Carotenoids from Guava (*Psidium guajava* L.): Isolation and Structure Elucidation

Adriana Z. Mercadante,*,† Andrea Steck,‡ and Hanspeter Pfander‡

Departamento de Ciência de Alimentos, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, CP 6121, CEP 13083-970, Campinas, Brazil, and Department of Chemistry and Biochemistry, University of Berne, Freiestrasse 3, Berne, CH-3012, Switzerland

Sixteen carotenoids were isolated from the flesh of Brazilian red guavas (*Psidium guajava* L.). Their structures were established by means of UV−visible, 400 and 500 MHz ^{1}H NMR, 120 and 125 MHz ^{13}C NMR, mass, and circular dichroism spectra. The carotenoids were identified as phytofluene, (*all-E*)-, (9*Z*)-, (13*Z*)-, and (15*Z*)-*β*-carotene, (*all-E*)-*γ*-carotene, (*all-E*)-, (9*Z*)-, (13*Z*)-, and (15*Z*)-lycopene, (*all-E*,3*R*)-*β*-cryptoxanthin, (*all-E*,3*R*)-rubixanthin, (*all-E*,3*S*,5*R*,8*S*)-cryptoflavin, (*all-E*,3*R*,3′*R*,6′*R*)-lutein, (*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*R*)-, and (*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*S*)-neochrome. Thirteen of the carotenoids identified are reported as guava carotenoids for the first time.

Keywords: *Carotenoids; isolation; LC/MS; NMR; guava; Psidium guajava L.*

INTRODUCTION

Guava, native to tropical America, is a tree which is now widely distributed throughout the tropic and subtropic areas, and its fruits are consumed fresh or processed. The major producers of guava are South Africa, India, Hawaii, Colombia, Puerto Rico, Jamaica, Brazil, and Israel. A great portion of guava products are consumed locally by the producing country. Guava fruits may be round, oval, or pear-shaped, 3−10 cm in diameter. The color of the peel of the ripe fruit is yellow, and the color of the flesh may be white, pink, yellow, salmon, or carmine, depending on the variety. The flesh contains numerous scleroids (stone cells) (Chan, 1993).

Due to many functions or actions attributed to carotenoids (Krinsky, 1994; Bendich, 1994), there is a worldwide interest in obtaining reliable analytical data on these compounds. The introduction of high-performance liquid chromatography (HPLC) for carotenoid analysis allowed the separation of a great number of carotenoids, especially epoxides and (*Z*)-isomers; therefore the identification based only on chromatographic behavior and UV−visible (UV−vis) spectra as published in many papers is insufficient. In fact, incomplete or conflicting information about the carotenoid composition of fruits is often found in the literature.

In previous studies on carotenoids from guava, the lycopene fraction (Nakasone et al., 1976; Gonzalez-Abreu et al., 1985), the total *β*-carotene content (Fonseca et al., 1969) or both (Wilberg and Rodriguez-Amaya, 1995) were determined. Gross (1987) found lutein as the single carotenoid in the peel of white guava. Padula and Rodriguez-Amaya (1986) characterized seven carotenoids in guavas IAC-4 from Brazil based on their chromatographic behavior, UV−vis spectra, and some chemical derivatizations. In the lattest study quantitative data were given, lycopene being the major carotenoid.

The present communication describes the complete identification of carotenoids from the flesh of red guavas from Brazil. For this purpose, besides UV−vis spectra and mass spectrometry (MS), nuclear magnetic resonance (NMR) techniques were employed to prove unequivocally the carotenoid structure, including the geometry of the double bonds and relative stereochemistry. Circular dichroism (CD) was performed in chiral carotenoids to determine the absolute configuration.

MATERIALS AND METHODS

Apparatus. HPLC analysis were performed with two Waters pumps, a Waters photodiode array detector (PDA) 990 and a Rheodyne injection valve with 100-*μ*L loop. The isolation of carotenes was carried out either on a C_{18} Vydac 218 TP 54 (Separations Group Inc., Hesperia, CA) or on a C_{30} YMC "carotenoid" (YMC Inc., Wilmington, NC) column, both with particle size of 5 *μ*m and 250 × 10 i.d. mm. For purification of xanthophylls, a nitrile Nucleosil (purchased from Macherey-Nagel, Zurich, Switzerland) column (5 *μ*m particle size, 250 × 10 i.d. mm) was employed. Optimization of mobile phases for isolation was performed in analytical columns (250 × 4.6 i.d. mm) with the same characteristics as the semipreparative columns described above. The UV−vis spectra were recorded on a PDA detector and compared with data given in the literature (Davies, 1976; Britton, 1995). Spectral fine structure was expressed as % III/II and relative intensity of the *cis*-peak as % A_B/A_{II} (Britton, 1995). Liquid chromatography−mass spectra (LC−MS) were recorded on a VG platform (Micromass, Manchester, U.K.) using negative or positive atmospheric pressure chemical ionization, corona voltage of −2 kV in negative mode and +3.2 kV in positive mode, cone voltage of −25 V, respectively, + 20 V, probe temperature of 400 °C, and source temperature of 120 °C. The CD spectra were measured with a modified Dichrograph CD6 (Jobin-Yvon, Paris, France) at −180 °C, with diethyl ether/isopentane/ethanol (5:5:2), known as EPA, as solvent. The NMR spectra were recorded on Bruker DRX-400 and DRX-500 instruments, both equipped with probeheads for inverse detection. All measurements were performed at 23 °C in deuterium chloroform (99.95%, Armar, Switzerland), passed twice through an aluminum oxide (Al_2O_3) minicolumn and under argon. Prior to NMR analysis, remaining traces of solvents were removed under high vacuum. Chemical shifts of ^{1}H and ^{13}C resonances (*δ*) were related to

* Author to whom correspondence should be addressed (fax 55 19 7887890; e-mail: mercadan@obelix.unicamp.br).

† Universidad Estadual de Campinus.

‡ University of Berne.

