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DIACETYLENIC CAROTENOIDS FROM *EUGLENA VIRIDIS**

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Key Word Index—*Euglena viridis*, Euglenophyceae, carotenoid analysis, new diacetylenic 3,4,7,8,3′,4′,7′,8′-octadehydro-β,β-carotene, esterified 7,8,3′,4′,7′,8′-hexadehydro-β,β-carotene-3-ol

Abstract—Quantitative carotenoid analysis revealed the presence of β,β-carotene (*ca* 5% of total carotenoid) mixed with some β,ε-carotene, the β,ε-carotene derived siphonein (siphonaxanthin 19-dodecenoate, 8%), the allenic neoxanthin (4%) and acetylenic carotenoids > 86% of total carotenoids. These were the monoacetylenic diatoxanthin (major, 61%), diadinoxanthin (rearranged to diadinochrome, 12%), heteroxanthin (1%) and the new diacetylenic 3,4,7,8,3′,4′,7′,8′-octadehydro-β,β-carotene (6%) and esterified 7,8,3′,4′,7′,8′-hexadehydro-β,β-caroten-3-ol (6%) The methods employed included TLC, HPLC, Vis, ^{1}H NMR (400 MHz) and MS The significance of the presence of siphonein and diacetylenic carotenoids for algal chemosystematics is briefly discussed.

INTRODUCTION

Recently the carotenoid composition of the marine euglenophyte *Eutreptiella gymnastica* has been studied in detail [1,2] Preliminary data on the carotenoids of another marine representative, now identified as *Euglena viridis*, have been reported [3] Further data are now presented

Previously the biosynthesis of diacetylenic carotenoids in algae has been considered as restricted to the class Cryptophyceae, for which alloxanthin (**1**, Scheme 1) has been considered a chemosystematic marker [4,5]

RESULTS AND DISCUSSION

Intact cells and a chloroplast preparation was available of *E. viridis* harvested from an annual natural bloom at La Jolla (California) sea shore Carotenoids possessing at least one carbon–carbon triple bond constituted more than 86% of the total carotenoids The monoacetylenic diatoxanthin (**2**, major carotenoid, 61% of total), diadinochrome (**3**, 12%), probably representing rearranged naturally occurring diadinoxanthin (**4**), and heteroxanthin (**5**, 1%) were characterized by co-chromatography with authentic samples and by visible and mass spectrometry.

Two new diacetylenic carotenoids were encountered, namely the highly unsaturated 3,4,7,8,3′,4′,7′,8′-octadehydro-β,β-carotene (**6**) and the monool 7,8,3′,4′,7′,8′-hexadehydro-β,β-caroten-3-ol (**7**)

