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CONFIGURATIONAL STUDIES ON RED ALGAE CAROTENOIDS*

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Key Word Index—Rhodophyceae; common carotenoids; chirality; CD; ^{1}H NMR; first documentation (3′*R*,6′*R*)-β,ε-caroten-3′-ol (α-cryptoxanthin).

Abstract—The configurations of (6′*R*)-β,ε-carotene, (3′*R*,6′*R*)-β,ε-caroten-3′-ol (α-cryptoxanthin), (3*R*,3′*R*,6′*R*)-β,ε-carotene-3,3′-diol (lutein), (3*R*)-β,β-caroten-3-ol (β-cryptoxanthin), (3*R*,3′*R*)-β,β-carotene-3,3′-diol (zeaxanthin) and all-*trans* (3*S*,5*R*,6*S*,3′*R*)-5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol (antheraxanthin) were established by CD and ^{1}H NMR studies. The red algal carotenoids consequently possessed chiralities at each chiral center (C-3, C-5, C-6, C-3′, C-6′), corresponding to the chiralities established for the same carotenoids in higher plants. Two *post mortem* artifacts from *Erythrotrichia carnea* were assigned the chiral structures (3*S*,5*R*,8*R*,3′*R*)-5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol [(8*R*)-mutatoxanthin] and (3*S*,5*R*,8*S*,3′*R*)-5,8-epoxy-5,8-dihydro-β,β-carotene-3,3′-diol [(8*S*)-mutatoxanthin]. This is the first well documented report of a naturally occurring β,ε-caroten-3′-ol (^{1}H NMR, CD, chemical derivatization).

INTRODUCTION

Characteristic red algae carotenoids are carotenes and xanthophylls with β- and ε- end groups [1–3]. No chiroptical properties for carotenoids from red algae are so far reported.

In recent years several cases have been reported where a carotenoid of given constitution occurs with a different chirality [4]. Particularly in view of the stereochemical variation of carotenoids possessing ε-rings [4–7] we now report CD and ^{1}H NMR data for common carotenoids of the Rhodophyceae, confirming the same absolute configurations as previously established for the respective carotenoids from higher plants.

RESULTS AND DISCUSSION

β,ε-Carotene (from *Antithamnion plumula* and *Ceramium rubrum*) exhibited a positive Cotton effect consistent with the 6′*R*-configuration (**1**, Fig. 1) [8].

A carotenol isolated from *A. plumula* and *C. rubrum*, co-chromatographed, had visible, ^{1}H NMR and mass spectra consistent with a β,ε-carotene-mono-ol and formed a monoacetate and an allylic methyl ether [1, 9]. The Cotton effect (Fig. 1) differed from that of (3*R*,6′*R*)-β,ε-caroten-3-ol (**2b**) [10] and was consistent with 3′-hydroxy substitution. Thus the Cotton effect was entirely positive as for (6′*R*)-β,ε-carotene (**1**). The chirality at C-3′ in the ε-rings is not revealed by the CD spectrum [7, 11–14], but followed from ^{1}H NMR data (δ0.85 *s* and δ1.00 *s* for Me-16′ and Me-17′, respectively, and δ2.42 *d*, $J = 8$ Hz, for H-6′, consistent with a 3′,6′-*trans* configuration) [7, 11–14]. The carotenol from *A. plumula* and *C. rubrum* consequently has the configuration (3′*R*,6′*R*)-β,ε-caroten-3′-ol (**2a**). This is the first well documented report of a 3′-hydroxylated β,ε-carotenol in Nature. The constitution of **2a** was previously assigned to physoxanthin from *Physalis alkekengi* [15], but subsequently revised to **2b** [10].§ We suggest that the trivial name zeinoxanthin [16] should have priority for β,ε-caroten-3-ol (**2b**) and the trivial name α-cryptoxanthin be reserved for β,ε-caroten-3′-ol (**2a**).

The Cotton effect of lutein (**3**) from *A. plumula* was compatible with the common 3′*R*,6′*R*-configuration (Fig. 1). A 3′,6′-*trans* configuration of the substituted ε-ring followed from the ^{1}H NMR spectrum as for α-cryptoxanthin, thus supporting 3*R*,3′*R*,6′*R* chirality. From our CD (Fig. 1) 3*S*-chirality cannot definitely be ruled out [7].

