Isolation and Identification of New Apocarotenoids from Annatto (*Bixa orellana*) Seeds

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Five apocarotenoids were isolated in trace amounts from the seed coat of the *Bixa orellana* L. fruits. Their structures were established by means of UV−visible, 400 and 500 MHz ^{1}H and 100 and 120 MHz ^{13}C NMR spectroscopy, and mass spectrometry. Methyl (7*Z*,9*Z*,9′*Z*)-apo-6′-lycopenoate, methyl (9*Z*)-apo-8′-lycopenoate, methyl (*all-E*)-apo-8′-lycopenoate are new carotenoids, and the other two, methyl (*all-E*)-8′-apo-*β*-caroten-8′-oate and methyl (*all-E*)-apo-6′-lycopenoate, have not been previously found in annatto.

Keywords: *Bixa orellana; annatto; apocarotenoids; structure elucidation*

INTRODUCTION

Among the naturally occurring colorants, annatto ranks second in economic importance (Ghiraldini, 1989). Since the demand for natural colorants is increasing, more stringent specifications for these products are imposed and also better understanding of their chemistry and biochemistry is required.

Annatto extracts are obtained from the seed coat of the *Bixa orellana* L. fruits. The rapidly growing, large tree *B. orellana* is native to tropical America. It bears clusters of brown or crimson capsular fruits that contain 10−50 seeds covered with a thin, highly colored resinous coating. The major producers of annatto seeds are Peru, Brazil, and Kenya, and the largest importers are U.S.A., Japan, and England.

Originally intended for the butter and cheese industry, annatto has also found use in coloring of margarine, fats, baked goods, snacks, ice-cream, salad dressings, yoghurts, drinks, meat, and fish.

It has been reported that more than 80% of the carotenoids in the *B. orellana* seed coat consists of bixin [methyl hydrogen (9′*Z*)-6,6′-diapocarotene-6,6′-dioate] (Preston and Rickard, 1980; Lauro, 1991). Other carotenoids have been detected in trace amounts. Tirimanna (1981) postulated the presence of *β*,*β*-carotene, cryptoxanthin, lutein, zeaxanthin, and methyl bixin on the basis of TLC behavior compared to standards. From UV−visible (UV−vis), mass (MS), and ^{1}H nuclear magnetic resonance (NMR) spectra, Jondiko and Pattenden (1989) established the structure of a new apocarotenoid, methyl (9*Z*)-8′-oxo-8′,6-diapocarotene-6-oate. Recently, Mercadante et al. (1996) isolated and identified methyl (9′*Z*)-apo-6′-lycopenoate by UV−vis, MS, and ^{1}H NMR spectra; C_{40} carotenes (phytoene, phytofluene, *ζ*-carotene, and neurosporene) were also characterized.

In continuation of our work on minor carotenoids from annatto, the present study reports the isolation and structure elucidation of three new apocarotenoids and two carotenoids isolated for the first time from annatto.

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MATERIALS AND METHODS

The procedure previously developed for the isolation of methyl (9′*Z*)-apo-6′-lycopenoate (Mercadante et al., 1996) was partially modified to avoid the use of large amounts of chlorinated solvents and to obtain reasonable amounts of the carotenoids for NMR measurement.

Apparatus. The final purification was performed by high-performance liquid chromatography (HPLC), with two or three Kontron pumps, and a Rheodyne injection valve with a 100 *μ*L loop. For detection, either a Waters PDA 990 or a PDA 991 photodiode array system was used. All separations were carried out with a 250 × 10 i.d. mm Nucleosil (Macherey-Nagel, Switzerland) reversed-phase (C_{18}), end-capped column of 3 *μ*m particle size, at a flow rate of 3 mL/min. UV−visible spectra were obtained with a Perkin-Elmer 554 spectrophotometer. Electron impact mass spectra were measured with an MS9 (AE, U.K.) equipped with a ZAB console (VG, U.K.) and an SS300 data system (Finnigan, Germany). The carotenoids were introduced by a direct probe inlet system at 70 eV, from 150 to 200 °C. The mass fragments obtained were compared with those described in the literature (Enzell et al., 1969; Enzell and Back, 1995). 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 $CDCl_3$ (99.95%) under argon that had been passed twice through an alumina minicolumn. 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 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 (correlated spectroscopy) experiments. The sample amounts of carotenoids **2**−**4** were insufficient to perform ^{13}C-detected experiments (^{13}C, DEPT 135), and therefore the *δ* values of the proton-bearing carbon nuclei were extracted from proton-detected HMQC (heteronuclear multiquantum coherence) experiment (optimized for $^1J_{CH}$) with a precision of ±0.2 ppm.

