Journal of Food Composition and Analysis 65 (2018) 1–5

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Original Research Article

Isolation of allene carotenoids from mamey

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ARTICLE INFO

Chemical compounds studied in this article:
Capsoneoxanthin (PubChem CID: 101078504)
Neoxanthin (PubChem CID: 5281247)
(9′*Z*)-Neoxanthin (PubChem CID: 101052392)

Keywords:
Food analysis
Food composition
Allene carotenoids
Capsoneoxanthin
Neoxanthin
Mamey
NMR analysis

ABSTRACT

The fruit of red mamey (*Pouteria sapota*) contains a wide variety of carotenoids, generally in high concentration, which makes possible even the isolation of minor components in measurable amounts. Carotenoids were extracted from red mamey with acetone, subsequent saponification resulted a crude extract, which was submitted to column chromatography using aluminium oxide (Al_2O_3) as adsorbent. By using consecutive chromatographic steps and crystallization allene carotenoids, such as neoxanthin, (9′*Z*)-neoxanthin and capsoneoxanthin, were isolated from the most polar fractions in milligram amounts and in high purity. The amount of capsoneoxanthin was found sufficient for the complete analysis of this rare carotenoid 15 years after its first isolation. The complete ^{1}H- and ^{13}C NMR assignments of neoxanthin and (9′*Z*)-neoxanthin using 2D techniques were achieved for the first time, as well. Electronic Circular Dichroism (ECD) spectra for the neoxanthin isomers were in good accordance with literature data, whereas the spectrum of capsoneoxanthin suggested aggregate formation in *n*-hexane solution.

1. Introduction

Carotenoids are tetraterpene pigments distributed in bacteria, fungi, algae, as well as in higher plants and animals. Until now, more than 700 carotenoids have been found in Nature, from which about 43 carotenoids contain the allene group (Britton et al., 2004). The principal allenic carotenoids are fucoxanthin in brown algae, diatoms and peridinin in dinoflagellates and neoxanthin (**1**) in higher plants and algae (Liaeen-Jensen, 1998; Dembitsky and Maoka, 2007). They serve as light-harvesting pigments in photosynthesis and occur in the form of carotenoid–chlorophyll–protein complexes in chloroplasts (Goodwin, 1980).

The first allene carotenoid, neoxanthin (**1**) was isolated from the green leaves of barley by Strain (1938) and was subsequently shown to be one of the principal xanthophylls in a wide variety of seed plants and spore-bearing plants. The structural elucidation of the allenic end group of neoxanthin (**1**) was performed by Cholnoky et al. (1969), and its structure was determined as 5',6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro-β,β-carotene-3,5,3'-triol. The absolute configuration of all-*E*- (**1**) and 9′*Z*-(3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-neoxanthin (**2**) was assigned and confirmed by chemical synthesis (Baumeler and Eugster, 1992; Baumeler et al., 1994). Neoxanthin (**1**) was converted to two stereoisomers of neochromes by acid catalyzed epoxy-furanoxide rearrangement, and these structures were characterized (Märki-Fischer et al., 1984), as well. The first preparation of (all-*E*,6*S*)- and (9′*Z*,6*S*)-neoxanthin was published in 2000 (Strand and Liaaen-Jensen, 2000).

The best known allene carotenoid (6*R*)-neoxanthin (**1**) can be found in relatively large amounts (up to 10%) together with its presumed natural precursor violaxanthin, especially in leafy vegetables, such as spinach. In natural sources the thermodynamically less stable (9′*Z*)-form (**2**) is predominant, which similarly to β-carotene or lutein, can participate in the photo-harvest system, whereas the all-*E* isomer (**1**) is usually present only in traces (Strand et al., 2000). The epoxy carotenoids violaxanthin and neoxanthin (**1**) are precursors of the important plant hormone abscisic acid in plants. In mammals, anticarcinogenic effect was attributed to them, as they act mostly by triggering apoptosis in certain tumor cell lines (Gagezl et al., 2012; Krinsky and Johnson 2005). From higher plants such as the petals of *Trollius europeus*, two C-6 epimers of pentahydroxy allenic carotenoids, neoflor and 6-epineoflor, were isolated (Marki-Fischer and Eugster, 1990).