10.1021/jf980405r CCC: $18.00
Published on Web 12/30/1998

residual solvent signals, and only relevant $^nJ_{HH}$ coupling constant values (J) are given. Complete proton line assignments were achieved by ^{1}H and H,H-COSY (*co*rrelated spectroscop*y*) experiments. When the sample amounts of carotenoids were insufficient to perform carbon-13 detected experiments (^{13}C, DEPT 135), the δ values of the proton-bearing carbon nuclei were extracted from proton-detected HMQC (*h*eteronuclear *m*ulti*q*uantum *c*oherence) experiment (optimized for $^1J_{CH}$) with a precision of ± 0.2 ppm.

Plant Material and Extraction. Fully ripe red guavas variety IAC-4 (*Psidium guajava* L.) were bought in Campinas, São Paulo State, Brazil. The yellow peel was removed and the edible portion (800 g) was lyophilized for 3 days and stored at −80 °C, under argon, until analysis.

The solvents used for extraction and column chromatography were purchased from Thommen (Berne, Switzerland) and hexane and ethyl acetate (EtOAc) distilled before use. All HPLC grade solvents were from Romil (England).

The carotenoids from lyophilized guava (92 g) were extracted in a high-performance mixer with *tert*-butyl methyl ether (*t*-BME) (800 mL), followed by EtOAc (800 mL) and methanol (MeOH) (800 mL). To avoid carotenoid oxidation/isomerization during this process, BHT (2,6-di-*tert*-butyl-*p*-cresol) and $CaCO_3$, both from Fluka (Switzerland), were added. The combined extracts were concentrated in a rotary evaporator (T < 35 °C), yielding 42 g, and dried overnight at high vacuum (21 g). The crude extract was dissolved in *t*-BME and saponified with 10% KOH in MeOH overnight at room temperature. Afterward, the carotenoids were transferred to *t*-BME and washed with water until free of alkali, yielding 10 g of saponified extract after evaporation of the solvent. An attempt to precipitate sterol was not successful due to lycopene crystallization.

Column Chromatography. The saponified extract was applied to a flash chromatography column (30 × 6 cm i.d.) packed with neutral Al_2O_3 (Camag, Muttenz, Switzerland) (activity grade III). Fraction 1 (carotenes, 8.9 g) was eluted with hexane, fraction 2 (principally lycopene, 0.2 g) with hexane/*t*-BME (7:3), fraction 3 (monohydroxy, 0.2 g) with hexane/*t*-BME (1:4), and fraction 4, containing the most polar carotenoids (0.1 g), with MeOH. The fractions were immediately concentrated and stored at −20 °C.

Isolation of Six Carotenes from Fraction 1. Due to the presence of large amounts of white material, the first fraction (8.9 g) was submitted to column chromatography on MgO (BDH, U.K.)/Celite (1:2) (21 × 5.5 cm). The main part of the white material was eluted with ca. 1.5 L of hexane and hexane/*t*-BME (8:2), and discarded. Four oily fractions (1.1−1.4) were separated: fraction 1.1 (32 mg) was eluted with 50 to 100% of *t*-BME in hexane, fraction 1.2 (124 mg) with 20 to 50% of EtOAc in *t*-BME, fraction 1.3 (316 mg) with 0 to 50% of MeOH in EtOAc and fraction 1.4 (215 mg) with H_2O. Despite the fact that fraction 1.1 still contained white material, it was purified by HPLC on a Vydac column, with MeOH at 4 mL/min as mobile phase to give phytofluene **(1)** (t_R = 16.1 min, 0.4 mg). Fraction 1.2 was applied on a silica TLC and developed with hexane as mobile phase, and the orange band (R_f = 0.26, 2.6 mg) was scraped off. Since HPLC analysis showed the presence of phytofluene and β-carotene (mixture of *E*/*Z*-isomers), fraction 1.2 was submitted to a MgO/kieselguhr TLC, with hexane/acetone (95:5) as mobile phase, to remove phytofluene. The final purification of fraction 1.2 (2.2 mg) was performed in two HPLC steps because separation on a Vydac column did not provide baseline separation of all *E*/*Z*-isomers of β-carotene. Thus, (*all-E*)-β-carotene **(2)** was collected from a Vydac column with MeOH/*t*-BME (98:2) at 3.5 mL/min (t_R = 16.7 min, 0.5 mg) as mobile phase and the (*Z*)-isomers were further separated on a C_{30} YMC column with MeOH/*t*-BME (8:2) at 4.7 mL/min, giving (15*Z*)-**(3)** (t_R = 18.5 min, < 0.1 mg), (13*Z*)-**(4)** (t_R = 19.6 min, 0.2 mg) and (9*Z*)-β-carotene **(5)** (t_R = 29.8 min, 0.1 mg). Cochromatography by HPLC (Vydac column, MeOH as mobile phase) with authentic ζ-carotene was performed with fraction 1.3. Fraction 1.4 was purified on a MgO/kieselguhr TLC, with hexane/acetone (4:1) as mobile phase (R_f = 0.60). The orange band (3.8 mg) was scraped off and (*all-E*)-γ-carotene **(6)** was purified by HPLC on a Vydac column with MeOH at 4 mL/min as mobile phase (t_R = 20.7 min, 0.2 mg).

Isolation of Four Carotenes from Fraction 2. HPLC separation of fraction 2 on a Vydac column, MeOH at 5 mL/min as mobile phase, gave 4 subfractions. Fractions 2.1 (t_R = 14.4 to 17.2 min, 3.6 mg) and 2.4 (t_R = 30.5 min, 22.6 mg) were discarded because further separation by HPLC on a C_{30} column showed that there was no major peak in these fractions. From fraction 2.2 (5.5 mg) (*all-E*)-lycopene **(7)** (t_R = 23.0 min, 0.5 mg) and (9*Z*)-lycopene **(8)** (t_R = 24.2 min, 0.4 mg) were isolated after HPLC purification on a Vydac column (MeOH, 4 mL/min). From fraction 2.3 (7.5 mg), (13*Z*)-**(9)** (t_R = 19.3 min, 0.5 mg) and (15*Z*)-lycopene **(10)** (t_R = 21.8 min, 0.3 mg) were isolated by HPLC (C_{30} column, MeOH/*t*-BME (65:35), 4.7 mL/min).

