A New *retro*-Carotenoid from the Petals of the Californian Yellow Poppy *Eschscholtzia californica*

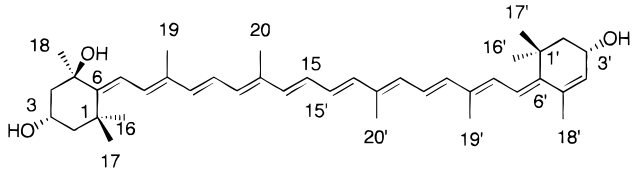
Takashi Maoka,*,† Yasuhiro Fujiwara,† Keiji Hashimoto,† Satomi Takeda,‡ Sanae Takaragaki,‡ and Kazuko Ida‡

Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8412, Japan, and Faculty of Science, Department of Environomental Sciences, Osaka Women's University, Daisen-cho, Sakai-shi, Osaka 590-0035, Japan

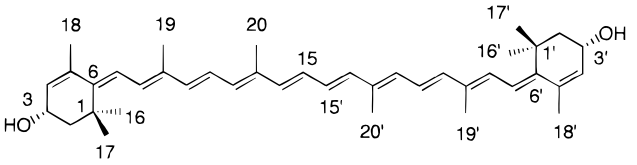
Received February 9, 2000

A new *retro*-carotenoid (**1**) isolated from the petals of the Californian yellow poppy, *Eschscholtzia californica*, was determined to be (all-*E*)-(3*S*,5*R*,3′*S*)-4′,5-*retro*-*β*,*β*-carotene-3,5,3′-triol by spectroscopic analysis.

In the course of our studies on plant carotenoids, [1,2] a new *retro*-carotenoid (**1**) was isolated from the petals of the Californian yellow poppy, *Eschscholtzia californica* (Papaveraceae) along with *(3S,3′S)*-eschscholtzxanthin (**2**).[3] This paper reports the isolation and structural elucidation of **1**.



1



2

The Me_2CO extract of the petals of *E. californica* was saponified with 5% KOH/MeOH, and unsaponifiable matter was chromatographed on silica gel using increasing percentages of Me_2CO in *n*-hexane. Successive purification by HPLC on silica afforded the new *retro*-carotenoid (**1**).

Compound **1** showed absorption maxima at 435, 459, and 489 nm. HRFABMS of **1** showed a molecular ion peak at *m*/*z* 584.4241 compatible with the formula $C_{40}H_{56}O_3$. The presence of two secondary hydroxy groups and one tertiary hydroxy group in **1** was revealed by HRFABMS and ^{13}C and ^1H NMR data.

The ^{13}C and ^1H NMR assignments for **1** in $CDCl_3$ are presented in the Experimental Section. Assignments were made by double quantum filtered (DQF)-COSY, NOESY, HSQC, and HMBC experiments and by comparing these data with those of eschscholtzxanthin (**2**).[4,5] The partial structures of the eschscholtzxanthin moiety (C-1′ to C-20′) and the *retro*-type polyene chain (C-6 to C-6′) including relative stereochemistry in **1** were characterized by ^{13}C, ^1H, and 2D NMR analysis. The structure of the remaining end group (C-1 to C-6) was also elucidated by 2D NMR experiments. DQF-COSY and HSQC experiments established the connectivities of C-2 to C-4 and the position of a secondary hydroxy group at C-3. The quaternary carbons at δ 36.95, δ 74.96, and δ 151.21 were assigned to C-1, C-5, and C-6, respectively, by HMBC experiment. Thus, the location of the remaining tertiary hydroxy group was deduced to be at C-5. ^{13}C and ^1H NMR signals of methyl groups at 16, 17, and 18 were also assigned by HSQC and HMBC spectra. Furthermore, the NOESY correlations between CH_3-16 and CH_3-17 to H-8 and CH_3-18 to H-7 revealed the 6*E* configuration. Thus, the structure of **1** was determined as 4′,5- *retro*-*β*,*β*-carotene-3,5,3′-triol.

The CD spectrum of **1** showed almost the same Cotton effect as that of *(3S,3′S)*-eschscholtzxanthin (**2**),[2,4] except the wavelength shift was 15 nm shorter than that of **2**, which is attributable to the lack of one double bond at C-4 in **1**. The 3*S*, 5*R*, 3′*S* chiralities for **1** was tentatively postulated on the basis of CD data described above and biosynthetic aspects.