Capsoneoxanthin (**3**) was previously isolated from *Asparagus falca-*

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http://dx.doi.org/10.1016/j.jfca.2017.04.004
Received 24 November 2016; Received in revised form 24 March 2017; Accepted 7 April 2017
Available online 09 April 2017

tus (Deli et al., 2000), but only a tentative NMR assignment could be achieved because of the small amount of the pigment. This compound was assumed to be the pinacol rearrangement product of neoxanthin, the formation of which is catalyzed by capsanthin-capsorubin synthase.

In our research group the carotenoid composition of the panamian red mamey (*Pouteria sapota*) fruit has been extensively studied over the past few years. This fruit is an especially rich source of carotenoids and generally the pigment content of the flesh of the fruit is very high, which means that minor components can be isolated in reasonable amounts, as well. From the nonpolar fraction of the mamey extract some new carotenoids with deoxy kappa end-group were isolated among other relatively rare carotenoids (Murillo et al., 2011, 2012; Gulyás-Fekete et al., 2013). Recently, we turned our attention to the polar fractions of the extract to isolate and characterize allene carotenoids, such as neoxanthin, (9′*Z*)-neoxanthin and capsoneoxanthin in full detail. In our present paper, the isolation and structure elucidation of these three carotenoids are described including the so far missing ^{13}C NMR data and ECD spectra.

2. Materials and methods

2.1. General experimental procedures

The UV/Vis spectra were recorded with a Jasco V-530 spectrophotometer in benzene. ECD spectra were recorded at room temperature with a J-810 Spectropolarimeter. Molar masses were obtained by an Autoflex II MALDI instrument (Bruker Daltonics) in positive mode.

2.2. NMR spectroscopy

All NMR experiments were carried out on a 600 MHz Varian DDR NMR spectrometer equipped with a 5 mm inverse-detection gradient (IDPFG) probehead. Standard pulse sequences available in VnmrJ 3.2C/Chempack 5.1 were used for structure identifications. The complete resonance assignments were established from direct ^{1}H–^{13}C, long-range ^{1}H–^{13}C, and scalar spin–spin connectivities using 1D ^{1}H, ^{13}C, ^{1}H–^{1}H *g*COSY, ^{1}H–^{1}H TOCSY, ^{1}H–^{1}H NOESY, ^{1}H–^{1}H ROESY, ^{1}H–^{13}C *g*HSQCAD (J = 140 Hz), ^{1}H–^{13}C *g*HMBCAD (J = 8 Hz) experiments, respectively. For the unequivocal resonance assignment of the overlapping resonances in the aliphatic and aromatic region band-selective HSQC and HMBC spectra aided the complete structure elucidation. Confirming the assignment and to extract coupling constants for the overlapping aromatic protons, selective 1D TOCSY experiment was utilized. The probe temperature was maintained at 298 K and standard 5 mm NMR tubes were used. The ^{1}H and ^{13}C chemical shifts were referenced to TMS (0.00 ppm). The deuterated chloroform was deacidified by passing through a Pasteur pipette column (10 cm) filled with neutral Al_2O_3 prior to the dissolution of the sample. $CDCl_3$ (99.8 atom% D) for NMR was purchased from VWR International.

2.3. HPLC-DAD measurement

Gradient pump Dionex P680; detector: Dionex PDA-100; detection wavelength, 450 nm; data acquisition was performed by Chromeleon 6.70 software. The HPLC separation was carried out on an endcapped C30 column (250 × 4.6 mm i.d.; YMC C30, 3 µm). Eluents: (A) MeOH:MTBE:H_2O(81:15:4); (B) MeOH:MTBE:H_2O = 6:90:4. The chromatography was performed in linear gradient from 100% A eluent to 50% B mixture in 45 min, with 1 cm^3/min flow (Turcsi et al., 2016).