Qualitative CD spectra of β-cryptoxanthin (**4**) and of zeaxanthin (**5**) corresponded to that of (3*R*,3′*R*)-zeaxanthin (**5**) [17], as expected for a 3*R*-configuration of **4** and 3*R*,3′*R*-configuration for **5**. ^{1}H NMR data (400 MHz) for zeaxanthin (**5**) was consistent with recent assignments [18].

A diol epoxide from *E. carnea* had spectroscopic and chemical properties consistent with a 5,6-epoxide of β,β-carotene-3,3′-diol, but differed in chromatographic properties from antheraxanthin from anthers of *Lilium tigrinum* [19]. ORD spectra of natural all-*trans* (3*S*,5*R*,6*S*,3′*R*)-zeaxanthin-5,6-epoxide (**6**) and of synthetic (3*S*,5*S*,6*R*,3′*R*)-zeaxanthin-5,6-epoxide (**7**) are reported [17]. Kronig-Kramers transformation [20, 21] of these ORD spectra into CD spectra showed agreement between the CD spectra of the epoxide from *E. carnea* and all-*trans* zeaxanthin-5,6-epoxide (**6**) (Fig. 2). For carotenoids with conservative CD spectra [17] a *cis*-bond in the polyene chain is known to change the sign of the Cotton effect. The calculated CD spectrum of mono-*cis* zeaxanthin-5,6-epoxide based on CD 1/2 violeoxanthin = 11-*cis* violaxanthin [22] + 1/2 zeaxanthin (**5**) [17]

*Part 29 in the series "Algal Carotenoids". For Part 28 see *Phytochemistry* **23**, 649.

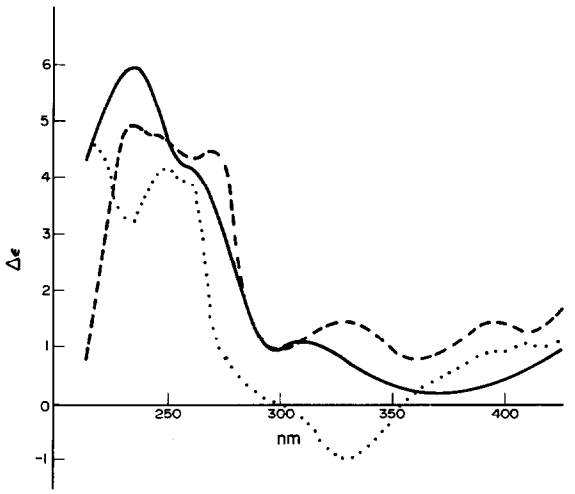
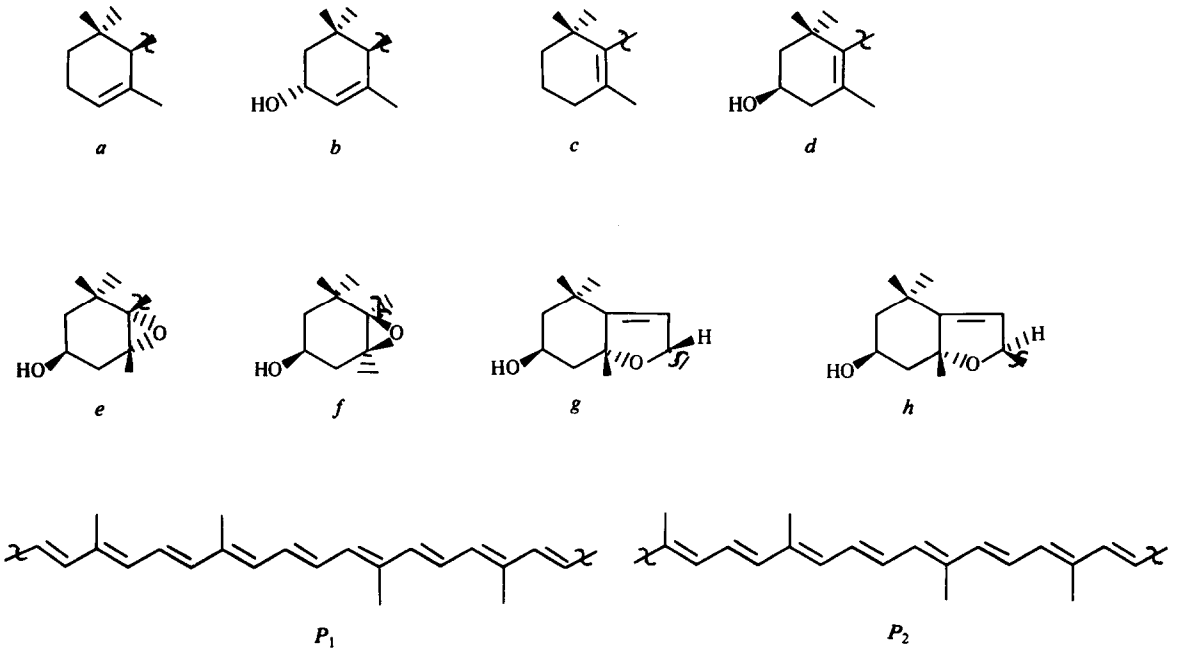


