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ALGAL CAROTENOIDS WITH NOVEL END GROUPS*†

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Key Word Index—*Eutreptiella gymnastica*; Euglenophyceae; new carotenoids; 3′,4′-anhydrodiatoxanthin; eutreptiellanone; siphonein.

Abstract—The structures of three previously unidentified carotenoids from *Eutreptiella gymnastica* are reported. These include siphonein with defined *n*-2-*trans*-2-dodecenoic esterifying acid and assigned 3*R*(?), 3′*R*,6′*R* chirality, (3*R*)-3′,4′-anhydrodiatoxanthin and eutreptiellanone (3,6-epoxy-3′,4′,7′,8′-tetradehydro-5,6-dihydro-β,β-caroten-4-one) with probable 3*S*,5*R*,6*S* chirality.

INTRODUCTION

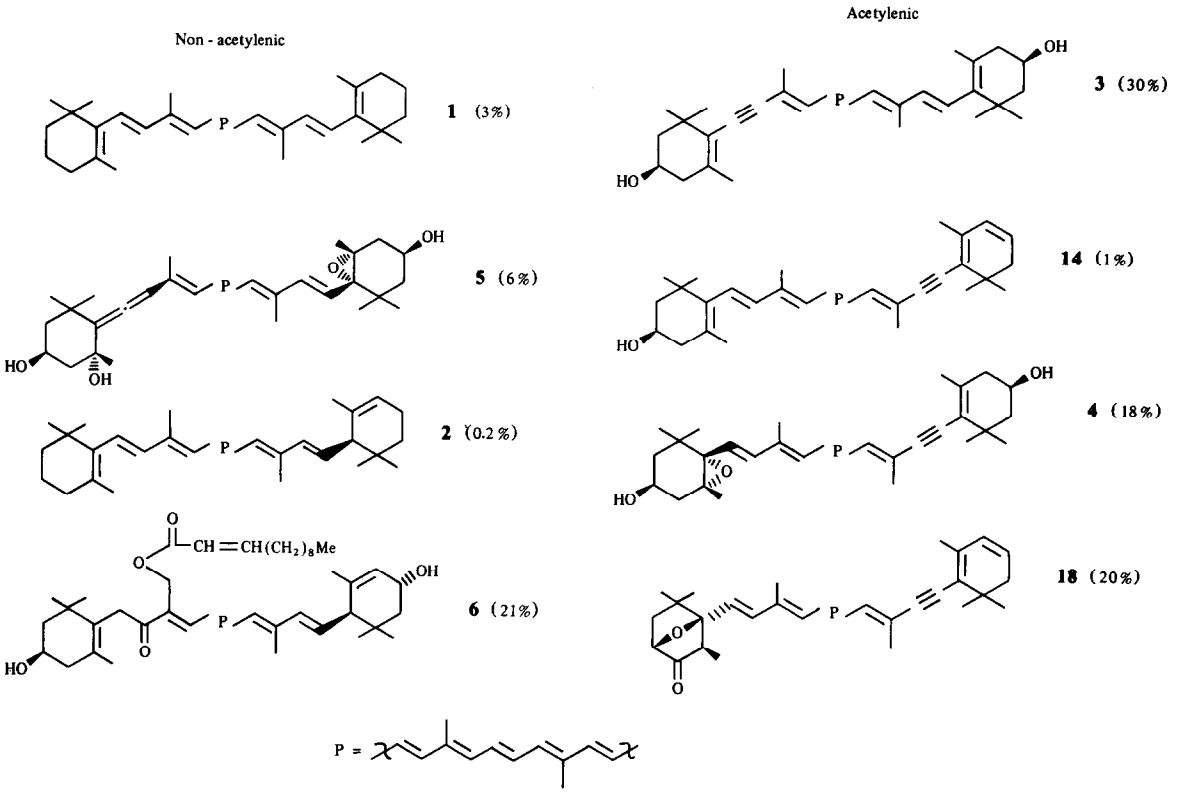
Bjørnland [1] recently characterized the carotenoids of the marine alga *Eutreptiella gymnastica* as β,β-carotene (**1**), β,ε-carotene (**2**), the acetylenic diatoxanthin (**3**) and diadinoxanthin (**4**) and the allenic neoxanthin (**5**) (Scheme 1). The chiralities established for these carotenoids from other sources [2–4] were assumed. We now report structural studies on three unidentified carotenoids [1]: unknown 1 (20% of total carotenoid), unknown 2 (1%) and unknown 3 (21%).