2.4. Plant material

Ripe fruits of red mamey (*Poutiera sapota*) were purchased on the Metropolitan public market in Panama City, Panama (Murillo et al., 2016).

2.5. 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 discoloration. The extract was diluted with a mixture of diethyl ether (Et_2O)/*n*-hexane (1:1), washed with H_2O to remove acetone, dried with anhydrous Na_2SO_4, and evaporated. The residue was dissolved in Et_2O and saponified with methanolic KOH. After saponification, the ethereal solution was washed to free from alkali and evaporated.

2.6. Column chromatography

The mamey extract was submitted to column chromatography on aluminium oxide (Murillo et al., 2016) the fraction containing neoxanthins and capsoneoxanthin was eluted with diethyl ether:methanol (97:3). 110 mg of this fraction was further purified on five parallel $CaCO_3$ columns (30 × 6 cm i.d., toluene:acetone 99:1, max. load for one column is approx. 20 mg) to give five fractions containing the three allene carotenoids and further polar pigments (Supplementary material Fig. S57). The red and polar Fraction 2 contained the capsoneoxanthin, which was obtained from this fraction in pure form using toluene:acetone 94:6 as eluent. Purification of the yellow Fraction 3 (toluene:acetone 96:4) gave the neoxanthin isomers. The pigments were crystallized from a toluene-*n*-hexane 1:3 mixture (Fig. 1).

2.7. (all-E)-Neoxanthin (1)

orange crystals, 1.5 mg, m.p. 146 °C. UV/Vis (benzene): λ_{max} 426, 451, and 482 nm, λ_{max} after acid treatment: 407, 430, 463 nm; ECD {MeOH, λ [nm] (Δε), $c = 2.08\times10^{-4}$ M}: 340 (0.16), 327 (−0.11), 314 (−0.25), 289sh (−0.30), 267sh (−2.40), 263 (−2.67), 255sh (−1.88), 249sh (−1.29), 226sh (−1.34), 219 (−1.42). ECD {*n*-hexane, λ [nm] (Δε), $c = 2.08\times10^{-4}$ M}: 268 (−0.23), 226 (−0.17). MS: *m/z* 601 ([M+H]$^+$), 583 ([M-H_2O + H]$^+$). t_R = 7.3 min on a C30 column.

2.8. (9′Z)-Neoxanthin (2)

orange crystals, 3.8 mg, m.p. 135 °C, UV/Vis (benzene): λ_{max} 423, 447, and 477 nm, λ_{max} after acid treatment: 404, 428, 457 nm; ECD {MeOH, λ [nm] (Δε), $c = 2.49\times10^{-4}$ M}: 327 (−0.53), 312sh

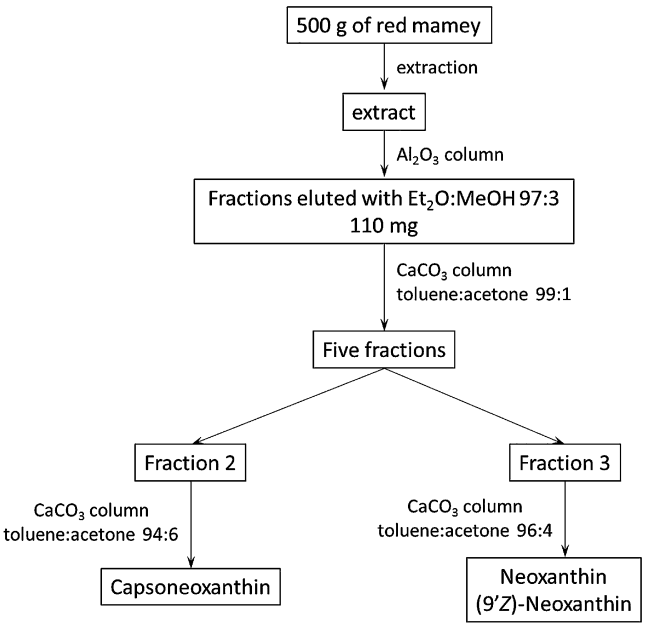

Fig. 1. Isolation of the allene carotenoids.

