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ISOLATION AND STRUCTURE ELUCIDATION OF MINOR CAROTENOIDS FROM ANNATTO (*BIXA ORELLANA* L.) SEEDS*

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Key Word Index—*Bixa orellana*; Bixaceae; annatto; carotenoids; dimethyl (9*Z*,9′*Z*)-6,6′-diapocarotene-6,6′-dioate; methyl (9*Z*)-10′-oxo-6,10′-diapocaroten-6-oate; methyl (9*Z*)-6′-oxo-6,5′-diapocaroten-6-oate; dimethyl (9*Z*)-6,6′-diapocarotene-6,6′-dioate; methyl (9*Z*)-8′-oxo-6,8′-diapocaroten-6-oate; methyl (4*Z*)-4,8-dimethyl-12-oxo-dodecyl-2,4,6,8,10-pentaenoate.

Abstract—From the seed coat of *Bixa orellana* fruits, six minor carotenoids and one C_{14}-carotenoid derivative were isolated by chromatography (CC, TLC, HPLC) and their structures elucidated by means of spectroscopy (UV-visible, MS, ^1H and ^{13}C NMR). Dimethyl (9*Z*,9′*Z*)-6,6′-diapocarotene-6,6′-dioate, methyl (9*Z*)-10′-oxo-6,10′-diapocaroten-6-oate, methyl (9*Z*)-6′-oxo-6,5′-diapocaroten-6-oate and methyl (4*Z*)-4,8-dimethyl-12-oxo-dodecyl-2,4,6,8,10-pentaenoate are new compounds, whereas methyl bixin and methyl (9*Z*)-8′-oxo-6,8′-diapocaroten-6-oate have previously been found in annatto.

INTRODUCTION

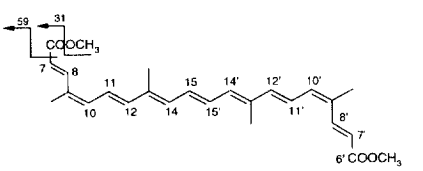
In the course of our previous studies [1, 2] on minor apocarotenoids from annatto (*Bixa orellana* L.) seeds, the structures of five new carotenoids and two carotenoids which have not previously been detected in annatto were assigned. In continuation of this work, the isolation and structure elucidation of another six diapocarotenoids and one C_{14}-carotenoid derivative, all present as minor compounds (in total *ca* 1% of the carotenoid content) in annatto seeds, are described.

RESULTS AND DISCUSSION

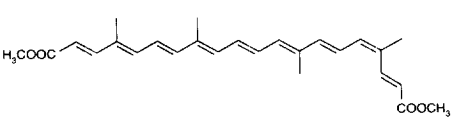
The ethyl acetate–*t*-BuOMe extract of *B. orellana* upon column chromatography, TLC and HPLC [2] afforded compounds **1**–**7**.

Dimethyl (9Z,9′Z)-6,6′-*diapocarotene*-6,6′-*dioate* (**1**) *and dimethyl* (9Z)-6,6′-*diapocarotene*-6,6′-*dioate* (**2**)

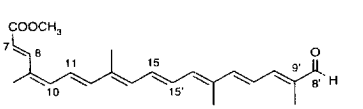
This is the first report on **1**, while the isomer **2** has previously been detected in annatto [3]. The UV-visible spectra of both compounds were similar (**1**: λ_{max} 350, 423, 448, 476 nm; **2**: λ_{max} 350, 427, 453, 483 nm), but in the case of the (9*Z*,9′*Z*)-diapocarotenoid **1**, a decreased spectral fine structure (%III/II 55) and a higher intensity of the *cis*-peak (% A_B/A_{II} 15) could be observed compared to the (9*Z*)-isomer **2** (%III/II 65 and % A_B/A_{II} 10). The iodine catalysed isomerization of **1** caused a bathochromic shift of 5 nm in the visible spectrum resulting in the same λ_{max} as for **2**. However, no shift of λ_{max} and no increase in the



Dimethyl (9*Z*,9′*Z*)-6,6′-diapocarotene-6,6′-dioate (**1**)



Methyl bixin (dimethyl (9*Z*)-6,6′-diapocarotene-6,6′-dioate) (**2**)



