Phytochemistry, 1972, Vol 11, pp 397 to 400 Pergamon Press Printed in England

SHORT COMMUNICATION

THE ISOLATION OF A POLY-*cis* ζ-CAROTENE FROM THE TANGERINE TOMATO[1]

LEONCIO C. RAYMUNDO and KENNETH L. SIMPSON
Department of Food and Resource Chemistry, University of Rhode Island,
Kingston, Rhode Island 02881 U S A

(*Received* 4 *January* 1971)

Abstract—A contaminant of the β-carotene fraction of Tangerine tomato fruit extract was isolated and identified as a poly-*cis* isomer of ζ-carotene The ripening fruit contains 0 8 μg/g dry weight of the pigment The possible role of poly-*cis* ζ-carotene in the biosynthesis of poly-*cis* carotenes is discussed

INTRODUCTION

TANGERINE tomato fruits contain as the characteristic pigments the poly-*cis* carotenes proneurosporene and prolycopene[2,3] and smaller amounts of *cis* isomers of phytofluene and ζ-carotene Compared with ordinary tomatoes, the Tangerine fruits have reduced levels of all-*trans* lycopene and β-carotene, but greatly increased amounts of the more saturated carotene precursors phytoene, phytofluene and ζ-carotene

The phytoene and phytofluene levels in the ripe Tangerine tomato are surpassed only by the levels of these polyenes in the *ghost* tomato [4,5] The all-*trans* ζ-carotene content which has been reported to amount to 414 4 μg/g dry weight[3] is the highest concentration of this pigment found in tomato fruits [6,7] The β-carotene content, on the other hand, is more typical of the values reported for green tomatoes [4]

In the present paper we report the identification of a poly-*cis* isomer of ζ-carotene from extracts of Tangerine tomatoes

RESULTS AND DISCUSSION

A contaminant of the β-carotene fraction eluted from the MgO: Hyflo Super Cel column was isolated during the purification of β-carotene by TLC on silica gel F plates The adsorption maxima of the yellow pigment were different from any of the carotenes whose fundamental band falls between the 370 and 420 nm region of the spectrum.

The pigment exhibited λ_{max} at 414, 390 and 369 nm which shifted to 422, 397 and 377 nm on the addition of catalytic amounts of iodine (Fig 1). The extinction of the fundamental region also increased concomitant with the bathochromic shift The absorption of the iodine catalyzed equilibrium mixture is identical to the spectral curve of the stereoisomers obtained upon iodine catalysis of the all-*trans* ζ-carotene A strong bathochromic shift and an in-

---

[1] Contribution Number 1383 of the Rhode Island Agricultural Experiment Station
[2] A L LEROSEN and L ZECHMEISTER, *J Am Chem Soc* **64**, 1075 (1942)
[3] R J H WILLIAMS, G BRITTON, J M CHARLTON and T W GOODWIN, *Biochem J* **104**, 767 (1967)
[4] G MACKINNEY, C M RICK and J A JENKINS, *Proc Nat Acad Sci U S* **42**, 404 (1956)
[5] M L TOMES, *Bot Gaz* **124**, 180 (1963)
[6] J W. PORTER and F P ZSCHEILE, *Arch Biochem* **10**, 537 (1946)
[7] J W PORTER and R E LINCOLN, *Arch Biochem* **27**, 390 (1950)

