# Isolation and Characterization of Novel Capsorubin-Like Carotenoids from the Red Mamey (*Pouteria sapota*)

by **Enrique Murillo**[a]), **Yesury Mosquera**[a]), **Tibor Kurtán**[b]), **Gergely Gulyás-Fekete**[c]), **Veronika Nagy**[c]), and **József Deli***[c])

[a]) Department of Biochemistry, Faculty of Exact Natural Sciences and Technology, University of Panama, Panama

[b]) University of Debrecen, Faculty of Sciences, Department of Organic Chemistry, Egyetem tér 1, H-4032, Debrecen

[c]) University of Pécs, Medical School, Department of Biochemistry and Medical Chemistry, Szigeti út 12, H-7624, Pécs (phone: +36-72-536356; fax: +36-72-536225; e-mail:jozsef.deli@aok.pte.hu)

From the ripe fruits of red mamey (*Pouteria sapota*), two new carotenoids, 3′-deoxycapsorubin and 3,3′-dideoxycapsorubin, were isolated and identified based on their UV/VIS, CD, $^1$H-NMR, and mass spectral data.

**Introduction.** – The most common carotenoids have six-membered rings as end-groups ($\beta$ or $\varepsilon$ rings), while carotenoids with a five-membered ring ($\kappa$-ring) are rare. Well-known carotenoids containing a $\kappa$ end-group, such as capsanthin, capsorubin, and cryptocapsin, are characteristic components of red paprika (*Capsicum annuum*) [1][2]. The $\kappa$ end-group of these carotenoids always bears a OH group. We have recently reported the complete isolation and characterization of sapotexanthin ($\beta$,$\kappa$-caroten-6′-one) containing a non-hydroxylated $\kappa$-end group, from red mamey (*Pouteria sapota*) pulp [3]. Besides, pulp of red mamey contains some other interesting red carotenoids with hydroxylated or non-hydroxylated $\kappa$-rings [4][5]. In this article, we present the isolation of two new diketo-di-$\kappa$-carotenoids structurally related to capsorubin.

**Results and Discussion.** – The acetone extract of ripe fruits of mamey (500 g) was saponified with 5% KOH/MeOH. The saponified residue was chromatographed on a column of aluminum oxide (*Brockmann III*) with an increasing percentage of $Et_2O$ in hexane. By repeated column chromatography, compounds **1**–**3** (*Fig. 1*) were separated, subsequently crystallized, and identified based on the analysis of their UV/VIS, CD, $^1$H-NMR, and mass spectra.

The UV/VIS spectra of the isolated compounds revealed maxima at 485 nm in EtOH (no *cis* peak), identical with the spectrum of capsorubin isolated from red paprika [1].

Reduction of compounds **1**–**3** with $NaBH_4$ gave mixtures of stereoisomeric alcohols with identical UV spectra. The UV spectra of these mixtures exhibited, as expected, an increased fine structure and a hypsochromic shift. The perfect superimposability of the spectra of compounds **1**–**3** and of those of their reduction products suggests substantial structural similarity between these compounds (*Fig. 2*). The 45-nm