The ^1H NMR of **1** also displayed the presence of a minor 6′*Z*-isomer, as well as the same *retro*-carotenoids such as eschscholtzxanthin (**2**),[4] eschscholtzxanthon,[2] and rhodoxanthin.[4,6] The ratio of 6′*E* and 6′*Z* isomers was determined to be 8:2 by the intensity of the corresponding ^1H NMR signals. This *retro*-carotenoid (**1**) was a key biosynthetic intermediate in the pathway from zeaxanthin via antheraxanthin to eschscholtzxanthin proposed by Williams et al.[4,7]

Experimental Section

General Experimental Procedures. The UV−vis spectrum was recorded in Et_2O with a Shimadzu UV-240 spectrophotometer. The FABMS spectrum was recorded using a JEOL SX 102 mass spectrometer with NBA (nitrobenzyl alcohol) as the matrix. The ^{13}C (125 MHz) and ^1H NMR (500 MHz) spectra were measured with a Varian UNITY INOVA 500 spectrometer in $CDCl_3$ with TMS as an internal standard. All two-dimensional spectra were recorded without spinning. DQF-COSY, NOESY, gHSQC, and gHMBC spectra were acquired using the standard Varian pulse programs. CD spectra were recorded in Et_2O at room temperature with a JASCO J-500 spectropolarimeter. HPLC was performed on a Shimadzu LC-6AD instrument with a Shimadzu SPD-6AV spectrophotometer set at 450 nm. The column used was a Shim-Pack PREP-SIL (Shimadzu, 20 mm × 250 mm, 5 μm) using *n*-hexane−Me_2CO (3:7) as the mobile phase.

Plant Material. *E. californica*. was grown in the fields of Osaka Women's University (Sakai City, Osaka, Japan) under well-watered conditions, and the petals were harvested in May−July. Voucher specimens have been deposited at Department of Natural Science, Osaka Women's University.

* To whom correspondence should be addressed. Tel.: +81-75-703-3810. Fax: +81-75-703-3811. Present address: Research Institute for Production Development, 15 Shimogamo-Morimoto-Cho, Sakyou-ku, Kyoto 607-0805, Japan.
† Kyoto Pharmaceutical University.
‡ Osaka Women's University.

10.1021/np0000670 CCC: $19.00
Published on Web 07/28/2000

Extraction and Isolation of Carotenoids. The Me_2CO extract of fresh petals (500 g) of *E. californica* was partitioned between *n*-hexanes−Et_2O (1:1) and aqueous NaCl. The organic layer was concentrated to dryness. The residue was saponified with 5% KOH−MeOH for 3 h at room temperature. Then unsaponifiable matter was extracted with *n*-hexanes−Et_2O (1:1) and washed with water. The organic layer was dried over Na_2SO_4 and then concentrated to dryness. The residue was subjected to CC on silica gel using an increasing percentage of Me_2CO in *n*-hexane. Compound **1** was eluted with Me_2CO−*n*-hexane (1:1) from a silica column and was further purified by HPLC on silica with *n*-hexane−Me_2CO (3:7) as the solvent to yield 5.0 mg (18% of the total carotenoid).

The following additional carotenoids were identified from the petals of *E. californica*: *β*-carotene (1% of the total carotenoid), eschscholtzxanthone (0.5%), eschscholtzxanthin (35%), lutein (1%), zeaxanthin (2%), antheraxanthin (10%), mutatoxanthin (6%), violaxanthin (10%), luteoxanthin (5%), auroxanthin (5%), and neoxanthin (2%). They were identified by vis, FABMS, ^{1}H NMR, and CD spectral data.