Isolation of Three Carotenoids from Fraction 3. Fraction 3 was submitted to silica (Macherey-Nagel, Switzerland) TLC, developed with hexane/EtOAc (6:4). To avoid 5,6- to 5,8-epoxide rearrangement, 0.2% of triethylamine (TEA, puriss. pa. > 99.5%, Fluka Switzerland) was added to the TLC mobile phase. Two major bands were scraped off (fraction 3.1, 3.1 mg, R_f = 0.51 and fraction 3.2, 7.5 mg, R_f = 0.38). Fraction 3.1 was further purified on MgO/kieselguhr plates, with EtOAc/hexane/*t*-BME (5:3:2) as mobile phase and two fractions were isolated (fractions 3.1.1 and 3.1.2). From fraction 3.1.1 (*all-E*,3*R*)-β-cryptoxanthin **(11)** was isolated by HPLC on a Vydac column with MeOH/*t*-BME (95:5) at 3 mL/min (t_R = 9.8 min, 1.1 mg) as mobile phase. Fraction 3.1.2 gave (*all-E*,3*R*)-rubixanthin **(12)** after HPLC purification on a nitrile column and hexane/EtOAc (9:1) at 4 mL/min (t_R = 13.8 min, 0.1 mg) as mobile phase. (*all-E*,3*S*,5*R*,8*S*)-Cryptoflavin **(13)** was purified from fraction 3.2 by HPLC (t_R = 16.9 min, 0.5 mg) with the same system employed for **12**.

Isolation of Three Carotenoids from Fraction 4. All HPLC separations were carried out on a nitrile column. This fraction was preseparated into three fractions (fractions 4.1, 4.2 and 4.3) by HPLC with hexane/EtOAc (7:3) at 5 mL/min as mobile phase. For further purification of fraction 4.1 (t_R = 6.3 to 10.1 min), the same system used above with a different solvent ratio (85:15) gave (*all-E*,3*R*,3′*R*,6′*R*)-lutein **(14)** (t_R = 20.0 min) as major compound. From fraction 4.2 (t_R = 12.9 min), (*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*R*)-neochrome **(15)** was isolated by HPLC with hexane/acetone (4:1) at 4 mL/min as mobile phase (t_R = 12.3 min). Two carotenoids were purified from fraction 4.3 (t_R = 14.8 to 18.4 min) by HPLC with hexane/acetone (85:15) at 5 mL/min as mobile phase. (*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*S*)-Neochrome **(16)** eluted at 21.7 min and the other carotenoid tentatively identified as (*all-E*)-neoxanthin eluted at 26.7 min.

RESULTS AND DISCUSSION

Sixteen carotenoids (Figure 1) were isolated from guava and fully characterized by their UV−vis, MS, CD, and NMR spectra. Based on its UV−vis spectrum and coelution with an authentic standard, ζ-carotene was also tentatively identified.

Phytofluene (1) (7,8,11,12,7′,8′-Hexahydro-ψ,ψ-carotene). The absorption maxima in the UV−vis spectrum (332, 348, and 366 nm in MeOH) and the spectral fine structure (% III/II 77) was consistent with an acyclic carotenoid with five conjugated double bonds. The mass spectrum showed the molecular ion at *m*/*z* 542, which is consistent with $C_{40}H_{62}$. The ^{1}H NMR spectrum confirmed the constitution (Table 1). The COSY spectrum had a very low signal-to-noise ratio in the olefinic region, as a consequence the cross-peaks of the spin system H−C(14)/H−C(15)/H−C(14′)/H−C(15′) could only be tentatively identified and therefore the geometry of the double bonds of **1** could not be assigned.

(*all-E*)- (2), (9*Z*)- (3), (13*Z*)- (4), and (15*Z*)-β,β-Carotene (5). The UV−vis spectra indicated that