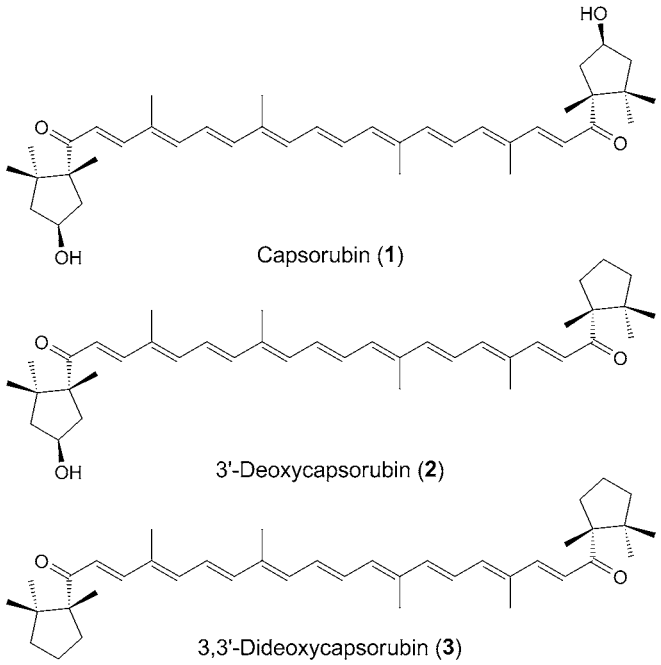


Fig. 1. *Structures of carotenoids with κ end-groups isolated from red mamey*

hypsochromic shift in the absorption maxima and the increase of the molecular mass of 4 units after the reduction show that compounds **1**–**3** contain two CO groups that are conjugated with the polyene chain consisting of nine conjugated C=C bonds. Acetylation of **1** ($M_w = 600$ g/mol) showed an increase of 84 mass units, which indicates that compound **1** contains two OH groups. Co-chromatography of **1** with capsorubin isolated from red paprika [1] in two different solvent systems by HPLC and TLC, as well as the results of the above described experiments, confirmed that compound **1** was capsorubin. The molecular weight of compound **2** was determined as 584 g/mol by MS ($C_{40}H_{56}O_3$). The reduction products of **2** showed a molecular weight of 586 g/mol ($C_{40}H_{58}O_3$), and the acetylation product of **2** had a molecular weight of 626 g/mol, which led to the conclusion that two conjugated C=O groups and only one OH group were present in the structure. Analogously, compound **3** was established not to contain a OH group, but the two keto groups conjugated to the polyene chain (*Table*).

The constitutions and configurations of the new compounds were established by NMR experiments. Because only very small amounts of samples were available, the $^1$H-assignments were based on simple $^1$H-, and in the case of **2**, also $^1$H,$^1$H-COSY experiments, analyzed with standard *Varian* software. The positions of the OH group in compound **2** could be easily established from the presence of the characteristic $^1$H chemical shifts (*e.g.*, H–C(3) ($\delta$(H) 4.47–4.55); $H_{ax}$–C(2) ($\delta$(H) 1.73); $H_{eq}$–C(2) ($\delta$(H) 2.00); $H_{ax}$–C(4) ($\delta$(H) 1.49); and $H_{eq}$–C(4) ($\delta$(H) 2.95)) and coupling constants in comparison with the respective $^1$H signals of the reference capsanthin 5,6-epoxide

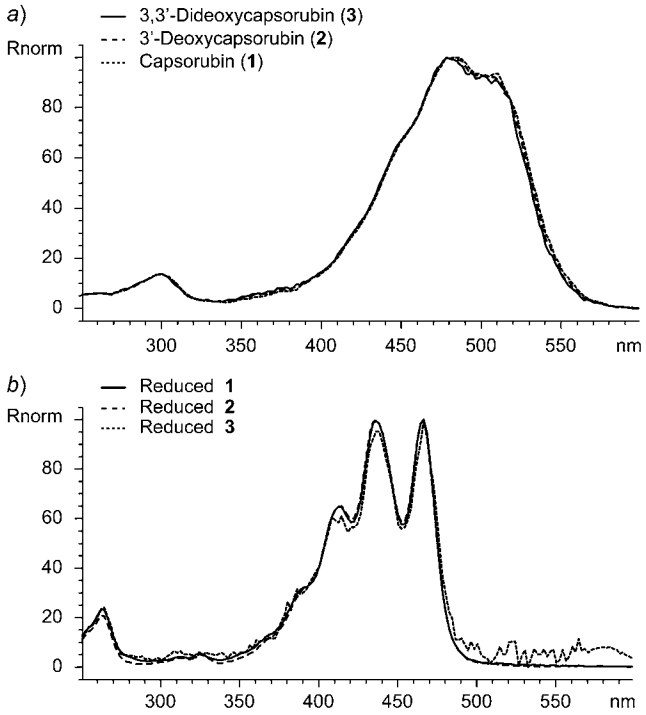


Fig. 2. a) *UV/VIS Spectra* (in HPLC eluent) *of* **1**–**3** *and* b) *UV/VIS spectra* (in HPLC eluent) *of reduction products of* **1**–**3**

Table. *UV/VIS, MS, HPLC, and TLC Data of* **1**–**3** *and Capsorubin* (isolated from paprika) *and Their Reduction and Acetylation Products*

| | Capsorubin | **1** | **2** | **3** |
|---|---|---|---|---|
| $\lambda_{max}$ EtOH | 485 | 485 | 485 | 486 |
| $\lambda_{max}$ ($C_{30}$) | 482 | 482 | 481 | 480 |
| $\lambda_{max}$ ($C_{18}$) | 477 | 477 | 477 | 476 |
| $\lambda_{max}$ red. ($C_{30}$) | 437 | 437 | 437 | 438 |
| MS ($M^+$) | 600 | 600 | 584 | 568 |
| MS ($M^+$, reduced) | 604 | 604 | 588 | 572 |
| MS ($M^+$, acetylated) | 684 | 684 | 626 | – |
| $t_R$ (HPLC ($C_{30}$))[a]) [min] | 14.67 | 14.67 | 20.13 | 26.81 |
| $t_R$ (HPLC ($C_{18}$))[a]) [min] | 2.73 | 2.73 | 3.17 | 3.66 |
| $R_f$ (TLC)[a]) | 0.08 | 0.08 | 0.31 | 0.89 |

