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ADDITIONAL OXA-BICYCLO[2.2.1]HEPTANE CAROTENOIDS FROM *EUTREPTIELLA GYMNASTICA**

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Key Word Index—*Eutreptiella gymnastica*; Euglenophyceae; new oxabicyclo[2.2.1]heptane carotenoids; diadinoxanthin; diatoxanthin; neoxanthin; taraxanthin; chirality.

Abstract—Two minor xanthophylls of the marine alga *Eutreptiella gymnastica* were assigned the structures (3*S*,5*R*,6*S*,3′*R*,6′*R*)-3′-hydroxy-3,6-epoxy-5,6-dihydro-β,ε-caroten-4-one (α-cryptoeutreptiellanone) and (3*S*,5*R*,6*S*,3′*R*)-3′-hydroxy-3,6-epoxy-7′,8′-didehydro-5,6-dihydro-β,β-caroten-4-one (β-cryptoeutreptiellanone) from spectroscopic and chemical evidence. Tentative chiralities are based on CD, ^{1}H NMR and biogenetic correlations. (3*S*,5*R*,6*R*,3′*R*)-Diadinoxanthin, (3*R*,3′*R*)-diatoxanthin and (3*S*,5*R*,6*R*,3′*S*,5′*R*,6′*S*)-neoxanthin had CD and ^{1}H NMR properties consistent with the chiralities assigned from other sources. A trace pigment had spectroscopic properties compatible with its identification as taraxanthin (lutein epoxide). Chemosystematic and phylogenetic considerations are made.

INTRODUCTION

Eutreptiella gymnastica is the first marine representative of the algal class Euglenophyceae examined for carotenoids [1, 2]. In the introductory work [1] β,β-carotene (**1**, Scheme 1), the acetylenic xanthophylls diatoxanthin (**2**) and diadinoxanthin (**3**) and the allenic neoxanthin (**4**) were detected, all of which are now considered common within the class [1, 3, 4]. In addition β,ε-carotene (**5**) was present as a minor component in the carotene fraction.

Recently, the structures of the three reported unknowns [1] were established as siphonein (**6**; previously not detected within the Euglenophyceae) and the two new acetylenic carotenoids anhydrodiatoxanthin (**7**) and eutreptiellanone (**8**) [2]. IR, ^{1}H NMR (including LIS experiments) and ^{13}C NMR spectra of **8** revealed the presence of a 3,6-oxa-bicycloheptane end group [2], which until then had not been encountered in naturally occurring carotenoids.

In earlier work on euglenophycean carotenoids diadinoxanthin (**3**) may have been confused with, among others, lutein (β,ε-carotene-3,3′-diol) ([5] and references in [1]). Other reports on β,ε-carotenoids within the class are scarce and are restricted to *Euglena gracilis* var. *bacillaris* [6], *E. gracilis* strain Z [7] and the heterotrophic *Astacia ocellata* [8], but the structures have not been firmly established with modern spectroscopic methods. Except for the less defined 'tri- or tetrahydroxy-α-carotene epoxide' [7], taraxanthin (**9**) is structurally the most complex of these insufficiently characterized pigments, and it has been isolated as a minor constituent from the freshwater alga *Euglena gracilis* var. *bacillaris* [6]. The identification of β, ε-carotene (**5**) (minor constituent) and siphonein (**6**) (major constituent) in *E. gymnastica* [1, 2] suggested that β, ε-carotenoids might be more frequent and structurally varied within the Euglenophyceae than documented hitherto.