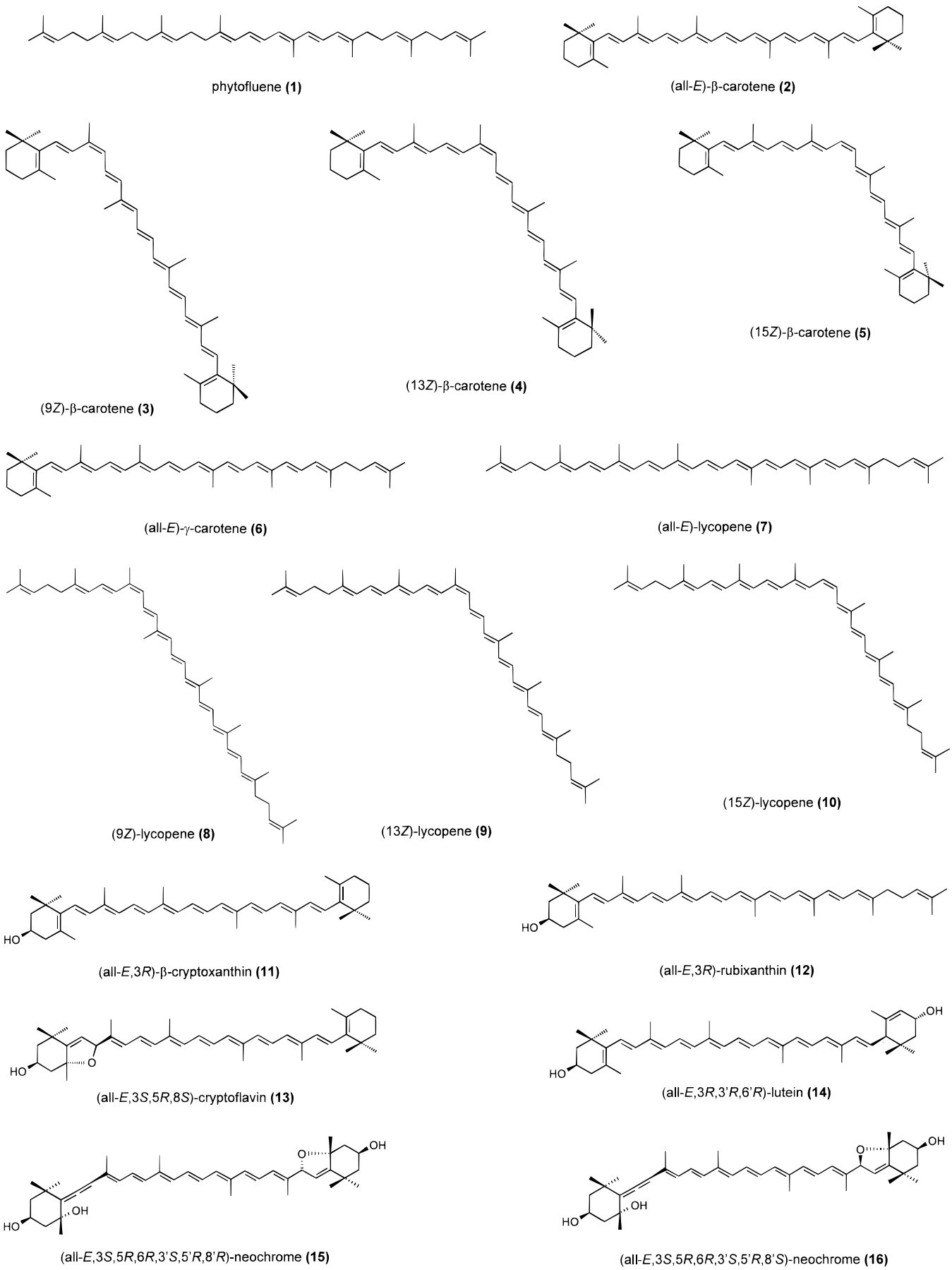

Figure 1. Carotenoids isolated and identified from guava.

carotenoids **2**, **3**, **4**, and **5** have β-rings at both ends of the molecule since the degree of spectral fine structure is relatively low. As expected the spectral fine structure decreased and the intensity of *cis* peak increased as the