[a]) See in the *General* section in the *Exper. Part.*

[6]. The $^1$H-NMR signals of $CH_2$ H-atoms in the non-hydroxylated $\kappa$-end group of **2** and **3**, except for $H_{eq}$–C(4′) ($\delta$(H) 2.47–2.56), were overlapped. Therefore, the H-atoms of $CH_2$(2′) and $CH_2$(4′) were identified on the basis of the similarity of the chemical shifts in compounds with non-hydroxylated $\kappa$ end-groups, such as sapotex-

anthin [3] and 3′-deoxycapsanthin [7]. The other $^1$H chemical shifts and the $J$(H,H) values corresponded well with literature values for the chemical shifts of substituted and unsubstituted $\kappa$ end-groups [3][6][7].

Compounds **2** and **3** each exhibited a CD spectrum with a positive *Cotton* effect at *ca.* 290 nm and negative *Cotton* effects at *ca.* 250 and 365 nm (*Fig. 3*), in agreement with the data for natural capsorubin (**1**) [8], which has the (3*S*,5*R*) configuration in the $\kappa$-ring. Thus, the (3*S*,5*R*) absolute configuration was postulated for the hydroxylated $\kappa$ end-group in **2**, and (5*R*) for the non-hydroxylated $\kappa$ end-groups in both compounds **2** and **3**. Therefore, compound **2** was defined as (3*S*,5*R*,5′*R*)-3-hydroxy-$\kappa$,$\kappa$-carotene-6,6′-dione (= 3′-deoxycapsorubin), and compound **3** as (5*R*,5′*R*)-$\kappa$,$\kappa$-carotene-6,6′-dione (= 3,3′-dideoxycapsorubin).

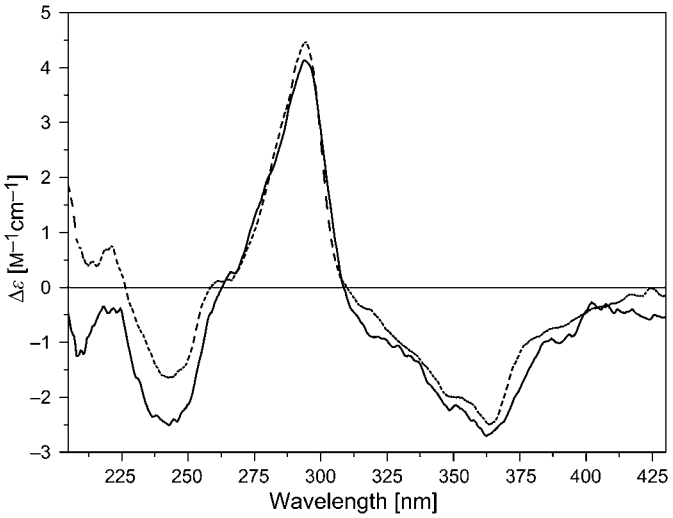


Fig. 3. *CD Spectra of 3′-deoxycapsorubin* (**2**; —) *and 3,3′-dideoxycapsorubin* (**3**; ----) *in hexane*

Concerning the biosynthesis of carotenoids in paprika, capsanthin-capsorubin synthase (CCS), the enzyme catalyzing the conversion of 5,6-epoxy end groups into 6-oxo-$\kappa$ end groups, was isolated from *C. annuum.* Thus, 3′-deoxycapsorubin (**2**) and 3,3′-dideoxycapsorubin (**3**) were assumed to be a product of the pinacollic rearrangement of $\beta$-cryptoxanthin-5,6,5′,6′-diepoxide and $\beta$-carotene-5,6,5′,6′-diepoxide, respectively, presumably catalyzed by CCS (*Scheme*). HPLC Analysis showed that significant amounts of the diepoxides of $\beta$-cryptoxanthin and $\beta$-carotene were present in the extract of mamey [4].