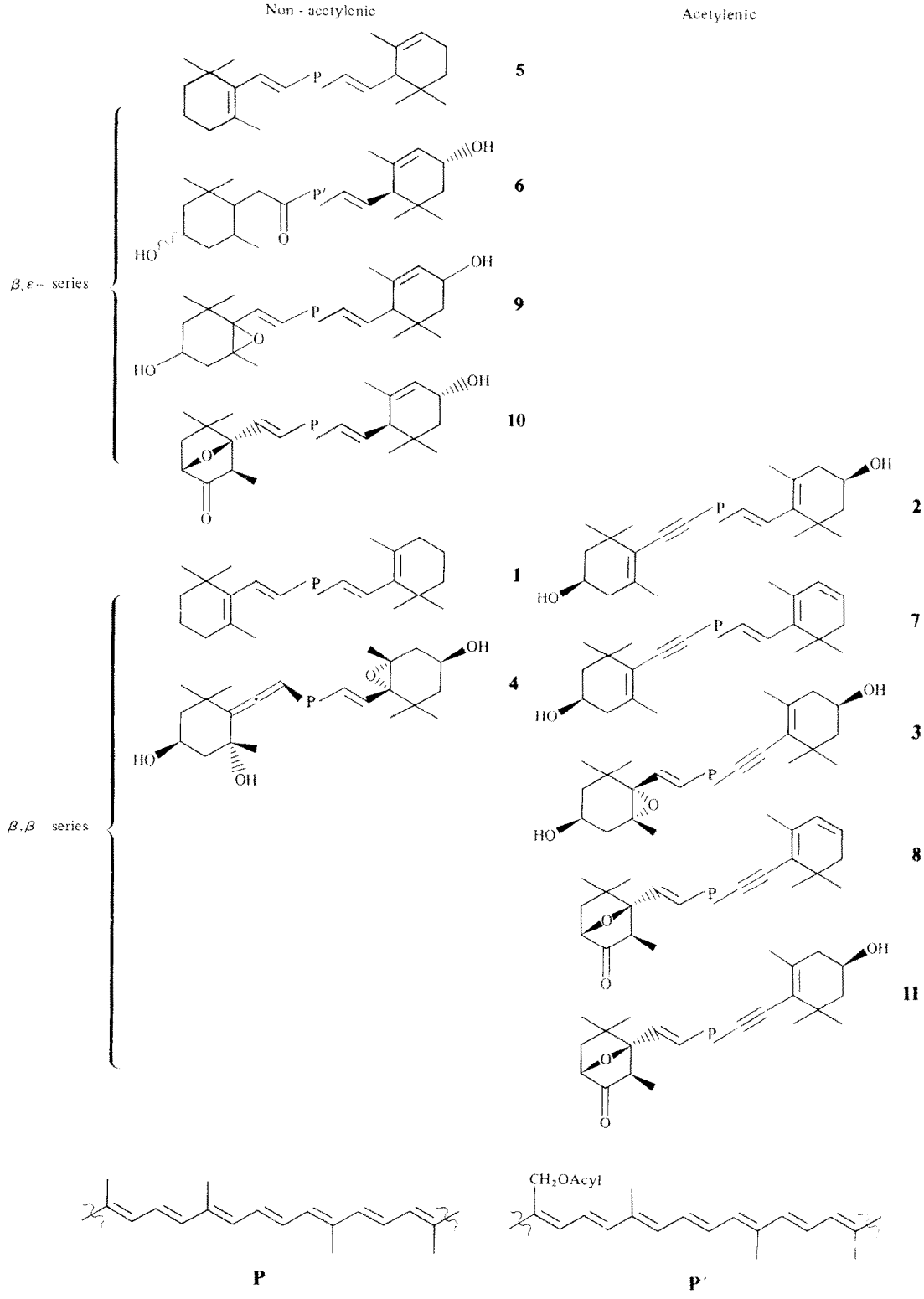
We now present data on the new minor xanthophylls α-cryptoeutreptiellanone (**10**) and β-cryptoeutreptiellanone (**11**) of *E. gymnastica* which are oxa-bicycloheptane derivatives structurally related to eutreptiellanone (**8**). The trivial names for **10** and **11** are suggested by analogy with α-cryptoxanthin and β-cryptoxanthin, respectively, with the hydroxylated end group in common. Formally β-cryptoeutreptiellanone (**11**) has a triple bond in the 7′,8′-position, also present in entreptiellanone (**8**), but absent in α-cryptoeutreptiellanone (**10**). One of the new xanthophylls (**10**) is a member of the β,ε-series.

