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Isolation and identification of sapotexanthin 5,6-epoxide and 5,8-epoxide from red mamey (*Pouteria sapota*)

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Abstract

Two new carotenoids, sapotexanthin 5,6-epoxide and sapotexanthin 5,8-epoxide, have been isolated from the ripe fruits of red mamey (*Pouteria sapota*). Sapotexanthin 5,6-epoxide was also prepared by partial synthesis via epoxidation of sapotexanthin, and the (5*R*,6*S*) and (5*S*,6*R*) stereoisomers were identified by high-performance liquid chromatography–electronic circular dichroism (HPLC-ECD) analysis. Spectroscopic data of the natural and semi-synthetic derivatives obtained by acid-catalyzed rearrangement of cryptocapsin 5,8-epoxide stereoisomers were compared for structural elucidation.

KEYWORDS

carotenoid, deoxy kappa end group, HPLC-ECD, semisynthesis, structure elucidation

1 | INTRODUCTION

Red mamey (*Pouteria sapota*) is a native fruit in Central America, featuring a pulp of intense red-orange color. It was previously demonstrated that the color can be attributed to the presence of carotenoids, mainly carotenoids with the kappa end group.[1-3] Carotenoids with κ end

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group are not so common in nature, and they usually contain a hydroxylated ring, as in capsanthin, capsorubin, and cryptocapsin. The main source of these carotenoids is the ripened fruit of red paprika (*Capsicum annuum*), but several other sources have been reported, as well.[4]

The detailed carotenoid analysis of red mamey (*P sapota*) has been achieved recently through the implementation of high-performance liquid chromatography–diode-array detector–mass spectrometry (HPLC-DAD-MS), chemical probes, and cochromatography using authentic samples in our laboratory.[3] Altogether, 47 components were found in the extract from which 34 were identified. The main carotenoids were cryptocapsin (**1**), sapotexanthin (**2**), and capsanthin 5,6-epoxide (**3**). Notably, the pulp of red mamey contains several other interesting red carotenoids associated with hydroxylated or nonhydroxylated κ-rings. We have reported earlier the isolation of sapotexanthin (**2**),[5] cryptocapsin 5,6-epoxide (**4**) and 5,8-epoxide (**5**),[6] 3′-deoxycapsanthin,[7] 3′-deoxycapsanthin 5,6-epoxide,[6] 3′-deoxycapsorubin, and 3,3′-dideoxycapsorubin (Figures 1 and 2).[8] Several precursors related to the above-mentioned carotenoids, specifically β-cryptoxanthin 5,6-epoxide and 5′,6′-epoxide[7] and