Fig. 1. CD spectra in EPA solution of: ———, (6′*R*)-β,ε-carotene (**1**) from *A. plumula* var. *bebbii*; ——, (3′*R*,6′*R*)-β,ε-caroten-3′-ol (**2a**) from *C. rubrum*; . . . . . , (3*R*,3′*R*,6′*R*)-lutein (**3**) from *A. plumula* var. *bebbii*.

(Fig. 2), shows an inverse Cotton effect relative to all-*trans* zeaxanthin-5,6-epoxide (**6**) and eliminates the presence of a *cis*-bond in zeaxanthin-5,6-epoxide (**6**) from *E. carnea*. Low-temperature CD data for all-*trans*-**6**, 9-*cis*-**6** and all-*trans*-**7** recently reported [23] are consistent with our calculated curves. The ^{1}H NMR (400 MHz) spectrum of the present epoxide, compared with published data [24, 25], corresponded to that of all-*trans* zeaxanthin-5,6-epoxide (**6**) and the coupling pattern supported the assignment of the hydroxy functions to C-3 and C-3′. ^{1}H NMR spectral data for the C-8 epimeric furanoid mixture obtained by acid rearrangement was compatible with data for (8*R*)-mutatoxanthin (**8**) (end group *g*) and (8*S*)-mutatoxanthin (**9**) (end group *h*) [26]. We therefore conclude that the major carotenoid of *E. carnea* is all-*trans* (3*S*,5*R*,6*S*,3′*R*)-antheraxanthin (**6**) of the same configuration as in the higher plants [24]. Antheraxanthin from anthers of *Lilium tigrinum* [27] is possibly the 9-*cis* isomer. Recently the 9-*cis* (3*S*,5*R*,6*S*,3′*R*)-configuration has been favoured for antheraxanthin from anthers of *Lilium candidum* [28].

Previous analyses of deep-frozen *E. carnea* stored for several weeks revealed the presence of two xanthophylls, both more polar than antheraxanthin (**6**) on alkaline TLC-plates [29]. These xanthophylls were not present in freshly extracted algal material [19] or in six samples extracted fresh after increasing dark periods [Bjørnland, T., unpublished results]. A large scale batch of *E. carnea* stored deep-frozen for five years was available for a re-investigation of these xanthophylls. UV-visible, CD, ^{1}H NMR and mass spectra of each xanthophyll were consistent with a mutatoxanthin structure. The less polar component on TLP-II was (8*R*)-mutatoxanthin (**8**) and the more polar component (8*S*)-mutatoxanthin (**9**) (CD,



1 *c* - P_1 - *a* β,ε - carotene
2a *c* - P_1 - *b* α - cryptoxanthin
2b *d* - P_1 - *a* zeinoxanthin
3 *d* - P_1 - *b* lutein
4 *d* - P_1 - *c* β - cryptoxanthin
5 *d* - P_1 - *d* zeaxanthin
6 *e* - P_1 - *d* zeaxanthin - 5,6 - epoxide (natural)
7 *f* - P_1 - *d* zeaxanthin - 5,6 - epoxide (synthetic)
8 *g* - P_2 - *d* (8*R*) - mutatoxanthin
9 *h* - P_2 - *d* (8*S*) - mutatoxanthin