Plant Material. Dried *B. orellana* seeds were purchased in Brazil and transported by air to Switzerland. The intact material was stored in the dark at room temperature until analysis.

Extraction. Carotenoid extract was prepared by stirring 650 g of whole dried annatto seeds five times with ethyl acetate (EtOAc) and three times with *tert*-butyl methyl ether (*t*-BME), in the dark, for around 24 h. The combined organic solvents were evaporated on a rotary evaporator under reduced pressure (T < 30 °C) to dryness, yielding 46 g of a deep-red oleoresin.

Flash Column Chromatography. The carotenoid extract was redissolved in 5 mL of dichloromethane and 50 mL of hexane/*t*-BME (1:1) and applied to a neutral aluminum oxide

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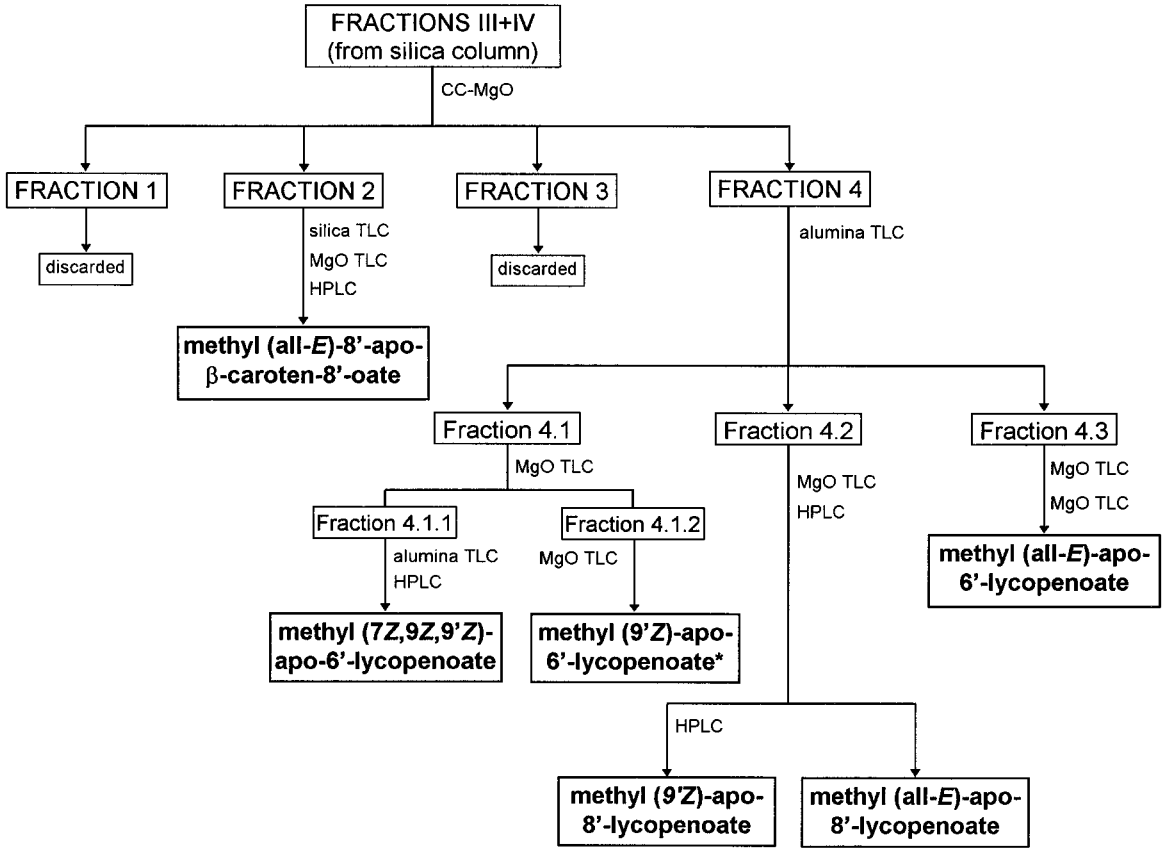
Scheme 1. Chromatographic Steps Employed for the Isolation of Five Carotenoids from Annatto