RESULTS AND DISCUSSION

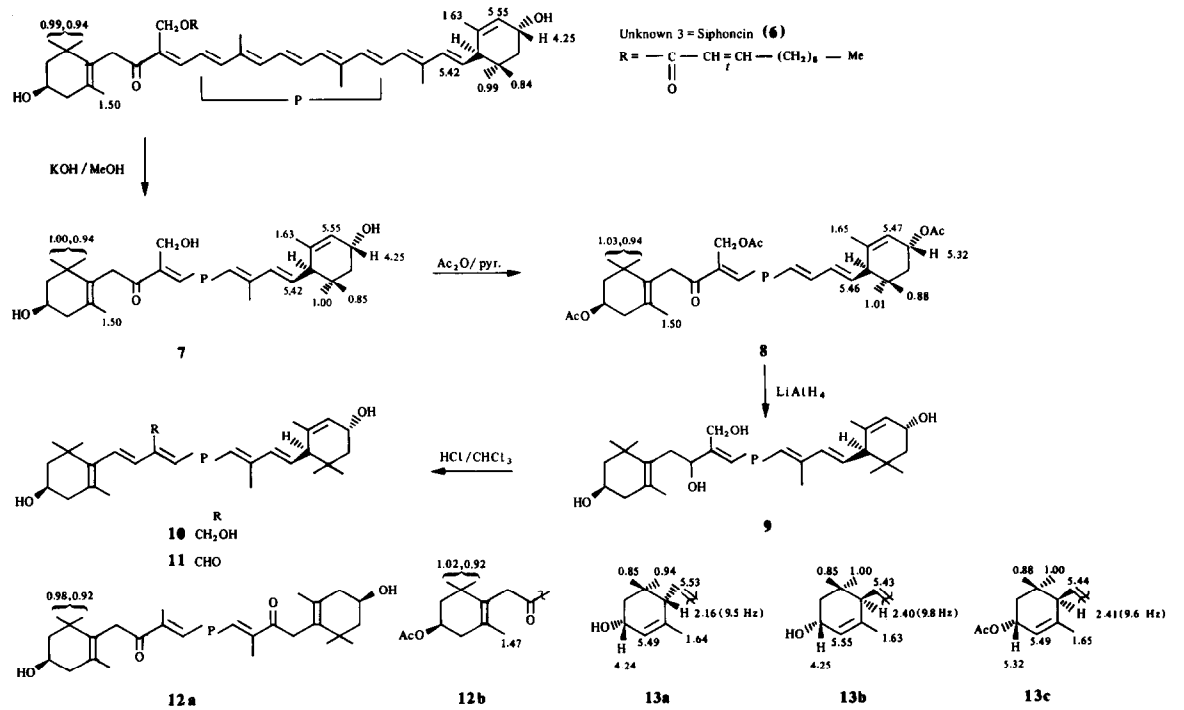
The most polar unknown 3 was identified as siphonein, an esterified carotenol previously encountered in Chlorophyceae (Siphonales) [5–8] and Prasinophyceae [9]. Siphonein has a currently accepted constitution [10, 11]. The esterifying acids have been identified as dodecenoic [10], lauric [6], mainly C_{13}- and C_{14}-unsaturated [8] and palmitic and stearic [11]. Our results for siphonein from *E. gymnastica* confirm the previous constitution [10, 11], define the esterifying acid as a conjugated *n*-dodecenoic acid and suggest 3*R*(?), 3′*R*,6′*R* chirality (**6**, Scheme 1). Our siphonein thus had ^{1}H NMR and mass spectral properties consistent with the constitution represented by **6** (Scheme 2). Comparative ^{1}H NMR and mass spectral data for siphonein (**6**) and its alkaline hydrolysis product siphonaxanthin (**7**, inseparable from an authentic standard) and NMR data for **6** defined the esterifying acid as *n*-2-*trans*-2-dodecenoic acid (^{1}H NMR H-2, δ 5.77 *d*, *J* = 15 Hz and ^{13}C NMR Me-12 δ 14.1), consistent with the findings of Walton *et al.* [10] for siphonein from *Codium fragile*. They reported a $C_{12:1}$ esterifying acid from mass spectral evidence. The chemical shift for CH_2-19 in siphonein (**6**, δ 5.10) and siphonaxanthin (**7**, δ 4.49) demonstrated the allocation of the ester moiety in **6** to a primary hydroxy function. The previously observed changes in the electronic spectra for **6** and **7**, ascribed to hydrogen-bonding in the α-ketol **7** [7], supported C-19 location of the acyloxy function. A conjugated keto group at C-8 further followed from the hypsochromic shift upon lithium aluminium hydride reduction to siphonaxanthol (**9**) [7, 10] and allylic dehydration of **9** to a loroxanthin-like product **10** [10]. By the latter conversion, a minor product with properties compatible with the aldehyde **11** (cf. loroxanthal [12]) was also obtained and was ascribed to allylic oxidation of **10** by air. Acetylation of siphonaxanthin (**7**) provided the triacetate **8** with the expected ^{1}H NMR and mass spectral properties. ^{1}H NMR assignments of siphonein (**6**), siphonaxanthin (**7**) and siphonaxanthin triacetate (**8**) were based on data for relevant models, including the synthetic dione **12a** prepared by Saucy and Weber (unpublished results) and its diacetate **12b**, and published data for the 3,6-*cis* ε-end group **13a** and the 3,6-*trans*-end group **13b** and its acetate **13c** [13, 14] (Scheme 2). A doublet at δ 2.16 (*J* = 7.4 Hz) in the ^{1}H NMR spectrum of siphonein (**6**), less apparent in the spectra of siphonaxanthin (**7**) and the triacetate **8**, is probably associated with the allylic methylene group in the esterifying acid in siphonein (**6**) and not the C-6 methine proton of **13a**. However, in the 100 MHz spectrum the δ 2.41 doublet of **13b** was obscured by signals caused by the C-4 methylene in the β-end group [13]. Also, considering the chemical shifts for H-4′ (δ 5.55 in **6** and **7** and 5.49 in **8**) and H-7′ (5.42 *d* in **6** and **7**) a 3,6-*trans* configuration is favoured for the ε-ring. It is well established that the C-6 centre largely determines the Cotton effect in carotenoids containing C-3 substituted ε-rings [14–17]. Since the CD spectrum of synthetic **12** had a very weak positive Cotton effect (Saucy, G. and Weber, G., unpublished results), the relatively strong Cotton effect of siphonaxanthin (**7**, Fig. 1) in favourable comparison with the CD spectra of the synthetic chiriquixanthins [14] with 3*R*,6′*R* chirality, siphonaxanthin (**7**) is assigned the 3′*R*,6′*R* configuration. The chirality of the ε-ring is then the same as for lutein with a well-established **13b** end group [14, 15]. The proposed 3*R* chirality for **7** is

*Part 28 in the series "Algal Carotenoids". For Part 27 see *Biochem. Syst. Ecol.* (in press).

†Dedicated to Professor E. Lederer on the occasion of his seventy-fifth birthday.



Scheme 1. Carotenoids of *Eutreptiella gymnastica*.



Scheme 2. Siphonein.