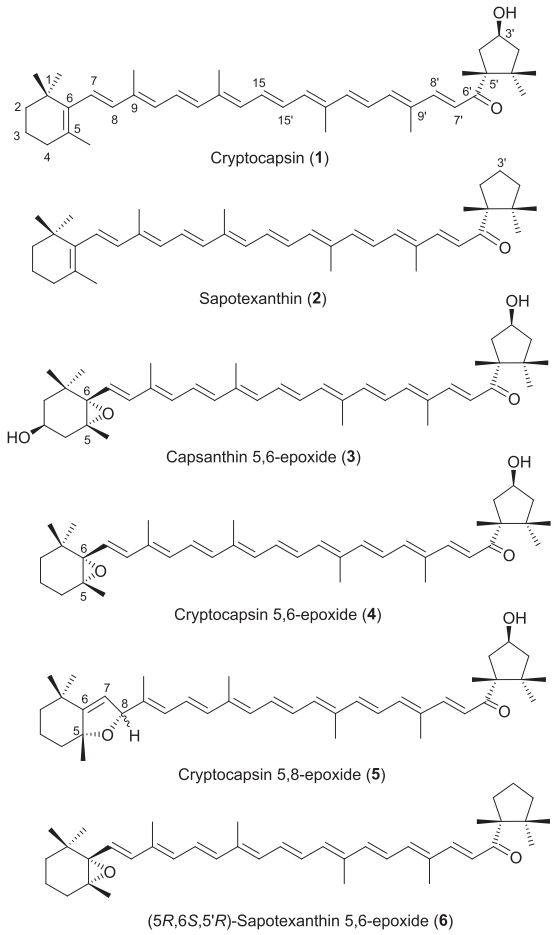

FIGURE 1 Structures of the carotenoids, part 1

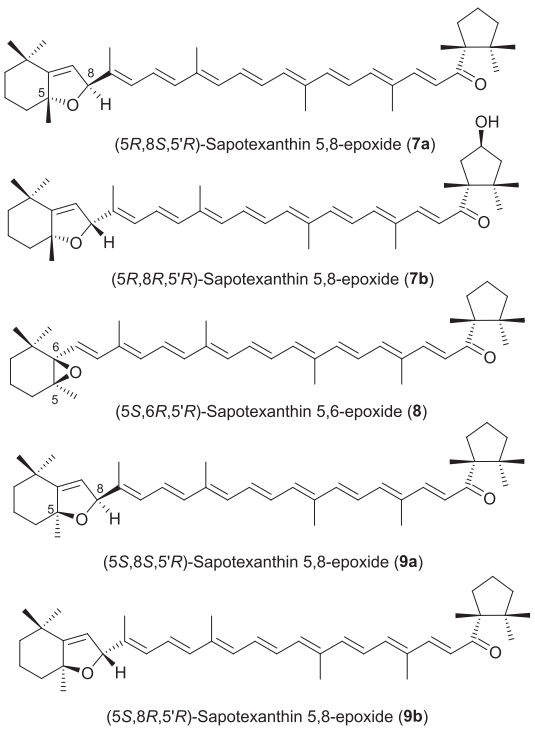

FIGURE 2 Structures of the carotenoids, part 2

5,6,5′,6′-diepoxide, were isolated in our laboratories.[8] For complete structural elucidation MS, electronic circular dichroism (ECD) and nuclear magnetic resonance (NMR) methods were used. The 5,6-epoxy carotenoids and their derivatives (5,8-epoxy carotenoids and kappa carotenoids) constitute nearly 99% of the total carotenoid content of red mamey.[3]

Bouvier et al showed that in paprika extract, kappa carotenoids are biosynthesized from 5,6-epoxy carotenoids and the reaction is catalyzed by the capsanthin-capsorubin synthase (CCS) enzyme.[9] The biosynthesis of capsanthin, capsorubin, and cryptocapsin (**1**) starts from antheraxanthin, violaxanthin, and β-cryptoxanthin 5,6-epoxide, respectively. 5,6-Epoxides of carotenoids with hydroxylated κ-rings are widespread in nature. However, carotenoids containing a 5,6-epoxide unit in the nonhydroxylated β-ring have rarely been reported.

On the level of biosynthesis, the presence of nonhydroxylated κ-rings can be caused by the relatively high activity of certain enzymes (a) that produces epoxides from nonhydroxylated β-rings and (b) that catalyzes the pinacol rearrangement of epoxides.

Since all precursors and products, indicating the presence of the CCS enzyme in connection with 5,6-epoxy sapotexanthin, have been found and reported,[3-8] we assumed the presence of this enzyme in red mamey. This paper reports the isolation and structural elucidation of the new carotenoid sapotexanthin 5,6-epoxide (**6**) and its 5,8-derivatives.

2 | MATERIALS AND METHODS

2.1 | General experimental procedures

The UV-Vis spectra were recorded using a Jasco V-530 and a Shimadzu UV-1203 spectrophotometer in methanol, hexane, and benzene. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured using a Jeol Ellippse +400 spectrometer. Chemical shifts are referenced to internal transcranial magnetic stimulation (TMS) (^{1}H) or to the residual solvent signals (^{13}C). ECD spectra were recorded at room temperature (RT) with a J-810 spectropolarimeter. A Hewlett-Packard liquid chromatograph model 1050, equipped with DAD, and HP ChemStation software was used for studies in the UV-Vis region. The molar masses were obtained by HPLC-APCI-MS in an Agilent 1100 HPLC chromatograph coupled to a JEOL MS LCmate mass spectrometer.

2.2 | Chiral HPLC and HPLC-ECD analysis

Chiral HPLC separations were carried out with a Jasco HPLC system on a Chiralpak IA column (0.46 × 25 cm, 5 μm) using *n*-hexane: *i*-PrOH = 9:1 at a flow rate of 0.5 mL/min for **5** and **7**. HPLC-UV and OR chromatograms were measured using a Jasco MD-910 multiwavelength and OR-2090Plus chiral detector, respectively. The baseline of the chromatograms was zeroed immediately following the beginning of each run, which supported the measurement of the relative absorbance or optical rotation. The HPLC-ECD traces were recorded at the specified wavelength using a Jasco J-810 ECD spectropolarimeter equipped with a 1-cm path length HPLC flow cell, and the baseline was zeroed following the beginning of each run. The online ECD and UV spectra were simultaneously recorded by stopping the flow at the UV absorption maximum of each peak. ECD ellipticity values (φ) were not corrected regarding concentration. For an HPLC-ECD spectrum, three consecutive scans were recorded and averaged with 2-nm bandwidth, 1-second response, and standard sensitivity. The HPLC-ECD spectrum of the eluent was identically recorded. The concentration of the injected sample was set in which the HT (voltage) value did not exceed 500 V in the HT channel.

2.3 | Isolation of the natural sapotexanthin and sapotexanthin 5,6-epoxide and 5,8-epoxide

2.3.1 | Extraction

Matured fruits were purchased from the Metropolitan public market in Panama City, Panama. Carotenoids were extracted from the fresh pulp of ripened red mamey. In reference to the extraction, the method proposed by Britton was used.[10] Two kilograms of the pulp of red mamey was homogenized and extracted several times using acetone until no color remained. The extract was concentrated in a vacuum at 35°C and was placed in a separatory funnel using diethyl ether: hexane 1:1, and it was extracted with 5% brine. The upper phase was concentrated and dissolved in 200 mL of diethyl ether, and 200 mL of 5% methanolic NaOH solution was added. After 1 hour standing in complete darkness, it was rinsed using 5 × 200 mL of 5% brine, dried, and evaporated.