Methyl (9*Z*)-8′-oxo-6,8′-diapocaroten-6-oate (**3**)

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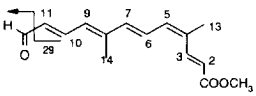
relative intensity of the *cis*-peak during the isomerization of **2** was observed. Therefore, after isomerization, carotenoids **1** and **2** showed the same wavelength maxima (429, 453 and 484 nm), spectral fine structure (%III/II 39) and relative intensity of the *cis*-peak (%A_B/A_{II} 13). These same features in the UV-visible spectrum of **2** were also reported by Zechmeister and Escue [4], who suggested that the great stability of the (*Z*)-double bond in **2** may cause a second (all-*E*) → (*Z*) isomerization giving substantial amounts of (di-*Z*)-methyl bixin instead of yielding significant amounts of the (all-*E*)-isomer. By comparison of the UV-visible data, it seems that neomethyl bixin C obtained by isomerization of methyl bixin [4] is the same compound as **1**. The mass spectra of **1** and **2** showed the $[M]^+$ at *m/z* 408, which is consistent with $C_{26}H_{32}O_4$, and characteristic fragments at *m/z* 377 $[M-31]^+$ and 349 $[M-59]^+$. In the NMR spectra, the chemical shifts of both end groups of **2** were in agreement with the data given previously for the same compound isolated from annatto [3]. No comparison of these data with our results with regard to the chemical shifts and coupling constants for the *olefinic* protons can be performed, since they were reported as multiplets. Compared to the NMR spectrum of **2** the spectrum of **1** exhibited shifts towards lower field for H-8 and H-11 of +0.57 and +0.23 ppm, respectively, and shifts towards higher field for H-10 and H-12 of −0.13 and −0.11 ppm, respectively. This is characteristic for (9*Z*)-isomers and in accordance with the data reported by Englert [5].

Methyl (9Z)-8′-*oxo*-6,8′-*diapocaroten*-6-*oate* (**3**)

This compound has been isolated previously from annatto. After iodine catalysed isomerization, the UV-visible spectrum of **3** showed a bathochromic shift of 3 nm of λ_{max}, a decreased spectral fine structure and an increased intensity of the *cis*-peak. The mass spectrum of **3** showed the $[M]^+$ at *m/z* 352 ($C_{23}H_{28}O_3$), as reported previously [3], and characteristic fragments at *m/z* 293 $[M-59]^+$ and at *m/z* 246 $[M-106]^+$ compatible with the elimination of toluene from the polyene chain. The ^1H and ^{13}C NMR data were in agreement with the literature [3], except for the ^1H values for H-7 and H-8 which are exchanged. Furthermore, the olefinic protons were reported as multiplets and the chemical shifts of the methyl groups, with the exception of the 6-$COOCH_3$ were not given.

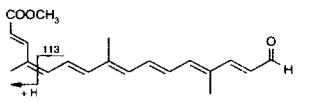
Methyl(4Z)-4,8-*dimethyl*-12-*oxo*-*dodecyl*-2,4,6,8,10-*pentaenoate* (**4**) *and methyl* (9Z)-10′-*oxo*-6,10′-*diapocaroten*-6-*oate* (**5**)

These are new compounds. According to the nomenclature rules [6], compound **4** is not a carotenoid since the two central methyl groups of the C_{40}-skeleton are not retained. However, it is probable that **4** is a degradation product of an annatto carotenoid such as bixin or another diapocarotenoid. The

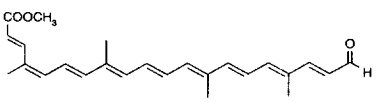


Methyl (4*Z*)-4,8-dimethyl-12-oxo-dodecyl-2,4,6,8,10-pentaenoate

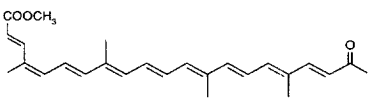
(**4**)



Methyl (9*Z*)-10′-oxo-6,10′-diapocarotene-6-oate (**5**)



Methyl (9*Z*)-6′-oxo-6,6′-diapocaroten-6-oate (**6**)



Methyl (9*Z*)-6′-oxo-6,5′-diapocaroten-6-oate (**7**)