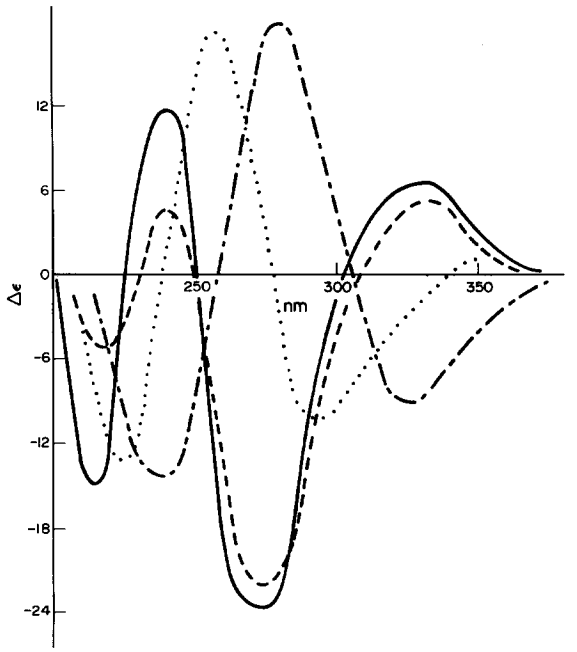


Fig. 2. CD spectra in EPA solution of: ——, (3*S*,5*R*,6*S*,3′*R*)-antheraxanthin (**6**) from *E. carnea*; — — —, **6**—calculated from the ORD spectrum [24]; . . . . . , synthetic (3*S*,5*S*,6*R*,3′*R*)-zeaxanthin-5,6-epoxide (**7**)—calculated from the ORD spectrum [17]; —·—·—, 9-*cis* (3*S*,5*R*,6*S*,3′*R*)-zeaxanthin-5,6-epoxide—calculated from the CD of 1/2 (3*R*,3′*R*)-zeaxanthin (**5**) [17] + 1/2 violeoxanthin [22]

^{1}H NMR-signals for Me-5, Me-9, H-7 and H-8) [26]. These xanthophylls were therefore *post mortem* artifacts ascribed to furanoid rearrangement of antheraxanthin (**6**) during storage.

EXPERIMENTAL

Biological material and culture methods. Antithamnion plumula (Ellis) Thur. in Le Jol. var. *plumula* was collected at Hoftøy, the Oslofjord. Female plants were grown at 18° in polystyrene dishes (Heger Plastics A/S 10100). The light sources were Philips fluorescent tubes (TL/55), the light intensity 8.6 $\mu E/m^2/sec^1$ (LI-188 integrating quantum photometer fitted with a LI-190s cosinus sensor; Lambda Instr. Corp.) and the LD regime 14:10. Each culture dish contained 140 ml of the enriched seawater medium IMR [30]. The salinity was 30‰, and the medium was renewed every second week. Yield from 16 dishes after 310 days was 2.52 g lipid-extracted dry wt (lot 1). *A. plumula* var. *bebbii* (Reinsch) J. Feldm. was collected at Hvaler, the Oslofjord. Female plants were grown as described above. Yield from 24 dishes after 290 days was 3.02 g lipid-extracted dry wt (lot 2). Both varieties were collected and brought into monoalgal culture by Jan Rueness, Section of Marine Botany, University of Oslo. Lot 1 and 2 (identical with the samples of isolates 3 and 4, respectively, in ref. [9]) were extracted fresh.

Ceramium rubrum (Huds.) C. Ag. was growing epilithic at 1–3 m depth at Ranvika, Tjølling, Vestfold. The material was collected in late August 1980 and cleaned from macroscopic biological contaminants. Dry wt after freeze-drying was 110 g. The material was stored at −20° for 2 weeks before extraction.

Erythrotrichia carnea (Dillw.) J. Ag. was isolated from the Oslofjord and brought into monoalgal culture by Jan Rueness. Mass cultures were grown as already described for *A. plumula* with minor modifications. The light sources were equal numbers of Philips fluorescent tubes TL/33 and TL/55 and the light intensity was 11 $\mu E/m^2/sec^1$. The salinity of the enriched seawater medium was 20‰, and the medium was renewed weekly. The yield from 100 polystyrene dishes after 10 months was 34.8 g dry wt (lot 1). Lot 1 was extracted fresh and is identical with the sample investigated in ref. [19].

A second lot (lot 2) was grown under the same conditions as lot 1. The yield from 108 polystyrene dishes after 12 months was 36.6 g dry wt. Lot 2 was stored freeze-dried at −20° for 5 years before extraction. Carotenoids for ^{1}H NMR were isolated from this batch.