2.3.2 | Open-column chromatography

The extract was dissolved in a small amount of diethyl ether:hexane 9:1 mixture and applied on a column of 24 × 5 cm packed with aluminum oxide Brockmann III. Using diethyl ether:hexane 9:1 as eluent and under suction, a fraction rich in sapotexanthin, 5,6-epoxy sapotexanthin, and 5,8-epoxy sapotexanthin was obtained. This fraction was rechromatografied on another Al_2O_3 column, with a gradient from 100% of hexane to 10% ether in hexane. The fraction that elutes with 3% ether contains primarily sapotexanthin (96%), the fraction that elutes with 6% ether is rich in 5,6-epoxy sapotexanthin, and the 5,8-epoxy sapotexanthin elutes with 8% ether.

2.3.3 | Semipreparative HPLC-DAD

Pure 5,6-epoxy sapotexanthin and 5,8-epoxy sapotexanthin were obtained by isocratic HPLC of the corresponding fractions. Column: 250 × 10.0-mm YMC C30; eluent: MeOH:MTBE:H_20 (60:36:4); flow rate: 2.5 mL/min, detection at 450 nm.

2.3.4 | Alkaline thin-layer chromatography

Silica gel thin-layer chromatography (TLC) plates (10 × 20, 0.25 μm) were immersed in a 1% methanolic NaOH solution for 10 minutes and were dried overnight in RT.[11] These slightly alkaline plates were used for the further purification of the epoxides to avoid furanoid formation. With the eluent *n*-hexane:ether 4:1, the pigment of 99% purity was achieved.

Following semipreparative HPLC and alkaline TLC, 225 μg of sapotexanthin 5,6-epoxide and 310 μg of sapotexanthin 5,8-epoxide were obtained, both at 99% purity.

All fractions were analyzed by analytical C30 HPLC-DAD using conditions previously described.[12]

2.4 | Semisynthesis of sapotexanthin epoxides

2.4.1 | Semisynthesis of sapotexanthin 5,6-epoxide

Nine milligrams of natural sapotexanthin (**2**) (96%) was used for epoxidation, in accordance to the Barua method (Figure 3).[13] It was dissolved in 30 mL of dry diethyl ether, and 5 mg of 77% 3-chloroperbenzoic acid (MCBA, Sigma) was added as an etherial solution of 5 mL. After 4 hours of stirring, the mixture was transferred into a separatory funnel, 10.0 mL of 5% aqueous NaOH was added, and it was extracted using 40 mL of diethyl ether. The ethereal phase was rinsed using 6 × 40 mL of water, dried, and stored under nitrogen, in a brown bottle. The 5,6-sapotexanthin epoxide was purified on a semi-preparative HPLC, as described in the next section, and obtained as a red powder, in 74% yield, and featuring a purity of 99.5% (HPLC).

Isolated (5*R*,6*S*,5′*R*)-sapotexanthin 5,6-epoxide (**6**): red crystals; UV-Vis (benzene): λ_{max} 480 and 505sh nm; λ_{max} after acid treatment: 464, 486 nm; ECD {*n*-hexane, λ [nm] ($\Delta\varepsilon$)}: 214.0 (2.18), 241.0 (1.75), 271.0sh (−1.85), 279.5 (−3.42), 333.0sh (1.12), 349.0 (1.48).

