(9*Z*)-Capsanthin-5,6-epoxide, a New Carotenoid from the Fruits of *Asparagus falcatus*

Péter Molnár,*,† József Deli,† Gyula Tóth,† Adrian Häberli,‡ Hanspeter Pfander,‡ and Kurt Bernhard§

Department of Medical Chemistry, Medical School, University of Pécs, P.O. Box 99, H-7601 Pécs, Hungary, Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland, and CaroteNature GmbH, CH-4419 Lupsingen, Switzerland

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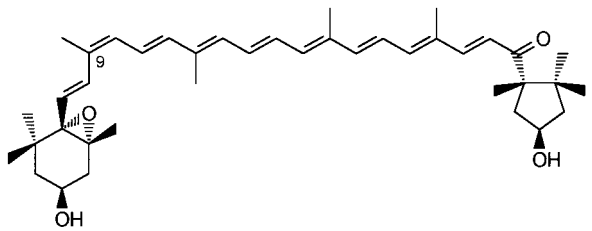
From the fruits of *Asparagus falcatus* a novel minor (*Z*)-carotenoid has been isolated and, on the basis of spectral data interpretation, characterized as (9*Z*)-capsanthin-5,6-epoxide [(9*Z*,3*S*,5*R*,6*S*,3′*S*,5′*R*)-5,6-epoxy-3,3′-dihydroxy-5,6-dihydro-*β*,*κ*-caroten-6′-one, (**1**)]. In addition, seven other (*Z*)-carotenoids [namely, (9*Z*)-, (9′*Z*)-, (13*Z*)-, and (13′*Z*)-capsanthins, (9*Z*)- and (13*Z*)-capsorubins, and (9*Z*)-violaxanthin], which have been previously described from other plants, were isolated and identified.

Previously, we have reported on the isolation of antheraxanthin, capsanthin, capsanthin-5,6-epoxide, capsanthone, capsochrome, capsoneoxanthin, capsorubin, *β*-carotene, *β*-cryptoxanthin, 5,6-diepikarpoxanthin, luteoxanthin, mutatoxanthin, violaxanthin, and zeaxanthin from the fruits of *Asparagus falcatus*; Aspfal (Liliaceae).[1,2] In continuation of these investigations, we describe in the present paper the isolation of (9*Z*)-capsanthin-5,6-epoxide (**1**), as a new minor, naturally occurring (*Z*)-carotenoid, and of seven other (*Z*)-carotenoids, which previously have been isolated from other natural sources.

The (*Z*)-carotenoids were separated by HPLC controlled column chromatography, on $CaCO_3$ with benzene−hexane (7:3) as eluting solvent. The separation was monitored by HPLC. After crystallization, the (*Z*)-isomers were characterized by their UV−vis, CD, ^{1}H NMR, and mass spectra. In addition, co-chromatography of the fully characterized compounds with authentic samples, on two different systems (TLC, HPLC), supported the identification. In addition to **1**, seven previously identified[3−5] (*Z*)-isomers, namely, (9*Z*)-capsanthin, (9′*Z*)-capsanthin, (13*Z*)-capsanthin, (13′*Z*)-capsanthin, (9*Z*)-capsorubin, (13*Z*)-capsorubin, and (9*Z*)-violaxanthin, were also fully identified.



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The hitherto unknown compound **1** had a UV−vis spectrum with maxima at 505, 475, 358 (*cis*-peak), and 323 nm (*cis*-peak) (in benzene). This corresponds to a hypsochromic shift of 5−6 nm compared to the (all-*E*)-isomer of **1**.[6−8] The weak intensities of the *cis*-peaks suggested that the (*Z*)-double bond was located at either C(9) or C(9′).

It is well known that in the acid-catalyzed reaction (C_6H_6−HCl) of (9*Z*)-epoxy carotenoids a simultaneous formation of the furanoid derivative and an isomerization of the (9*Z*)-double bond takes place.[9−11] In contrast, the same reaction of the corresponding (9′*Z*)-isomer gives the furanoid derivative without isomerization of the (9′*Z*)-double bond. The newly isolated (*Z*)-carotenoid **1** gave, in the presence of acid, a product with a UV−vis spectrum with maxima at 487 and 462 nm (in benzene), characteristic of the (all-*E*)-furanoid (5,8-epoxide)-isomer of **1**. This corresponds to a bathochromic shift of 4−5 nm compared to the spectrum of the product given, under the same conditions, by the (9′*Z*)-isomer (obtained by iodine-catalyzed photoisomerization of the (all-*E*)-capsanthin-5,6-epoxide; UV−vis maxima in benzene: 482 and 458 nm). These findings strongly support the (9*Z*)-position of the double bond.