UV spectrum of **4** exhibited λ_{max} at 367 nm, with spectral fine structure (%III/II 55) and no *cis*-peak. The iodine catalysed isomerization caused a bathochromic shift of 2 nm and an increased spectral fine structure (% III/II 63), which gives an indication that the (4*Z*)-double bond was transformed to the (*E*)-double bond. In the mass spectra of **4**, the $[M]^+$ appeared at *m/z* 246 compatible with $C_{15}H_{18}O_3$, and characteristic fragment ions were detected at *m/z* 217 $[M-29]^+$, 199 $[M-47]^+$ and 185 $[M-61]^+$, and at *m/z* 215 $[M-31]^+$ and 187 $[M-59]^+$. The NMR data of **4** confirm the (4*Z*)-double bond and are in agreement with the proposed structure.

With seven conjugated carbon–carbon double bonds (c.d.b.), carotenoid **5** exhibited a UV-visible spectrum with the most prominent fine structure (% III/II 82) among the isolated compounds. An analogous observation has been reported for C_{40}-carotenes, where the degree of fine structure increases from phytoene (3 c.d.b.) to ξ-carotene (7 c.d.b.) and then decreases as the chromophore is extended [7]. A bathochromic shift of 3 nm and an increased spectral fine structure (% III/II 95) was observed after isomerization, indicating a (*Z*) → (*E*)-isomerization. The mass spectrum of **5** showed the $[M]^+$ at *m/z* 312 ($C_{20}H_{24}O_3$); characteristic fragment ions due to the loss of the aldehyde (*m/z* 283 $[M-29]^+$, 265 $[M-47]^+$) and the methyl ester (*m/z* 253 $[M-31]^+$, 199 $[M-113]^+$) end groups were detected. The NMR data of **5** were in agreement with the structure proposed.

In addition to the above compounds, two new carotenoids, methyl (9*Z*)-6′-oxo-6,6′-diapocaroten-6′-oate (**6**) and methyl (9*Z*)-6′-oxo-6,5′-diapocaroten-6-

oate (**7**), were isolated. Characteristic differences in the UV-visible spectra of **6** and **7** can be observed, although both compounds contain the same chromophore. The UV-visible spectrum of **6** exhibited λ_{max} at longer wavelength (459 nm) and a decreased spectral fine structure (% III/II 57) compared to the keto carotenoid **7** (455 nm and % III/II 67). After iodine catalysed isomerization, both carotenoids showed a bathochromic shift of λ_{max} of 1 nm, a decreased spectral fine structure and an increased relative intensity of the *cis*-peak. The mass spectrum of **6** showed the $[M]^+$ at *m/z* 378, consistent with $C_{25}H_{30}O_3$, and fragment ions at *m/z* 209 $[M-59-18-92]^+$ and 195 $[M-59-18-106]^+$ due to the combined losses of the methyl ester, H_2O (from the aldehyde) and elimination of toluene and xylene, respectively. The $[M]^+$ of **7** appeared at *m/z* 392 ($C_{26}H_{32}O_3$), characteristic ions at *m/z* 349 $[M-43]^+$, 109 and 43 due to the loss of the keto group and at *m/z* 286 due to the elimination of toluene from the polyene chain were observed. The ^1H and ^{13}C chemical shifts confirmed the presence of the aldehyde and keto group in **6** and **7**, respectively, and are in agreement with the proposed structure [1, 5].

Although no alkali was used during the extraction and isolation procedure, the presence of the keto carotenoid **7** is most likely an artifact arising from an aldol condensation of carotenoid **3** with acetone from the mobile phase used in the TLC on MgO.

Each of the minor isolated compounds contain the same (9*Z*)-methyl ester end group as bixin, which is the main carotenoid accounting for more than 80% of the total carotenoid content of *B. orellana* seeds. Apparently diapocarotenoids are formed by oxidative degradation of C_{40}-carotenoids, although no detailed evidence for bixin has been presented until now.

In fact, these minor carotenoids might be considered as natural metabolites derived from C_{40}-carotenes by enzymatic oxidative cleavage. It seems that the preferred reaction is a cleavage at the $C_{5,6}$- and $C_{5',6'}$-double bonds, respectively, at both ends of the molecule (carotenoids **1**, **2** and **6**) yielding structures similar to bixin. To a smaller extent, cleavage at the $C_{5,6}$-double bond plus a cleavage at different positions at the other end, e.g. at double bond $C_{7,8}$ (carotenoid **3**), $C_{9',10'}$ (resulting in carotenoid **5**) and $C_{13',14'}$ (resulting in carotenoid-derivative **4**) also seems to occur.