*Isolation described previously (Mercadante et al., 1996).

flash column (activity between grades III and IV). The fraction eluted with hexane/*t*-BME (1:1) was collected, under a slight pressure on the top of the column, whereas the polar carotenoids (mostly bixin) remained on the column. The eluted fraction was concentrated to dryness (12.3 g) on a rotary evaporator and redissolved in petroleum ether. Further separation was performed on a silica flash column. The elution was carried out by increasing concentration of toluene in hexane (bands I and II) from 0 to 100% followed by 0 to 80% of EtOAc in hexane (bands III to VI). The third and fourth fractions (III + IV), which eluted together with hexane/EtOAc (80:20), are the subject of this communication. Scheme 1 gives a synopsis of the chromatographic steps employed for isolation of the carotenoids from fraction III + IV. This fraction was separated into four fractions on a MgO (BDH, U.K.):Celite (1:2) flash column. Fractions 1 and 3, eluted with hexane/*t*-BME (80:20) and acetone/water (95:5), respectively, were discarded due to the presence of a high amount of oil. Fractions 2 and 4 were eluted with acetone/hexane (60:40) and acetone/water (50:50), respectively.

Final Isolation. The second fraction (fraction 2, Scheme 1) was applied to a silica gel thin-layer chromatography (TLC) plate (0.25 mm), which was developed with hexane/*t*-BME (90:10). The major band ($R_f = 0.45$) was scraped off, desorbed with EtOAc, and submitted to a MgO:Kieselguhr TLC plate (0.5 mm), with hexane/acetone (85:15) as mobile phase. In order to separate *E*/*Z* isomers, the final purification was performed by HPLC, using MeOH/*t*-BME (95:5) as mobile phase. The carotenoid was eluted at $t_R = 15.1$ min.

As shown in Scheme 1, fraction 4 was separated in three bands on an aluminum oxide TLC plate ($R_f = 0.59, 0.54, 0.49$), using hexane/dichloromethane (60:40) as mobile phase. The first band (fraction 4.1) was applied to a MgO:Kieselguhr TLC plate and developed with hexane/acetone (1:1), where two fractions were separated. Fraction 4.1.1 was applied to an aluminum oxide TLC plate [mobile phase: hexane/*t*-BME (80:20)], the main band was isolated, and the final pure apocarotenoid was obtained by HPLC with MeOH:2-isopropyl alcohol (2-propOH) (80:20) as mobile phase ($t_R = 12.28$ min). The isolation and identification of fraction 4.1.2 [methyl (9′*Z*)-apo-6′-lycopenoate] are described elsewhere (Mercadante et al., 1996). Fraction 4.2 (Scheme 1) was submitted to a MgO:Kieselguhr TLC plate (acetone/hexane (70:30) as mobile phase) from where the main band was scraped off. The HPLC [mobile phase: MeOH/2-propOH (85:15)] analysis of this fraction showed two peaks of similar intensities with $t_R = 13.5$ and 14.8 min. The third band (fraction 4.3) was purified twice by TLC with MgO:Kieselguhr, with acetone/hexane (80:20) and EtOAc/acetone/hexane (50:30:20) as mobile phases.

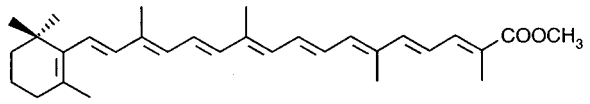
Isomerization Reaction. A few drops of an iodine solution in hexane were added to the pigments dissolved in *t*-BME (Davies, 1976). This mixture was exposed to light, and the reaction was followed spectrophotometrically at 5, 15, and 30 min.