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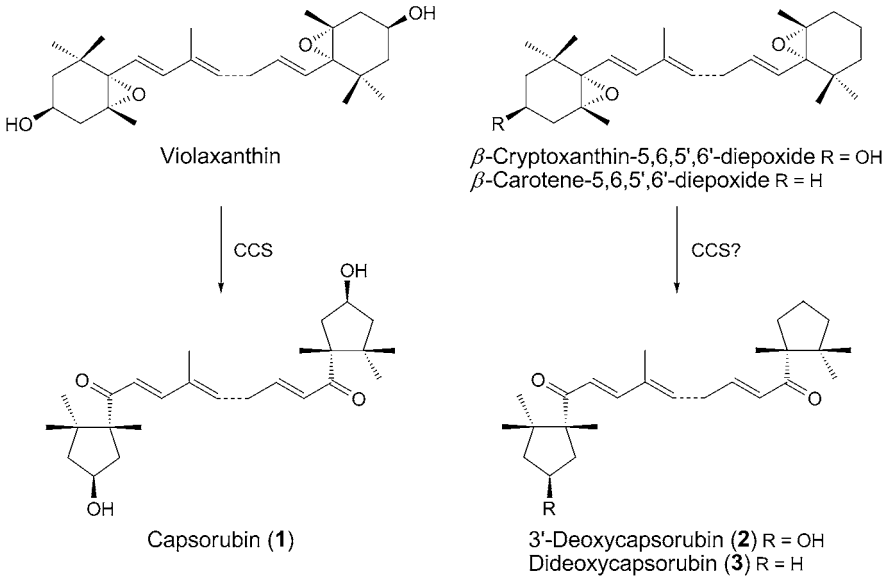
Scheme. *Proposed Mechanism for the Formation of Compounds* **1**–**3**




## Experimental Part

*General.* HPLC: *Hewlett-Packard* chromatograph model *1050* equipped with diode array detector and *HP* ChemStation software; the molar masses were obtained by HPLC APCI-MS in an *Agilent 1100 HPLC* chromatograph coupled to a *JEOL MS LCmate* mass spectrometer. HPLC Conditions for $C_{30}$ column: 250 × 4.6 mm i.d.; *YMC* $C_{30}$, 5 μm, linear gradient from methyl *tert*-butyl ether (MTBE)/$H_2O$/MeOH 15 : 4 : 81 to MTBE/$H_2O$/MeOH 90 : 4 : 6 during 90 min. $C_{18}$ column: *Spherisorb ODS2 HP* (250 × 4.6 mm i.d. 5 μm), isocratic elution, MeCN/$CH_2Cl_2$/MeOH 82 : 13 : 5; flow rate, 1.00 ml/min. TLC: silica plate (*Merck*), eluent $Et_2O$/hexane 1 : 1. UV Spectra: *Jasco V-530* spectrophotometer, in benzene or EtOH; $\lambda$ in nm. CD Spectra: *J-810* spectropolarimeter. $^1$H-NMR Spectra: *Varian UNITY INOVA 400-WB* (400 MHz) spectrometer in $CDCl_3$ with TMS as an internal standard. HR-ESI-TOF-MS: *Waters Q-TOF Premier* mass spectrometer (*Waters Corporation*, Milford, MA, USA); the sample was dissolved in MeOH and measured in positive-ion electrospray ionization mode.

*Plant Material.* Matured fruits were purchased at the Metropolitan public market in Panama City, Panama.

*Extraction and Isolation.* The pulp of red mamey (500 g) was homogenized in a porcelain mortar with 50 g of $NaHCO_3$ and extracted with acetone until no more color was observed. The extract was diluted with a mixture of $Et_2O$/hexane 1 : 1, washed with $H_2O$ to remove acetone, dried ($Na_2SO_4$), and evaporated. The residue was dissolved in $Et_2O$ and saponified with methanolic KOH. After saponification, the $Et_2O$ soln. was washed to alkali-free, and then evaporated. The residue was subjected to open CC ($Al_2O_3$, *Brokman grade III*) using an increasing percentage of $Et_2O$ in hexane. Capsorubin (**1**) was isolated in pure form after additional CC of *Fr. 11* (eluted with 100% $Et_2O$), 3′-deoxycapsorubin (**2**) from *Fr. 8* (eluted with 65% $Et_2O$), and 3,3′-dideoxycapsorubin (**3**) from *Fr. 1* (eluted with 4% $Et_2O$). The purity of the compounds was verified by HPLC-DAD and HPLC/MS.