Table 1. ^{1}H NMR Data of Some Carotenoids Isolated from Guava

	δ (ppm), signal multiplicity, coupling constant (Hz)									
H-no.	**1**	**2**	**3**	**4**	**5**	**6**	**11**	**13**	**15**	**16**
H-2ax	5.10, m	1.47, m	1.48, m	1.47, m	1.47, m		1.46, m	1.47, m	1.34, ψt ($J \sim 12$; 12)	1.34, dd ($J = 12.4$; 12.0)
H-2eq									1.95, ddd ($J = 12$; 4.1)	1.95, ddd ($J = 12.4$; 5.3)
H-3	2.05, m	1.62, m	1.62, m	1.62, m	1.62, m		1.62, m	1.62, m	4.32, m	4.32, m
H-4ax	1.98, m	2.02, t ($J = 6.3$)	2.03, m	2.02, t	2.03, t ($J = 6.3$)	2.03, m	2.02, t ($J = 6.4$)	2.02, t ($J = 6.1$)	1.41, ψt ($J \sim 14$; 14)	1.41, ψt ($J \sim 14.1$; 14.1)
H-4eq									2.26, ddd ($J \sim 14$; 5.3)	2.26, ddd ($J = 14.1$; 5.2)
H-6	5.11, m									
H-7	2.08, m	~6.16, AB system ($J = 16.2$)	6.18, d ($J = 16.1$)	~6.16, AB system ($J = 16.1$)	~6.16, AB system ($J \sim 16$)	6.13, AB system ($J = 16.4$)	~6.16, AB system ($J = 16.1$)	6.16, AB system ($J = 16.1$)		
H-8	1.96, m	~6.15, AB system ($J = 16.2$)	6.67, d ($J = 16.1$)	~6.15, AB system ($J = 16.1$)	~6.15, AB system ($J \sim 16$)	6.15, AB system ($J = 16.4$)	~6.15, AB system ($J = 16.1$)	6.15, AB system ($J = 16.1$)	6.03, s	6.03, s
H-10	5.13, m	6.15, d ($J = 10.8$)	6.05, d ($J = 11.6$)	6.20, d ($J = 11.4$)	~6.15, d ($J = 11.3$)		6.15, d ($J = 11.3$)	6.15, d ($J = 11.2$)	6.11, dq ($J = 11.1$)	6.11, dq ($J = 11.2$; 1.0)
H-11	2.14, m	6.65, dd ($J = 10.8$; 14.8)	6.75, dd ($J = 11.6$; 15.1)	6.65, dd ($J = 11.4$; 15.0)	6.69, dd ($J = 11.3$; 15.0)	6.64, dd ($J = 15.1$)	6.65, dd ($J = 11.3$; 14.6)	6.64, dd ($J = 11.2$; 15.1)	6.54, dd ($J = 11.1$; 14.8)	6.54, dd ($J = 11.2$; 14.8)
H-12	2.23, m	6.35, d ($J = 14.8$)	6.29, d ($J = 15.1$)	6.88, d ($J = 15.0$)	6.43, d ($J = 15.0$)	6.35, d ($J = 15.1$)	6.35, d ($J = 14.6$)	6.35, d ($J = 15.1$)	6.33, d ($J = 14.8$)	6.33, d ($J = 14.8$)
H-14	6.37,[a] d ($J = 10.5$)	6.25, m	6.25, m	6.10, d ($J = 12.2$)	6.64, m	6.26, m	6.25, m	6.25, m	6.25, m	6.25, m
H-15	6.45,[a] dd ($J = 10.5$)	6.63, m	6.63, m	6.79, d ($J = 12.2$; 13.6)	6.36, m		6.63, m	6.62, m	6.62, m	6.61, m
H-16	1.678, s	1.030, s	1.041, s	1.034,[b] s	1.040,[b] s	1.027, s	1.029, s	1.026, s	1.331, s	1.332, s
H-17	1.591, s	1.030, s	1.041, s	1.034,[b] s	1.036,[b] s	1.027, s	1.029, s	1.026, s	1.066, s	1.066, s
H-18	1.600, s	1.718, s	1.755, s	1.723, s	1.673, s	1.717, s	1.718, s	1.715, s	1.347, s	1.348, s
H-19	1.621, s	1.972, s	1.960, s	1.975, s	1.980, s	1.969, s	1.971,[b] s	1.968, s	1.798, s	1.799, s
H-20	1.794, s	1.972, s	1.971, s	1.992, s	1.980, s	1.982, s	1.973, s	1.948, s	1.954, s	1.951, s
H-2′ax	5.10, m	1.47, m	1.48, m	1.47, m	1.47, m	5.11, m	1.48, ψt ($J = 11.6$; 11.6)	1.80, ddd ($J = \sim 15$; 5.4; 1.7)	1.76, dd ($J = 16.1$; 4.9)	1.80, dd ($J = 14.1$; ~4)
H-2′eq							1.77, ddd ($J = 11.6$; 3.0; 1.9)	1.48, m	1.52, dd ($J = 16.1$; 3.3)	1.48, dd ($J = 14.1$; ~4)
H-3′	2.05, m	1.62, m	1.62, m	1.62, m	1.62, m	2.11, m	4.00, m	4.25, m	4.25, m	4.24, m
H-4′ax	1.98, m	2.02, t ($J = 6.3$)	2.03, m	2.02, t	2.03, t ($J = 6.3$)	2.11, m	2.04, dd ($J = 17.2$; 9.5)	2.11, ddd ($J = 13.0$; 4.2; 1.7)	2.13, ddd (14.2; 1.2)	2.11, ddd (14.4; 1.6)
H-4′eq							2.39, ddd ($J = 17.2$; 4.8; 1.9)	1.90, dd ($J = 13.0$; 4.9)	1.99, dd ($J = 14.2$; 4.2)	1.90, dd ($J = 14.4$; 5.1)
H-6′	5.11, m					5.95, d				
H-7′	2.08, m	~6.16, AB system ($J = 16.2$)	~6.15, AB system ($J \sim 17$)	~6.16, AB system ($J = 16.1$)	~6.16, AB system ($J \sim 16$)	6.48, dd	6.10, AB system ($J = 17.6$)	5.30, d ($J = 1.8$)	5.25, d	5.30, d ($J = 1.9$)
H-8′	1.96, m	~6.15, AB system ($J = 16.2$)	~6.14, AB system ($J \sim 17$)	~6.15, AB system ($J = 16.1$)	~6.15, AB system ($J \sim 16$)		6.16, AB system ($J = 17.6$)	5.07, dq ($J = 1.8$; 1.3)	5.17, d	5.07, m
H-10′	5.93, d ($J = 10.8$)	6.15, d ($J = 10.8$)	6.15, d ($J = 10.7$)	~6.15, d ($J = 11.5$)	~6.15, d ($J = 11.3$)	6.21, d ($J \sim 11$)	6.16, d ($J \sim 11$)	6.18, dq ($J = 11.1$)	6.19, d ($J = 10.8$)	6.19, d ($J = 10.9$)
H-11′	6.49, dd ($J = 10.8$; 15.3)	6.65, dd ($J = 10.8$, 14.8)	6.65, dd ($J = 10.7$; 15.0)	6.64, dd ($J = 11.5$; 14.9)	6.69, dd ($J = 11.3$; 15.0)		6.64, dd ($J \sim 11$; 14.9)	6.54, dd ($J = 11.1$; 14.9)	6.49, dd ($J = 10.8$; 15.1)	6.50, dd ($J = 10.9$; 15.0)
H-12′	6.26, d ($J = 15.3$)	6.35, d ($J = 14.8$)	6.35, d ($J = 15.0$)	6.35, d ($J = 14.9$)	6.43, d ($J = 15.0$)		6.36, d ($J = 14.9$)	6.32, d ($J = 14.9$)	6.32, d ($J = 15.1$)	6.32, d ($J = 15.0$)
H-14′	6.53,[a] d ($J = 11.1$)	6.25, m	6.25, m	6.23, d ($J = 12.4$)	6.64, m	6.24, m	6.25, m	6.23, m	6.23, m	6.23, m
H-15′	6.21,[a] dd ($J = 11.1$)	6.63, m	6.63, m	6.55, dd ($J = 12.4$; 13.6)	6.36, m		6.63, m	6.62, m	6.62, m	6.62, m
H-16′	1.678, s	1.030, s	1.027, s	1.028,[b] s	1.040,[b] s	1.686, d ($J = 1.1$)	1.074, s	1.194, s	1.172, s	1.193, s
H-17′	1.591, s	1.030, s	1.027, s	1.028,[b] s	1.036,[b] s	1.616, d ($J = 2.2$)	1.074, s	1.343, s	1.331, s	1.343, s
H-18′	1.600, s	1.718, s	1.721, s	1.717, s	1.673, s	1.821, s	1.737, s	1.679, s	1.617, s	1.680, s
H-19′	1.821, s	1.972, s	1.971, s	1.969, s	1.980, s	1.969, s	1.973,[b] s	1.805, d ($J = 1.3$)	1.714, s	1.803, s
H-20′	1.903, s	1.972, s	1.971, s	1.963, s	1.980, s	1.969, s	1.973, s	1.948, s	1.944, s	1.951, s

[a] Tentative assignment. [b] Assignment may be interchanged for values in the same column.

(*Z*)-double bond moved from a peripherical position to the center of the molecule as from the (*all-E*)- (% III/II 22, % A_B/A_{II} 0) to (9*Z*)- (% III/II 20, % A_B/A_{II} 10), to (13*Z*)- (% III/II 4, % A_B/A_{II} 45), and to the (15*Z*)-isomer (% III/II 0, % A_B/A_{II} 56). Compound **2** displayed UV−vis, in MeOH, at λ_{max} 450, 475 nm, **3** at 342, 446, 472 nm, **4** at 338, 444, (468) nm, and **5** at 338, 448, (472) nm. All β-carotene isomers showed similar MS and molecular ion at *m*/*z* 536, which is consistent with $C_{40}H_{56}$. The ^{1}H NMR data (Table 1) of the end groups and of the polyenic chain are in agreement with the data given by Englert (1995) and by Koyama et al. (1989) for these parts of structure, respectively. The isomerization shift values ($\Delta\delta = \delta_{(Z)} - \delta_{(all-E)}$) confirmed the configurations assigned for these carotenoids, according to values presented by Koyama et al. (1989) and Englert (1995).