RESULTS AND DISCUSSION

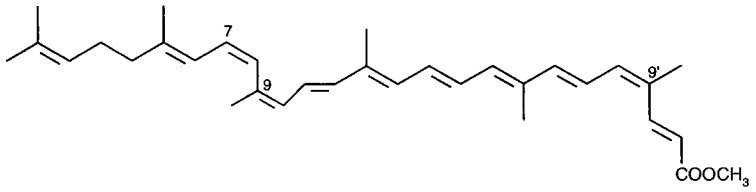
Figure 1 shows the structures of the five carotenoids isolated from annatto. The carotenoids **2**−**4** have not yet been described so far in the literature (Straub, 1987; Kull and Pfander, 1995), and **1** and **5** are known carotenoids which have not previously been isolated from annatto.

The identification of the isolated carotenoids (**1**, 0.3 mg; **2**, 0.9 mg; **3**, 0.7 mg; **4**, 0.7 mg; **5**, 0.6 mg) was based on UV−vis, mass, and NMR spectra. An isomerization reaction catalyzed by iodine was also performed.

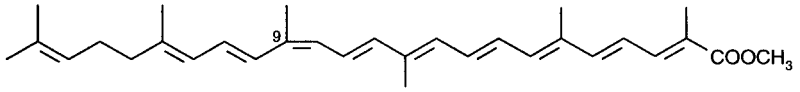
One major carotenoid was isolated from fraction 2. According to its UV−vis, mass, and 400 MHz ^{1}H-NMR spectra the apocarotenoid was identified as methyl (*all-E*)-8′-apo-*β*-caroten-8′-oate **(1)**. Compound **1** displayed UV−vis λ_{max} at 442 and 462 nm in *t*-BME, with no significant fine structure, as reported for the same compound isolated previously from *Staphylococcus aureus* 209P (Taylor and Davies, 1983). After isomerization with I_2, an hypsochromic shift of 1 nm was



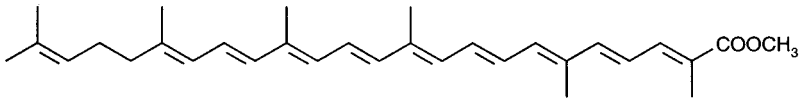
methyl (all-*E*)-8'-apo-β-caroten-8'-oate **(1)**



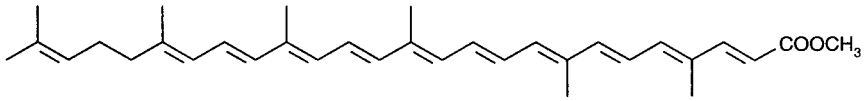
methyl (7*Z*,9*Z*,9'*Z*)-apo-6'-lycopenoate **(2)** or
methyl (7*Z*,9*Z*,9'*Z*)-6'-apo-ψ-caroten-6'-oate



methyl (9*Z*)-apo-8'-lycopenoate **(3)** or
methyl (9*Z*)-8'-apo-ψ-caroten-8'-oate



methyl (all-*E*)-apo-8'-lycopenoate **(4)** or
methyl (all-*E*)-8'-apo-ψ-caroten-8'-oate



methyl (all-*E*)-apo-6'-lycopenoate **(5)** or
methyl (all-*E*)-6'-apo-ψ-caroten-6'-oate

Structures of the apocarotenoids isolated from annatto seeds.