RESULTS AND DISCUSSION

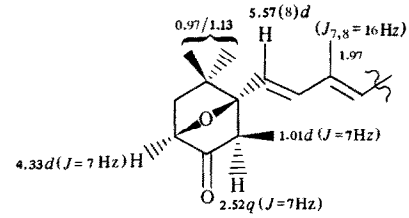
The new xanthophylls α-cryptoeutreptiellanone (**10**) and β-cryptoeutreptiellanone (**11**) may be easily overlooked by TLC on silica gel due to their small amounts (each 0.06% of total carotenoids) and because they are partly masked by chlorophylls *a* and *b*. Both xanthophylls retained their chromatographic behaviour after saponification and are therefore unesterified.

The oxa-bicycloheptane carotenoid **10** exhibited a molecular ion at *m/z* 582, compatible with the molecular formula $C_{40}H_{54}O_3$, and the visible spectrum revealed an aliphatic nonaene chromophore. Its ^{1}H NMR spectrum (400 MHz) showed the characteristic signals for end group **A** (Scheme 2) of eutreptiellanone (**8**) [2]. The assignments were partly confirmed by spin decoupling. The second end group had ^{1}H NMR signals compatible with a 3,6-*trans* hydroxylated ε-end group **B** (Scheme 2) [9] confirmed by the formation of a monoacetate upon acetylation of **10**, and an allylic monomethyl ether upon methylation of **10** with acidified methanol [10]. In-chain

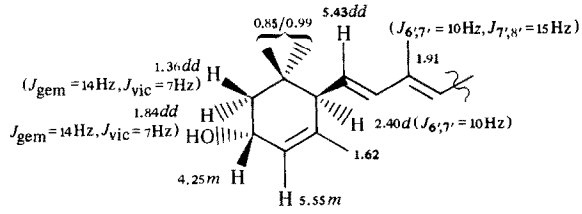
*Part 35 in the series "Algal Carotenoids". For Part 34 see *Phytochemistry* **25**, 119.


Non - acetylenic
Acetylenic
β,ε– series
β,β– series
5
6
9
10
1
4
2
7
3
8
11
P
P'
CH2OAcyl

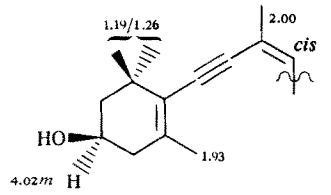
Scheme 1.



A



B



C

Scheme 2.

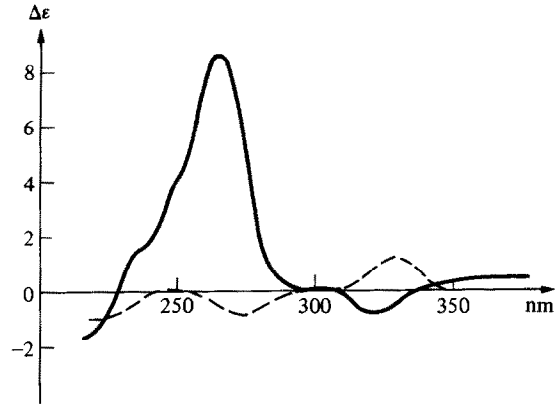


Fig. 1. CD of 3'-hydroxy-3,6-epoxy-5,6-dihydro-β,ε-caroten-4-one (**10**) and 3'-hydroxy-3,6-epoxy-7',8'-didehydro-5,6-dihydro-β,β-caroten-4-one (**11**). ——, **10** (in MeOH); – – – –, **11** (in EPA: Et_2O–*iso*-pentane–EtOH, 5:5:2).

methyl groups and olefinic protons had ^{1}H NMR properties consistent with structure **10** for the natural ketone.