Semisynthetic mixture of (5*R*,6*S*,5′*R*)-sapotexanthin 5,6-epoxide (**6**) and (5*S*,6*R*,5′*R*)-sapotexanthin 5,6-epoxide (**8**): red crystals; UV-Vis (benzene): λ_{max} 480 and 505sh nm; λ_{max} after acid treatment: 464, 486 nm. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.85 (s, 3H, Me-16′), 0.94 (s, 3H, Me-17), 1.11 (s, 3H, Me-16), 1.15 (s, 3H, Me-17′), 1.18 (s, 3H, Me-18), 1.26 (s, 3H, Me-18′), 1.43 (m, 1H, H-3), 1.48 (m, 1H, Hax-4′), 1.50 (m, 1H, Heq-2), 1.57 (m, 1H, Heq-2′), 1.68 (m, 3H, Hax-2′, H-3′), 1.76 (m, 1H, Hax-4), 1.88 (m, 1H, Heq-4), 1.94 (s, 3H, Me-19), 1.96 (s, 3H, Me-19′), 1.98 (s, 6H, Me-20, Me-20′), 2.51 (m, 1H, Heq-4′), 5.90 (d, $J_{7,8}$ = 15.7 Hz, 1H, H-7), 6.19 (d, $J_{10,11}$ = 11.2 Hz, 1H, H-10), 6.27 (d, $J_{14,15}$ = 9.3 Hz, 1H, H-14), 6.29 (d, $J_{8,7}$ = 14.7 Hz, 1H, H-8), 6.34 (d, $J_{14',15'}$ = 10.7 Hz, 1H, H-14′), 6.37 (d, $J_{12,11}$ = 14.2, Hz 1H, H-12), 6.48 (d, $J_{7',8'}$ = 14.2 Hz, 1H, H-7′), 6.51 (d, $J_{12',11'}$ = 12.7 Hz, 1H, H-12′), 6.54 (d, $J_{10',11'}$ = 11.7 Hz, 1H, H-10′), 6.58 (dd, $J_{11',10'}$ = 8.3 Hz, $J_{11',10'}$ = 11.7 Hz, 1H, H-11′), 6.62 (d, $J_{11,10}$ = 9.7 Hz, 1H, H-11), 6.64 (m, 1H, H-15), 6.64 (m, 1H, H-15′), 7.32 (d, $J_{8',7'}$ = 15.1 Hz, 1H, H-8′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 12.7 (CH_3, C-20), 12.8 (CH_3, C-19, 20′), 13.0 (CH_3, C-19′), 17.1 (CH_2, C-3), 19.5 (CH_2, C-3′), 20.9 (CH_3, C-18′), 21.1 (CH_3, C-18), 24.6 (CH_3, C-17′), 25.6 (CH_3, C-16′), 25.9 (CH_3, C-17), 26.0 (CH_3, C-16), 30.1 (CH_2, C-4), 33.8 (C, C-1), 34.4 (CH_2, C-4′), 35.8 (CH_2, C-2), 40.5 (CH_2, C-2′), 44.0 (C, C-1′), 58.9 (C, C-5′), 65.5 (C, C-5), 71.4 (C, C-6), 121.4 (CH, C-8′), 124.2 (CH, C-11′), 124.4 (CH, C-7), 125.3 (CH, C-11), 129.8 (CH, C-15), 131.5 (CH, C-15′), 131.8 (CH, C-10), 132.5 (CH, C-14), 133.8 (C, C-9′), 134.9 (C, C-9), 135.0 (CH, C-14′), 136.0 (C, C-13), 137.3 (CH, C-8), 137.4 (C, C-13′), 137.8 (CH, C-12), 140.3 (CH, C-12′), 141.7 (CH, C-10′), 146.4 (CH, C-7′), 203.8 (C, C-6′).

HPLC-ECD of **6** {*n*-hexane/*i*-PrOH (9:1), λ [nm] (ϕ)}: 210.0sh (−10.84), 214.0 (−15,71), 241.0 (9.38), 271.0sh (−12.32), 279.5 (−22.44), 333.0sh (6.21), 349.0 (8.42).

HPLC-ECD of **8** {*n*-hexane/*i*-PrOH (9:1), λ [nm] (ϕ)}: 207.0sh (4.78), 214.0 (13.73), 241.0 (−14.28), 270.0sh (16.89), 279.5 (31.08), 333.0sh (−6.48), 349.0 (−9.69).

2.4.2 | Semisynthesis of 5,8-epoxy sapotexanthin

Three milligram of the synthetic 5,6-epoxy sapotexanthin was dissolved in 50 mL of methanol, and five drops of 10% HCl in methanol were added. After 5 minutes, 50 mL of hexane and 50 mL of water were added, and the phases were separated. The organic phase was rinsed three more times using water, dried, and evaporated. The isomers of 5,8-epoxy sapotexanthin were purified by semipreparative HPLC (see Section 2.3.3). Under the given conditions, no resolution of the isomers could be achieved. The product appeared as a red powder, in 92% yield, and a purity of 99.5% (HPLC).

Mixture of (5*R*,8*R*/*S*,5′*R*)-(**7a** and **7b**) and (5*S*,8*R*/*S*,5′*R*)-sapotexanthin 5,8-epoxides (**9a** and **9b**): red crystals; UV-Vis (benzene): λ_{max} 464, 486 nm. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.84 (s, 3H, Me-16′), 1.11 (s, 6H, Me-17′, 16), 1.15 (s, 3H, H-17), 1.18 (s, 3H, H-18′), 1.43 (s, 3H, H-18), 1.48 (m, 1H, Hax-4′), 1.54 (m, 1H, Heq-2′),