*3′-Deoxycapsorubin* (=*(3*S*,5*R*,5′*R*)-3-Hydroxy-κ,κ-carotene-6,6′-dione*; **2**). Red crystals. UV (EtOH): 485. CD (hexane): 429 (−0.55), 362 (−2.70), 327sh (−1.08), 294 (4.13), 280sh (1.98), 243 (−2.51). $^1$H-NMR (400 MHz, $CDCl_3$): 0.84 (*s*, Me(16)); 0.85 (*s*, Me(16′)); 1.11 (*s*, Me(17′)); 1.18 (*s*, Me(18′)); 1.21 (*s*, Me(17)); 1.37 (*s*, Me(18)); 1.49 (*dd*, $J_{gem}$ = 14.5, $J(4_{ax},3)$ = 3.1, $H_{ax}$–C(4)); 1.48–1.55 (*m*, $H_{ax}$–C(4′)); 1.55–1.59 (*m*, $H_{eq}$–C(2′)); 1.66–1.71 (*m*, $H_{ax}$–C(2′), $CH_2$(3′)); 1.73 (*dd*, $J_{gem}$ = 13.7, $J(2_{ax},3)$ =

3.2, $H_{ax}$–C(2)); 1.97 (*s*, Me(19), Me(19′)); 1.98 (*s*, Me(20)); 1.99 (*s*, Me(20′)); 2.00 (*dd*, $J_{gem}$ = 13.7, $J(2'_{eq},3)$ = 7.8, $H_{eq}$–C(2)); 2.47–2.56 (*m*, $H_{eq}$–C(4′)); 2.95 (*dd*, $J_{gem}$ = 14.5, $J(4_{eq},3)$ = 8.7, $H_{eq}$–C(4)); 4.47–4.55 (*m*, H–C(3)); 6.32–6.37 (*m*, H–C(14), H–C(14′)); 6.44 (*d*, *J*(7,8) = 15.1, H–C(7)); 6.48 (*d*, *J*(7′,8′) = 15.0, H–C(7′)); 6.51 (*dd*, *J*(12,11) = 14.6, H–C(12)); 6.52 (*d*, *J*(12′,11′) = 13.2, H–C(12′)); 6.56 (*d*, *J*(10,11) = 11.4, H–C(10)); 6.57 (*d*, *J*(10′,11′) = 9.7, H–C(10′)); 6.59–6.64 (*m*, H–C(11)); 6.63 (*dd*, *J*(11′,10′) = 9.7, *J*(11′,12′) = 13.2, H–C(11′)); 6.66–6.72 (*m*, H–C(15), H–C(15′)); 7.32 (*d*, *J*(8,7) = 15.1, *J*(8′,7′) = 15.0, H–C(8), H–C(8′)). HR-ESI-TOF-MS: 585.4308 ($[M+H]^+$, $C_{40}H_{57}O_3^+$; calc. 585.4308).

*3,3′-Dideoxycapsorubin* (= (5R,5′R)*-κ,κ-Carotene-6,6′-dione*; **3**). Red crystals. UV (EtOH): 485. CD (hexane): 416 (–0.19), 363 (–2.37), 337sh (–1.33), 293 (4.10), 282sh (2.29), 243 (–1.56). $^1$H-NMR (400 MHz, $CDCl_3$): 0.85 (*s*, Me(16), Me(16′)); 1.11 (*s*, Me(17), Me(17′)); 1.18 (*s*, Me(18), Me(18′)); 1.66–1.72 (*m*, $H_{ax}$–C(2), $H_{ax}$–C(2′), $CH_2$(3), $CH_2$(3′)); 1.99 (*s*, Me(19), Me(19′)); 2.01 (*s*, Me(20), Me(20′)); 2.46–2.55 (*m*, $H_{eq}$–C(4), $H_{eq}$–C(4′)); 6.33–6.36 (*m*, H–C(14), H–C(14′)); 6.49 (*d*, *J*(7,8) = *J*(7′,8′) = 15.1, H–C(7), H–C(7′)); 6.51 (*d*, *J*(12,11) = *J*(12′,11′) = 14.6, H–C(12), H–C(12′)); 6.55 (*d*, *J*(10,11) = *J*(10′,11′) = 11.1, H–C(10), H–C(10′)); 6.64 (*dd*, *J*(11,10) = *J*(11′,10′) = 11.1, *J*(11,12) = *J*(11′,12′) = 14.6, H–C(11), H–C(11′)); 6.67–6.71 (*m* H–C(15), H–C(15′)); 7.32 (*d*, *J*(8,7) = *J*(8′,7′) = 15.1, H–C(8), H–C(8′)). HPLC-MS: 586 ($M^+$).

*Capsorubin* (= *(3*S,*3′*S,*5*R,*5′*R*)-3,3′-Dihydroxy-κ,κ-carotene-6,6′-dione*; **1**). UV (EtOH): 485 nm. CD (dioxane): see [8]. HPLC-MS: 600 ($M^+$).

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