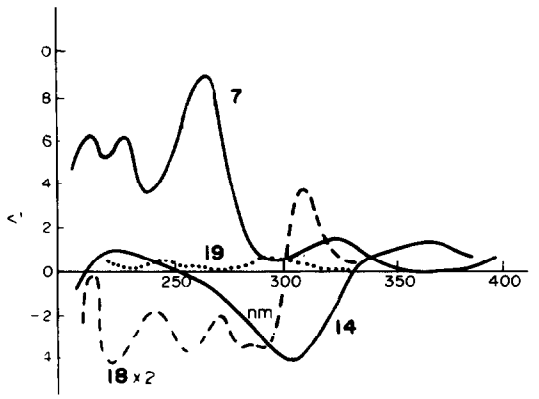


Fig. 1. CD spectra in EPA solution of siphonaxanthin (**7**), 3',4'-anhydrodiatoxanthin (**14**), eutreptiellanone (**18**) and eutreptiellanol (**19**).

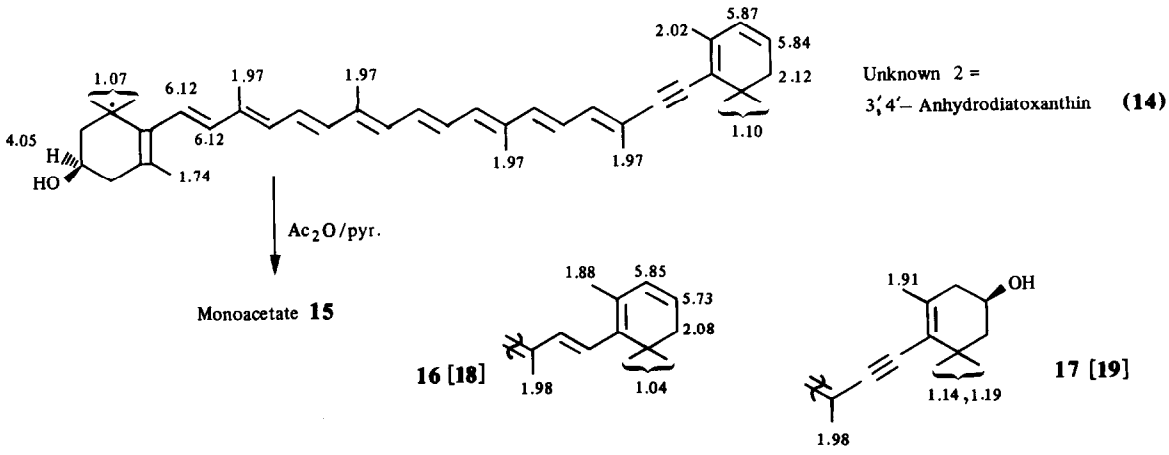
based on analogy with all other 3-hydroxy-β-type carotenoids, but is not proved.

The stereochemical relationship between loroxanthin [12] and product **10** needs comment. According to an early ^{1}H NMR spectrum [12], loroxanthin appears to be 3',6'-*trans*, and the chirality of loroxanthin has recently been unequivocally established [18] as a 19'-hydroxylated 3*R*,3'*R*,6'*R*(3',6'-*trans*)-lutein. Product **10** should consequently be identical with loroxanthin, from which it was not separated by TLC. In conclusion, the configuration 3*R*(?),3'*R*,6'*R* is proposed for siphonein (**6**) from *E. gymnastica*.

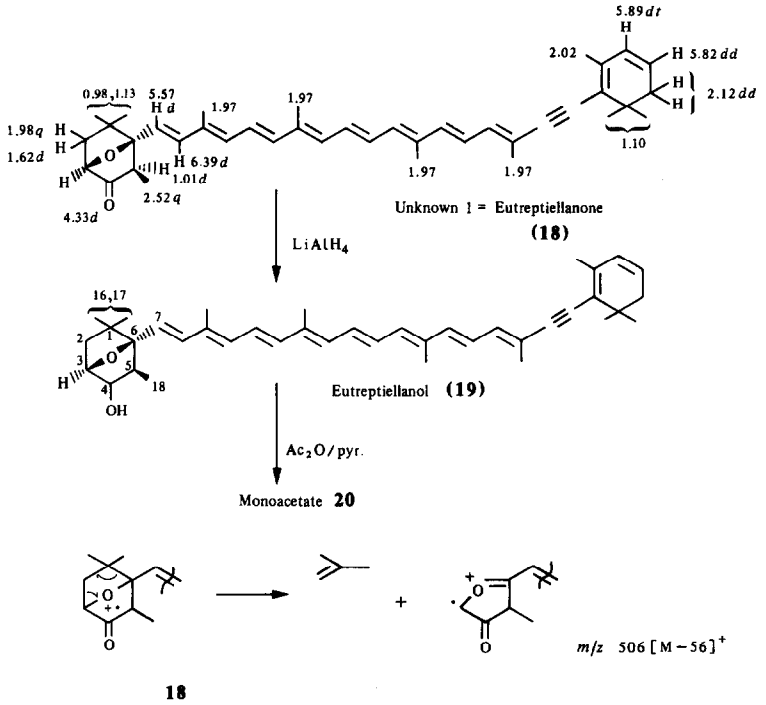
Unknown 2 was assigned the 3',4'-anhydrodiatoxanthin [(3*R*)-3',4',7',8'-tetradehydro-β,β-caroten-3-ol, **14**] structure, possessing a novel acetylenic end group. The molecular formula $C_{40}H_{52}O$ was determined by high precision mass spectrometry, and the oxygen function was shown by acetylation to a monoacetate (**15**) to be a primary or secondary hydroxy group. A new chromophore was indicated by the electronic spectrum. ^{1}H NMR signals (Scheme 3) consistent with a 3-hydroxy β-end group were identified by LIS.