Carotenoid isolation. The algal material was extracted repeatedly with Me_2CO and Me_2CO–MeOH (7:3) in a Waring blendor (*A. plumula* in a mortar) until no more pigments were released. The extracts were evaporated to dryness and saponified with 5% KOH in MeOH at 20° for 12 hr. The carotenoids were separated by TLC on silica gel G–$CaCO_3$ (1:1) (TLP-I) and silica gel G–$Ca(OH)_2$–MgO–$CaSO_4$ (10:4:3:1) (TLP-II) [1]. The developing solvents were appropriate mixtures of petrol, Me_2CO, *iso*-PrOH and MeOH.

Chemical and physical data. CD spectra were recorded in EPA soln (Et_2O–*iso*-pentane–EtOH, 5:5:2) at room temp on a Roussel-Jouan Dichrographe. ^{1}H NMR spectra were obtained with a Jeol JNM-FX 100 FT or a Bruker WM-400 instrument (100 and 400 MHz, respectively) and mass spectra with an AEI MS 902 instrument with direct inlet system at 70 eV, 190–210°.

(6′*R*)-*β*,*ε*-*Carotene* (**1**). R_f: 1.00 (TLP-I; petrol–Me_2CO, 7:3) and 0.84 (TLP-II; petrol–Me_2CO, 97:3); **1** co-chromatographed with *β*,*ε*-carotene from *Daucus carota* on TLP-II.

A. plumula var. *plumula* (lot 1): yield 0.77 mg (18% of total carotenoids); crystallized from Me_2CO; UV-visible: λ_{max}^{Petrol} nm: 420, 444 and 473, III/II (%) [32] = 68.

A. plumula var. *bebbii* (lot 2): yield 1.2 mg (15%); crystallized from Me_2CO; UV-visible: λ_{max}^{Petrol} nm: 421, 444 and 474, III/II (%) = 71; MS (see ref. [9]); CD (EPA) Fig. 1.

C. rubrum: yield 5.8 mg (14%); crystallized from Me_2CO; UV-visible: λ_{max}^{Petrol} nm: 420, 444 and 474, III/II (%) = 73; MS *m/z* (rel. int.): 536 $[M]^+$ (53), 480 $[M-56]^+$ (2), 444 $[M-92]^+$ (12) and 430 $[M-106]^+$ (2); ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): *δ*0.82 (3H, *s*, Me-1′), 0.90 (3H, *s*, Me-1′), 1.03 (6H, *s*, Me-1, 1), 1.58 (3H, *br s*, Me-5′), 1.72 (3H, *s*, Me-5), 1.91 (3H, *s*, Me-9′), 1.96 and 1.97 (9H, *s*, Me-9, 13, 13′), 2.18 (1H, *d*, J = 9Hz, H-6′), 5.41 (1H, *m*, H-4′), 5.52 (1H, *dd*, $J_{H\text{-}6'}$ = 9Hz, $J_{H\text{-}8'}$ = 15.5 Hz, H-7′), 6.14 (2H, *s*, H-7, 8) and 6.08–6.68 (*ca.* 11H, *m*, conj. olefinic); CD (EPA) nm (Δε): 238 (4.2), 266 (4.0), 298 (0.4), 330 (1.1), 362 (0.1) and 400 (2.0).

(3′*R*,6′*R*)-*α*-*Cryptoxanthin* (**2**). R_f: 0.60 (TLP-I; petrol–Me_2CO, 7:3) and 0.52 (TLP-II; petrol–Me_2CO–*iso*-PrOH, 84:15:1).

A. plumula var. *plumula* (lot 1): yield 0.14 mg (4%); UV-visible: λ_{max}^{petrol} nm: 421, 444 and 472, III/II (%) = 51; contaminating colourless lipids partly eliminated by conversion of **2** to its acetate by standard procedure [32]. The acetate was chromatographed on TLP-I, yield 0.10 mg (67%); UV-visible: λ_{max}^{petrol} nm: (420), 442 and 471, III/II (%) = 47.

A. plumula var. *bebbii* (lot 2): yield 0.41 mg (5%); UV-visible and MS (see ref. [9]). 2-Acetate; UV-visible; $\lambda_{max}^{Me_2CO}$ nm: (425), 447 and 475, III/II (%) = 43; MS (see ref. [9]). 2-Me ether; MS (see ref. [9]).