EXPERIMENTAL

Spectroscopy. UV-visible spectra: *tert*-butyl methyl ether (*t*-BuOMe). Spectral fine structure is expressed as %III/II and relative intensity of *cis*-peak as % A_B/A_{II} [7, 8]. The isomerization reaction catalysed by I_2 and light was performed in *t*-BuOMe as solvent by addition of four drops of an I_2 soln in the same solvent and monitored after 5 and 30 min [8]. Mass spectra were obtained by an AE MS 9 instrument with a VG console and a Finnigan MAT SS300 data system. The samples were introduced using a direct inlet system at 70 eV, 140–210°. The ^1H NMR (400.13 MHz) and ^{13}C NMR (100.25 MHz) spectra were recorded on a Bruker DRX-400 instrument at 23° in $CDCl_3$ (99.95%). Chemical shifts of ^1H and ^{13}C resonances were related to residual solvent signals, and only relevant $^nJ_{HH}$ values are given. For all compounds, complete proton assignments were achieved by ^1H and $^1H^1H$ COSY experiments. The sample amounts of compounds **4**, **6** and **7** were insufficient to perform carbon-13 experiments (^{13}C, DEPT 135), and therefore δ values of the proton-bearing carbon nuclei were extracted from proton detected HMQC experiments (optimized for $^1J_{CH}$) with a precision of ± 0.3 ppm.

Plant material and chromatographic systems. See refs [1, 2]. Several chromatographic systems including CC, TLC and HPLC were used. The TLC systems used were: TLC-1: silica gel, EtOAc–hexane (1:4); TLC-2: silica gel, EtOAc–hexane (2:3); TLC-3: MgO–kieselguhr (0.5 mm), Me_2CO–hexane (1:1); TLC-4: MgO–kieselguhr (0.5 mm), Me_2CO–hexane (3:2); TLC-5: MgO–kieselguhr (0.5 mm), Me_2CO–hexane (4:1); TLC-6: MgO–kieselguhr (0.5 mm), Me_2CO–CH_2Cl_2 (9:1). The HPLC systems all used RP-Nucleosil-3RP-18 (250 × 10 mm) at a flow rate of 3 ml min^{-1} with the following mobile phases: HPLC-1: MeOH–*iso*-PrOH (9:1); HPLC-2: MeOH–EtOAc–H_2O (16:1:3); HPLC-3: MeOH–EtOAc–H_2O (12:3:5); HPLC-4: MeOH–EtOAc–H_2O (68:7:25).

Isolation. Carotenoid extraction and subsequent two flash CC steps were performed according to Mercadante *et al.* [2]. The subject of this communication is the sixth band (Fr. VI) eluted from the silica gel column with EtOAc–hexane (3:2). Fr. VI was applied on a second flash column on silica gel, resulting in two frs (fr. 1 and fr. 2) eluted with EtOAc–hexane (1:1) and EtOAc, respectively.