(*all-E*)-γ-Carotene (6) ((*all-E*)-β,ψ-Carotene). As expected, the maxima wavelengths at (434), 460, 490 nm in MeOH and spectral fine structure (% III/II 48) of **6**, with one β ring and a ψ end group, fits between the values obtained for β-carotene, with two β rings, and lycopene, which shows two ψ end groups. The molecular ion in the mass spectrum appeared at *m*/*z* 536, consistent with $C_{40}H_{56}$. Most of the signals in the ^{1}H NMR spectrum (Table 1) were partially or totally overlapped because the sample concentration was too low, and also impurities or degradation products were present. The COSY spectrum had a signal-to-noise ratio too low for complete analysis. However, most of the end group resonances (especially the methyl signals) and some characteristic in-chain resonances were identified, and are in agreement with the values given by Englert (1995). As a result, carotene **6** was assigned as (*all-E*)-γ-carotene without doubt.

(*all-E*)- (7), (9*Z*)- (8), (13*Z*)- (9), (15*Z*)- (10) Lycopene (ψ,ψ-Carotene). The maximum absorption and the high degree of spectral fine structure in the UV−vis spectra indicated that carotenoids **7**, **8**, **9**, and **10** have 11 conjugated double bonds, with no steric hindrance. As observed for the isomers of β-carotene, the degree of spectral fine structure decreased and the intensity of the *cis* peak increased as the *Z* double bond moved from a peripherical position to the center of the molecule as from the (*all-E*)-lycopene (% III/II 65, % A_B/A_{II} 8) to (9*Z*)- (% III/II 55, % A_B/A_{II} 11), to (13*Z*)- (% III/II 54, % A_B/A_{II} 58), and to (15*Z*)-isomer (% III/II 43, % A_B/A_{II} 74). Carotenoid **7** displayed UV−vis, in MeOH, at λ_{max} 362, 444, 470, 500 nm, **8** at 360, 442, 466, 496 nm, **9** at 360, 440, 466, 496 nm, and **10** at 360, 444, 470, 500 nm. As expected, all lycopene isomers showed similar MS and molecular ion at *m*/*z* 536, which is consistent with $C_{40}H_{56}$. The ^{1}H NMR data of lycopene isomers (carotenoids **7**, **8**, **9**, and **10**) and the ^{13}C δ of carotenoids **7** and **10** were identical with the values from the literature (Hengartner et al., 1992; Englert, 1995). The ^{13}C NMR data of (9*Z*)- and (13*Z*)-lycopene are presented in Table 2. The isomerization shift values ($\Delta\delta = \delta_{(Z)} - \delta_{(all-E)}$) were in agreement with the values of Hengartner et al. (1992) and Englert (1995), confirming the configurations assigned for these carotenoids.

(*all-E*,3*R*)-β-Cryptoxanthin (11) ((*all-E*,3*R*)-β,β-Caroten-3-ol). As expected, the UV−vis spectrum of **11** showed similar absorption maxima (445, 474 nm in *t*-BME (% III/II 14); 449, 476 nm in MeOH/*t*-BME (95:5) (% III/II 22)), shape and degree of spectral fine structure as the spectrum of (*all-E*)-β-carotene **(2)**. The molecular ion was observed at *m*/*z* 552, which is consistent with $C_{40}H_{56}O$. The ^{1}H and ^{13}C chemical shift values (Tables 1 and 2) of the end groups were identical with data given by Englert (1995), confirming the structure assigned. The shape of the conservative CD spectrum of **11** was similar to authentic (*all-E*,3*R*)-β-cryptoxanthin (Noack and Thomson, 1979). Carotenoid **11** displayed CD spectrum at 200 (+0.7), 208 (+14.5), 215 (0.0), 226 (−23.9), 235 (0.0), 251 (+19.2), 268 (0.0), 290 (−20.7), 323 (0.0), 354.0 (+5.4), 385 (0.0), 421 (0.0), 459.0 (+1.2), 468 (0.0), 498 (+2.5), 505 (0.0).

Table 2. ^{13}C NMR Data of Some Carotenoids Isolated from Guava

	δ (ppm)						
C-no.	**8**	**9**	**11**	**13**	**14**	**15**	**16**
C-1		131.22	34.28				
C-2	123.9	123.93	39.66	39.5	48.4	49.5	49.6
C-3	26.6	26.69	19.27	19.0	64.9	70.5	
C-4	40.3	40.23	33.12	32.8	42.4	48.7	48.9
C-5		139.76	129.40				
C-6		125.70	137.92				
C-7	126.1	125.14	126.70	126.5	125.3		
C-8		135.30	137.76	137.5	138.4	103.1	103.2
C-9		136.37	136.08				
C-10		131.48	130.82	126.4	131.2	128.6	128.5
C-11		125.09	125.11		124.6	124.5	
C-12	135.4[a]	129.23	137.20	136.8	137.5	137.6	137.4
C-13		135.30	136.37				
C-14	132.6	130.93	132.38	132.1	132.4	132.5	132.5
C-15	130.0	128.77	129.92	129.8	129.9	130.0	129.8
C-16	25.8	25.70	29.69	28.7	28.4	29.2	29.4
C-17	17.6	17.71	29.69	28.7	30.1	32.1	32.1
C-18	16.9	16.96	21.76	21.2	21.5	31.2	31.6
C-19	20.8	12.90[b]	12.81	12.5	12.6	13.7	14.2
C-20	12.8	20.72	12.75	12.5	12.6	12.8	12.7
C-1′		131.22	37.13				
C-2′	123.9	123.93	48.45		44.6	46.4	47.4
C-3′	26.6	26.69	65.10	67.6	65.8	67.8	68.0
C-4′	40.3	40.23	42.57		124.3	47.3	47.4
C-5′		139.76	126.15				
C-6′	125.7	125.71	137.92		54.9		
C-7′	124.8	124.74	125.55	118.6	128.4	120.0	118.5
C-8′	135.5	135.41	138.51	88.2	137.5	87.5	88.4
C-9′		136.37	134.99				
C-10′	131.5	131.54	131.33	125.7	130.6	127.2	125.9
C-11′	125.0	125.01	124.85		124.5	124.1	124.2
C-12′	137.4	137.38	137.60	136.7	137.5	137.6	137.4
C-13′		136.56	136.59				
C-14′	132.6	132.62	132.64	132.1	132.4	132.3	132.5
C-15′	130.0	129.35	130.14	129.8	129.9	130.0	129.8
C-16′	25.8	25.70	28.98	31.1	24.1	31.2	31.2
C-17′	17.6	17.71	30.26	27.8	29.4	29.1	28.0
C-18′	17.0	16.96	21.62	30.4	22.7	29.0	30.5
C-19′	12.8	12.90[b]	12.81	13.1	12.9	12.3	13.2
C-20′	12.8	12.77[b]	12.75	12.5	12.6	12.8	12.7

[a] Tentative assignment. [b] Assignment may be interchanged.