The exact agreement in chemical shifts for end group **A** supports the same relative stereochemistry as favoured for eutreptiellanone (**8**) [2]. End group **A** is known to cause only a weak Cotton effect [2]. According to the additivity hypothesis [11, 12] the relatively strong positive Cotton effect ($\Delta\varepsilon$ = 8.5 at 265 nm) must consequently be caused by the ε-ring **B**. The 6'-*R*-chirality is concluded from chiroptical correlation with (6*R*)-ε,ψ-carotene (δ-carotene) with entirely positive Cotton effect ($\Delta\varepsilon$ = 4.1 at 282 nm) [13]. The chiral C-3' centre is known to have no significant influence on the CD spectra of ε-type carotenoids [12]. However, in combination with the ^{1}H NMR data the 3'*R*,6'*R*-configuration for ketone **10** may be assigned, and the tentative 3*S*,5*R*,6*S*-configuration follows from ^{1}H NMR and biogenetic analogy with eutreptiellanone (**8**) [2].

The second new oxa-bicycloheptane carotenoid **11** exhibited the molecular ion at *m/z* 580, compatible with the molecular formula $C_{40}H_{52}O_3$. The presence of end group **A** (Scheme 2) was again evident from the ^{1}H NMR data, and IR absorption at 1770 cm^{-1} confirmed the presence of an unconjugated keto group in a strained ring system (cf. [2]). Acetylenic carotenoids are known to undergo facile *cis*-isomerization [14] during isolation and ^{1}H NMR spectral data for the second end group was compatible with the acetylenic 9-*cis* end group **C** (Scheme 2) [15]. Structure **A**–**P**–**C** for compound **11** was consistent with the visible spectrum, including its double *cis*-peak at 325, 338 nm, and the absence of an [M − 106] ion [16] in the mass spectrum. In accordance with this assignment compound **11** provided a monoacetate which could not be silylated. The chirality tentatively assigned to **11** is for end group **A** based on ^{1}H NMR and biogenetic correlations and for end group **C** on biosynthetic considerations, cf. chiralities for the acetylenic diatoxanthin (**2**) and diadinoxanthin (**3**) below, and it is compatible with the weak Cotton effect observed for compound **11**, eutreptiellanone (**8**) [2] and for 9,9'-di-*cis* alloxanthin [17] with end groups **C**. The ketone **11** is one likely biosynthetic precursor of eutreptiellanone (**8**), which is formally its dehydration product.

The chiralities of (3*R*,3'*R*)-diatoxanthin (**2**), (3*S*,5*R*,6*R*,3'*R*)-diadinoxanthin (**3**) and (3*S*,5*R*,6*R*,3'*S*,5'*R*,6'*S*)-neoxanthin (**4**) from other sources have been established [11, 18]. A comparison between present and previous CD [18] and ^{1}H NMR [19] data favour the same chiralities also for these carotenoids *ex E. gymnastica*.

A minor epoxidic carotenoid had chromatographic properties, visible, ^{1}H NMR (cf. [13, 20]) and mass spectra (cf. [16]) compatible with taraxanthin (**9**, lutein epoxide) [21], a possible biosynthetic precursor of the new oxa-bicycloheptane carotenoid **10**. Taraxanthin (**9**) would thereby possess an analogous biosynthetic relationship to compound **10** as previously postulated for diadinoxanthin (**3**) to eutreptiellanone (**8**) [2].

The present and previous examinations of *E. gymnastica* [1, 2], revealed several carotenoid structural elements applicable in algal chemotaxonomy and phylogeny [22] (cf. Scheme 1). These include a large proportion of carotenoids (22% of total) with a terminal ε-ring. The identification of siphonein (**6**) in *E. gymnastica* [2] represents an important chemosystematic contribution as **6** is the structurally most complex β,ε-carotenoid in several Chlorophyceae and Prasinophyceae as well [3]

(but see also [23]). The complement of β,ε-derivatives has now been extended to include also a member (**10**) of the 3,6-oxa-bicycloheptane carotenoids.

Among the non-acetylenic β,β-carotenoids (10% of total) the structurally most complex xanthophyll is neoxanthin (**4**), and again this xanthophyll is also the most complex β,β-derived carotenoid of the Chlorophyceae and Prasinophyceae [3]. Together with chlorophyll *b*, the common occurrence of siphonein (**6**) and neoxanthin (**4**) (chiralities not considered) should, from a chemosystematic point of view, reflect a close phylogenetic connection between these three algal classes.