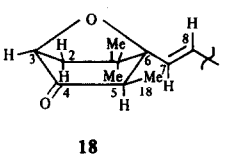
Furthermore, ^{1}H NMR signals of the cyclohexadiene end group were correlated with those of 3,4,3',4'-tetradehydro-β,β-carotene [13], and the chemical shift of Me-18' was compatible with its in-chain nature and neighbouring triple bond, cf. models **16** [13] and **17** [19] (Scheme 3). Allowing for the longer chromophore of 3',4'-anhydrodiatoxanthin, the similarity in its bathochromically shifted CD spectrum (Fig. 1) relative to that of (3*R*)-β,β-caroten-3-ol leads to the 3*R* configuration for the new acetylenic carotenol (**14**).

Unknown 1 [1], now called eutreptiellanone (**18**, Scheme 4), represents a new class of naturally occurring carotenoids with a substituted 3,6-oxa-bicycloheptane ring system. Related 2,5-oxa-bicycloheptane carotenoids have recently been prepared by partial synthesis [20]. Eutreptiellanone, $C_{40}H_{50}O_2$ by high precision mass spectrometry, could not be acetylated, silylated, or dehydrated with $POCl_3$, contained a non-conjugated carbonyl group (ν_{max} 1770 cm^{-1}) and was stable towards alkali and acid. Eutreptiellanone (**18**) was reduced with lithium aluminium hydride to eutreptiellanol, $C_{40}H_{52}O_2$ (**19**), with a similar chromophore, providing eutreptiellanol monoacetate (**20**) upon acetylation. These data, besides the carbonyl frequency in the IR spectrum, were compatible with a four-ring or strained five-ring ketone [21] and an inert, second oxygen function. In the ^{1}H NMR spectra of eutreptiellanone (**18**) and eutreptiellanol (**19**), signals not influenced by the reduction could be associated with the acetylenic end group of 3',4'-anhydrodiatoxanthin (**14**). The novel bicyclic end group assignment of eutreptiellanone (**18**) was based on the ^{1}H NMR (Scheme 4), including LIS experiments, and ^{13}C NMR spectra of **18** and **19**. Figure 2 shows the LIS ^{1}H NMR data for eutreptiellanone (**18**), demonstrating decreasing induced shifts for H-5, H-3, H-2, Me-18, Me-17, H-7 and Me-16, compatible with the proximity of these protons to the complexing site (primarily the keto function at C-4). Deviation from linear shifts may be ascribed to the presence of moisture and a final increase in sample volume by 35%. The observed ^{1}H NMR coupling constants for the bicyclic ring system were compatible with the dihedral angles revealed by models (Scheme 4) and with ^{1}H NMR data for related oxa-bicycloheptane derivatives [20–23] and for 2-methyl-4-oxa-



Scheme 3. 3',4'-Anhydrodiatoxanthin.





18

Dihedral angles

∠ H−3(eq)/H−2(eq) ~ 20° ; J = 7.5 Hz
∠ H−3(eq)/H−2(ax) ~ 100° ; J = 0 Hz

Observed couplings (18)

$J_{H\text{-}2eq,\,H\text{-}2ax}$ = 14 Hz
$J_{H\text{-}2eq,\,H\text{-}3eq}$ = 7.5 Hz
$J_{H\text{-}2ax,\,H\text{-}3eq}$ = 0 Hz
$J_{H\text{-}5,\,Me\text{-}18}$ = 7.5 Hz
$J_{H\text{-}7,\,H\text{-}8}$ = 16 Hz *(trans)*
$J_{H\text{-}2'eq,\,H\text{-}2'ax}$ = 0 Hz
$J_{H\text{-}2',\,H\text{-}3'}$ = 4 Hz
$J_{H\text{-}2',\,H\text{-}4'}$ = 1.5 Hz
$J_{H\text{-}3',\,H\text{-}4'}$ = 9.5 Hz *(cis)*

Scheme 4. Eutreptiellanone.

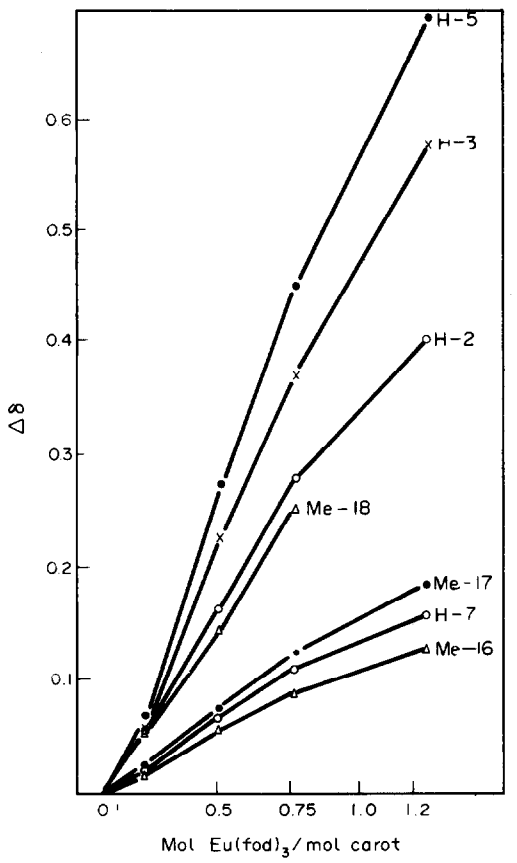


Fig. 2. Induced ^{1}H NMR shifts ($\Delta\delta$) upon stepwise addition of $Eu(fod)_3$ to eutreptiellanone (**18**) in $CDCl_3$ at 100 MHz.

cyclopentanone [24]. The large *gem.*-coupling (14 Hz) of H-2(ax) and H-2(eq) of eutreptiellanone (**18**) was consistent with five- and six-rings [24], and $J_{7,8}$ (16 Hz) demonstrated *trans*-configuration for the Δ^7-bond. Improved ^{1}H NMR data for the acetylenic end group, also present in 3′,4′-anhydrodiatoxanthin (**14**), were obtained at 250 MHz for eutreptiellanone (**18**) (see coupling constants cited in Scheme 4).