Dimethyl (9Z,9′Z)-6,6′-*diapocarotene*-6,6′-*dioate* (**1**). (*ca* 1.6 mg). Obtained from fr. 1 by TLC-1 (R_f 0.58) and purified by TLC-5 and HPLC-1 (*R*, 8.5 min). UV-vis λ_{max} nm: 350, 423, 448, 476 (%III/II 55, $\%A_B/A_{II}$ 15), after I_2: 350, 429, 453, 484 (%III/II 44, A_B/A_{II} 13); EI-MS *m/z* (rel. int.): 408 $[M]^+$ (27), 339 $[M-69]^+$ (2), 313 $[M-95]^+$ (5), 236 (11), 145 (69), 69 (100); ^1H NMR (400 MHz, $CDCl_3$): δ 1.958 (6H, *s*, H-19 and H-19′), 1.999 (6H, *s*, H-20 and H-20′), 3.787 (6H, *s*, 6-$COOCH_3$ and 6′-$COOCH_3$), 5.91 (2H, *d*, J = 15.5 Hz, H-7 and H-7′), 6.31 (2H, M and M′ part of AA′MM′, H-14 and H-14′), 6.37 (2H, *d*, J = 11.7 Hz, H-10 and H-10′), 6.41 (2H, *d*, J = 14.8 Hz, H-12 and H-12′), 6.66 (2H, A and A′ part of AA′MM′, H-15 and H-15′), 6.85 (2H, *dd*, J = 11.7 and 14.8 Hz, H-11 and H-11′), 7.96 (2H, *d*, J = 15.5 Hz, H-8 and H-8′); ^{13}C NMR (100.25 MHz, $CDCl_3$): δ 12.97 (C-20 and C-20′), 20.28 (C-19 and C-19′), 51.60 (Me of C-6 and C-6′), 117.41 (C-7 and C-7′), 123.15 (C-11 and C-11′), 130.85 (C-15 and C-15′), 131.42 (C-9 and C-9′), 134.31 (C-14 and C-14′), 136.71 (C-13 and C-13′), 138.00 (C-10 and C-10′), 140.46 (C-8 and C-8′), 140.49 (C-12 and C-12′), 168.00 (C-6 and C-6′).

Dimethyl (9Z)-6,6′-*diapocarotene*-6,6′-*dioate* (**2**).

Isolated from fr. 1 by TLC-1 (R_f 0.54) followed by TLC-4 and TLC-6. UV-vis λ_{max} nm: 350, 427, 453, 483 (%III/II 65, A_B/A_{II} 10), after I_2: 349, 428, 453, 484 (%III/II 40, A_B/A_{II} 13); EI-MS *m/z* (rel. int.): 408 $[M]^+$ (43), 377 $[M-31]^+$ (1), 349 $[M-59]^+$ (2), 302 $[M-106]^+$ (9), 145 (100); ^1H NMR (400.13 MHz, $CDCl_3$): δ 1.944 (3H, *s*, H-19), 1.958 (3H, *s*, H-19′), 1.984 (3H, *s*, H-20′), 2.005 (3H, *s*, H-20), 3.76 (3H, *s*, 6′-$COOCH_3$), 3.78 (3H, *s*, 6-$COOCH_3$), 5.88 (1H, *d*, J = 15.5 Hz, H-7), 5.91 (1H, *d*, J = 15.3 Hz, H-7′), 6.32 (1H, *m*, H-14), 6.35 (1H, *m*, H-14′), 6.37 (1H, *d*, J = 11.4 Hz, H-10′), 6.41 (1H, *d*, J = 14.5 Hz, H-12′), 6.50 (1H, *d*, J = 11.3 Hz, H-10), 6.52 (1H, *d*, J = 13.7 Hz, H-12), 6.62 (1H, *dd*, J = 11.3 and 13.7 Hz, H-11), 6.68 (1H, *m*, H-15′), 6.69 (1H, *m*, H-15), 6.86 (1H, *dd*, J = 11.4 and 14.5 Hz, H-11′), 7.39 (1H, *d*, J = 15.5 Hz, H-8), 7.96 (1H, *d*, J = 15.3 Hz, H-8′).