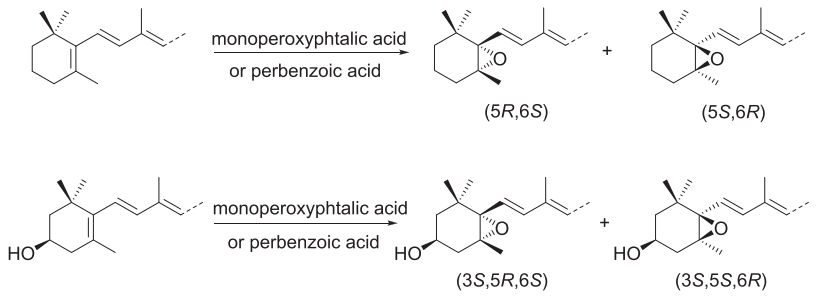

FIGURE 3 Epoxidation of hydroxylated and nonhydroxylated β-rings

1.66 (m, 4H, H-3, H-3′), 1.68 (m, 1H, Hax-2′), 1.95 (brs, 9H, Me-19′, 20, 20′), 1.97 (brs, 2H, H-4), 2.51 (m, 1H, Heq-4′), 5.08 (brs, 1H, H-8S) 5.15 (brs, 1H, H-7R), 5.17 (brs, 1H, H-8R), 5.22 (brs, 1H, H-7S), 6.19 (d, $J_{10,11}$ = 13.1 Hz, 1H, H-10), 6.24 (d, $J_{14,15}$ = 9.9 Hz, 1H, H-14), 6.32 (d, $J_{12,11}$ = 10.2 Hz, 1H, H-12), 6.34 (d, $J_{14',15'}$ = 10 Hz, 1H, H-14′), 6.47 (d, $J_{7',8'}$ = 15.1 Hz, 1H, H-7′), 6.51 (d, $J_{10',11'}$ = 11.7 Hz, 1H, H-10′), 6.52 (m, 1H, H-11), 6.54 (d, $J_{12',11'}$ = 12.2 Hz, 1H, H-12′), 6.62 (m, 1H, H-11′), 6.63 (m, 2H, H-15, 15′), 7.31 (d, J = 15.1 Hz, 1H, H-8′) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ: 12.6 (CH_3, C-19), 12.7 (CH_3, C-20), 12.8 (CH_3, C-19′), 12.9 (CH_3, C-20), 19.6 (CH_2, C-3), 20.4 (CH_2, C-3′), 20.9 (CH_3, C-18′), 24.6 (CH_3, C-17′), 25.6 (CH_3, C-16′), 26.0 (CH_3, C-16, 18), 30.7 (CH_3, C-17), 34.4 (CH_2, C-4′), 34.6 (C, C-1), 40.6 (CH_2, C-2′), 41.2 (CH_2, C-4), 44.0 (C, C-1′), 58.9 (C, C-5′), 77.2 (C, C-5), 87.6 (CH, C-8R), 88.1 (CH, C-8S), 117.8 (CH, C-7S), 118.8 (CH, C-7R), 121.4 (CH, C-7′), 124.1 (CH, C-11′), 125.0 (CH, C-11), 127.1 (CH, C-10), 129.6 (CH, C-15′), 131.5 (CH, C-15), 132.0 (CH, C-14), 133.7 (C, C-9′), 135.0 (CH, C-14′), 135.8 (C, C-9), 137.3 (CH, C-12), 137.4 (C, C-13), 138.8 (C, C-13′), 140.3 (CH, C-12′), 141.7 (CH, C-10′), 146.4 (CH, C-8′), 154.6 (C, C-6), 203.8 (C, C-6′).

2.4.3 | Reduction of 5,6-sapotexanthin epoxides

Several $NaBH_4$ crystals were added to 5 mL of the methanolic solution of approximately 20 μg of epoxides.[14] The mixture was kept at RT for 50 minutes. Then, solid NaOH was added to the solution to decompose the complex. The mixture was diluted with benzene, the organic phase washed with H_2O (five times), dried (Na_2SO_4), and evaporated, and the residue was dissolved in benzene. Both the isolated natural and the mixture of semisynthetic epoxides were reduced. The reaction mixtures were analyzed using HPLC-DAD.

3 | RESULTS AND DISCUSSION

The extraction of mamey pulp was accomplished with published procedures.[10] Column chromatography of the extract on Al_2O_3 and semipreparative HPLC yielded 9-mg sapotexanthin (**2**), 0.2-mg sapotexanthin 5,6-epoxide (**6**), and 0.3-mg sapotexanthin 5,8-epoxide (**7a** and **7b**).

3.1 | Structural elucidation of sapotexanthin 5,6-epoxide

The structure of the isolated sapotexanthin 5,6-epoxide (**6**) was determined by UV-Vis, ECD, and ^{1}H and ^{13}C NMR data of the semisynthetic compound. The UV-Vis spectrum (λ_{max}: 480 and 505sh nm in benzene) showed the presence of a conjugated carbonyl group. The reduction of sapotexanthin 5,6-epoxide (**6**) by $NaBH_4$ delivered two diastereomeric alcohols in a 1:1 ratio. In the UV spectrum, a fine structure appeared, and also, a hypsochromic shift (λ_{max}: 426, 451, 481 nm in benzene) was observed. Upon treatment of sapotexanthin 5,6-epoxide (**6**) with HCl/HOAc, the appearance of 464- and 486-nm λ_{max} values of the product hinted the presence of the 5,6-epoxide in the starting material.

