sub>2</sub> catalysis. The spectrum of poly-*cis*- $\gamma$ -carotene exhibits two maxima which do not shift; however, a third absorption peak is formed and the spectrum was then identical to that of the stereoisomers obtained upon I<sub>2</sub> catalysis of  $\gamma$ -carotene extracted from carrots and high-beta tomatoes (Table 1). The wavelength maxima for both  $\gamma$ -carotene and poly-*cis*- $\gamma$ -carotene are lower than the literature values [4–7], but the purified  $\gamma$ -carotene isolated from Tangerine tomatoes was identical to a sample isolated from Summer Sunrise or high-beta tomatoes and carrots.

No cyclic poly-*cis* carotenes have been reported previously. It remains to be seen if pro- $\gamma$ -carotene from *Pyracantha angustifolia* is identical with this poly-*cis*- $\gamma$ -carotene and what biosynthetic relationship exists between this poly-*cis* carotene and its possible precursors [3,8].

\*Contribution No. 1551 of Rhode Island Agricultural Experiment Station.

Table 1.  $\lambda_{\max}$  of  $\gamma$ -carotene, poly-*cis*- $\gamma$ -carotene and some natural and synthetic isomers [4–7]

Isomer	Source	$\lambda_{\max}$ I <sub>2</sub> Catalysis	
		Before	After
all- <i>trans</i> - $\gamma$ -Carotene	<i>Mimulus longiflora</i>	436, 461–2, 493	434, 458, 486–7
pro- $\gamma$ -Carotene	<i>Pyracantha angustifolia</i>	434, 457	434, 457, 487–8
<i>cis</i> -15-15'- $\gamma$ -Carotene	Synthesis	432, 457, 486	
$\gamma$ -Carotene	High-beta tomato*	431, 456, 486	428, 453, 482
$\gamma$ -Carotene	Carrots	431, 456, 486	428, 454, 482
$\gamma$ -Carotene	Tangerine tomato*	431, 456, 486	428, 454, 482
poly- <i>cis</i> - $\gamma$ -Carotene	Tangerine tomato*	428, 453	428, 453, 482

\* *Lycopersicon esculentum*

The conversion of *cis*- $\zeta$ -carotene to *trans*- $\zeta$ -carotene, pro-neurosporene, prolycopene, neurosporene, lycopene and  $\gamma$ - and  $\beta$ -carotene by a soluble enzymic system from tangerine tomatoes [9] shows possible conversions which might occur.

#### EXPERIMENTAL

**Pigment extraction and purification.** Fresh Tangerine tomato fruits were macerated with Me<sub>2</sub>CO–light petrol mixture (bp 30–50°) (1:1) under N<sub>2</sub>. The Me<sub>2</sub>CO was quickly washed out and the upper layer saponified with 20% KOH in MeOH in the dark under N<sub>2</sub> at room temp. overnight. The extract was transferred to light petrol, washed with H<sub>2</sub>O, dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated *in vacuo*. Carotenes were chromatographed on Sea Sorb 43 Hyflo–SuperCel (1:2, w/w) with increasing concentrations of Me<sub>2</sub>CO in light petrol (up to 15%). The eluate containing the pink band was rechromatographed on the same adsorbent using 1, 2, 4 and 6% Me<sub>2</sub>CO in light petrol, the band separating into 2 components. The upper band was  $\gamma$ -carotene and the lower poly-*cis*- $\gamma$ -carotene. Pigments were eluted from the extruded adsorbent with Me<sub>2</sub>CO–light petrol, evaporated to dryness, rechromatographed on alumina II with increasing concentrations of Et<sub>2</sub>O in light petrol, and on TLC precoated aluminum oxide (Type E) F<sub>254</sub> plates with 2% Et<sub>2</sub>O in light petrol. Poly-*cis*- $\gamma$ -carotene was finally purified on alumina II with 20% C<sub>6</sub>H<sub>6</sub> and 4% Et<sub>2</sub>O in light petrol and TLC on precoated Si gel F<sub>254</sub> plates as described. Gamma carotene was identified by its absorption spectrum, and by comparison with authentic samples of  $\gamma$ -carotene. Poly-*cis*- $\gamma$ -carotene was identified by its position on the column below  $\gamma$ -carotene, its absorption spectrum before and after I<sub>2</sub> catalysis and comparison to the literature [4–6].

**Absorption spectra.** Absorption spectra of carotenes were reported from solns in light petrol (bp 30–50°). Quantitative estimation of poly-*cis*- $\gamma$ -carotene was made from the equilibrium mixture of stereoisomers after the poly-*cis* pigment was subject to I<sub>2</sub> catalysis using the  $E_{1\%}^{1\text{cm}}$  value of 3100 for the all-*trans*  $\gamma$ -carotene at 462 nm [10].

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### BACTERIOSTATIC ACTIVITY OF SOME COUMARIN DERIVATIVES

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**Key Word Index.**—Bacteriostatic activity; hydroxycoumarins; furanocoumarins and -glucosides.

**Abstract.**—The bacteriostatic effect of some mono- and dihydroxy-coumarins and isopropylidihydrofuranocoumarins have been examined and compared with other simple phenols. Most of the coumarins proved to be far more bacteriostatic than the simple phenols.

Antimicrobial properties of coumarins are of interest in connection with the possible activity of these compounds as phytoalexins [1–3]. Since vaginidiol and its derivatives are often present in umbelliferous plants we were interested in the bacteriostatic activity of these compounds and specially of apterin [4, 5] and its hydrolysis products, vaginidiol, oroselol and oroselon. Two other derivatives were not investigated: oroselolglucosid because we could not detect it *in vivo* or *in vitro*, and the dimer of oroselon [6, 7] because its formation in a dilute, weakly acidic aqueous solution is very slow. In this paper a compari-

son of the bacteriostatic activity of some coumarin derivatives with some common bacteriostatic phenols is reported (Table 1). The furanocoumarins proved to be more bacteriostatic than the phenols and also showed more activity than that recorded by other workers [2].

#### EXPERIMENTAL

Laboratory strains of *E. coli* and *B. subtilis* were used. Overnight cultures of the bacteria on nutrient agar (Difco) were washed once in sterile peptone-saline soln and the cells were resuspended in peptone-saline. Suitable dilutions were made.