Methyl (9Z)-8′-*oxo*-6,8′-*diapocarotene*-6-*oate* (**3**). (*ca* 1.2 mg). From fr. 2 by TLC-1 (R_f 0.29) and subsequent purification by TLC-3 and HPLC-1 (R_t 6.3 min). UV-vis λ_{max} nm: 337, 415, 441, 470 (%III/II 79, A_B/A_{II} 9), after I_2: 337, (418), 444, 472 (%III/II 68, A_B/A_{II} 13); EI-MS *m/z* (rel. int.): 352 $[M]^+$ (100), 293 $[M-59]^+$ (3), 246 $[M-106]^+$ (19), 209 (13), 145 (45), 197 (18), 105 (50), 91 (85); ^1H NMR (400.13 MHz, $CDCl_3$): δ 1.905 (3H, *d*, J = 1.0 Hz, H-19′), 1.965 (3H, *s*, H-19), 2.014 (3H, *s*, H-20′), 2.019 (3H, *s*, H-20), 3.790 (3H, *s*, 6-$COOCH_3$), 5.92 (1H, *d*, J = 15.5 Hz, H-7), 6.34 (1H, *d*, J = 11.6 Hz, H-14), 6.37 (1H, *d*, J = 11.7 Hz, H-10), 6.41 (1H, *d*, J = 14.9 Hz, H-12), 6.45 (1H, *d*, J = 12.0 Hz, H-14′), 6.68 (1H, *dd*, J = 12.0 and 14.5 Hz, H-15′), 6.70 (1H, *m*, A part of ABM, H-11′), 6.72 (1H, *m*, B part of ABM, H-12′), 6.77 (1H, *dd*, J = 11.6 and 14.5 Hz, H-15), 6.89 (1H, *dd*, J = 11.7 and 14.9 Hz, H-11), 6.95 (1H, *m*, M part of ABM, H-10′), 7.96 (1H, *d*, J = 15.5 Hz, H-8), 9.459 (1H, *s*, H-8′); ^{13}C NMR (100.25 MHz, $CDCl_3$): δ 9.67 (C-19′), 12.75 (C-20′), 13.06 (C-20), 20.32 (C-19), 51.63 (Me of C-6), 117.69 (C-7), 123.07 (C-11′), 123.78 (C-11), 130.32 (C-15′), 131.93 (C-9), 132.57 (C-15), 133.91 (C-14), 135.95 (C-13′), 136.99 (C-9′), 137.22 (C-13), 137.78 (C-14′), 137.93 (C-10), 140.38 (C-8), 167.96 (C-6), 194.57 (C-8′).

The second band (fr. 2.2), obtained from fr. 2 by TLC-1 (R_f 0.21), gave two zones on TLC-4 (R_f 0.49 and 0.1). Two compounds (**4** and **5**) were isolated from the upper zone, while carotenoids **6** and **7** from the lower band.

Methyl (4Z)-4,8-*dimethyl*-12-*oxo*-*dodecyl*-2,4,6,8,10-*pentaenoate* (**4**). (*ca* 0.4 mg). Sepd from carotenoid **5** by HPLC-3 (R_t 8.1 min) and again purified by HPLC-4 (R_t 10.2 min). UV-vis λ_{max} nm: 350, 367, 387 (%III/II 55), after I_2: 351, 369, 390 (%III/II 63); EI-MS *m/z* (rel. int.): 246 $[M]^+$ (100), 231 $[M-15]^+$ (8), 217 $[M-29]^+$ (29), 215 $[M-31]^+$ (10), 199 $[M-29-18]^+$ (13), 187 $[M-59]^+$ (33), 185 $[M-28-18-15]^+$ (38), 171 (30), 157 (73), 143 (54), 91 (47); ^1H NMR (400.13 MHz, $CDCl_3$): δ 1.99 (3H, *s*, H-13), 2.13 (3H, *d*, J = 1.0 Hz, H-14), 3.80 (3H, *s*, 1-$COOCH_3$), 5.98 (1H, *d*, J = 15.5 Hz, H-2), 6.21 (1H, *dd*, J = 11.6 and 15.2 Hz, H-11), 6.38 (1H, *d*, J = 11.7 Hz, H-5), 6.40 (1H, *d*, J = 11.6 Hz, H-9), 6.44 (1H, *d*, J = 15.1 Hz, H-7), 7.07 (1H, *dd*, J = 11.7 and 15.1 Hz, H-6). 7.51 (1H, *dd*, J = 11.6 and 15.2 Hz, H-10), 7.94 (1H, *d*, J = 15.5 Hz, H-3), 9.64 (1H, *d*, J = 15.2 Hz, H-12); ^{13}C NMR (100.25 MHz, $CDCl_3$): δ 13.2 (C-14), 20.3 (C-13), 51.6 (Me of C-1), 118.7 (C-2), 126.9 (C-6), 130.0 (C-9), 136.6 (C-5), 138.6 (C-7), 139.7 (C-3), 146.7 (C-10), 193.5 (C-12).