HREIMS of **1** gave the molecular ion at *m*/*z* 600.4167 corresponding to $C_{40}H_{56}O_4$ (calculated for $C_{40}H_{56}O_4$, 600.41786), and the EIMS gave fragment ions at *m*/*z* 600 ($[M]^+$, 73), 494 (36), 221 (22), 181 (15), 109 (100), and 43 (100).[12] The 400 MHz ^{1}H NMR spectrum showed signals at 5.95 ppm [H−C(7)], 6.08 ppm [H−C(10)], and 6.84 ppm [H−C(8)] and was in agreement with the data previously reported for the (9*Z*)-epoxy end group.[5,14] In addition, the presence of a (9′*Z*)-double bond could be excluded unambiguously because of the lack of the signal at 7.95 ppm, characteristic for H-C(8′) of the (9′*Z*)-*κ*-end group.[3,4]

The CD spectrum of (9*Z*)-capsanthin-5,6-epoxide (**1**) exhibited positive maxima at 220, 240, 260, 270, 288, and 343 nm and negative maxima at 350, 380, 396, and 490 nm ($\Delta\epsilon$ values are indicated in the Experimental Section). Compared to the (all-*E*)-isomer, all signs between 220 and 400 nm are reversed, which is a characteristic feature of (mono-*Z*)-isomers,[13] so the (3*S*,5*R*,6*S*,3′*S*,5′*R*)-configuration of (9*Z*)-capsanthin-5,6-epoxide (**1**) was confirmed.

The occurrence of (9*Z*)-capsanthin 5,6-epoxide (**1**) in the fruits of *Asparagus falcatus* supports our earlier suggestion that in higher plants (*Lilium candidum*, *Lilium tigrinum*, *Helianthus annuus*, *Taraxacum officinale*) the (9*Z*)-isomers of unsymmetrical epoxy carotenoids such as antheraxanthin or lutein-5,6-epoxide occur together with the (13*Z*)- and (13′*Z*)-isomers without any detectable amounts of the corresponding (9′*Z*)-isomers.[4,9−11,14] This confirms the particular stability of the (9*Z*)-configuration of 5,6-epoxy carotenoids and indicates that, in nature, the formation of **1** is a stereospecific process.

* To whom correspondence should be addressed. Tel: +36-72-536 001/1698. Fax: +36-72-536 225. E-mail: Peter.Molnar@aok.pte.hu.
† University of Pécs.
‡ University of Bern.
§ CaroteNature GmbH.

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Experimental Section

General Experimental Procedures. UV−vis spectra were recorded on a Beckman DU-65 spectrophotometer. CD spectra were obtained with a Jobin-Yvon Dichrograph-6 in EPA (Et_2O−isopentane−EtOH, 5:5:2) at room temperature and −180 °C. ^{1}H NMR spectra were recorded on a Bruker DRX 400 spectrophotometer in $CDCl_3$ at 20 °C; chemical shifts (δ) are given in ppm relative to Me_4Si; coupling constants (J) are given in Hz. EIMS were acquired with a Varian MAT-CH 7A spectrometer and HREIMS with a Finnigan MAT 95QS magnetic tandem spectrometer.

Plant Material. The fruits of *Asparagus falcatus* were obtained from a commercial garden in Pécs (southern Hungary) in October 1998. The plant material was identified by Prof. Dr. L. Gy. Szabó, Dr. D. Kovács, and Dr. B. Kevey, Department of Botany, University of Pécs, Hungary. A voucher specimen (no. 56818) has been deposited at the Botanical Department of the Hungarian Natural History Museum, Budapest, Hungary.

Extraction and Isolation. Conditions of extraction and isolation procedures have been described previously.[1,2] The hypophasic carotenoids, after partition between hexane and MeOH−H_2O (9:1), were separated by column chromatography on $CaCO_3$ (Biogal, Debrecen, Hungary), with benzene−hexane (1:1) as developing solvent. The fraction with lowest mobility contained **1** and the other (*Z*)-carotenoids and was obtained by extruding the column and recovering the adsorbed carotenoids with MeOH. The individual carotenoids were isolated from this fraction by repeated column chromatography on $CaCO_3$, with benzene−hexane (7:3) as developing solvent. In order of increasing mobility, the compounds obtained were (13*Z*)-capsorubin (1.8 mg), (13*Z*)-capsanthin (1.3 mg), (13′*Z*)-capsanthin (1.0 mg), (9*Z*)-capsorubin (0.8 mg), (9*Z*)-capsanthin-5,6-epoxide (**1**; 1.5 mg), (9*Z*)-capsanthin (2.3 mg), and (9′*Z*)-capsanthin (2.0 mg). The purity of the isolated compounds, as determined by HPLC and comparison with authentic samples, was >95%. The structures were confirmed by UV−vis, ^{1}H NMR, CD, and MS.