***retro*-Carotenoid 1:** vis (Et_2O) λ max 435, 459 and 489 nm. (% III/II = 42); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.25 (3H, s, H-16′), 1.37 (3H, s, H-17), 1.41 (3H, s, H-16), 1.45 (3H, s, 17′), 1.51 (1H, dd, J = 12, 8 Hz, H-2′ax), 1.54 (3H, s, H-18), 1.56 (1H, dd, J = 13.5, 7.5 Hz, H-2ax), 1.65 (1H, dd, J = 13.5, 7.5 Hz, H-4ax), 1.81 (1H, dd, J = 12, 5 Hz, H-2′eq), 1.87 (1H, ddd, J = 13.5, 5, 2 Hz, H-2eq), 1.95 (6H, s, H-19, 18′), 1.97 (12H, s, H-20, 19′, 20′), 2.18 (1H, ddd, J = 13.5, 6.5, 2 Hz, H-2eq), 4.25 (1H, m, H-3), 4.35 (1H, m, H-3′), 5.75 (1H, br. s, H-4′), 6.22 (1H, d, J = 11 Hz, H-12), 6.23 (1H, d, J = 11 Hz, H-12′), 6.40 (4H, m, H-14, 15, 14′, 15′), 6.42 (1H, d, J = 15 Hz, H-10), 6.43 (1H, d, J = 15 Hz, H-10′), 6.46 (1H, d, J = 12 Hz, H-8), 6.48 (1H, d, J = 12 Hz, H-7′), 6.65 (1H, dd, J = 15, 11 Hz, H-11), 6.66 (1H, dd, J = 15, 11 Hz, H-11′), 6.73 (1H, d, J = 12, H-8′), 6.76 (1H, d, J = 12, H-7), signals of a minor 6′ *Z*-isomer of **1**, δ 1.10 (ca. 0.6 H, s, H-16′), 1.25 (s, H-17′), 5.67 (ca. 0.2 H, br s, H-4′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.2 (C-20′), 12.8 (C-19, 19′, 20′), 21.4 (C-18′), 27.3 (C-16′), 30.5 (C-17), 31.6 (C-18), 31.7 (C-17′), 32.9 (C-16), 35.6 (C-1′), 37.0 (C-1), 47.8 (C-4), 49.8 (C-2), 50.4 (C-2′), 64.1 (C-3), 65.6 (C-3′), 75.0 (C-5), 120.3 (C-7), 121.9 (C-7′), 125.0 (C-11, 11′), 129.2 (C-15, 15′), 129.5 (C-8), 131.6 (C-4′), 132.6 (C-12, 12′), 131.6 (C-8′), 134.8 (C-5′), 135.4 (C-13′), 136.1 (C-13, C-9′), 137.0 (C-9), 137.4 (C-14, 14′), 138.6 (C-10, 10′), 144.1 (C-6′), 151.2 (C-6); CD (Et_2O) λ ext 370 ($\Delta\epsilon$ 0), 365 (8), 357 (4), 349 (5), 325 (0), 295 (−9), 266 (0), 254 (12), 230 (0); HRFABMS m/z [M^+] 584.4241 ($C_{40}H_{56}O_3$ requires 584.4222).

***(3S,3′S)*-Eschscholtzxanthin (2)**: ^{1}H NMR, see refs 4, 5; ^{13}C NMR ($CDCl_3$, 125 MHz) δ 12.2 (C-20, 20′), 12.8 (C19, 19′), 21.4 (C-18, 18′), 25.2 (C-18, 18′ signal for minor *Z*-isomer), 27.3 (C-16, 16′), 27.5 (C-16, 16′ *Z*-isomer), 29.9 (C-17, 17′ *Z*-isomer), 31.7 (C-17, 17′), 35.5 (C-1, 1′), 38.6 (C-1, 1′ *Z*-isomer), 47.3 (C-2, 2′ *Z*-isomer), 50.4 (C-2, 2′), 65.6 (C-3, 3′), 66.2 (C-3, 3′ *Z*-isomer), 120.5 (C-7, 7′ *Z*-isomer), 121.9 (C-7,7′), 125.0 (C-11, 11′), 129.2 (C-15, 15′), 130.9 (C-8, 8′ *Z*-isomer), 131.6 (C-8, 8′), 132.6 (C-4, 12, 4′, 12′), 134.8 (C-5, 5′), 135.4 (C-13, 13′), 136.1 (C-9, 9′), 137.4 (C-14, 14′), 138.6 (C-10, 10′) and 144.1 (C-6, 6′); CD (Et_2O) λ ext 400 ($\Delta\epsilon$ 0), 381 (19), 373 (8), 365 (13), 339 (0), 312 (−17), 280 (0), 264 (24), 244 (0).

Supporting Information Available: Figures indicating HMBC and NOESY correlations of **1** and a possible biosynthetic pathway of zeaxanthin to carotenoids **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Ida, K.; Masamoto, K.; Maoka, T.; Fujiwara, Y.; Takeda, S.; Hasegawa, E. *J. Plant Res.* **1995**, *108*, 369−376.
(2) Maoka, T.; Ito, Y.; Fujiwara, Y.; Hashimoto, K. *J. Jpn. Oil Chem. Soc.* **1996**, *45*, 641−646.
(3) Strain, H. H. *J. Biol. Chem.* **1938**, *123*, 425−437.
(4) Andrews, A. G.; Englert, G.; Borch, G.; Strain, H. H.; Liaaen-Jensen, S. *Phytochemistry* **1979**, *63*, 303−309.
(5) Englert, G. NMR spectroscopy. In *Carotenoids Vol. 1B*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhauser: Basel, 1994; pp 163−198.
(6) Englert, G.; Vecchi, M. *J. Chromatogr.* **1982**, *235*, 197−203.
(7) Williams, R. J. H.; Britton, G.; Goodwin, T. W. *Biochim. Biophys. Acta* **1966**, *124*, 200−203.

NP0000670