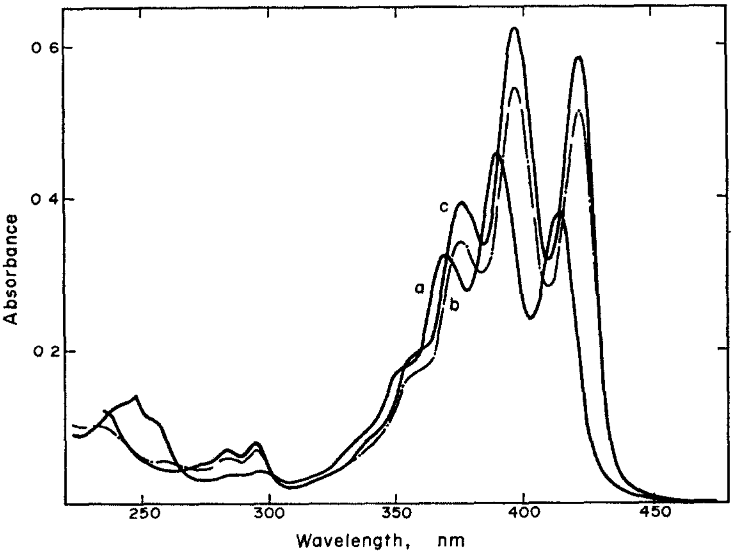

FIG 1 SPECIFIC EXTINCTION CURVES OF POLY-*cis* ζ-CAROTENE, IN PETROLEUM ETHER a, FRESH SOLUTION, b, MIXTURE OF STEREOISOMERS AFTER CATALYSIS BY IODINE, AND c, MIXTURE OF STEREOISOMERS AFTER CATALYSIS OF ALL-*trans* ζ-CAROTENE BY IODINE

crease in the extinction at the fundamental region on iodine catalysis are characteristic properties exhibited by all poly-*cis* carotenoids [8] These data are consistent with the conclusion that the isolate is a poly-*cis* isomer of ζ-carotene A comparison of the absorption maxima of the poly-*cis* ζ-carotene with the spectrum of some synthetic and natural isomers of ζ-carotene is given in Table 1

In the ripening fruit the poly-*cis* ζ-carotene was found at a concentration of 0 8 μg/g dry weight which is about 0 4% of the total ζ-carotene content of 189 2 μg/g dry weight.

The structure of poly-*cis* carotenoids has not been satisfactorily elucidated Based on spectral studies of *cis-trans* carotenoids and related polyenes, ZECHMEISTER[8] suggested that

TABLE 1 λ_{max} OF THE POLY-*cis* ζ-CAROTENE AND SOME NATURAL AND SYNTHETIC ISOMERS

Isomer	Source	λ_{max} (nm)						Reference
all-*trans* ζ-Carotene	Carrot oil	425	400	378	(361)			9
all-*trans* ζ-Carotene	Synthetic	425	401	380				10
unsym ζ-Carotene	Synthetic	420	395	375	(356)			9, 10
unsym ζ-Carotene (7,8,11,12-Tetrahydrolycopene)	*Rhodospirillum rubrum*	418 5	394 5	374	(354)			11
"neo A" ζ-Carotene	Carrot oil	419	396	375	(357)	296	285	9
cis-15- ζ-Carotene	Synthetic	422	398	378	(359)	296	286	9, 10
cis ζ-Carotene	Tangerine tomato	423	398	378				3
poly-*cis* ζ-Carotene	Tangerine tomato	414	390	369	(352)	296	285	

---

[8] L ZECHMEISTER, cis-trans *Isomeric Carotenoids, Vitamins A and Arylpolyenes*, Springer, Vienna (1963)

[9] J B DAVIS, L M JACKMAN, P T SIDDONS and B C L WEEDON, *J Chem Soc* (C) 2154 (1966)

[10] J B DAVIS, L M JACKMAN, P T SIDDONS and B C L WEEDON, *Proc Chem Soc* **261** (1961)

prolycopene has a symmetrical structure with *cis* configuration at the central double bond and 4 other sterically unhindered double bonds. The unhindered positions are the methylated carbon-to-carbon double bonds in the carotenoid molecule [12] Introduction of hindered *cis* double bond(s) results in the loss of fine structure in the fundamental region [8,13,14]

The absence of a strong *cis*-peak in the near UV region of the poly-*cis* ζ-carotene spectrum suggests that the molecule does not have a bent shape The central mono-*cis* isomer, e g central mono-*cis* isomers of β-carotene and lycopene, has the strongest *cis*-peak because of its bent shape, conversely, the spectral curve of the all-*trans* isomer has a relatively flat, although slightly elevated *cis*-peak region [8]

In view of our present isolation of a poly-*cis* ζ-carotene, we suggest that the proposed pathway of carotenoid biosynthesis of Porter and Anderson[15] be modified to include this new compound as an intermediate The poly-*cis* ζ-carotene could be formed from the all-*trans* ζ-carotene. The existence of 3 poly-*cis* polyenes would lead one to suggest that poly-*cis* phytoene and phytofluene might also be formed in the system (Fig. 2). These compounds, however, have not been reported If they were formed their poly-*cis* nature would be difficult to detect since most of the unhindered *cis* positions would be out of conjugation Such a parallel pathway would be similar to one proposed by Malhotra *et al* [17] for the biosynthesis of 1,2-dihydro carotenes from 1,2-dihydro phytoene in *Rhodopseudomonas viridis*