Assignment of the ^{13}C NMR signals of eutreptiellanone (**18**) was consistent with data for alloxanthin (acetylenic end groups of **3**) [25], violaxanthin (epoxidic end groups of **5**) [25], 3,4,3′,4′-tetradehydro-β,β-carotene [26] and the synthetic 2,5-oxabicycloheptane carotenoid derivative [20]. A mixture of all-*trans*- and 9′-*cis*- eutreptiellanone (**18**) upon storage was concluded from the observed C-7′, C-8′ and C-19′ signals [19]. LIS ^{13}C NMR revealed approximately equal induced shifts for C-2, C-3, C-5 and C-6. Consideration of models predicts rather similar amounts of 4*R*- and 4*S*-eutreptiellanol (**19**) upon lithium aluminium hydride reduction of **18**. Possible concomitant 9′-*cis*-isomerization further complicated the ^{13}C NMR assignments of **19**. However, $Eu(fod)_3$ shifted the C-2, C-3, C-5 and C-6 signals to an equal extent.

Unexpectedly, different C-3 and C-6 signals were not identified for eutreptiellanone (**18**) or for eutreptiellanol (**19**), even in the presence of $Eu(fod)_3$. The intensity of the δ 81 signals suggested that it might account for two carbons. Alternatively, one signal was hidden by the $CDCl_3$ signals. Consistent with the presence of a tetrahydrofurane ring, the oxygen bridge of eutreptiellanone (**18**) could not be opened with lithium aluminium hydride, even under forcing conditions [27]. As shown by the ^{1}H NMR spectrum, the α-positions of the keto group of **18** could not be deuterated in the presence of base. An enolic Δ^3-double bond would violate Bredt's rule and a

Δ^4-double bond would imply a more strained ring system. Moreover, the acidity of H-5 was reduced by the positive inductive effect of Me-18. A strong molecular ion upon electron impact and minor fragment ions at $[M-15]^+$ (methyl radical), $[M-92]^+$ (toluene), no $[M-106]^+$ (elimination of xylene prevented by the 7′,8′-triple bond and the bulky dicyclic ring system) and a $[M-56]^+$ fragment ion (rationalized in Scheme 4) were compatible with the constitution assigned to eutreptiellanone (**18**) [28]. Better spectral fine-structure in the visible spectrum of eutreptiellanone (**18**) than for eutreptiellanol (**19**) indicated better planarity in the chromophore of **18** and hence a steric interaction of Me-18 and the polyene chain in **19**, also suggested by their λ_{max}.

Concerning the configuration of the bicyclic end group of eutreptiellanone (**18**, Scheme 4), the 3,6-oxygen bridge determines the relative configuration of C-3 and C-6. Moreover, models reveal that the methyl group at C-5 is most likely to be equatorial in order to prevent a 1,3-diaxial methyl–methyl interaction. The chirality of the bicyclic ring system is consequently expected to be as represented by **18** (Scheme 4) or its enantiomer. Preference for the 3*S*,5*R*,6*S* configuration (**18**) rests on biogenetic considerations. Diadinoxanthin (**4**, Scheme 1) is a plausible precursor. Nucleophilic attack by the C-3 hydroxy group at C-6 would determine the chirality at C-3. Also CD considerations by two different approaches lead to the same preference. The Cotton effect (Fig. 1) of eutreptiellanone (**18**) is negative below 300 nm ($\Delta\varepsilon = ca -2$) as for ε-type carotenoids with the 6*S* configuration [14, 15], cf. the positive Cotton effect for siphonaxanthin (**7**) with opposite chirality. However, the weak positive Cotton effect of eutreptiellanol (**19**) may suggest that the Cotton effect of eutreptiellanone (**18**) is mainly determined by the keto group in chiral surroundings. Application of the octant rule [29], if valid, again would predict a negative Cotton effect, as observed [5]. Although the validity of these models may be debated, the result is in favour of the biogenetic hypothesis.

EXPERIMENTAL

Biological material. The isolate of *Eutreptiella gymnastica* Throndsen was the same as published [1].

Culture methods. The alga was grown in three series of aerated 5 l. Erlenmeyer flasks at 16°. Series 1 consisted of 28 × 3.0 l., series 2 of 32 × 3.0 l. and series 3 of 31 × 3.5 l. The culture medium was IMR [30] based on 25‰ natural seawater. Series 3 (contrary to 1 and 2) was further enriched with $NaNO_3$ (50 mg per l.) and KH_2PO_4 (6.8 mg per l.) 3 times at 48 hr intervals towards the end of the culture period. The flasks were continuously illuminated from above with Philips fluorescent tubes (TL/32). The light intensity was 35 $\mu E/m^2$ per sec as measured with a LI-188 integrating quantum photometer fitted with a LI-190s cosinus sensor (Lambda Instruments Corp.). The alga was harvested by continuous centrifugation (Kahlsico model 903-1S) after 14–16 days. The dry wts of the lipid-extracted cells were 2.1, 2.8 and 8.0 g for series 1, 2 and 3, respectively.

Extraction and chromatography. The previous procedure [1] was used. The total carotenoid content corresponded to *ca* 0.6% of the dry wt of the extracted cells; total yield of chromatographically pure unknown 1 (**18**) 24 mg, unknown 2 (**14**) 0.9 mg and unknown 3 (**6**) 8 mg. Chromatography of derivatives was carried out by TLC (silica gel, 0.25 mm); R_f values refer to 40% Me_2CO in hexane if not otherwise specified.