Other carotenoid structural elements reveal, however, that the carotenoid parameter of *E. gymnastica* is both dualistic and unique in a phylogenetic context. The β,β-derived acetylenic carotenoids (68% of total) have a general occurrence also among other photosynthetic Euglenophyceae, but are completely lacking within the Chlorophyceae and Prasinophyceae [3]. This structural element would rather indicate a relationship with classes of the Chromophyta [22]. Moreover, the oxa-bicycloheptane carotenoids, now demonstrated to be members of both the β,ε-series and the acetylenic part of the β,β-series, have hitherto not been detected in other algal classes. TLC (TLP-I) indicated that the three oxa-bicycloheptane carotenoids of *E. gymnastica* were also present in a species to be described as *Eutreptiella eupharyngea* Moestrup [Øjvind Moestrup, personal communication; Bjørnland, T., unpublished], and that eutreptiellanone (**8**) was absent in *Euglena gracilis* strain Z [1]. Accordingly, these carotenoids may have a restricted occurrence even within the photosynthetic Euglenophyceae, and thereby represent a potential chemosystematic marker on the sub-class level.

Except for the chloroplast parameters other cell properties of the Euglenophyceae are highly characteristic for this class and do not support a close phylogenetic connection with the Chlorophyceae/Prasinophyceae [24–26]. The accumulating evidence that the euglenophycean chloroplasts have an endosymbiotic origin may represent a plausible solution to this enigma. The different host/endosymbiont systems hypothesized to give rise to the photosynthetic Euglenophyceae have been discussed by Gibbs [26]. In this perspective the chloroplast pigmentation would turn up as an unreliable parameter in vertical euglenophycean phylogeny and would be restricted to the origin of the chloroplasts by horizontal evolution.

The earlier view on euglenophycean carotenoids has been based on a too limited number of species and genera (cf. [1]). Reconfirmation of earlier carotenoid data and detailed studies on further species are still needed, with special emphasis on the identity and distribution of β,ε-derived and oxa-bicycloheptane carotenoids. Studies along these lines are in progress [27].

EXPERIMENTAL

Biological material and culture methods. The isolate of *Eutreptiella gymnastica* Throndsen (Euglenophyceae) previously applied [1, 2] was cultured as described elsewhere [2]. The yield from 290 l. of culture medium was 12.9 g dry wt of lipid-extracted cells.

Pigment isolation. The total extract was separated into main fractions by TLC on silica gel G–$CaCO_3$ (2:1) (TLP-I) with petrol–Me_2CO (4:1) as a developing solvent. Saponification (5% KOH in MeOH) of the chlorophyll *a,b*-fraction and rechromatography in the same TLC-system revealed two yellow pigments with unchanged chromatographic polarity (R_f: 0.54 (**10**) and 0.49 (**11**); chlorophyll *a*: 0.49). Each carotenoid was further purified by TLC on silica gel G–Kieselguhr–$Ca(OH)_2$–MgO (14:16:9:9) (TLP-II) with petrol–Me_2CO–*iso*-PrOH (35:14:1) as the developing solvent.

Chromatographic behaviour and authentic pigments. R_f-values given below refer to the following TLC-system: stationary phase: TLP-I; developing solvent: petrol–Me_2CO (41:9). R_f-values of authentic pigments in the same systems: echinenone (synthetic, Hoffmann-La Roche): 0.66; canthaxanthin (synthetic, Hoffmann-La Roche): 0.38 and chlorophyll *a* (*E. gymnastica*): 0.25.

Chemical reactions. Acetylation (Ac_2O–pyridine) [28], silylation (SYLON BTZ, Supelco Inc.) [28], epoxide–furanoide rearrangement (HCl–Et_2O) [28] and allylic methylation (HCl–MeOH) [10] were carried out by standard procedures on the ≤ 0.2 mg scale.