Methyl (9Z)-10′-*oxo*-6,10′-*diapocaroten*-6-*oate* (**5**). Isolated (*ca* 0.8 mg) by HPLC-2 (R_t 11.2 min). UV-vis λ_{max} nm: 398, 420, 445 (%III/II 82), after I_2: (402), 423, 449 (%III/II 95); EI-MS *m/z* (rel. int.): 312 $[M]^+$ (100), 283 $[M-29]^+$ (11), 281 $[M-31]^+$ (4), 265 $[M-29-18]^+$ (2), 253 $[M-59]^+$ (8), 223 $[M-59-28]^+$ (13), 209 (15), 199 $[M-113]^+$ (11), 145 (54); ^1H NMR (400.13 MHz, $CDCl_3$): δ 1.981 (6H, *s*, H-19 and H-20), 2.045 (3H, *s*, H-20′), 3.801 (3H, *s*, 6-$COOCH_3$), 5.95 (1H, *d*, J = 15.3 Hz, H-7), 6.21 (1H, *dd*, J = 7.9 and 15.4 Hz, H-11′), 6.35 (1H, X part of ABMX, $J \approx$ 12.3 Hz, H-14)*, 6.38 (1H, *d*, J = 11.7 Hz, H-10), 6.42 (1H, *d*, J = 15.1 Hz, H-12), 6.62 (1H, M part of ABMX, $J \approx$ 12.0 Hz, H-14′)*, 6.67 (1H, B part of ABMX, $J \approx$ 12.9 and $\approx$14.5 Hz, H-15′)*, 6.87 (1H, A part of ABMX, $J \approx$ 12.3 and $\approx$14.5 Hz, H-15)*, 6.93 (1H, *dd*, J = 11.7 and 15.1 Hz, H-11), 7.18 (1H, *d*, J = 15.4 Hz, H-12′), 7.97 (1H, *d*, J = 15.3 Hz, H-8), 9.61 (1H, *d*, J = 7.9 Hz, H-10′); ^{13}C NMR (100.25 MHz, $CDCl_3$): δ 12.77 (C-20), 13.15 (C-20′), 20.34 (C-19), 51.66 (Me of C-6), 117.95 (C-7), 124.46 (C-11), 127.53 (C-11′), 129.53 (C-15′), 132.44 (C-9), 133.41 (C-14), 134.41 (C-13), 134.85 (C-15), 137.57 (C-10), 139.30 (C-13′), 139.90 (C-12), 140.29 (C-8), 140.77 (C-14′), 156.37 (C-12′), 167.92 (C-6), 193.71 (C-10′).

Methyl (9Z)-6′-*oxo*-6,6′-*diapocaroten*-6′-*oate* (**6**). (*ca* 0.8 mg). Sepd from carotenoid **7** by TLC-2 (R_f 0.48) and purified by HPLC-2 (R_t 28.0 min). UV-visible λ_{max} nm: 434, 459, 490 (%III/II 57); after I_2: (436), 460, 491 (%III/II 18); EI-MS *m/z* (rel. int.): 378 $[M]^+$ (100), 209 $[M-59-18-92]^+$ (2), 195 $[M-59-18-106]^+$ (3), 157 (31), 183 (4), 169 (6), 145 (65), 43 (97); ^1H NMR (400.13 MHz, $CDCl_3$): δ 1.962 (3H, *s*, H-19), 1.986 (3H, *s*, H-19′), 2.001 (3H, *s*, H-20), 2.025 (3H, *s*, H-20′), 3.790 (3H, *s*, 6-$COOCH_3$), 5.92 (1H, *d*, J = 15.1 Hz, H-7), 6.19 (1H, *dd*, J = 7.7 and 15.2 Hz, H-7′), 6.33 (1H, X part of ABMX, H-14), 6.37 (1H, *d*, J = 11.8 Hz, H-10), 6.39 (1H, M part of ABMX, H-14′), 6.41 (1H, *d*, J = 14.4 Hz, H-12), $\approx$6.60 (3H, spin system of higher order, H-10′, H-11′ and H-12′), $\approx$6.62 (1H, B part of ABMX, H-15′), 6.70 (1H, A part of ABMX, H-15), 6.87 (1H, *dd*, J = 11.8 and 14.4 Hz, H-11), 7.18 (1H, *d*, J = 15.2 Hz, H-8′), 7.96 (1H, *d*, J = 15.1 Hz, H-8), 9.59 (1H, *d*, J = 7.7 Hz, H-6′); ^{13}C NMR (100.25 MHz, $CDCl_3$): δ 12.8 (C-19, C-19′ and C-20), 20.2 (C-19), 51.5 (Me of C-6), 117.6 (C-7), 123.3 (C-11), 123.9 (C-11′), 131.9 (C-15), 134.1 (C-

* Coupling constant values were estimated by simulation.