Spectroscopy. Electronic spectra were recorded in Et_2O. Spectral fine-structure is defined as % III/II [31]. Concns were calculated using $E^{1\%}_{1cm} = 2500$ at λ_{max}. IR spectra were recorded in KBr discs. Mass spectra were obtained with an AEI MS 902 instrument with direct inlet system at 70 eV, 190–210°. The base peak was chosen > *m/z* 180 and diagnostically useful or prominent peaks only are cited. ^{1}H NMR spectra were recorded with a Jeol JNM-FX 100 FT instrument (100 MHz), except for a 250 MHz spectrum of eutreptiellanone (**18**) obtained with a Bruker WM 250 instrument. ^{13}C NMR spectra were obtained with the Jeol instrument. NMR spectra were recorded in $CDCl_3$ with TMS as standard. CD spectra were recorded on a Rousell–Juane Dichrograph in EPA (Et_2O–*iso*-pentane–EtOH, 5:5:2) at room temp.

Chemical derivatizations. Normal precautions for work with carotenoids were taken [32]. Alkali treatment (saponification), acetylation, silylation and epoxide–furanoxide rearrangements were carried out by general procedures [31]. Reduction with $LiAlH_4$ was carried out in dry Et_2O, under forcing conditions as previously described [27]. Allylic dehydration [33] and attempted dehydration with $POCl_3$ [27] followed normal procedures. ^{2}H-exchange of **18** was attempted by addition of 40% NaOD/D_2O (2 drops) to the carotenoid dissolved in $CD_3OD/CDCl_3$; the reaction being monitored by ^{1}H NMR. Other reactions were monitored by TLC.

Siphonein (**6**). R_f 0.40; VIS λ_{max} nm: 448; ^{1}H NMR: δ 0.84 *s* and 0.99 *s* (3 + 3H, H_3-16′, H_3-17′), 0.94 *s* and 0.99 *s* (3 + 3H, H_3-16, H_3-17), 1.50 *s* (3H, H_3-18), 1.63 *s* (3H, H_3-18′), 1.92 *s* (3H, H_3-19′), 1.99 *s* (6H, H_3-20, H_3-20′), 2.16 *d* (J = 7.4 Hz), *ca* 2.4 *m*, 3.46 *s* (2H, H_2-7), 5.10 *s* (2H, H_2-19), 5.55 (1H, H-4′), 5.77 *d* (J = 15 Hz, 1H, olefinic, α to C=O in ester moiety), *ca* 7.0 *m* (1H, olefinic, β to C=O in ester moiety), 6.0–7.0 *m* (*ca* 11H, olefinic); ^{13}C NMR δ 12.9 (C-20, C-20′), 13.2 (C-19′), 14.1 (Me ester moiety), 22.9 (C-18′), 24.3 and 29.4 (C-16′, C-17′), 28.0 and 29.1 (C-16, C-17), 29.4 (CH_2 in ester moiety), 34.0 (C-1′), 37.0 (C-1), 42.0 (C-4), 44.6 (C-2′), 48.0 (C-2), 55.0 (C-6′), 57.6 (C-19), 65.3 (C-3), 65.9 (C-3′), 166.6 (C=O ester moiety), 196.9 ((C=O)-8); unassigned sp^3 C signals: 20.4, 22.7, 28.3, 31.9, 32.2 and 37.3; remaining sp^2 C signals not assigned: 120.8, 122.5, 124.6, 126.0, 127.9, 129.3, 130.6, 130.8, 132.1, 132.3, 133.1, 135.4, 135.9, 137.2, 137.7, (v.s.) 137.8, 138.5, 142.6, 147.5 and 150.2 MS *m/z* (rel. int.): 780 $[M]^+$ (40), 762 $[M-18]^+$ (12), 752 $[M-28]^+$ (5), 734 $[M-18-28]^+$ (2), 688 $[M-92]^+$ (2), 674 $[M-106]^+$ (1), 600 $[M-180]^+$ (7), 584 $[M-196]^+$ (45), 582 $[M-198]^+$ (28), 564 $[M-18-198]^+$ (29), 181 (100).

Siphonaxanthin (**7**). Obtained by alkaline hydrolysis of **6**, R_f 0.20, inseparable from authentic **7** from naturally occurring *Laurencia filiformis*; VIS λ_{max} nm: 441, (464); ^{1}H NMR: δ 0.85 *s* and 1.00 *s* (3 + 3H, H_3-16′, H_3-17′), 1.00 *s* and 0.94 *s* (3 + 3H, H_3-16, H_3-17), 1.50 *s* (3H, H_3-18), 1.63 *s* (3H, H_3-18′), 1.92 *s* (3H, H_3-19), 2.00 *s* (6H, H_3-20, H_3-20′), 2.1–2.4 *m*, 3.50 *s* (2H, H_2-7), 4.22 *m* (1H, H-3′), 4.49 *s* (2H, H_2-19), 5.43 (1H, H-7′), 5.55 (1H, H-4′), 6.0–6.8 *m* (*ca* 11H, olefinic); MS *m/z* (rel. int.): 600.4186 (calc. 600.4177 for $C_{40}H_{56}O_4$) $[M]^+$ (100), 584.4219 (calc. 584.4228 for $C_{40}H_{56}O_3$) $[M-O]^+$ (9), 582.4080 (calc. 582.4072 for $C_{40}H_{54}O_3$) $[M-H_2O]^+$ (19), 572 $[M-28]^+$ (8), 564 $[M-18-18]^+$ (4), 494 $[M-106]^+$ (1); CD, see Fig. 1, cf. [16].