(9*Z*)-Capsanthin-5,6-epoxide (1). This new minor carotenoid was obtained as dark red microcrystals (benzene−hexane, 1:4): 1.5 mg; mp 108−110 °C; UV−vis (benzene) λ_{max} (log ϵ) 358 (4.16), 475 (4.97), 505 (4.87) nm, λ_{max} after acid treatment 463, 487 nm; CD (EPA) λ_{max} ($\Delta\epsilon$) 220 (+0.47), 240 (+0.47), 260 (+0.98), 270 (+0.86), 288 (+1.60), 343 (+0.14), 350 (−0.17), 380 (−0.50), 396 (−0.36), 490 (−1.10) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (1H, d, $J_{8',7'}$ = 15.0 Hz, H-8′), 6.84 (1H, d, $J_{8,7}$ = 15.8 Hz, H-8), 6.75 (1H, dd, $J_{11,10}$ = 11.4 Hz, $J_{11,10}$ = 14.8 Hz, H-11), 6.69 (1H, m, H-15′), 6.63 (1H, m, H-15), 6.61 (1H, dd, $J_{11',10'}$ = 11.4 Hz, $J_{11',12'}$ =14.6 Hz, H-11′), 6.56 (1H, d, $J_{10',11'}$ = 11.4 Hz, H-10′), 6.51 (1H, d, $J_{12',11'}$ = 14.6 Hz, H-12′), 6.44 (1H, d, $J_{7',8'}$ = 15.0 Hz, H-7), 6.30 (1H, d, $J_{12,11}$ = 13.0 Hz, H-12), 6.25 (1H, m, H-14), 6.08 (1H, d, $J_{10,11}$ = 11.4 Hz, H-10), 5.95 (1H, d, $J_{7,8}$ = 15.6 Hz, H-7), 4.51 (1H, m, H-3′), 3.92 (1H, m, H_{ax}-3), 2.96 (1H, dd, J_{gem} = 14.4 Hz, $J_{4'eq,3'}$ = 8.5 Hz, H_{eq}-4′), 2.39 (1H, ddd, J_{gem} = 14.2 Hz, $J_{4eq,3}$ = 4.9 Hz, $J_{4eq,2}$ = 1.6 Hz, H_{eq}-4), 2.00 (1H, dd, J_{gem} = 13.7 Hz, $J_{2'eq,3'}$ = 7.8 Hz, H_{eq}-2), 1.98 (3H, s, Me-20′), 1.96 (3H, s, Me-20), 1.96 (3H, s, Me-19′), 1.93 (3H, s, Me-19), 1.71 (1H, dd, J_{gem} = 13.7 Hz, $J_{2'ax,3'}$ = 4.6 Hz, H_{ax}-2′), 1.63 (1H, dd, J_{gem} = 14.2 Hz, $J_{4ax,3}$ = 8.8 Hz, H_{ax}-4), 1.62 (1H, ddd, J_{gem} = 14.7 Hz, $J_{2eq,3}$ = 3.6 Hz, $J_{2eq,4}$ = 1.7 Hz, H_{eq}-2), 1.49 (1H, dd, J_{gem} = 14.4 Hz, $J_{4'ax,3'}$ = 3.2 Hz, H_{ax}-4′), 1.37 (3H, s, Me-18′), 1.25 (1H, dd, J_{gem} = 14.7 Hz, $J_{2ax,3}$ = 10.2 Hz, H_{ax}-2), 1.21 (3H, s, Me-17′), 1.21 (3H, s, Me-18), 1.17 (3H, s, Me-16), 1.01 (3H, s, Me-17), 0.84 (3H, s, Me-16′); EIMS *m*/*z* 600 $[M]^+$ (73), 494 (36), 221 (22), 181 (15), 109 (100), 43 (100); HREIMS *m*/*z* 600.4167 $[M]^+$ (58), 584.1871 (12), 520.1887 (10), 508.1855 (15), 494.2699 (50), 221.1538 (100).

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