3′-Hydroxy-3,6-epoxy-5,6-dihydro-β,ε-caroten-4-one (**10**). Available amount: 0.32 mg (0.025 mg/g lipid-extracted dry wt, *ca* 0.06% of total carotenoids); R_f 0.28; crystallized from Me_2CO–MeOH; UV-visible λ_{max}^{petrol} nm: 414, 438 and 468; III/II (%) [29] = 105 (cryst.) $\lambda_{max}^{Me_2CO}$ nm: 417, 441 and 470; III/II (%) = 103 (cryst.); MS *m/z* (rel. int.): 582 $[M]^+$ (5), 567 $[M-15]^+$ (1), 564 $[M-18]^+$ (15) and 43 (100); ^1H NMR (400 MHz, $CDCl_3$, D-locked): δ0.85 *s* (3H, Me-1′), 0.97 *s* (3H, Me-1), 0.99 *s* (3H, Me-1′), 1.01 *d* (J = 7 Hz, 3H, Me-5), 1.13 *s* (3H, Me-1), 1.36 *dd* (J_{gem} = 14 Hz, $J_{2',3'}$ = 7 Hz, *ca* 1H, H-2′), 1.62 *s* (3H, Me-5′), 1.84 *dd* (J_{gem} = 14 Hz, $J_{2',3'}$ = 7 Hz, *ca* 1H, H-2′), 1.91 *s* (3H, Me-9′), 1.94 *s* (3H, Me-13′), 1.97 *s* (6H, Me-9, 13), 2.40 *d* (J = 10 Hz, 1H, H-6′), 2.52 *q* (J = 7 Hz, 1H, H-5), 4.25 *m* (1H, H-3′), 4.33 *d* (J = 7 Hz, 1H, H-3), 5.43 *dd* ($J_{6',7}$ = 10 Hz, $J_{7',8'}$ = 15 Hz, 1H, H-7′), 5.55 *m* (1H, H-4′), 5.57 *d* ($J_{7,8}$ = 16 Hz, 1H, H-7) and 6.12–6.7 *m* (*ca* 12H, conj. olefinic); decoupling expt (first figure denotes point of irradiation, second figure signal effected) (δ-values) 2.52 (*q*)/1.01 *d* → *s*; CD (MeOH) nm (Δε) 228 (0), 265 (8.5), 302 (0), 322 (−0.8) and 337 (0) (cryst.); the epoxide test was negative.

10-Ac was obtained from **10** (0.18 mg) by a standard procedure; yield 0.12 mg (67%, 11% of **10** recovered); R_f 0.54; precipitated from Me_2CO–MeOH; UV-visible λ_{max}^{petrol} nm: 414, 438 and 468; III/II (%) = 99 (semi-cryst.); $\lambda_{max}^{Me_2CO}$ nm: 417, 441 and 470; III/II (%) = 97 (semi-cryst.); MS *m/z* (rel. int.): 624 $[M]^+$ (3), 564 $[M-60]^+$ (16), 472 $[M-60-92]^+$ (1) and 43 (100); the silylation test was negative (TLC).

10-Me was obtained from **10** (0.10 mg) by a standard procedure; yield: 0.04 mg (40%, 8% of a red degradation product, R_f *ca* 0.40); R_f 0.59; UV-visible λ_{max}^{petrol} nm: 315, 329, 413, 436 and 466; III/II (%) = 68 (non-cryst.); MS *m/z* (rel. int.): 596 $[M]^+$ (6), 564 $[M-32]^+$ (0.4), 504 $[M-92]^+$ (0.5) and 57 (100); the epoxide test was negative.

