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Sapotexanthin, an A-Provitamin Carotenoid from Red Mamey (*Pouteria sapota*)

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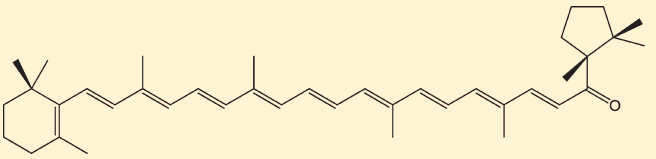
Supporting Information

ABSTRACT: From the ripe fruits of red mamey (*Pouteria sapota*) sapotexanthin, a new carotenoid, was identified as (all-*E*,5′*R*)-β,κ-caroten-6′-one.



The well-known carotenoids containing the κ end group, such as capsanthin, capsorubin, and cryptocapsin, are characteristic of red paprika (*Capsicum annuum*).[1,2] Capsanthin has also been found in the pollen anthers of *Lilium tigrinum* and in the fruit of *Berberis* spp. and *Asparagus officinalis*.[3] Capsorubin has also been isolated from the integument of *Encephalartos altensteinil*, the petals of *Cajophora lateritia*, and the fruits of *A. officinalis*.[3]

Generally, the κ-ring of carotenoids is hydroxylated, as in capsanthin and capsorubin, but Maoka and his co-workers[4] identified two carotenoids from red paprika with a nonhydroxylated κ-ring.

A survey of local plants in Panama has revealed the presence of ketocarotenoids in a range of species. Examples with high concentrations of ketocarotenoids were found among fruits, e.g., "mamey" (*Pouteria sapota*), "maracuya chino" (*Cionosicyos macrunthus*), and "jipijapa" (*Carludovica palmata*), and in young red-brown leaves and red seeds of *Zamia dressleri*. Mamey, known sometimes as mamey sapote or just sapote, is a tropical fruit native to the Caribbean and Central America. It is cultivated in tropical regions in the Americas including Southern Florida and is a popular fruit with many Caribbean and Central American people.

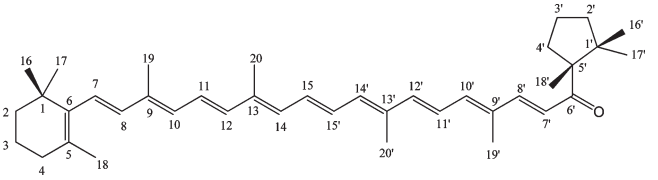
Several studies indicated that the orange pulp color of mamey is mainly due to carotenoids, but the specific carotenoids were not separated and identified.[5,6]

In the course of studies on the carotenoids of Panamian fruits[7] a new carotenoid that possesses a 6-oxo-κ end group was isolated. This paper reports the isolation and structural elucidation of this new carotenoid.

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 diethyl ether in *n*-hexane. A major compound that chromatographed between β-carotene and cryptocapsin on the Al_2O_3 column was further investigated. By repeated column chromatography, this carotenoid was isolated and crystallized from benzene/*n*-hexane (8 mg, red crystals; mp 135 °C). Its UV spectrum revealed maxima at 483 nm in benzene and 475 nm in ethanol (no *cis* peak), identical with the spectrum of capsanthin and cryptocapsin, but no reaction took place with acetic anhydride, indicating that there was no hydroxy group present. Reduction of the compound with $NaBH_4$ gave a mixture (ca. 1:1) of two products with identical UV spectra, but they could be separated by HPLC. This behavior is characteristic of stereoisomeric alcohols. The UV spectrum of this mixture exhibited, as expected, an increased fine structure and a hypsochromic shift to 487, 458, and 433 nm in benzene.

The mass spectrum gave a molecular mass of 552 ($C_{40}H_{56}O$), and the reduction products a molecular mass of 554 ($C_{40}H_{58}O$), showing that the conjugated keto group was the only oxygen function in the structure.