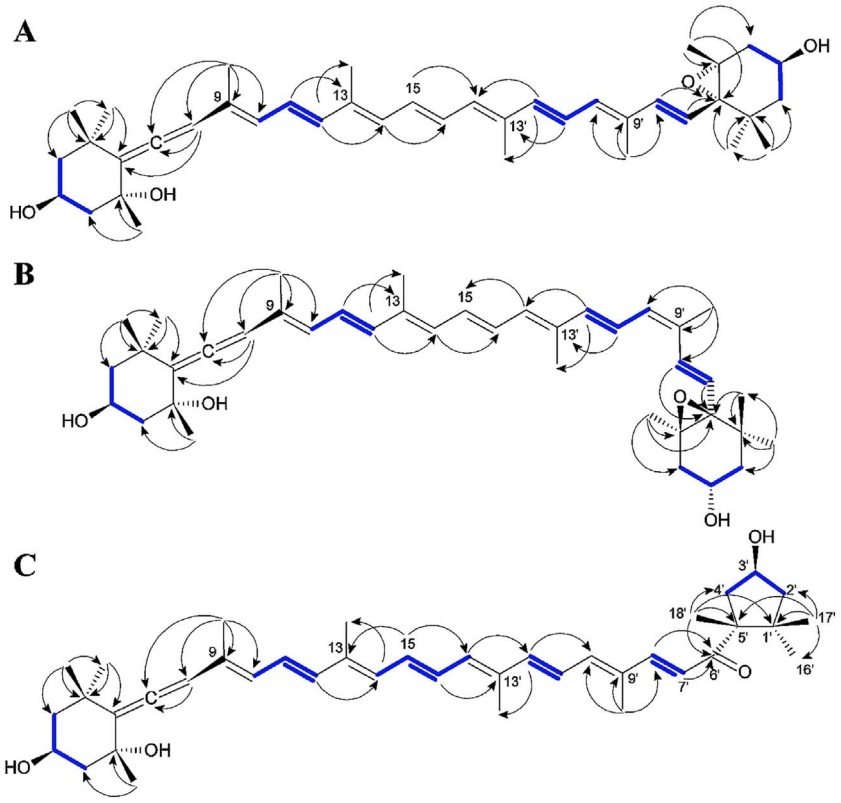

Fig. 2. Key HMBC (→) and ^{1}H-^{1}H COSY (▬) correlation of all-*E*-neoxanthin (**1**, A), (9′*Z*)-neoxanthin (**2**, B) and capsoneoxanthin (**3**, C).

(−0.44), 301sh (−0.23), 266 (1.14), 257sh (0.71), 229 (−2.70), 206 (−1.79). ECD {*n*-hexane, λ [nm] (Δε), $c = 2.49 \times 10^{-4}$ M}: 322 (−0.04), 266 (0.19), 229 (−0.44). MS: *m/z* 601 ($[M+H]^+$), 583 ($[M\text{-}H_2O + H]^+$). t_R = 9.1 min on a C30 column.

2.9. Capsoneoxanthin (3)

red crystals, 2.1 mg, m.p. 182 °C, UV/Vis (benzene): λ_{max} 480, 510 nm, ECD {MeOH, λ [nm] (Δε), $c = 1.364 \times 10^{-4}$ M}: 352sh (0.58), 338 (0.61), 287 (−0.36), 242 (0.33), 214 (−0.65). ECD {*n*-hexane, λ [nm] (Δε), $c = 1.364 \times 10^{-4}$ M}: 524sh (3.62), 483sh (4.07), 451sh (4.31), 388 (6.76), 342sh (−5.79), 321sh (−6.01), 262 (−4.11), 234sh (−1.76). MS: *m/z* 601 ($[M + H]^+$), 583 ($[M\text{-}H_2O + H]^+$). t_R = 7.7 min on a C30 column.