C. rubrum: yield 1.0 mg (2.5%); crystallized from Me_2CO–MeOH; UV-visible: λ_{max}^{petrol} nm: 420, 444 and 473, III/II (%) = 73; MS *m/z* (rel. int.): 552 $[M]^+$ (85), 550 $[M-2]^+$ (11), 534 $[M-18]^+$ (100), no $[M-56]^+$, 460 $[M-92]^+$ (2), 442 $[M-18$

$-92]^+$ (3), 267 $[M-18]^{2+}$ (8) and 105 (55); ^{1}H NMR (100 MHz, $CDCl_3$, TMS): δ0.85 (3H, *s*, Me-1'), 1.00 (3H, *s*, Me-1'), 1.03 (6H, *s*, Me-1, 1), 1.63 (3H, *s*, Me-5'), 1.72 (3H, *s*, Me-5), 1.90 (3H, *s*, Me-9'), 1.97 (9H, *s*, Me-9, 13, 13'), 2.42 (1H, *d*, J = 8 Hz, H-6'), 4.24 (1H, *m*, H-3'), 5.55 (1H, *m*, H-4'), 6.15 (*ca* 2H, *s*, H-7, 8) and 5.4–7 (*ca* 12H, *m*, conj. olefinic); CD (EPA) Fig. 1. Compound **2** was converted to its acetate by standard procedure [32]; yield 0.67 mg (67%; 6% **2** recovered); **2**-acetate crystallized from Me_2CO–MeOH; UV-visible: λ_{max}^{petrol} nm: 421, 445 and 473, III/II (%) = 73; MS *m/z* (rel. int.): 594 $[M]^+$ (2), 534 $[M-60]^+$ (31), 442 $[M-60-92]^+$ (7), 428 $[M-60-106]^+$ (2) and 105 (100).

(3*R*,3'*R*,6'*R*)-*Lutein* (**3**). R_f: 0.45 (TLP-I; petrol–Me_2CO, 7:3) and 0.53 (TLP-II; petrol–Me_2CO–*iso*-PrOH, 73:25:2); **3** co-chromatographed with lutein from *Medicago sativa* on TLP-II.

A. plumula var. *plumula* (lot 1): yield 3.1 mg (72%); crystallized from Me_2CO–MeOH; UV-visible: λ_{max}^{Petrol} nm: 419, 445 and 473, III/II (%) = 68.

A. plumula var. *bebbii* (lot 2): yield 5.9 mg (72%); crystallized from Me_2CO–MeOH; UV-visible, MS and ^{1}H NMR (see ref. [9]), also 2.42 (1H, *d*, J = *ca* 8 Hz, H-6'); CD (EPA) nm ($\Delta\varepsilon$): 232 (3.3), 248 (4.2), 260 (4.0), 300 (0), 328 (−1.0), 355 (0) and 415 (1.0). Compound **3** was converted to 3-3'-Me ether by standard procedure [33]; yield 2.9 mg (83%); UV-visible: $\lambda_{max}^{Me_2CO}$ nm: (425), 447 and 474, III/II (%) = 46; MS (see ref. [9]). Acetylation of 3-3'-Me ether by standard procedure [32] gave a mono-acetate: yield 1.1 mg (76%); UV-visible: $\lambda_{max}^{Me_2CO}$ nm: (424), 446 and 474, III/II (%) = 46; MS (see ref. [9]).

(3*R*)-*β*-*Cryptoxanthin* (**4**). R_f: 0.60 (TLP-I; petrol–Me_2CO, 7:3) and 0.33 (TLP-II; petrol–Me_2CO–*iso*-PrOH, 84:15:1); **4** co-chromatographed with *β*-cryptoxanthin from the calyx of *Physalis alkekengi* on TLP-II.

E. carnea (lot 1): yield 3.7 mg (7%); crystallized from Me_2CO–MeOH; UV-visible: λ_{max}^{Petrol} nm: (425), 446 and 473, III/II (%) = 33; MS (see ref. [19]); CD(EPA) nm ($\Delta\varepsilon$): 215 (0), 225 (− max.), 235 (0), 248 (+ max.), 262 (0), 285 (− max.), 315 (0) and 338 (+ max).