The isolated sapotexanthin 5,6-epoxide (**6**) showed positive Cotton effects (CEs) at 241 and 349 nm and negative effects at 214 and 279.5 nm, which agree well with the ECD data of the natural (5*R*,6*S*)-cryptocapsin 5,6-epoxide (**4**) and (3*S*,5*R*,6*S*)-capsanthin 5,6-epoxide (**3**).[6,15] Thus, the ECD spectra clearly indicated the (5*R*,6*S*) configuration of natural sapotexanthin 5,6-epoxide (**6**) (Figure 4).

Since the natural 5,6-epoxide of sapothexanthin (**6**) was found as a very minor carotenoid, we were unable to obtain sufficient material to carry out a conclusive structural determination. For the identification of minor carotenoids, at least three criteria must be fulfilled[16]: (a) The UV-Vis spectrum should be the same, (b) on HPLC cochromatography with an authentic sample is needed, and (c) a proper mass spectrum.

In order to aid the identification of sapotexanthin 5,6-epoxide (**6**), the compound was prepared semisynthetically from sapotexanthin (**2**) by oxidation with 3-chloroperbenzoic acid. The epoxidation of sapotexanthin with 3-chloroperbenzoic acid was not diastereoselective, since the two diastereomeric epoxides formed in equal amounts. The C-5′ chirality center is too remote to have a chiral induction. In the biosynthesis,

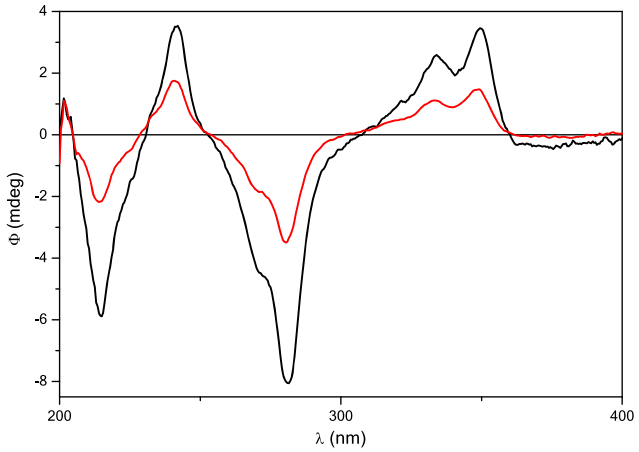

FIGURE 4 Electronic circular dichroism (ECD) spectra of (5*R*,6*S*,5′*R*)-sapotexanthin 5,6-epoxide (**6**, red) and (5*R*,6*S*,5′*R*)-cryptocapsin 5,6-epoxide (**4**, black)

the epoxydation is carried out by enzymes, which provide the chiral nonracemic environment for the diastereoselective epoxidation.

Carotenoids containing β-rings with or without substituents in the 3,3′-positions are readily epoxidized under controlled conditions with perbenzoic acid or monoperoxyphthalic acid. The epoxidation produced two diastereomeric 5,6-epoxides with (5*R*,6*S*) and (5*S*,6*R*) absolute configurations (Scheme 1). The separation of anti-(3*S*,5*R*,6*S*) and syn-(3*S*,5*S*,6*R*) diastereomers of 3-hydroxy-5,6-epoxy carotenoids can be achieved readily by classical column chromatography or HPLC on C18 or C30 stationary phases.[17] The epoxidation of the non-hydroxylated κ-ring also offers two diastereomeric 5,6-epoxides with (5*R*,6*S*) and (5*S*,6*R*) absolute configurations.[6,7] Usually, an unsubstituted β-ring makes the separation of such mixtures tedious. In our case, the separation of the diastereomers could not be accomplished by any chromatographical method including HPLC.

Finally, the separation of the diastereomeric (5*R*,6*S*)-sapotexanthin 5,6-epoxide (**6**) and (5*S*,6*R*)-sapotexanthin 5,6-epoxide (**8**) was achieved on a Chiralpak IA HPLC column; nearly equal amounts of the two diastereomers was observed (Figure 5). The OR-detected HPLC chromatogram showed that the two diastereomers had negative optical rotation, although this parameter cannot be used to assign the stereochemistry. Online HPLC-ECD measurements can be efficient in the structure elucidation of natural products,[6] and this technique was applied for **6** and **8**.

Online HPLC-ECD spectra were recorded by stopping the flow of the eluent in the HPLC-ECD flow cell at the maximum concentrations of the diastereomers. The diastereomers had characteristic near mirror image ECD curves above 280 nm that allowed us to assign the configurations unambiguously (Figure 6).