Siphonaxanthin triacetate (**8**). Prepared by acetylation of siphonaxanthin (**7**), R_f 0.60; VIS λ_{max} nm: 448; ^{1}H NMR: δ 0.88 *s* and 1.01 *s* (3 + 3H, H_3-16′, H_3-17′), 0.94 *s* and 1.03 *s* (3 + 3H, H_3-16, H_3-17), 1.50 *s* (3H, H_3-18), 1.65 *s* (3H, H_3-18′), 1.92 *s* (3H, H_3-19′), 2.00 *s* (6H, H_3-20, H_3-20′), 2.03 *s* (3H, acetate Me at C-19), 2.04 *s* (6H, acetate Me at C-3, C-3′), *ca* 2.4 *m*, 3.46 (2H, H_2-7), 5.03 *s* (2H, H_2-19), 5.32 (1H, H-3′), 5.47 (1H, H-4′), 6.0–6.8 *m* (*ca* 12H, olefinic); MS *m/z* (rel. int.): 726 $[M]^+$ (52), 666 $[M-60]^+$ (19), 634 $[M-92]^+$ (3), 620 $[M-106]^+$ (3), 606 $[M-60-60]^+$ (8), 574 $[M-60-92]^+$ (6), 560 $[M-60-106]^+$ (5), 472 [M

$-42-60-60-92]^+$ (30), 454 $[M-60-60-60-92]^+$ (27), 412 (100), 394 (73).

Siphonaxanthol (**9**). Prepared by $LiAlH_4$ reduction of siphonaxanthin triacetate (**8**), R_f 0.15; VIS λ_{max} nm: 396, 419, 447, % III/II = 71; MS *m/z* (rel. int.): 602 $[M]^+$ (26), 600 $[M-2]^+$ (5), 586 $[M-16]^+$ (100), 584 $[M-18]^+$ (63), 568 $[M-16-18]^+$ (6), 566 $[M-18-18]^+$ (41), 550 $[M-16-18-18]^+$ (6), 476 $[M-16-18-92]^+$ (3), 431 (26).

Loroxanthin (**10**). Obtained by acid treatment [32] of siphonaxanthol (**9**), R_f 0.25, inseparable from authentic **10** [12]. VIS λ_{max} nm: 442, (465); MS *m/z* (rel. int.): 584 $[M]^+$ (18), 582 $[M-2]^+$ (24), 564 $[M-2-18]^+$ (36), 363 (100).

Loroxanthal (**11**). Obtained after acid treatment of siphonaxanthol (**9**), R_f 0.45; UV/VIS λ_{max} nm: 465.

3',4'-Anhydrodiatoxanthin (**14**). R_f 0.50; VIS λ_{max} nm: (447), 461, 488, % III/II = 17; ^1H NMR: δ 1.07 *s* (6H, H_3-16, H_3-17), 1.10 *s* (6H, H_3-16', H_3-17'), 1.74 *s* (3H, H_3-18), 1.97 *s* (12H, H_3-19, H_3-20, H_3-19', H_3-20'), 2.02 *s* (3H, H_3-18'), 2.12 *d* (J = 4 Hz, 2H, H-2'), 4.05 *m* (1H, H-3), 5.84 (1H, H-3'), 5.87 (1H, H-4'), 6.12 *s* (2H, H-7, H-8), 6.1–6.8 *m* (*ca* 10 H, olefinic), upon addition of $Eu(fod)_3$ (0.5 mol/mol carotenoid) δ 1.07 shifted downfield to δ 1.10; MS *m/z* (rel. int.): 548.4021 (calc. 548.4018 for $C_{40}H_{52}O$) $[M]^+$ (100), 546 $[M-2]^+$ (3), 533 $[M-15]^+$ (5), 530 $[M-18]^+$ (1), 456 $[M-92]^+$ (3); CD, see Fig. 1. Acid treatment [32] caused no shift in the VIS spectrum. Compound **14** was stable towards alkali (5% KOH).

3',4'-Anhydrodiatoxanthin monoacetate (**15**). Prepared by acetylation of **14**, R_f 0.63; VIS λ_{max} nm: (445), 460, 487; MS *m/z* (rel. int.): 590 $[M]^+$ (28), 575 $[M-15]^+$ (25), 530 $[M-60]^+$ (7), 515 $[M-15-60]^+$ (6), 368 (impurity, 100).