E. carnea (lot 2): yield 0.29 mg (3%); non-crystalline; ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ1.03 (6H, *s*, Me-1', 1'), 1.07 (6H, *s*, Me-1, 1), 1.71 (3H, *s*, Me-5'), 1.74 (3H, *s*, Me-5) and 1.97 (12H, *s*, Me-9, 13, 9', 13').

(3*R*,3'*R*)-*Zeaxanthin* (**5**). R_f: 0.45 (TLP-I; petrol–Me_2CO, 7:3) and 0.25 (TLP-II; petrol–Me_2CO–*iso*-PrOH, 73:25:2); **5** co-chromatographed with zeaxanthin from the calyx of *Physalis alkekengi* on TLP-II.

E. carnea (lot 1): yield 1.8 mg (3%); crystallized from Me_2CO–MeOH; UV-visible: λ_{max}^{Petrol} nm: (427), 448 and 475, III/II (%) = 32; MS (see ref. [19]); CD (EPA) nm ($\Delta\varepsilon$): 230 (− max.), 235 (0), 248 (+ max.), 262 (0), 285 (− max.), 315 (0) and 338 (+ max.).

E. carnea (lot 2): yield 0.57 mg (6%); ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ1.07 (12H, *s*, Me-1, 1, 1', 1'), 1.73 (6H, *s*, Me-5, 5'), 1.968 and 1.970 (12H, *s*, Me-9, 13, 9', 13'), 2.05 (2H, *dd*, H_{ax}-4, 4'), 2.39 (2H, *dd*, $H_{eq.}$ −4, 4') and 4.0 (2H, *m*, H-3, 3').

(3*S*,5*R*,6*S*,3'*R*)-*Antheraxanthin* (**6**). R_f: 0.37 (TLP-I; petrol–Me_2CO, 7:3) and 0.48 (TLP-II; petrol–Me_2CO–*iso*-PrOH, 73:25:2); **6** co-chromatographed with antheraxanthin from *Lactuca sativa* and green grass but was less polar than antheraxanthin from the anthers of *Lilium tigrinum* on TLP-II.

E. carnea (lot 1): yield 35 mg (65%); crystallized from Me_2CO–MeOH; UV-visible: λ_{max}^{Petrol} nm: 421, 443 and 472, III/II (%) = 67; MS (see ref. [19]); ^{1}H NMR (100 MHz, $CDCl_3$, TMS): δ0.98 (3H, *s*, Me-1), 1.08 (6H, *s*, Me-1', 1'), 1.15 (3H, *s*, Me-1), 1.20 (3H, *s*, Me-5), 1.73 (3H, *s*, Me-5'), 1.93 (3H, *s*, Me-9), 1.97 (9H, *s*, Me-13, 9', 13'), 3.95 (2H, *m*, H-3, 3') and 5.93–6.85 (*ca* 14H, *m*, conj. olefinic); CD (EPA) Fig. 2. The furanoid rearrangement product (2.7 mg) prepared by standard acid treatment [32] had UV-visible: λ_{max}^{Hexane} nm: (406), 426 and 453, III/II (%) = 61; ^{1}H NMR (100 MHz, $CDCl_3$, TMS) for signals associated with (8*R*)-mutatoxanthin (**8**) [26]: δ1.16 (Me-1), 1.33 (Me-1), 1.61 (Me-5), 5.18 (H-8), 5.28 (H-7), signals for (8*S*)-mutatoxanthin (**9**) [26]: *ca* 1.20 (Me-1), 1.39 (Me-1), *ca* 1.70 (Me-5'), 5.07 (H-8), 5.28 (H-7) and with the common end group 1.07 (Me-1', 1') and 1.72 (Me-5').

E. carnea (lot 2): yield 2.9 mg (29%); crystallized from Me_2CO–MeOH; UV-visible: λ_{max}^{Petrol} nm: 424, 445 and 474, III/II (%) = 75; ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ0.98 (3H, *s*, Me-1), 1.07 (6H, *s*, Me-1', 1'), 1.15 (3H, *s*, Me-1), 1.19 (3H, *s*, Me-5), 1.73 (3H, *s*, Me-5'), 1.93 (3H, *s*, Me-9), 1.96 (9H, *s*, Me-13, 9', 13'), 2.05 (*ca* 1H, *dd*, J_{gem} = 17 Hz, $J_{ax,ax}$ = 9 Hz, H_{ax}-4'), 2.39 (*ca* 1H, *dd*, J_{gem} = 17 Hz, $J_{ax,eq}$ = 6 Hz, $H_{eq.}$ = 4'), 3.93 (1H, *m*, H-3), 4.02 (1H, *m*, H-3'), 5.87 (1H, *d*, J = 16 Hz, H-7), 6.12 (2H, *br s*, H-7', 8'), 6.28 (1H, *d*, J = 16 Hz, H-8) and 6.14–6.68 (*ca* 11H, *m*, conj. olefinic).