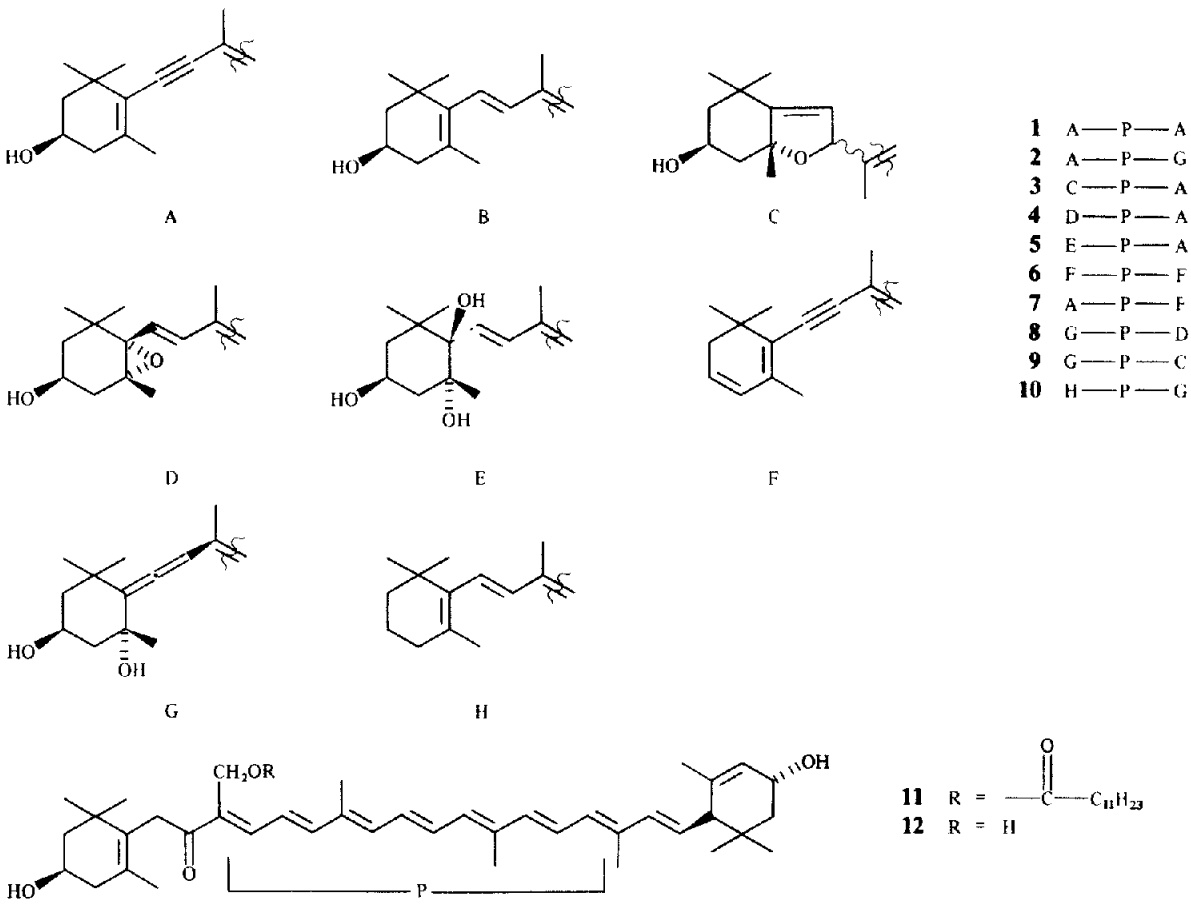
The diacetylenic carotene was isolated as a mixture of the all-*trans* (**6a**), 9-*cis* (**6b**) and 9,9′-di*cis* (**6c**) isomers Crystals were enriched in all-*trans* (**6a**) and the mother liquor in 9-mono*cis* (**6b**) according to HPLC. The iodine catalysed stereomutation mixture contained 9,9′-di*cis* (**6b**, 55% of total), 9-mono*cis* (**6b**, 38%) and all-*trans* (**6a**, 10%), consistent with earlier isomerization studies on diacetylenic carotenoids [6] In view of the steric instability of all-*trans* **6a**, the *cis* isomers **6b** and **6c** are likely isolation artifacts. The ^{1}H NMR spectra (400 MHz) of the individual HPLC purified isomers **6a–6c** were in accordance with those of eutreptiellanone and anhydrodiatoxanthin [1], possessing the same strongly unsaturated end group *F*(see Scheme 1).

In the all-*trans* isomer **6a** the *gem* dimethyl groups give rise to a singlet at δ 1.10, shifted to 1 15 in the 9(9′)-*cis* isomers. The H-2, H-3 and H-4 signals are also shifted downfield by 0.02 p.p m in the 9(9′)-*cis* isomers The assignment of the Me-18 signal in the all-*trans* and 9-*cis* end group was based on the lack of allylic coupling in comparison with the Me-19 and Me-20 signals Signals for the in-chain methyl groups were assigned on the basis of published data for acetylenic 9-*cis* and 9-*trans* models [7]. Visible and mass spectral data were consistent with the structure deduced It should be mentioned that the same carotene was subsequently isolated from a marine sponge [8].