Figure 1. Structures of the apocarotenoids isolated from annatto seeds.

observed. The mass spectrum (EI, 70 eV) exhibited peaks at *m*/*z* 446 (34), 354 (2), 339 (3), 69 (100). The molecular ion peak appeared at *m*/*z* 446 (consistent with $C_{31}H_{42}O_2$), and characteristic fragments were detected at *m*/*z* 354 $[M - 92]^+$, due to the elimination of toluene from the polyene chain, and at *m*/*z* 339 $[M - 92 - 15]^+$. The δ and relevant *J* values for the ^{1}H NMR (400.13 MHz, $CDCl_3$) spectrum of **1** were as follows: 1.03 (6H, s, H-16 and H-17), 1.44 (2H, m, H-2), 1.61 (2H, m, H-3), 1.71 (3H, s, H-18), 1.96 (12H, s, H-19, H-20, H-19′ and H-20′), 2.02 (2H, m, H-4), 3.76 (3H, s, 8′-$COOCH_3$), 6.13 (1H, B part of AB system, $J = 16.2$ Hz, H-8), 6.15 (1H, d, $J \approx 11$, H-10), 6.19 (1H, A part of AB system, $J = 16.2$ Hz, H-7), 6.25 (1H, d, $J = 11.7$ Hz, H-14), 6.34 (1H, d, $J \approx 15$, H-12), 6.35 (1H, d, $J = 11.2$ Hz, H-14′), 6.50 (1H, dd, $J = 11.2$, 14.6 Hz, H-11′), 6.61 (1H, d, $J = 14.6$ Hz, H-12′), 6.63 (1H, dd, $J = 11.2$, 13.5 Hz, H-15′), 6.68 (1H, dd, $J \approx 11$, ≈15 Hz, H-11), 6.71 (1H, dd, $J = 11.7$, 13.5 Hz, H-15), 7.28 (1H, dd, $J = 11.2$, 1.2 Hz, H-10′). The chemical shifts of the β-ionone end group were identical to the corresponding data from the literature (Englert, 1995). The singlet for the methyl protons of the ester group appeared at 3.76 ppm as for methyl (9′*Z*)-apo-6′-lycopenoate (Mercadante et al., 1996). This is the first time that this carotenoid was isolated from *B. orellana* seeds. It has previously been found only in the bacteria *S. aureus* (Taylor and Davies, 1983).

Four carotenoids were isolated from fraction 4. From fraction 4.1 two carotenoids could be separated and purified. The apocarotenoid methyl (9′*Z*)-apo-6′-lycopenoate was previously isolated from annatto and characterized by Mercadante et al. (1996), whereas the other was unequivocally identified as methyl (7*Z*,9*Z*,9′*Z*)-apo-6′-lycopenoate (**2**) by means of UV−vis, mass, and 500 MHz ^{1}H-NMR spectra. The (7*Z*,9*Z*,9′*Z*) isomer has not been reported as a natural constituent. The UV−vis spectrum of apocarotenoid **2** showed a single broad band at λ_{max} 438 nm in *t*-BME. A bathochromic shift of 20 nm was observed after isomerization catalyzed by I_2, indicating the presence of more than one conjugated (*Z*) double bond. The MS-EI (70 eV) data of **2** were as follows: 472 (46), 446 (3), 403 (7), 380 (2), 366 (28), 297