The near mirror image ECD curves of (5*R*,6*S*,5′*R*)-**6** and (5*S*,6*R*,5′*R*)-**8** clearly suggest that the ECD spectra are determined by the absolute configuration of the epoxide moiety and the C-5′ chirality center has only minor influence. In solution, these sapotexanthin 5,6-epoxides may form weakly bonding aggregates, which is also reflected in the ECD spectra. The absolute configuration of the epoxide moiety may have a larger influence on the chirality of the aggregate and hence on the ECD spectra. The optical rotation values recorded online during the chiral HPLC separation had the same negative sign for both diastereomers. The opposite CEs of natural (anti, **6**) and semisynthetic (syn, **8**) sapotexanthin 5,6-epoxides above 250 nm reflected the opposite configuration of the 5,6-epoxy end groups. These findings corroborated well the values of anti-capsanthin and syn-capsanthin 5,6-epoxide[15] and cryptocapsin 5,6-epoxide found in the literature.[6]

The natural and the semisynthetic sapotexanthin 5,6-epoxide and their reduced or acid-treated derivatives showed similar UV-Vis spectra in different solvents and similar chromatographic retention values with different chromatographic conditions (Tables 1 and 2). For the structure elucidation, the semisynthetic compounds were used.

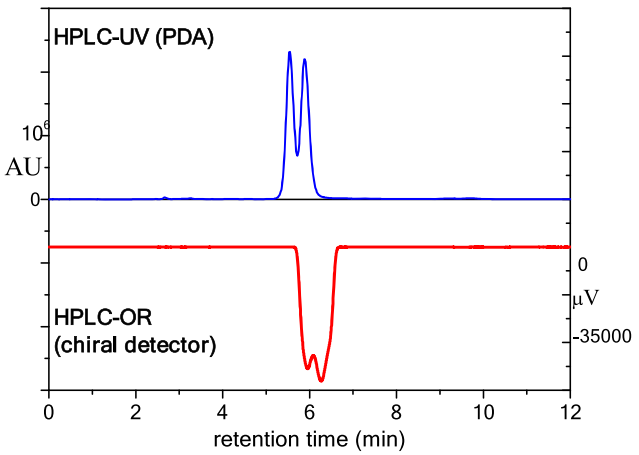

FIGURE 5 High-performance liquid chromatography (HPLC)–UV (upper blue curve) and HPLC-OR (lower red curve) chromatograms of the separated (5*R*,6*S*,5′*R*)-sapotexanthin 5,6-epoxide and (5*S*,6*R*,5′*R*)-sapotexanthin 5,6-epoxide diastereomers (**6** and **8**) monitored at 480 nm (Chiralpak IA, *n*-hexane/*i*-PrOH 90:10)

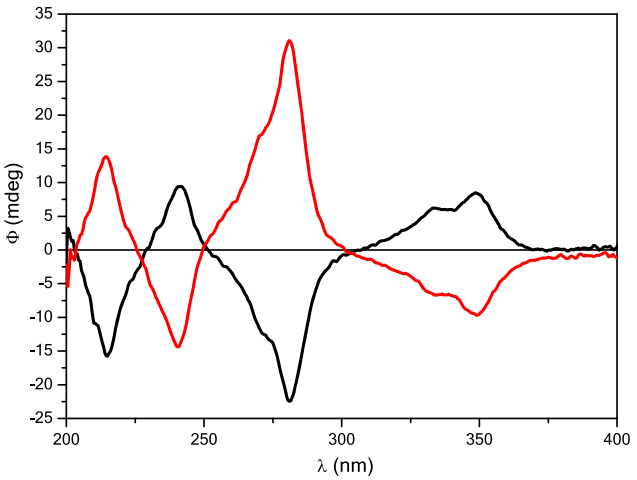

FIGURE 6 Online high-performance liquid chromatography–electronic circular dichroism (HPLC-ECD) spectra of diastereomeric (5*R*,6*S*,5′*R*)-sapotexanthin 5,6-epoxide (**6**, black, first eluting diastereomer) and (5*S*,6*R*,5′*R*)-sapotexanthin 5,6-epoxide (**8**, red, second eluting diastereomer) recorded after separation and by stopping the flow of the eluent at the UV maximum

TABLE 1 UV-Vis data of natural and semisynthetic sapotexanthin 5,6-epoxides in different solvents

Solvent	λ_{max}, nm			III/II, %
	1	2	3	
Hexane	(440)	464	495	55
Ethanol	-	469	-	-
Benzene	-	479	508	15
MTBE:MeOH:H_2O	-	463	-	-
MeCN:CH_2Cl_2:MeOH	-	454	(486)	-

One-dimensional and 2D NMR analyses were performed for the diastereomeric mixtures of (5*R*,6*S*)-sapotexanthin 5,6-epoxide and (5*S*,6*R*)-sapotexanthin 5,6-epoxide **6** and **8** using methods such as ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC, and DEPT 135. A comparison was made between the chemical shifts of the mixture of **6** and **8** and those of sapotexanthin (**2**)[5] and cryptocapsin 5,6-epoxide (**4**).[6] The measurements showed the presence of an all *E* polyene, a 5,6-epoxy β end group, and a nonhydroxylated 6-oxo κ end group (Supporting Info is available).