14), 137.6 (C-14′), 137.7 (C-10), 140.7 (C-12), 140.8 (C-7′), 141.3 (C-12′), 143.3 (C-10′), 193.5 (C-6′).

Methyl (9Z)-6′-*oxo*-6,5′-*diapocaroten*-6-*oate* (**7**). (*ca* 0.5 mg). Isolated by TLC-2 (R_f 0.45) and HPLC-2 (R_t 31.7 min). UV-vis λ_{max} nm: 430, 455, 486 (%III/II 67), after I_2: (432), 456, 488 (%III/II 34); EI-MS *m/z* (rel. int.): 392 $[M]^+$ (65), 349 $[M-43]^+$ (6), 286 $[M-106]^+$ (7), 145 (100), 109 (44), 43 (98); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 1.958 (6H, *s*, H-19 and H-19′), 1.991 (3H, *s*, H-20), 2.007 (3H, *s*, H-20′), 2.31 (3H, *s*, H-5′), 3.79 (3H, *s*, 6-$COOCH_3$), 5.91 (1H, *d*, J = 15.5 Hz, H-7), 6.17 (1H, *d*, J = 15.7 Hz, H-7′), 6.32 (1H, N part of ABMN, H-14′), 6.37 (1H, *d*, J = 11.8 Hz, H-10), 6.37 (1H, M part of ABMN, H-14), 6.41 (1H, *d*, J = 14.9 Hz, H-12), 6.54 (1H, *d*, J = 15.4 Hz, H-12′), 6.55 (1H, *d*, J = 11.0 Hz, H-10′), 6.64 (1H, *dd*, J = 11.0 and 15.4 Hz, H-11′), 6.67 (1H, B part of ABMN, H-15), 6.71 (1H, A part of ABMN, H-15′), 6.86 (1H, *dd*, J = 11.8 and 14.9 Hz, H-11), 7.23 (1H, *d*, J = 15.7 Hz, H-8′), 7.96 (1H, *d*, J = 15.5 Hz, H-8); ^{13}C NMR (100.25 MHz, $CDCl_3$): δ $\approx$12.6 (C-19′), $\approx$12.7 (C-20), 12.9 (C-20′), 20.2 (C-19), 27.5 (C-5′), 51.5 (Me of C-6), 117.3 (C-7), 123.4 (C-11), 124.2 (C-11′), 125.6 (C-7′), 130.7 (C-15), 131.5 (C-15′), 134.1 (C-14′), 135.1 (C-14), 137.9 (C-10), 140.3 (C-8 and C-10′), 140.4 (C-12), 142.1 (C-12′), 147.8 (C-8′).

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REFERENCES

1. Mercadante, A. Z., Steck, A., Rodriguez-Amaya, D., Pfander, H. and Britton, G., *Phytochemistry*, 1996, **41**, 1201.
2. Mercadante, A. Z., Steck, A. and Pfander, H., *Journal of Agriculture and Food Chemistry*, 1997, **45**, 1050.
3. Jondiko, I. J. O. and Pattenden, G., *Phytochemistry*, 1989, **28**, 3159.
4. Zechmeister, L. and Escue, R. B., *Journal of the American Chemical Society*, 1944, **66**, 322.
5. Englert, G., in *Carotenoids* Vol. 1B: *Spectroscopy*, ed. G. Britton, S. Liaaen-Jensen and H. Pfander. Birkhäuser, Basel, 1995, p. 147.
6. IUPAC Commission on the Nomenclature of Organic Chemistry and IUPAC-IUB Commission on Biochemical Nomenclature, *Pure and Applied Chemistry*, 1975, **41**, 407.
7. Britton, G., in *Carotenoids* Vol. 1B: *Spectroscopy*, ed. G. Britton, S. Liaaen-Jensen and H. Pfander. Birkhäuser, Basel, 1995, p. 13.
8. Ke, B., Imsgard, F., Kjosen, H. and Liaaen-Jensen, S., *Biochemica et Biophysica Acta*, 1970, **120**, 139.