(14), 145 (100), 119 (99), 69 (91). As expected, the mass spectrum was similar to that of the (9′*Z*) isomer (Mercadante et al., 1996) and of the (*all-E*) isomer (Kjrsen and Liaaen-Jensen, 1969), with the molecular ion at *m*/*z* 472 ($C_{33}H_{44}O_2$). Characteristic loss of 69 mass units from the molecular ion and a peak at *m*/*z* 69 indicated the presence of a ψ-end group. The differences between these three isomers could be easily determined by the NMR spectra. The δ and relevant *J* values from ^{1}H NMR (500.13 MHz, $CDCl_3$) of **2** were the following: 1.56 (3H, s, H-17), 1.65 (3H, s, H-16), 1.80 (3H, s, H-18), 1.85 (3H, s, H-20), 1.95 (3H, s, H-19′), 1.98 (3H, s, H-20′), 2.00 (3H, s, H-19), 2.06 (2H, m, H-3), 2.07 (2H, m, H-4), 3.79 (3H, s, 6′-$COOCH_3$), 5.07 (1H, m, H-2), 5.90 (1H, d, *J* = 15.5 Hz, H-7′), 6.02 (1H, d, *J* = 12.6 Hz, H-8), 6.03 (1H, d, *J* = 11.8 Hz, H-10), 6.10 (1H, d, *J* = 11.6 Hz, H-6), 6.22 (1H, X part of ABMX system, *J* = 11.1 Hz, H-14), 6.29 (1H, d, *J* = 15.2 Hz, H-12), 6.30 (1H, M part of ABMX system, *J* = 11.6 Hz, H-14′), 6.31 (1H, dd, *J* = 11.6, 12.6 Hz, H-7), 6.36 (1H, d, *J* = 12.2 Hz, H-10′), 6.40 (1H, d, *J* = 14.5 Hz, H-12′), 6.50 (1H, dd, *J* = 11.8, 15.2 Hz, H-11), 6.61 (1H, B part of ABMX system, H-15′), 6.65 (1H, A part of ABMX system, H-15), 6.82 (1H, dd, *J* = 12.2, 14.5, H-11′), 7.96 (1H, d, *J* = 15.5 Hz, H-8′). ^{13}C NMR data (125.77 MHz, $CDCl_3$): 12.6 (C-20 and C-20′), 16.5 (C-18), 17.6 (C-17), 20.1 (C-19′), 24.6 (C-19), 25.5 (C-16), 26.5 (C-3), 40.3 (C-4), 51.4 (6′-$COOCH_3$), 117.0 (C-7′), 122.1 (C-6), 122.4 (C-11′), 123.7 (C-2), 125.7 (C-8), 126.0 (C-7 and C-14′), 126.5 (C-11), 129.2 (C-15′), 129.6 (C-10), 131.2 (C-15), 131.6 (C-14), 134.3 (C-12), 138.0 (C-10′), 140.3 (C-8′), 140.5 (C-12′). The ^{1}H and ^{13}C isomerization shift values ($\Delta\delta$) of methyl (7*Z*,9*Z*,9′*Z*)-apo-6′-lycopenoate compared to methyl (9′*Z*)-apo-6′-lycopenoate (^{1}H) (Mercadante et al., 1996), lycopene (^{13}C) and (7*Z*,9*Z*,7′*Z*,9′*Z*)-lycopene (^{1}H, ^{13}C) (Hengartner et al., 1992) were in good agreement with the values reported by Englert (1995), proving the (7*Z*,9*Z*,9′*Z*) configuration of compound **2**. The *J* value of 12.6 Hz found between the vicinal protons H-C(7) and H-C(8) was typical of a (*Z*) arrangement since the (*E*) form should present *J* around 15 Hz. This was an additional argument for the assigned apocarotenoid configuration.