3. Results and discussion

Column chromatography of the mamey extract on aluminium oxide and calcium carbonate resulted in 1.5 mg of neoxanthin, **1**, 3.2 mg of (9′*Z*)-neoxanthin, **2**, and 2.1 mg of capsoneoxanthin, **3**. UV–vis spectroscopic and mass spectrometric properties, melting points of all three pigments were identical as found in the literature. NMR analysis resulted in minor amendments and expansions in the previous assignations of neoxanthin and (9′*Z*)-neoxanthin, whereas for capsoneoxanthin the amount of the compound made possible a detailed NMR analysis.

The ^{1}H NMR spectrum of **1** showed typical signals of two oxymethine [δ 4.32 (1H, m, H-3) and 3.91 (1H, m, H-3′)], thirteen olefinic protons, and ten tertiary methyl protons [δ 1.07 (H-16), 1.33 (H-17), 1.35 (H-18), 1.80 (H-19), 1.96 (H-20), 0.98 (H-16′), 1.15 (H-17′), 1.19 (H-18′), 1.80 (H-19′), and 1.96 (H-20′), each 3H, s]. The ^{13}C NMR spectrum revealed forty carbon signals, including ten methyl, four methylene, thirteen methine, and eleven quaternary carbons confirmed by HSQC and HMBC experiments. In addition, the presence of an allene group was indicated by the low-field ^{13}C NMR signal at δ 202.29 (C-7′).

The complete resonance assignment of **1** was established by using the combination of HSQC, HMBC and COSY spectra. The routine ^{1}H–^{1}H COSY experiment provided the connectivities for the protons of the C-2/C-3/C4, C-10/C-11/C-12, C-10′/C-11′/C-12′, and C-2′/C-3′/C-4′ spin systems, respectively (highlighted in blue on Fig. 2). The epoxy group at C-5′/C-6′ was identified by detecting HMBC correlations between H-18′ (δ 1.19) and C-4′ (δ 41.02)/C-5′ (δ 66.96)/C-6′ (δ 70.33). A detailed analysis of all HMBC correlations led to the planar structure of **1** (Fig. 2A). The relative configurations (axial and equatorial arrangements) were deduced by ROESY experiments.

The complete NMR resonance assignment of **2** followed the same strategy. Since the ^{1}H chemical shifts of H-14, 14'and H-15, 15' resonances were identical, COSY experiment failed to provide site-specific assignment. However, subtle differences in the ^{13}C chemical shifts offered the possibility to record band-selective HMBC experiments and thereby provided unequivocal resonance assignment. Comparing the chemical shifts of **1** and **2**, remarkable differences were detected for H-8′ and C-8′. The coupling pattern and the significant downfield shift observed for H-8' (from 6.29 ppm to 6.84 ppm) and the remarkable upfield shift for C-8' (from 137.35 ppm to 129.40 ppm) also revealed the 9′*Z* arrangement (Britton et al., 2004). The chemical shift changes for C-9' and C-10' also confirmed the structure of **2** (Fig. 2B).

The resonance assignment of the allenic moiety of **3** was established by combining COSY, HSQC and HMBC data, similarly to **1** and **2**. In the case of **3,** the spin system of H-14/H-15/H-15'/H-14' was identified by the COSY experiment (Fig. 2C). The first bottleneck in the assignment was the identification of the entry point into the spin system containing H-7'. In order to clarify the ^{1}H and ^{13}C resonances, band-selective versions of both HSQC and HMBC were utilized. The diagnostic H-8' resonance at 7.33 ppm revealed a conjugation with a carbonyl group (Britton et al., 2004). The presence of a characteristic carbonyl resonance (C-6') at 202.91 ppm served as a crucial HMBC "pillar" connecting the kappa end to the core polyene chain through H-18′. Further difficulties also arose in the case of the ^{13}C resonance assign-

Table 1
Comparative NMR data of the three allene carotenoids (δ in ppm) in $CDCl_3$.