Structural information about the constitution and configuration of the new compound was deduced from NMR experiments. The ^1H and ^{13}C NMR chemical shifts and the $J_{H,H}$ coupling constants corresponded with literature values for the chemical shifts of unsubstituted β and κ end groups.[4,8]



Sapotexanthin (β,κ-carotene-6′-one) (**1**)

The ^{13}C and ^1H NMR data of **1** were similar to those of cryptocapsin except for the signals of the κ end group (C-1′ to

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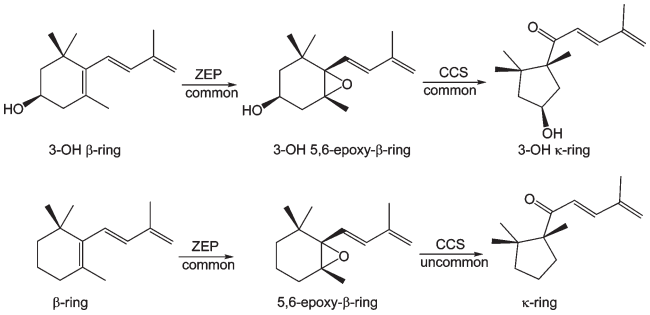
Scheme 1. Proposed Mechanism for the Formation of the κ-Ring



C-6′ including C-16′, C-17′, and C-18′). One oxygen function was assigned to a carbonyl group (δ_C 203.8) on the basis of ^{13}C NMR data. The partial structures of a nonhydroxylated β end group and an all-*E* polyene chain in **1** were confirmed from 2D NMR spectra. The remaining end group has nine carbons including three methyls (δ_C 20.9, 24.6, and 25.6), three methylenes (δ_C 19.6, 34.3, and 40.5), and three quaternary carbons (δ C 44.0, 58.9, and 203.8). The ^{1}H NMR signals of methylene protons in this end group except for H-4′$_{eq}$ (δ_H 2.52) overlapped. Therefore, the connections of protons from H-2′ to H-4′ were confirmed on the basis of the similarity of the chemical shifts in the κ end group **1** and 3′-deoxycapsanthin.[4] All other ^{1}H and ^{13}C NMR data were in good accordance with literature data.[4,8]

Compound **1** exhibited a CD spectrum with positive Cotton effects at 212 and 291 nm and negative Cotton effects at 239 and 365 nm, in agreement with the data for natural capsorubin, capsanthin, and cryptocapsin,[9] which have a 3′*S*,5′*R* configuration of the κ-ring. The influence of additional substituents such as OH groups at C-3 or C-3′ is rather small. The 5′*R* absolute configuration was postulated for **1**, which was defined as (5′*R*)-β,κ-carotene-6′-one, and the compound was named sapotexanthin.

Concerning the biosynthesis of carotenoids in paprika, the gene corresponding to capsanthin−capsorubin synthase (CCS), an enzyme that catalyzes the conversion of a 3-hydroxy-5,6-epoxy-β end group into a 3-hydroxy-6-oxo-κ end group, was isolated from *C. annuum*.[10] It seems likely that sapotexanthin is biosynthesized from β-carotene via β-carotene 5,6-epoxide in an analogous way to the formation of capsanthin from zeaxanthin via antheraxanthin (Scheme 1.). HPLC analysis showed that significant amounts of β-carotene epoxides were present in the extract of mamey.[11]

In some countries of Central America people have considerable vitamin A deficiency. Mamey contains significant amounts of sapotexanthin, a vitamin A provitamin, which makes it an important source of this vitamin. As it is an edible fruit, mamey and other tropical fruits with high sapotexanthin content could play an important role in the diet of this region as natural vitamin A sources.

EXPERIMENTAL SECTION

General Experimental Procedures. The UV spectra were recorded with a Jasco V-530 spectrophotometer in benzene and EtOH. CD spectra were recorded at room temperature with a J-810 spectropolarimeter. The exact mass measurements (HRESITOFMS) were performed using a Waters Q-TOF Premier mass spectrometer (Waters Corporation, Milford, MA, USA). The sample was solved in MeOH and measured in positive electrospray ionization mode. The ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured with a Jeol Ellipse +400 and a Varian UNITY INOVA 400-WB spectrometer in $CDCl_3$ with TMS as an internal standard. A liquid Hewlett-Packard chromatograph model 1050, equipped with diode array detector (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.