Two apocarotenoids, both reported for the first time in the literature, namely methyl (9*Z*)-apo-8′-lycopenoate (**3**) and methyl (*all-E*)-apo-8′-lycopenoate (**4**) were obtained from fraction 4.2. The (9*Z*) isomer displayed UV−vis maxima in *t*-BME at λ 446 and 471 nm and showed a bathochromic shift of 3 nm after isomerization with I_2. The (*all-E*) isomer showed λ_{max} at 451 and 475 nm (*t*-BME), a hypsochromic shift of ∼4 nm after isomerization. Both UV−vis spectra had no significant fine structure. Mass (EI, 70 eV) spectra of apocarotenoid **3** presented peaks at *m*/*z* 446 (8), 285 (2), 147 (33), and 69 (100), and the mass spectra of compound **4** displayed peaks at *m*/*z* 446 (55), 377 (8), 285 (9), 147 (62), and 69 (100). As expected, both isomers showed similar mass spectra, with the molecular ion at *m*/*z* 446 (corresponding to $C_{31}H_{42}O_2$). The characteristic loss of 69 mass units from the molecular ion and from $[M - 92]^+$ and a peak at *m*/*z* 69 indicated the presence of a ψ-end group. The δ and relevant *J* values from 400 MHz ^{1}H NMR ($CDCl_3$) of **3** were the following: 1.622 (3H, s, H-17), 1.684 (3H, s, H-16), 1.845 (3H, s, H-18), 1.961 (3H, s, H-19), 1.972 (3H, s, H-20′), 1.992 (3H, d, *J* = 1.3 Hz, H-19′), 1.997 (3H, s, H-20), ∼2.12 (4H, m, H-3 and H-4), 3.77 (3H, s, 8′-$COOCH_3$), 5.14 (1H, tt, *J* = 7.1, 1.4, H-2), 6.02 (1H, d, *J* = 11.0 Hz, H-6), 6.04 (1H, d, *J* = 11.7 Hz, H-10), 6.25 (1H, d, *J* = 12.1 Hz, H-14), 6.28 (1H, d, *J* = 14.8 Hz, H-12), 6.36 (1H, dd, *J* = 11.3, 0.5 Hz, H-14′), 6.51 (1H, dd, *J* = 11.3, 15.1 Hz, H-11′), 6.52 (1H, dd, *J* = 11.0, 15.2 Hz, H-7), 6.61 (1H, dd, *J* = 11.3, 14.0 Hz, H-15′), 6.62 (1H, d, *J* = 15.1 Hz, H-12′), 6.72 (1H, dd, *J* = 12.1, 14.0, H-15), 6.76 (1H, d, *J* = 15.2 Hz, H-8), 6.83 (1H, dd, *J* = 11.7, 14.8 Hz, H-11), 7.29 (1H, dq, *J* = 11.3, 1.3 Hz, H-10′). ^{13}C NMR data for **3** (100.62 MHz, $CDCl_3$): 12.6 (C-20′), 12.8 (C-20 and C-19′), 16.6 (C-18), 17.5 (C-17), 20.6 (C-19), 25.5 (C-16), 26.6 (C-3), 40.0 (C-4), 51.7 (8′-$COOCH_3$), 122.9 (C-11′), 123.8 (C-2), 124.4 (C-11), 126.3 (C-6 and C-7), 126.6 (C-8), 129.2 (C-15′), 129.6 (C-10), 131.8 (C-14), 131.9 (C-15), 135.7 (C-14′), 136.2 (C-12), 138.9 (C-10′), 143.9 (C-12′). ^{1}H NMR data (400.13 MHz, $CDCl_3$) for carotenoid **4**: 1.623 (3H, s, H-17), 1.686 (3H, s, H-16), 1.828 (3H, s, H-18), 1.959 (3H, s, H-19), 1.974 (3H, s, H-20′), 1.997 (3H, d, *J* = 1.2 Hz, H-19′), 1.985 (3H, s, H-20), ∼2.12 (4H, m, H-3 and H-4), 3.77 (3H, s, 8′-$COOCH_3$), 5.13 (1H, m, H-2), 5.94 (1H, d, *J* = 11.4 Hz, H-6), 6.19 (1H, d, *J* = 11.8 Hz, H-10), 6.25 (1H, d, *J* = 15.0 Hz, H-8), 6.27 (1H, d, *J* ≈ 11.7 Hz, H-14), 6.36 (1H, d, *J* = 14.9 Hz, H-12), 6.36 (1H, d, *J* ≈ 10.2 Hz, H-14′), 6.51 (1H, dd, *J* = 11.4, 15.0 Hz, H-7), 6.51 (1H, dd, *J* ≈ 11.3, 15.3 Hz, H-11′), 6.62 (1H, d, *J* = 15.3 Hz, H-12′), 6.62 (1H, dd, *J* ≈ 10.2, 14.2 Hz, H-15′), 6.67 (1H, dd, *J* = 11.8, 14.9 Hz, H-11), 6.72 (1H, dd, *J* ≈ 11.7, 14.2, H-15), 7.29 (1H, dq, *J* ≈ 11.3, 1.2 Hz, H-10′). ^{13}C NMR data for **4** (100.62 MHz, $CDCl_3$): 12.6 (C-19′ and C-20′), 12.7 (C-19 and C-20), 16.7 (C-18), 17.4 (C-17), 25.6 (C-16), 26.4 (C-3), 40.0 (C-4), 51.6 (8′-$COOCH_3$), 122.9 (C-11′), 123.8 (C-2), 125.0 (C-7), 125.8 (C-11), 126.3 (C-6), 129.3 (C-15′), 131.2 (C-10), 131.9 (C-15), 132.0 (C-14), 135.2 (C-8), 135.7 (C-14′), 136.9 (C-12), 138.8 (C-10′), 143.7 (C-12′). The ^{1}H and ^{13}C $\Delta\delta$ values of the (9*Z*) isomer compared to the (*all-E*) isomer were in good agreement with the data reported by Englert (1995).