3′-Hydroxy-3,6-epoxy-7′,8′-didehydro-5,6-dihydro-β,β-caroten-4-one (**11**). Available amount: 0.31 mg (0.024 mg/g lipid-extracted dry wt, *ca* 0.06% of total carotenoids); R_f 0.25; UV-visible λ_{max}^{petrol} nm: 325, 338, 420, 442 and 472; III/II (%) = 54; $\lambda_{max}^{Me_2CO}$ nm: 424, 446 and 475; III/II (%) = 56; IR: ν_{max}^{KBr} cm^{-1} (contaminated): 3400 *w* (OH); 2960 *s*, 2930 *s* and 2860 *m* (CH); 1770 *s* (C=O); 1470 *m* and 1455 *m* (CH_2); 1375 *s* (Me); 1175 *w*, 1055 *w* and 1030 *w* (*sec.* -OH) and 970 *s* (C=C); MS *m/z* (rel. int.): 580 $[M]^+$ (23), 562 $[M-18]^+$ (17), 488 $[M-92]^+$ (2) and 41 (100); ^1H NMR (400 MHz, $CDCl_3$, D-locked): δ0.97 *s* (3H, Me-1), 1.01 *d* (J = 7 Hz, 3H, Me-5), 1.13 *s* (3H, Me-1), 1.19 *s* (3H, Me-1′), 1.26 (Me-1′; obscured by lipids), 1.93 *s* (3H, Me-5′), 1.94 *s* (3H, Me-13′), 1.97 *s* (6H, Me-9, 13), 2.00 *s* (3H, Me-9′), 2.52 *q* (J = 7 Hz, 1H, H-5), 4.02 *m* (1H, H-3′), 4.33 *d* (J = 7 Hz, 1H, H-3), 5.58 *d* (J = 16 Hz, 1H, H-7) and 6.2–6.7 (*ca* 11H, conj. olefinic); CD (EPA) nm (Δε) < 246 (neg.), *ca* 246 (0), 273 (−0.9), 300 (0), 328 (1.3) and 350 (0) (non-cryst.).

11-Ac was obtained from **11** (0.11 mg) by a standard procedure; yield 0.079 mg (72%); R_f 0.51; UV-visible λ_{max}^{petrol} nm: 416, 439 and 468; III/II (%) = 58; MS *m/z* (rel. int.): 622 $[M]^+$ (38), 564 $[M-58]^+$ (6), 562 $[M-60]^+$ (9) and 57 (100); ^{1}H NMR (200 MHz, $CDCl_3$, D-locked): δ0.98 *s* (3H, Me-1), 1.01 *d* (*J* = 7 Hz, 3H, Me-5), 1.13 *s* (3H, Me-1), 1.22 *s* (3H, Me-1'), 1.26 (Me-1'; obscured by lipids), 1.93 *s* (3H, Me-5'), 1.95 *s* (3H, Me-13'), 1.96 *s* (6H, Me-9, 13), 2.00 *s* (3H, Me-9'), 2.05 *s* (3H, acetyl-Me at C-3'), 2.52 *q* (*J* = 7 Hz, 1H, H-5), 4.32 *d* (*J* = 7 Hz, 1H, H-3) and 5.57 *d* (*J* = 16 Hz, 1H, H-7); decoupling expt (400 MHz, $CDCl_3$, D-locked) (δ-values) 2.52 (*q*)/1.01 *d* → *s*; the epoxide test and the silylation test (TLC) were both negative.

Diatoxanthin (**2**). Available amount: *ca* 25 mg; R_f: see ref. [1]; **2** was crystallized from MeOH; UV-visible λ_{max}^{petrol} nm: 428, 450 and 479; III/II (%) = 50 (cryst.); $\lambda_{max}^{Me_2CO}$ nm: 432, 454 and 483, III/II (%) = 45 (cryst.); MS: see ref. [1]; ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ1.07 *s* (6H, Me-1', 1'), 1.14 *s* (3H, Me-1), 1.20 *s* (3H, Me-1), 1.73 *s* (3H, Me-5'), 1.92 *s* (3H, Me-5), 1.95 *s* (3H, Me-13), 1.97 *s* (3H, Me-13'), 1.98 *s* (3H, Me-9'), 2.00 *s* (3H, Me-9), 4.01 *m* (2H, H-3, 3') and 6.1–6.7 *m* (*ca* 12H, conj. olefinic); CD (EPA) nm ($\Delta\varepsilon$) 220 (−3.7), 232 (0), 247 (4.7), 261 (0), 285 (−9.0), 323 (0) and 345 (3.3) (cryst.)