	Neoxanthin (**1**)		(9′*Z*)-Neoxanthin (**2**)		Capsoneoxanthin (**3**)	
No.	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
1	–	35.83	–	35.82	–	35.84
1′	–	35.36	–	35.31	–	43.98
2eq	1.95 (m, 1H)	49.47	1.95 (m, 1H)	49.47	1.95 (m, 1H)	49.47
2ax	1.33 (m, 1H)		1.34 (dd, *J* = 11.8 Hz, 1H)		1.34 (m, 1H)	
2′eq	1.63 (m, 1H)	47.20	1.64 (m, 1H)	47.21	2.00 (dd, *J* = 13.6, 7.8 Hz, 1H)	50.88
2′ax	1.25 (m, 1H)		1.26 (dd, *J* = 12.9, 10.5 Hz, 1H)		1.71 (dd, *J* = 13.6, 4.7 Hz, 1H)	
3	4.32 (m, 1H)	64.34	4.32 (ddd, *J* = 11.2, 7.0, 4.3 Hz, 1H)	64.34	4.32 (ddd, *J* = 15.0, 10.8, 3.9 Hz, 1H)	64.33
3′	3.91 (m, 1H)	64.32	3.93 (m, 1H)	64.31	4.51 (tt, *J* = 8.0, 3.9 Hz, 1H)	70.38
4eq	2.26 (m, 1H)	48.92	2.26 (ddd, *J* = 13.0, 4.3, 2.2 Hz, 1H)	48.91	2.26 (ddd, *J* = 13.0, 4.3, 2.2 Hz, 1H)	48.92
4ax	1.41 (t, *J* = 12.0 Hz, 1H)		1.41 (dd, *J* = 12.9, 11.2 Hz, 1H)		1.41 (dd, *J* = 12.8, 11.2 Hz, 1H)	
4′eq	2.39 (ddd, *J* = 14.2. 5.1. 1.8 Hz, 1H)	41.02	2.41 (ddd, *J* = 14.2, 5.2, 1.8 Hz, 1H)	41.01	2.96 (dd, *J* = 14.4, 8.5 Hz, 1H)	45.32
4′ax	1.63 (m, 1H)		1.64 (dd, *J* = 14.3, 8.8 Hz, 1H)		1.49 (dd, *J* = 14.4, 3.3 Hz, 1H)	
5	–	73.02	–	73.03	–	73.02
5′	–	66.96	–	67.07	–	58.95
6	–	117.66	–	117.65	–	117.71
6′	–	70.33	–	70.51	–	202.91
7	–	202.29	–	202.27	–	202.36
7′	5.88 (d, *J* = 15.5 Hz, 1H)	123.84	5.93 (d, *J* = 15.4 Hz, 1H)	125.93	6.44 (d, *J* = 15.0 Hz, 1H)	120.91
8	6.03 (s, 1H)	103.26	6.03 (s, 1H)	103.27	6.03 (s, 1H)	103.25
8′	6.29 (d, *J* = 15.5 Hz, 1H)	137.35	6.84 (d, *J* = 15.4 Hz, 1H)	129.40	7.33 (d, *J* = 15.0 Hz, 1H)	146.85
9	–	131.91	–	131.83	–	132.36
9′	–	134.27	–	132.76	–	133.66
10	6.11 (d, *J* = 11.4 Hz, 1H)	128.51	6.11 (d, *J* = 11.3 Hz, 1H)	128.53	6.12 (d, *J* = 11.3 Hz, 1H)	128.44
10′	6.20 (d, *J* = 11.4 Hz, 1H)	132.26	6.07 (d, *J* = 11.5 Hz, 1H)	130.78	6.55 (d, 1H)	140.67
11	6.55 (dd, *J* = 15.1, 11.3 Hz, 1H)	124.84	6.54 (dd, *J* = 15.0, 11.3 Hz, 1H)	124.74	6.58 (dd, 1H)	125.37
11′	6.60 (dd, *J* = 15.1, 11.3 Hz, 1H)	124.64	6.76 (dd, *J* = 14.9, 11.5 Hz, 1H)	123.60	6.61 (dd, 1H)	124.11
12	6.33 (d, *J* = 15.0 Hz, 1H)	137.31	6.34 (d, *J* = 15.0 Hz, 1H)	137.35	6.34 (d, *J* = 14.9 Hz, 1H)	137.15
12′	6.37 (d, *J* = 15.0 Hz, 1H)	138.20	6.29 (d, *J* = 15.0 Hz, 1H)	137.44	6.52 (d, *J* = 14.6 Hz, 1H)	141.94
13	–	136.47	–	136.31	–	137.46
13′	–	136.31	–	136.48	–	135.92
14	6.24 (d, *J* = 10.8 Hz, 1H)	132.51	6.24 (m, 1H)	132.54	6.26 (d, *J* = 11.5 Hz, 1H)	132.33
14′	6.26 (d, *J* = 10.8 Hz, 1H)	132.88	6.22 (m, 1H)	132.59	6.35 (d, *J* = 11.4 Hz, 1H)	135.21
15	6.63 (m, 1H)	130.24	6.62 (m, 1H)	130.11	6.70 (dd, *J* = 14.2, 11.5 Hz, 1H)	131.59
15′	6.62 (m, 1H)	130.00	6.62 (m, 1H)	129.99	6.64 (m, 1H)	129.71
16	1.07 (s, 3H)	32.16	1.33 (s, 3H)	29.36	1.33 (s, 3H)	29.36
16′	0.98 (s, 3H)	24.92	1.17 (s, 3H)	29.57	0.84 (s, 3H)	25.88
17	1.33 (s, 3H)	29.36	1.07 (s, 3H)	32.17	1.07 (s, 3H)	32.16
17′	1.15 (s, 3H)	29.59	1.01 (s, 3H)	24.96	1.21 (s, 3H)	25.10
18	1.35 (s, 3H)	31.40	1.35 (s, 3H)	31.40	1.35 (s, 3H)	31.39
18′	1.19 (s, 3H)	20.01	1.21 (s, 3H)	20.00	1.37 (s, 3H)	21.31
19	1.80 (s, 3H)	13.97	1.80 (s, 3H)	13.97	1.81 (s, 3H)	14.00
19′	1.93 (s, 3H)	13.01	1.93 (s, 3H)	21.01	1.96 (s, 3H)	12.85
20	1.96 (s, 3H)	12.80	1.96 (s, 3H)	12.80	1.98 (s, 3H)	12.88
20′	1.96 (s, 3H)	12.81	1.96 (s, 3H)	13.02	1.98 (s, 3H)	12.75