From fraction 4.3, the apocarotenoid methyl (*all-E*)-apo-6′-lycopenoate (**5**) was isolated. It has previously been found as major carotenoid in ripe berries of *Shepherdia canadensis* (Kjøsen and Liaaen-Jensen, 1969). Carotenoid **5** showed λ_{max} at 464 nm, with no fine structure, in *t*-BME. Surprisingly, the same compound isolated by Kjøsen and Liaaen-Jensen (1969) showed different λ_{max} [(448), 471, and 503 nm] and shape in petroleum ether. Methyl (*all-E*)-apo-6′-lycopenoate gave a mass spectrum similar to that described above for the (7*Z*,9*Z*,9′*Z*) isomer and similar to those reported previously for the same compound from *S. canadensis* (Kjøsen and Liaaen-Jensen, 1969) and for the (9′*Z*) isomer (Mercadante et al, 1996). ^{1}H NMR data for carotenoid **5** (400.13 MHz, $CDCl_3$): 1.614 (3H, s, H-17), 1.687 (3H, s, H-16), 1.822 (3H, s, H-18), 1.974 (6H, s, H-19 and H-20), 1.939 (3H, s, H-19′), 1.983 (3H, s, H-20′), ∼2.11 (4H, m, H-3 and H-4), 3.76 (3H, s, 6′-$COOCH_3$), 5.11 (1H, m, H-2), 5.87 (1H, d, *J* = 15.5 Hz, H-7′), 5.95 (1H, d, *J* = 10.2 Hz, H-6), 6.18 (1H, d, *J* = 11.1 Hz, H-10), 6.25 (1H, d, *J* = 15.1 Hz, H-8), 6.26 (1H, m, *J* ≈ 11.3 Hz, H-14), 6.35 (1H, d, *J* ≈ 14.9 Hz, H-12), 6.35 (1H, m, *J* ≈ 11.0 Hz, H-14′), 6.49 (1H, d, *J* ≈ 14.3 Hz, H-12′), 6.51 (1H, dd, *J* = 10.2, 15.1 Hz, H-7), 6.51 (1H, d, *J* ≈ 10.5 Hz, H-10′), 6.60 (1H, dd, *J* ≈ 10.5, 14.3 Hz, H-11′), 6.62 (1H, m, H-15′), 6.67 (1H, dd, *J* = 11.1, ∼14.9 Hz, H-11), 6.70 (1H, m, H-15), 7.39 (1H, d, *J* = 15.5 Hz, H-8′). The values for the methyl protons of the ester group and the ψ-end group were in good agreement with data from the literature (Kjøsen and Liaaen-Jensen, 1969; Mercadante et al., 1996; Englert,

1995). The poor resolution of the proton spectrum previously obtained for the (*all-E*)-isomer isolated from *S. canadensis* (Kjøsen and Liaaen-Jensen, 1969) did not allow a complete data comparison with regard to chemical shift and coupling constant values of the olefinic proton signals.

The present investigation contributes a step forward in the structure elucidation of the carotenoids present in the commonly used food colorant annatto. As to the natural occurrence of the isolated apocarotenoids, it cannot be excluded that they are formed during the drying process. On the other hand, our experimental evidence, such as chromatograms of the whole extract and the known relative stability of carotenoids under the applied experimental conditions, shows that it is highly unlikely that the isolated compounds are artifacts that are formed during the isolation procedure. Furthermore, it should be considered that bixin, a (Z)-diapocarotenoid, is the main pigment in annatto.

In sum, the knowledge gained from the structure elucidation of new minor carotenoids in annatto could lead to better comprehension of the bixin biosynthesis process in annatto.

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