Diadinoxanthin (**3**). Available amount: *ca* 15 mg; R_f: see ref. [1]; **3** was crystallized from MeOH; UV-visible λ_{max}^{petrol} nm: 422, 446 and 476; III/II (%) = 78 (cryst.); $\lambda_{max}^{Me_2CO}$ nm: 428, 448 and 478; III/II (%) = 77 (cryst.); MS: see ref. [1]; ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ0.98 *s* (3H, Me-1), 1.14 *s* (6H, Me-1, 1'), 1.19 *s* (3H, Me-5), 1.20 *s* (3H, Me-1'), 1.92 *s* (6H, Me-9, 5'), 1.95 *s* (3H, Me-13), 1.97 *s* (3H, Me-13'), 2.00 *s* (3H, Me-9'), 3.92 *m* (1H, H-3), 4.01 *m* (1H, H-3'), 5.88 *d* ($J_{7,8}$ = 15.5 Hz, 1H, H-7) and 6.18–6.7 *m* (*ca* 11H, conj. olefinic); CD (EPA) nm ($\Delta\varepsilon$) 225 (0), 239 (4.6), 249 (0), 280 (−8.5), 297 (0) and 237 (4.7) (cryst.).

Neoxanthin (**4**). Available amount: *ca* 5 mg; R_f: see ref. [1]; UV-visible λ_{max}^{hexane} nm: 394, 416, 440 and 469; III/II (%) = 93 (non-cryst.); MS: see ref. [1]; ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ0.98 *s* (3H, Me-1'), 1.07 *s* (3H, Me-1), 1.15 *s* (3H, Me-1'), 1.19 *s* (3H, Me-5'), 1.33 *s* and 1.35 *s* (3+3H, Me-1, 5) (compare ref. [30]), 1.80 *s* (3H, Me-9), 1.93 *s* (3H, Me-9'), 1.96 *s* (6H, Me-13, 13'), 2.26 *br d* (*J* = 13 Hz, 1H, H-4), 2.39 *dd* (J_1 = 14 Hz, J_2 = 1.6 Hz, 1H, H-4'), 3.91 *m* (1H, H-3'), 4.32 *m* (1H, H-3), 5.88 *d* ($J_{7,8}$ = 15.5 Hz, 1H, H-7'), 6.03 *s* (1H, H-8), 6.11 *d* ($J_{10,11}$ = 11 Hz, 1H, H-10) and 6.15–6.7 *m* (*ca* 10H, conj. olefinic); CD (EPA) nm ($\Delta\varepsilon$) 219 (0), 225 (−1.8), 243 (−0.7), 265 (−2.8), 293 (−0.6), 311 (−0.8) and *ca* 350 (+0.1) (non-cryst.).

Taraxanthin (**9**). Available amount: 0.13 mg (0.01 mg/g lipid-extracted dry wt, *ca* 0.02% of total carotenoids); chromatographic properties: co-chromatographed with diadinoxanthin (**3**) on TLP-I, but was less polar than **3** on TLP-II; UV-visible $\lambda_{max}^{Me_2CO}$ nm: 418, 441 and 471; III/II (%) = 84 (non-cryst.); MS *m/z* (rel. int.): 584 $[M]^+$ (1), 568 $[M-16]^+$ (0.8), 566 $[M-18]^+$ (9), 548 $[M-18-18]^+$ (7), 504 $[M-80]^+$ (2), 486 $[M-18-80]^+$ (6), 474 $[M-18-92]^+$ (2), 221 (36), 181 (27) and 91 (100); ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ0.85 *s* (3H, Me-1'), 0.98 *s* (3H, Me-1'), 1.00 *s* (3H, Me-1), 1.16 *s* (3H, Me-1), 1.19 *s* (3H, Me-5), 1.62 *s* (3H, Me-5'), 1.92 *s* (3H, Me-9'), 1.94 *s* (3H, Me-9), 1.97 *s* (6H, Me-13, 13'), 2.40 *d* (*ca* 1H, H-6'), 3.93 *m* (1H, H-3'), 5.43 *dd* (1H, J_1 = 10 Hz, J_2 = 16 Hz, H-7'), 5.56 (1H, H-4'), 5.90 *d* (1H, *J* = 15 Hz, H-7) and 6.0–6.6 *m* (conj. olefinic); the epoxide test was positive.

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