Eutreptiellanone (**18**). R_f 0.80; VIS λ_{max} nm: (434), 457, 483, % III/II = 48; IR ν_{max} cm^{-1} 3040 (w), 2965, 2930 (s), 2870 (s), 1770 (s), 1565 (w), 1450 (s), 1370 (s), 970 (s); ^1H NMR (250 MHz): δ 0.98 *s* and 1.13 *s* (3 + 3H, H_3-16, H_3-17), 1.01 *d* (J = 7.5 Hz, 3H, H_3-18), 1.10 *s* (6H, H_3-16', H_3-17'), 1.62 *d* ($J_{gem.}$ = 14 Hz, 1H, H_{ax}-2), 1.98 *dd* ($J_{gem.}$ = 14 Hz, $J_{2,3}$ = 7.5 Hz, 1H, H_{eq}-2), 1.97 *s* (12H, H_3-19, H_3-20, H_3-19', H_3-20'), 2.02 *s* (3H, H_3-18'), 2.12 *dd* ($J_{2',3'}$ = 4 Hz, $J_{2',4'}$ = 1.5 Hz, 1H, H-2'), 2.52 *q* (J = 7.5 Hz, 1H, H-5), 4.33 *d* ($J_{2,3}$ = 7.5 Hz, 1H, H-3), 5.57 *d* ($J_{7,8}$ = 16 Hz, 1H, H-7), 5.82 *dd* ($J_{2',3'}$ = 4 Hz, $J_{3',4'}$ = 9.5 Hz, 1H, H-3'), 5.89 *dt* ($J_{3',4'}$ = 9.5 Hz, $J_{2',4'}$ = 1.5 Hz, 1H, H-4'), 6.39 *d* ($J_{7,8}$ = 16 Hz, 1H, H-8), 6.1–6.8 *m* (*ca* 10H, olefinic); decoupling expts where the first figure denotes the point of irradiation and the second figure indicates the signal effected (δ) 2.52 (*q*)/1.01 *d* → *s*, 1.01 (*d*)/2.52 *q* → *s*, 4.33 (*d*)/1.98 *dd* → changed pattern, 1.98 (*dd*)/4.33 *d* → *s*, 5.57 (*d*)/6.39 *d* → *s*, 6.39 (*d*)/5.57 *d* → *s*; ^1H NMR LIS expt, Fig. 2; ^{13}C NMR tentative assignments: δ 12.8 (C-19, C-20, C-20'), 18.1 (C-19'), 20.7 (C-18'), 24.3 (C-18), 27.2 (C-16', C-17'), 29.1 and 29.7 (C-16, C-17), 33.0 (C-1'), 37.4 (C-1), 38.3 (C-2'), 42.6 (C-5), 45.2 (C-2), 80.5 (C-3, C-6), 92.7 (C-7'), 102.4 (C-8'), 119.3 (C-9'), 124.7 (C-3'), 126.5 (C-5'), 130.3 (C-4'), 214.9 (C-4, C=O); remaining sp^2 C signals not assigned: 121.7, 124.4, 128.1, 132.6, 133.0, 133.4, 134.2, 135.3, 136.0, 131.4, 136.5, 137.1, 138.1, 138.4; unassigned sp^3 C: 42.5. ^{13}C NMR LIS expt: Addition of $Eu(fod)_3$ (0.51 mol/mol carotenoid) shifted δ values were observed: 42.9 (C-5), 45.4 (C-2), 80.7 (C-3, 6) and an extra signal δ 23.5 (C-19', 9-*cis*), reduced signal at δ 18.1 (C-19', all-*trans*) and δ 92.5 (C-7', all-*trans*) and lacking signal δ 102.4 (C-8', all-*trans*); MS *m/z* (rel. int.): 562.3808 (calc. 562.3811 for $C_{40}H_{50}O_2$) $[M]^+$ (100), 547 $[M-15]^+$ (6), 506.3188 (calc. 506.3185 for $C_{36}H_{42}O_2$) $[M-C_4H_8]^+$ (2), 491.2956 (calc. 491.2950 for $C_{35}H_{39}O_2$) $[M-Me-C_4H_8]^+$ (2), 470 $[M-92]^+$ (2), 455 $[M-15-92]^+$ (2), 430 (impurity ?, 2), 329 (impurity ?, 7), 261 (12); CD, see Fig. 1.

Eutreptiellanone (**18**) could not be acetylated, silylated or dehydrated with $POCl_3$, and was recovered unchanged after alkali treatment (5% KOH) and acid treatment (0.03 M HCl in $CHCl_3$). Attempted ^2H-exchange caused no change in ^1H NMR (δ 2.52 *q* and 4.33 *d*).

Eutreptiellanol (**19**). Prepared by $LiAlH_4$ reduction of **18**, R_f 0.70; VIS λ_{max} nm: (430), 454, 482, % III/II = 23; ^1H NMR: δ 0.89 *s* and 1.18 *s* (3 + 3H, H_3-16, H_3-17), 1.13 *d* (J = 5 Hz, 3H, H_3-18) 1.10 *s* (6H, H_3-16', H_3-17'), 1.94 *s* (3H, H_3-19), 1.96 *s* (9H, H_3-20, H_3-19', H_3-20'), 2.03 *s* (3H, H_3-18'), 2.18 *t* (J = 1.5 Hz, 2H, H-2'), 4.33 *m* (*ca* 2H, H-3, H-4), 5.57 *d* (J = 16 Hz, 1H, H-7), 5.8–5.9 *m* (2H, H-3', 4'), 6.0–6.9 *m* (*ca* 11H, olefinic); ^{13}C NMR tentative assignments: δ 12.8 (C-20, C-20'), 15.3 (C-19), 18.1 (C-19'), 20.6 (C-18'), 22.7 and 25.7 (C-16, C-17), 23.7 (C-18), 27.2 (C-16', C-17'), 32.8 (C-1'), 37.5 (C-1), 38.4 (C-2'), 42.4 (C-2), 43.0 (C-5), 63.1 and 65.9 (C-4, *R/S*), 80.8 (C-3, C-6), 94.4 (C-7'), 102.5 (C-8'), 119.0 (C-9'); other sp^2 C signals not assigned: 123.8, 124.3, 125.0, 126.5, 128.1, 130.0, 130.5, 131.5, 132.6, 133.5, 134.6, 135.3, 136.2, 136.7, 137.7 and 138.1; unassigned sp^3 C signals: 18.3, 19.8, 24.5, 24.8. ^{13}C NMR LIS expt: Additon of $Eu(fod)_3$ (0.96 mol/mol carotenoid) shifted δ values were observed: 24.2 (C-18), 43.1 (C-2), 43.6 (C-5), 79.0, 84.2, 85.0 (C-4 *R/S* and C-3, C-6), 109.2 (C-8'); MS *m/z* (rel. int.): 564 $[M]^+$ (100), 549 $[M-15]^+$ (9), 472 $[M-92]^+$ (3), 457 $[M-15-92]^+$ (2), 430 (impurity ?, 54), 329 (impurity ?, 11), 282 (17); CD, see Fig. 1. Eutreptiellanol (**19**) gave no elimination products upon treatment with acid (0.03 M HCl in $CHCl_3$) or $POCl_3$, and gave no new product upon treatment with $LiAlH_4$ at forcing conditions.

Eutreptiellanol monoacetate (**20**). Prepared upon acetylation of **19**, R_f 0.80; VIS λ_{max} nm: (428), 452, 480, % III/II = 20; MS *m/z* (rel. int.): 606 $[M]^+$ (100), 591 $[M-15]^+$ (6), 547 $[M-59]^+$ (11), 531 $[M-15-60]^+$ (3), 514 $[M-92]^+$ (2), 455 (7), 445 (6), 430 (impurity?, 8), 329 (impurity?, 19), 221 (28).

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