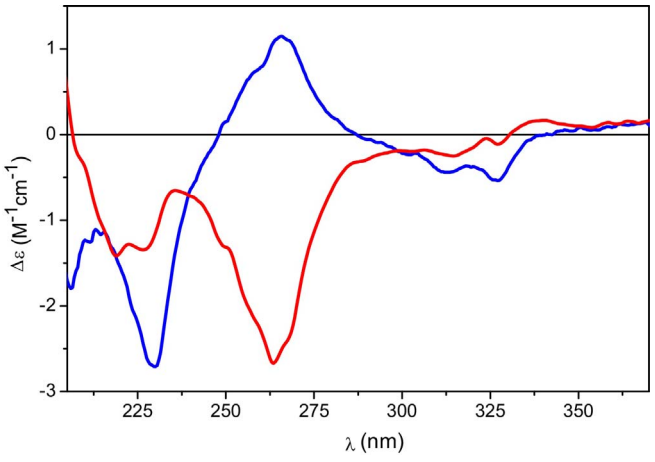

Fig. 3. ECD spectra of (all-*E*)-neoxanthin (**1**, red curve) and (9′*Z*)-neoxanthin (**2**, blue curve) in MeOH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ment of the quaternary carbons C-5' and C-1' at the kappa end. The heteronuclear coupling constants in the kappa end did not allow a clear distinction of two- and three-bond correlations of H-18' and H-17'/H-16' to the quaternary carbons (C-5' and C-1') in the HMBC spectrum. The close proximity of the electron withdrawing C-6' carbonyl suggested that C-5' exhibit the higher chemical shift at 58.95 ppm, while the quaternary carbon signal at 43.98 ppm could be assigned to C-1'. This assignment was also confirmed by chemical shift predictions.