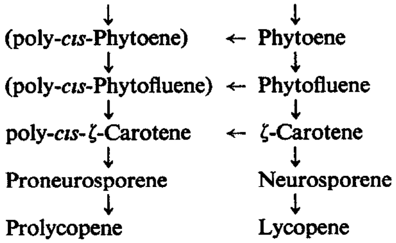

FIG 2 POSSIBLE PATHWAYS LEADING TO THE FORMATION OF PROLYCOPENE

EXPERIMENTAL

Materials Field-ripened Tangerine tomato fruits were used in this study The seeds were a gift from Dr M L Tomes of the Department of Botany and Plant Pathology, Purdue University, Lafayette, Indiana, U S A

Pigment extraction and purification Thirty-g aliquots of the fruit were extracted with acetone-light petroleum (b p 30–50°) in a Waring Blender under a continuous stream of N_2 The extract was washed immediately with H_2O to remove the acetone The extract was either chromatographed after drying (Na_2SO_4) without prior saponification or saponified with 10% KOH in MeOH (w/v) Saponification was accomplished by evaporating the solvent in a flash evaporator to transfer the pigment extract into the MeOH solution The

---

[11] B H DAVIES, *Biochem J* **116,** 93 (1970)

[12] L PAULING, *Fortschr Chem organ Naturstoffe* **3,** 203 (1939)

[13] C H EUGSTER, C F GARBERS and P KARRER, *Helv Chim Acta* **36,** 1378 (1953)

[14] O VON ISLER, L H CHOPARD-DIT-JEAN, M MONTAVON, R RUEGG and P ZELLER, *Helv Chim Acta* **40,** 1256 (1957)

[15] J W PORTER and D G ANDERSON, *Arch Biochem Biophys* **97,** 520 (1962)

[16] J W PORTER and D G ANDERSON, *Ann Rev Plant Physiol* **18,** 197 (1967)

[17] H C MALHOTRA, G BRITTON and T W GOODWIN, *Internat Z Vit Forschung* **40,** 315 (1970)

extract was transferred to petrol after 15 min following the usual procedure,[18] dried (Na_2SO_4) and chromatographed on a 22 × 360 mm column of MgO Hyflo Super Cel (1 2, w/w) The chromatogram was developed with 2, 5 and finally 10% acetone in petrol The column plug was extruded and the zone below the *cis* ζ-carotene band was cut together with the β-carotene band The pigments were eluted from the adsorbent with acetone-petrol, evaporated to dryness and rechromatographed on a 12 × 150 column of alumina II The column was developed with increasing concentrations of Et_2O in petrol The poly-*cis* ζ-carotene was eluted from the column together with the β-carotene band with 5% Et_2O The eluate was evaporated to dryness and purified by TLC on precoated silica gel F_{254} plates with petrol as developing solvent The yellow band adsorbed below the β-carotene band is the poly-*cis* β-carotene band Alternatively, the eluate from the MgO Hyflo Super Cel column was first purified by TLC on precoated silica gel F_{254} plates to remove the β-carotene The poly-*cis* ζ-carotene band was eluted from the adsorbent and rechromatographed on a short column of alumina II to remove a contaminating β-zeacarotene-like pigment

Absorption spectra The absorption spectra of the carotenes were recorded from solutions in petrol with a Cary 15 recording spectrophotometer Quantitative estimation of the poly-*cis* ζ-carotene was made from the equilibrium mixture of stereoisomers after the poly-*cis* pigment was subjected to I_2 catalysis using the $E^{1\%}_{1cm}$ value of 2270 for the all-*trans* ζ-carotene at 400 nm[19]

Acknowledgments—This investigation was supported by the Public Health Service Research Grant No. 1 RO1/NB 08516-01 of the National Institute of Neurological Diseases and Stroke The assistance of Dr A E Griffiths is gratefully acknowledged

---

[18] L C RAYMUNDO, A E GRIFFITHS and K L SIMPSON, *Phytochem* **6**, 1527 (1967)

[19] B H DAVIES, In *Chemistry and Biochemistry of Plant Pigments* (edited by T W GOODWIN), p 489, Academic Press, New York (1965)

Key Word Index—Lycopersicum esculentum, Solanaceae, tangerine tomato, poly *cis*-ζ-carotene