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

Extraction and Isolation of Carotenoids. 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 obtained. The extract was diluted with a mixture of Et_2O/*n*-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 etheral solution was washed free from alkali and evaporated. The residue was subjected to column chromatography (Al_2O_3 Brokman grade III) using an increasing percentage of ether in *n*-hexane. The sapotexantin was isolated in pure form from fraction 4, eluted with 3% ether. The purity of the compound was verified by HPLC-DAD and HPLC-MS. The fraction was crystallized from benzene/hexane to give 8 mg of **1**.

Sapotexanthin (1): red crystals; mp 135 °C; UV (benzene) λ_{max} 483 nm, (EtOH) λ_{max} 475 nm; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.85 (s, 3H, H-16′), 1.03 (s, 6H, H-16, H-17), 1.11 (s, 3H, H-17′), 1.18 (s, 3H, H-18′), 1.47 (2H, H-2), 1.48 (m, 1H, H$_{ax}$-4′), 1.57 (m, 1H, H$_{eq}$-2′), 1.62 (2H, H-3), 1.68 (m, 3H, H$_{ax}$-2′, H-3′), 1.71 (s, 3H, H-18), 1.96 (s, 3H, H-19), 1.97 (s, 6H, H-20, H-19′), 1.98 (s, 3H, H-20′), 2.03 (2H, H-4, J = 5.7), 2.52 (m, 1H, H$_{eq}$-4′), 6.14 (1H, H-8), 6.17 (1H, H-7), 6.17 (d, 1H, H-10, $J_{10/11}$ = 12.7), 6.27 (1H, H-14), 6.34 (d, 1H, H-14′, J = 10.9), 6.35 (d, 1H, H-12, $J_{12/11}$= 14.4), 6.48 (d, 1H, H-7′, $J_{7'/8'}$ = 15.0), 6.53 (d, 1H, H-12′, $J_{12'/11'}$ = 15.5), 6.56 (d, 1H, H-10′, $J_{10'/11'}$ = 11.0), 6.63 (m, 2H, H-15, H-15′), 6.63 (dd, 1H, H-11′, $J_{11'/10'}$ = 11.0, $J_{11'/12'}$ = 15.5), 6.66 (dd, 1H, H-11, $J_{11/10}$= 12.7, $J_{11/12}$ = 14.4), 7.32 (d, 1H, H-8′, $J_{8'/7'}$ = 15.0); ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.7 (CH_3, C-20), 12.8 (CH_3, C-19, 20′), 12.9 (CH_3, C-19′), 19.3 (CH_2, C-3), 19.6 (CH_2, C-3′), 20.9 (CH_3, C-18′), 21.8 (CH_3, C-18), 24.6 (CH_3, C-17′), 25.6 (CH_3, C-16′), 29.0 (CH_3, C-16), CH_3, C-17), 33.1 (C, C-1), 34.3 (CH_2, C-4′), 34.4 (CH_2, C-4), 39.7 (CH_2, C-2), 40.5 (CH_2, C-2′), 44.0 (C, C-1′), 58.9 (C, C-5′), 121.4 (CH, C-8′), 124.1 (CH, C-11′), 125.6 (CH, C-11), 129.6 (CH, C-15′), 126.9 (CH, C-7), 129.5 (C, C-5), 130.7 (CH, C-15), 131.6 (CH, C-10), 132.1 (CH, C-14), 133.7 (C, C-9′), 135.1 (CH, C-14′), 135.8 (C, C-9), 136.5 (C, C-13), 137.0 (CH, C-12), 137.6 (C, C-13′), 137.7 (CH, C-8), 137.9 (C, C-6), 140.3 (CH, C-12′), 141.7 (CH, C-10′), 146.4 (CH, C-7′), 203.8 (C, C-6′); CD (dioxane, λ [nm] ($\Delta\varepsilon$): 399sh (−0.15), 391sh (−0.18), 382sh (−0.25), 365 (−0.46), 331sh (−0.14), 318 (0), 291 (1.12), 261 (0), 254sh (−0.14), 239 (−0.25), 231 (0), 212 (0.47); HRESITOFMS *m*/*z* 552.4338 (calcd for $C_{40}H_{56}O$, 552.4331).

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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