Based on the 2D-NMR analysis of capsoneoxanthin (**3**), the following changes were observed compared to the previous assignation (Deli et al., 2000): the chemical shifts of H-15 and H-15' should be exchanged, the right values can be found in Table 1. H-12 and H-14' are not "exchangeable" anymore, the assignation is unambiguous in 600 MHz. Contrary to the previous article the carbons C-20, C-19' and C-20' could be clearly assigned. The chemical shifts for C-16 and C-17 were amended, and all the quaternary carbons were assigned, as well. In the case of all-*E*- and (9'*Z*)-neoxanthin we could provide the most extensive NMR data to date (Supplementary material Figs. 1–52).

Additionaly, ECD spectra of the pure pigments were compared (*vide infra*). The ECD spectrum of the (all-*E*)-neoxanthin (**1**) was in accordance with reported ECD data in ethanol (Baumeler and Eugster, 1992) and it showed an intense negative Cotton effect (CE) at 263 nm in

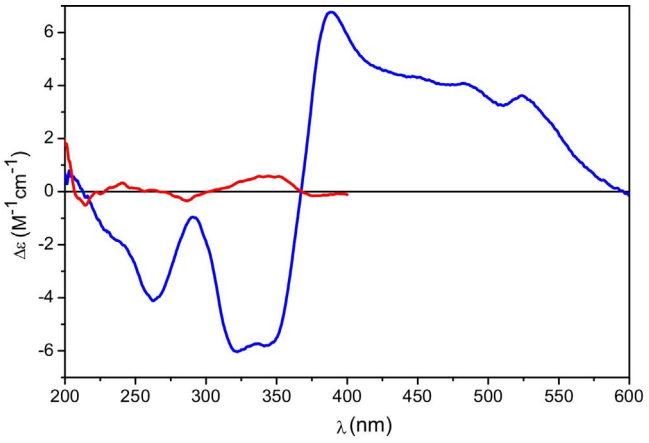

Fig. 4. ECD spectra of capsoneoxanthin (**3**) in methanol (red) and *n*-hexane (blue). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

methanol, which was changed to a positive CE (226 nm) in the ECD spectrum of the (9'*Z*)-neoxanthin (Fig. 3). The ECD spectra of **1** and **2** measured in *n*-hexane had the same ECD pattern as those recorde in methanol but intensities were smaller, which may be attributed to the lower solubility in *n*-hexane.

The ECD spectrum of capsoneoxanthin (**3**) (Fig. 4) showed weak CEs in the 200–400 nm wavelength range, characteristic of the individual solvated molecule, while intensities significantly increased and new bands appeared in *n*-hexane, which suggested the formation of supramolecular aggregates. Interestingly, this behaviour was observed only for capsoneoxanthin (**3**), while the ECD spectra of **1** and **2** in *n*-hexane were found similar to those measured in methanol (Supplementary material Figs. 53–56).

4. Conclusions

Mamey contains allene carotenoids in a relatively high amount. Because of the high general pigment content of the fruit these carotenoids can be isolated in milligram amounts and in crystalline form by using succesive chromatographic steps, which allowed the full ^{1}H and ^{13}CNMR assignation of both isomers of neoxanthin and the first measurement of the 2D and ^{13}C NMR spectra of the minor carotenoid component capsoneoxanthin.

Acknowledgements

This study was financed by the Hungarian Scientific Research Fund (Grants: OTKA 115931, K120181 and PD 109373). The financial support from ÚNKP-16-4 New National Excellence Program of the Ministry of Human Capacities for S. B. is highly appreciated. The authors are grateful to Mrs. Judit Rigó, Ms. Zsuzsanna Götz and Mr. Roland Lukács for their skillful assistance. The present scientific contribution is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jfca.2017.04.004.

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