(*all-E*,3*R*)-Rubixanthin (12) ((*all-E*,3*R*)-β,ψ-Caroten-3-ol). As expected, the UV−vis spectrum of **12** exhibited the same absorption maxima (λ_{max} at 433, 460, 492 nm in hexane/EtOAc (9:1), % III/II 40) and characteristics (shape and degree of spectral fine structure) as the spectrum of (*all-E*)-γ-carotene **(6)**. The molecular ion was observed at *m*/*z* 552, which is consistent with $C_{40}H_{56}O$. The ^{1}H NMR chemical shifts and coupling constants were compatible with values given by Märki-Fisher et al. (1983). Chirality was suggested by analogy with **11**.

(*all-E*,3*S*,5*R*,8*S*)-Cryptoflavin (13) ((*all-E*,3*S*,5*R*,8*S*)-5,8-Epoxy-5,8-dihydro-β,β-caroten-3-ol). The presence of one 5,8-epoxide group in **13** was indicated by a hypsochromic shift of ca. 20 nm (403, 425, 452 nm in *t*-BME (% III/II 62); 404, 428, 452 nm in hexane/EtOAc

(9:1) (%III/II 64)) compared to the maxima absorption of (*all-E*)-*β*-carotene **(2)** in the UV−vis spectrum. The mass spectrum showed the molecular ion at *m*/*z* 568, which is consistent with $C_{40}H_{56}O_2$. The assignment of the (8*S*) stereochemistry was based on NMR spectra, where the ^{1}H and ^{13}C chemical shifts (Tables 1 and 2) of H−C(7) and H−C(8) appeared at 5.30 and 118.6 ppm, and at 5.07 and 88.2 ppm, characteristic for this stereoisomer (Englert, 1995). The CD spectrum showed signs at 200 (−5. 9), 206 (−11.9), 215 (0.0), 228 (+3.6), 246 (+0.2), 247 (0.0), 261 (−1.0), 271 (0.0), 293 (+2.0), 303 (+2.3), 465 (+1.3), 470 (0.0).

Although TEA was added during chromatography on silica to avoid epoxide−furanoxide rearrangement, the presence of **13** in fresh guavas should be taken with caution.

(*all-E*,3*R*,3′*R*,6′*R*)-Lutein (14) ((*all-E*,3*R*,3′*R*,6′*R*)-*β*,*ε*-Carotene-3,3′-diol). The maxima wavelengths (422, 444, 474 nm in hexane/EtOAc (85:15)) and spectral fine structure (% III/II 63) were in agreement with the data of the literature (Britton, 1995). The mass spectrum exhibited the molecular ion at *m*/*z* 568, consistent with $C_{40}H_{56}O_2$, and fragment at *m*/*z* 550 corresponding to the loss of a hydroxy group. The ^{1}H NMR values were in agreement with published values (Mayer and Rüttimann, 1980; Khachik et al., 1992) and ^{13}C δ values (Table 2) of the end groups were identical with data given by Englert (1995). The chirality was suggested by biosynthetic analogy.

(*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*R*)-Neochrome (15) ((*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*R*)-5′,8′-Epoxy-6,7-didehydro-5,6,5′,8′-tetrahydro-*β*,*β*-carotene-3,5,3′-triol) and (*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*S*)-Neochrome (16) ((*all-E*,3*S*,5*R*,6*R*,3′*S*,5′*R*,8′*S*)-5′,8′-Epoxy-6,7-didehydro-5,6,5′,8′-tetrahydro-*β*,*β*-carotene-3,5,3′-triol). The presence of one 5,8-epoxide group in **15** and **16** was indicated by a hypsochromic shift of ca. 20 nm compared to the maxima absorption of (*all-E*)-*β*-carotene in the UV−vis spectrum. Carotenoid **15** displayed UV−vis at λ_{max} 395, 418, 445 nm in *t*-BME (% III/II 75); 400, 424, 451 nm in hexane/acetone (8:2) (% III/II 91) and carotenoid **16** UV−vis at λ_{max} 375, 420, 446 nm in *t*-BME (% III/II 86), and 400, 424, 451 nm in hexane/acetone (85:15) (% III/II 93). The mass spectrum showed the molecular ion at *m*/*z* 600, which is consistent with $C_{40}H_{56}O_4$. The assignment and distinction between the 8′*R*- and 8′*S*-isomers **15** and **16** was based on their NMR spectra (data in Tables 1 and 2), where a shift difference of 0.1 for ^{1}H and 0.9 ppm for ^{13}C in H−C(8′) was observed. The shape of the CD spectra of **15** and **16** were similar with the spectra reported at room temperature by Märki-Fischer et al. (1984) for these compounds, confirming the *R*- and *S*-configuration at the C-8′, respectively. The neochromes are formed by an acid-catalyzed reaction of neoxanthin. This reaction may occur either during extraction/isolation (Schiedt and Liaaen-Jensen, 1995) or during the aging process in living plants (Eugster and Märki-Fischer, 1991). Carotenoid **15** displayed CD spectrum at 200 (+7.0), 207 (+5.5), 209 (+5.7), 225 (+1.3), 231 (+1.5), 252 (+3.7), 263 (+2.2), 280 (+3.0), 283 (+3.2), 351 (0.0), 366 (−0.3), 424 (0.0), 427 (+0.2), 428 (0.0), 452 (0.0), 458 (+0.8), 460 (0.0) and the CD spectrum of carotenoid **16** showed signs at 200 (−3.1), 211 (−6.9), 231 (−0.5), 233 (−0.4), 252 (−3.0), 260 (0.0), 265 (+0.4), 279 (+0.7), 317 (−0.5), 426 (+1.6), 457 (+2.3).