The ^{13}C and ^{1}H NMR data of mixture of **6** and **8** were similar to those of cryptocapsin 5,6-epoxide (**4**) but not for κ end-group signals (C-1′ to C-6′ and C-16′, C-17′, and C-18′). The chemical shift at 203.8 ppm in ^{13}C NMR data was assigned to a carbonyl group. All nine carbons of the κ end group could be identified: three methyl groups (δ C 20.9, 24.6, and 25.6 ppm), three methylene groups (δ C 19.5, 34.4, and 40.5 ppm), and three quaternary carbons (δ C 44.0, 58.9, and 203.8 ppm). There was an overlap in the ^{1}H NMR signals of methylene protons of the κ end group except for one proton H-4′$_{eq}$ (δ H 2.51 ppm). These latter methylene signals were assigned based on the similarity with sapotexanthin (**2**).[5] The signal assignment for the nonhydroxylated β end group and the polyene chain was confirmed with 2D NMR spectra. The proton chemical shifts of the β end groups (H-7 at δ 5.90 ppm and H-8 at δ 6.29 ppm) and the $^3J_{H,H}$ coupling constants ($J_{7,8}$ = 15.7 Hz) were identical with the corresponding data of the natural (5*R*,6*S*)-cryptocapsin 5,6-epoxide (**4**).[6] All ^{1}H and ^{13}C NMR data were in complete accordance with the currently published literature data.[5,6]

3.2 | Structural elucidation of sapotexanthin 5,8-epoxide

The UV-Vis spectrum of isolated sapotexanthin 5,8-epoxide (**7**) (λ_{max}: 464 and 486 nm in benzene) showed the presence of nine double bonds conjugated with a carbonyl group. In HPLC-MS, the parent ion was found at *m/z* 584.4 ($C_{40}H_{56}O_3$). On the HPLC chromatogram, two peaks appeared with identical UV-Vis spectra that corresponded to the two stereoisomers with (5*R*,8*S*) and (5*R*,8*R*) absolute configurations. Separation was only possible on HPLC. The two isomers had the same retention properties on $CaCO_3$ or Al_2O_3 open columns.

Baseline separation of (5*R*,8*S*)-epimers and (5*R*,8*R*)-epimers was achieved on Chiralpak IA and Chiralcel OD columns; however, HPLC-ECD analysis could not be carried out due to the minor amount of the sample. In reference to the complete NMR investigation, the authentic samples of sapotexanthin 5,8-epoxide were synthesized. Acid-catalyzed rearrangement of mixture of the semisynthetic sapotexanthin 5,6-epoxides **6** and **8** provided a mixture of four stereoisomers of sapotexanthin 5,8-epoxides (**7** and **9**). The four stereoisomers were the 5*R*,8*R* and 5*R*,8*S* (5*R*,8*RS*, **7**) and the 5*S*,8*R* and 5S,8S (5*S*,8*RS*, **9**) isomers, which could not be separated. Due to the complexity of the NMR signals, they were only partially assigned. The ^{1}H chemical shifts, the ^{13}C chemical shifts, and the coupling constants of the 5,8-epoxy β end group were identical with the data previously reported.[18,19] The presence of both 8*R* and 8*S* stereoisomers was confirmed by the characteristic signals at 5.16 and 5.24 ppm for H-7 and at 5.18 and 5.08 ppm for H-8. It showed an approximately 3:1 *R*/*S* ratio. In the ^{13}C spectrum, C-8 (87.6 ppm for 8*R* and 88.1 ppm for 8*S*) and C-7 (118.8 ppm for 7*R*

TABLE 2 Retention values of sapotexanthin 5,6-epoxide and its derivatives on different chromatographic conditions

Carotenoid	HPLC C30 R_t, min	HPLC C18 R_t, min	TLC Silica Gel R_f
Sapotexanthin 5,6-epoxide natural	18.31	8.87	0.84
Sapotexanthin 5,6-epoxide semisynthetic	18.31	8.87	0.84
Sapotexanthin 5,8-epoxide natural	20.92	9.58	0.69
Sapotexanthin 5,8-epoxide semisynthetic	20.92	9.58	0.69
Sapotexanthinol 5,6-epoxide (a)	14.25	18.08	0.64
Sapotexanthinol 5,6-epoxide (b)	11.80	27.60	0.46

Abbreviations: HPLC, high-performance liquid chromatography; TLC, thin-layer chromatography.

and 117.8 ppm for 7*S*) showed characteristic values for the 5,8-epoxide stereoisomers.

4 | CONCLUSION

Our results confirm our recent findings regarding the biosynthesis of carotenoids in mamey. Two enzymes must be especially active in mamey: the one that catalyzes the epoxidation of the nonhydroxylated β-rings and the one that is responsible for the rearrangement of the epoxides. Thus, κ carotenoids without hydroxyl groups can be produced in nature.[5,6,9] Our findings confirm the presence of 5*R*,6*S* configuration in the natural 5,6-epoxides, both in hydroxylated and in nonhydroxylated β-rings. Additionally, our study has effectively demonstrated that the 5*S*,6*R* and 5*R*,6*S* epoxide stereoisomers can only be separated on a chiral column in the case that a nonhydroxylated ring is present.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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