(8*R*)-*Mutatoxanthin* (**8**) a *post mortem* product formed during storage of *E. carnea* (lot 2). R_f: 0.17 (TLP-II; petrol–Me_2CO–MeOH, 55:42:3); **8** co-chromatographed with partially synthetic mutatoxanthin (less polar component) from **6** *ex E. carnea* (lot 2) on TLP-II. Yield 3.8 mg (38%); crystallized from Me_2CO–MeOH and from Et_2O–petrol; UV-visible: λ_{max}^{Petrol} nm: 404, 427 and 453; III/II (%) = 63 (non-cryst.); MS: *m/z* (rel. int.): 584 $[M]^+$ (6), 566 $[M-18]^+$ (4), 550 $[M-34]^+$ (2), 504 $[M-80]^+$ (7), 492 $[M-92]^+$ (2), 221 (40), 181 (26), and 43 (100); ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ1.07 (6H, *s*, Me-1', 1'), 1.17 (3H, *s*, Me-1), 1.33 (3H, *s*, Me-1), 1.62 (*ca.* 3H, *s*, Me-5), 1.71 (3H, *s*, Me-9), 1.73 (3H, *s*, Me-5'), 1.94 (3H, *s*, Me-13), 1.97 (6H, *s*, Me-9', 13'), 4.02 (1H, *m*, H-3'), 4.27 (1H, *m*, H-3), 5.17 (1H, *s*, H-8), 5.25 (1H, *s*, H-7), 6.11 (2H, *br s*, H-7', 8') and 6.15–6.68 (*ca* 10H, *m*, conj. olefinic); CD (EPA) nm ($\Delta\varepsilon$): 248 (5.6), 282 (0), 314 (−3.2) and 338 (0).

(8*S*)-*Mutatoxanthin* (**9**) a *post mortem* product formed during storage of *E. carnea* (lot 2). R_f: 0.11 (TLP-II; petrol–Me_2CO–MeOH, 55:42:3); **9** co-chromatographed with partially synthetic mutatoxanthin (more polar component) from **6** *ex E. carnea* (lot 2) on TLP-II. Yield 1.5 mg (15%); crystallized from Me_2CO–MeOH and from Et_2O–petrol; UV-visible: λ_{max}^{Petrol} nm: 405, 427 and 453; III/II (%) = 75; MS: *m/z* (rel. int.): 584 $[M]^+$ (4), 566 $[M-18]^+$ (4), 550 $[M-34]^+$ (2), 504 $[M-80]^+$ (5), 486 $[M-18-80]^+$ (1), 221 (34), 181 (14) and 43 (100); ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ1.07 (6H, *s*, Me-1', 1'), 1.19 (3H, *s*, Me-1). 1.34 (3H, *s*, Me-1), 1.68 (*ca*, 3H, *s*, Me-5), 1.73 (3H, *s*, Me-5'), 1.80 (3H, *s*, Me-9), 1.95 (3H, *s*, Me-13), 1.97 (6H, *s*, Me-9', 13'), 4.02 (1H, *m*, H-3'), 4.26 (1H, *m*, H-3), 5.07 (1H, *br s*, H-8), 5.30 (1H, *d*, J = 1.6 Hz, H-7), 6.107 and 6.114 (2H, *s*, H-7', 8') and 6.14–6.7 (*ca* 10H, *m*, conj. olefinic); CD (EPA) nm ($\Delta\varepsilon$): 228 (−0.5), 258 (−5.0), 313 (−0.2), 330 (−0.4) and 350 (0).

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NOTE ADDED IN PROOF

Structure **2a**, disregarding chirality, was also assigned to a minor carotenoid from *Capsicum annuum* [Cholnoky, L., Szabolcs, J. and Nagy, E. (1958) *Ann. Chem.* **616**, 207], but subsequently revised to **2b** [24].