Of the 16 carotenoids identified in the present study, *β*-carotene, *γ*-carotene, and lycopene were also previously isolated by Padula and Rodriguez-Amaya (1986) from red guavas from Brazil. These authors mentioned that the lycopene fraction contained small amounts of (*Z*)-isomers, but did not identify them. (9*Z*)- **(3)**, (13*Z*)- **(4)**, (15*Z*)-*β*-carotene **(5)**, (9*Z*)- **(8)**, (13*Z*)- **(9)**, (15*Z*)-lycopene **(10)**, *β*-cryptoxantin **(11)**, rubixanthin **(12)**, cryptoflavin **(13)**, lutein **(14)**, and the neochromes (**15** and **16**) are being reported for the first time as carotenoids from guava.

LITERATURE CITED

Bendich, A. Recent advances in clinical research involving carotenoids. *Pure Appl. Chem.* **1994**, *66*, 1017−1024.

Britton, G. UV/Visible Spectroscopy. In *Carotenoids Vol 1B: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; pp 13−62.

Chan, H. T., Jr. Passion Fruit, Papaya and Guava Juices. In *Fruit Juice Processing Technology*; Nagy, S., Chen, C. S., Shaw, P. E., Eds.; Agscience Inc.: Auburndale, 1993; pp 334−377.

Davies, B. H. Carotenoids. In *Chemistry and Biochemistry of Plant Pigments*; Goodwin, T. W., Ed.; Academic Press: London, 1976; Vol. 2, pp 38−165.

Englert, G. NMR Spectroscopy. In *Carotenoids Vol 1B: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds; Birkhäuser: Basel, 1995; pp 147−260.

Eugster, C. H.; Märki-Fisher, E. The chemistry of the colorants in roses. *Angew. Chem.* **1991**, *103*, 671.

Fonseca, H.; Nogueira, J. N.; Marcondes, A. M. S. Teor de ácido ascórbico e *β*-caroteno em frutas e hortaliças brasileiras (Ascorbic acid and *β*-carotene contents of Brazilian fruits and vegetables). *Arch. Latinoamer. Nutr.* **1969**, *19*, 9−16.

Gonzalez-Abreu, A.; Moreira, T.; Villegas, M. I. Preliminary determination of the main carotenes in several fruit pulps. *Rev. Cienc. Biol.* (*Havana*) **1985**, *16*, 1−6.

Gross, J. *Pigments in Fruits*; Academic Press: London, 1987; pp 143−144.

Hengartner, U.; Bernhard, K.; Meyer, K.; Englert, G.; Glinz, E. Synthesis, isolation, and NMR-spectroscopic characterization of fourteen (*Z*)-isomers of lycopene and of some acetylenic didehydro- and tetradehydrolycopenes. *Helv. Chim. Acta* **1992**, *75*, 1848−1865.

Khachik, F.; Englert, G.; Daitch, C. E.; Beecher, G. R.; Tonucci, L.; Lusby, W. R. Isolation and structural elucidation of the geometrical isomers of lutein and zeaxanthin in extracts from human plasma. *J. Chromatogr.* **1992**, *582*, 153−166.

Krinsky, N. I. The biological properties of carotenoids. *Pure Appl. Chem.* **1994**, *66*, 1003−1010.

Koyama, Y.; Hosomi, M.; Hashimoto, H.; Shimamura, T. ^{1}H NMR spectra of the all-*trans*, 7-*cis*, 9-*cis*, 13-*cis* and 15-*cis* isomers of *β*-carotene: elongation of the double bond and shortening of the single bond toward the center of the conjugated chain as revealed by vicinal coupling constants. *J. Mol. Struct.* **1989**, *193*, 185−201.

Märki-Fischer, E.; Marti, U.; Buchecker, R.; Eugster, C. H. Das Carotinoidspektrum der Hagebutten von Rosa pomifera: Nachweis von (5*Z*)-Neurosporin; Synthese von (3*R*,15*Z*)-Rubixanthin (Carotenoids from hips of *Rosa pomifera*: discovery of (5*Z*)-neurosporene; synthesis of (3*R*,15*Z*)-rubixanthin). *Helv. Chim. Acta* **1983**, *66*, 494−513.

Märki-Fischer, E.; Buchecker, R.; Eugster, C. H. Trennung und absolute Konfiguration der C(8)-epimeren (all-E)-Neochrome (Trollichrome) und -Dinochrome (Separation and absolute configuration of the C(8)-epimeric (*all-E*)-neochromes (trollichromes) and -dinochromes). *Helv. Chim. Acta* **1984**, *67*, 461−466.

Mayer, H.; Rüttimann, A. Synthese von optisch aktiven, natürlichen Carotenoiden und strukturell verwandten Naturprodukten. IV. Synthese von (3R,3′R,6′R)-Lutein (Synthesis of optically active natural carotenoids and structurally related compounds. IV. Synthesis of (3*R*,3′*R*,6′*R*)-lutein). *Helv. Chim. Acta* **1980**, *63*, 1451−1455.

Nakasone, H. Y.; Brekke, J. E.; Cavaleto, C. G. Fruit and yield evaluation of 10 clones of guava (*Psidium guajava* L.). Research Report, Hawaii Agricultural Experimental Station, 1976; no. 218, 17 pp.

Noack, K.; Thomson, A. L. Conformation and optical activity of all-*trans*, mono-*cis*, and di-*cis* carotenoids: temperature-dependent circular dichroism. *Helv. Chim. Acta* **1979**, *62*, 1902−1921.

Padula, M.; Rodriguez-Amaya, D. B. Characterisation of the carotenoids and assessment of the vitamin A value of Brasilian guavas (*Psidium guajava* L.). *Food Chem.* **1986**, *20*, 11−19.

Schiedt, K.; Liaaen-Jensen, S. Isolation and Analysis. In *Carotenoids Vol 1A: Isolation and Analysis*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; pp 81−108.

Wilberg, V. C.; Rodriguez-Amaya, D. B. HPLC quantitation of major carotenoids of fresh and processed guava, mango and papaya. *Food Sci. Technol.* **1995**, *28*, 474−480.

Received for review April 22, 1998. Revised manuscript received October 6, 1998. Accepted October 7, 1998. We thank FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) for the postdoctoral grant to A.Z.M., Swiss National Foundation for financial support, and F. Hoffmann-La Roche for financial support, standards, and for measuring the CD spectra.

JF980405R