The related diacetylenic monool (**7**) was only obtained after alkaline hydrolysis and presumably occurs esterified The natural ester was probably covered by chlorophyll on silica TLC plates ^{1}H NMR, visible and mass spectral data and acetylation evidence for the monool were compatible with the structure (**7**) proposed The allocation of the 3-hydroxy group followed from comparative ^{1}H NMR data for the four methyl groups associated with the hydroxylated 9-*cis* and 9-*trans* end groups A[7]

Non-acetylenic carotenoids encounted were the allenic neoxanthin (**8**, 3%), its furanoid rearrangement product neochrome (**9**, 1%) and β,β-carotene (**10**, ≤ 6%). An [M-56]$^+$ fragment ion in the mass spectrum of the β,β-carotene fraction, compatible with RDA fragmentation of an ε-end group [9], indicates the presence of some β,ε-carotene The only β,ε-carotene derivative isolated in the pure state was siphonein (**11**, 8%), which was converted to siphonaxanthin (**12**) upon alkaline hydrolysis. Siphonein (**11**) and siphonaxanthin (**12**) were characterized by co-chromatography with authentic standards, the visible

*Part 38 in the series Algal Carotenoids For part 37 see *Phycologia* (1987) **86**, 142



Scheme 1

and mass spectra and ^{1}H NMR data (**12** only) Siphonein has previously been considered characteristic for green algae of the order Siphonales [10–12] and class Prasinophyceae [13,14]. However, siphonein (**11**) was recently encountered in *Eutreptiella gymnastica* (Euglenophyceae) [1] Mass spectrometric data for the ester supports the identification of dodecenoic acid as the esterfying acid in siphonein *ex E viridis*, as previously reported for siphonein *ex E gymnastica* [1]. A mixture of other fatty acids including dodecanoic (lauric) acid seems to be involved as esterifying acids in siphonein from Siphonales [10,11]. The isolation of siphonein (**11**) from these two marine euglenophytes might be taken as support for the symbiont theory and green-algal origin of the chloroplasts in Euglenophyceae [15] However, acetylenic carotenoids are not encountered in green algae (*cf* a recent discussion [2])

No chiroptical measurements were carried out in the present study. The chiralities of the carotenoids dealt with were considered to be those commonly encountered in algal carotenoids [5], including the recently revised chirality of heteroxanthin (**5**) [16] (see Scheme 1). There was no difference between the carotenoid composition of whole cells and chloroplast preparation

Less than 2% of the total carotenoid represented unidentified minor carotenoids No oxabicycloheptane derivatives [1,2] were identified Otherwise the carotenoid distribution pattern resembles that of *E gymnastica*[1,2]

The present data demonstrate that diacetylenic carotenoids in algae are not restricted to Cryptophyceae, but are also synthesized to a lesser extent by Euglenophyceae

EXPERIMENTAL

Biological material A unialgal harvest from La Jolla sea shore, California, was used The alga was identified by Mag art Jahn Trondsen (Department of Biology, University of Oslo), as *Euglena viridis* Ehrenberg (1830) A choroplast preparation was also provided via Prof F T Haxo (Scripps Institution of Oceanography, University of California, La Jolla) The intact cells were stored lyophilized and the chloroplast preparation kept in a frozen suspension

Methods These were as commonly employed in our laboratory and summarized elsewhere [1] TLC was carried out on (i) silica gel, eluent Me_2CO in hexane (% AH as specified) and (ii) on special alkaline plates [17] with Me_2CO–MeOH–hexane (4 1 5) as eluent if not specified HPLC was performed using a spherisorb (silica gel, 5 μm) 4 6 × 250 mm column, eluent hexane (isocratic), flow 1 2 ml/min, detection at 465 nm

If not specified visible spectra were recorded in Et_2O Spectral fine-structure is expressed as % III/II [18] Only prominent or diagnostically useful peaks with $m/z > 180$ are reported for the mass spectra Saponification, acetylation and furanoid rearrangement with acid was carried out by general procedure [19]

Isolation. Three Me_2CO–MeOH (3:1) extracts were analysed. (i) from intact lyophilized cells, no saponification step involved. Dry cells (2·8 g) provided in total 11.5 mg carotenoids (0.41% of the lipid-extracted residue), (ii) From intact dried cells, saponified extract and (iii) from the chloroplast preparation, 1·4 mg carotenoids (0.10%) was isolated from 1·5 g lipid-extracted residue

Individual carotenoids These are treated in order of increasing adsorption upon TLC (silica gel, 40% AH)

β,β-Carotene (**10**), R_f = 1·00, inseparable from an authentic standard upon co-chromatography in hexane, Vis λ_{max} nm. (423), 448, 473, MS *m/z* (rel.int.) 536 $[M]^+$ (100), 480 $[M-56]^+$ (2), 444 $[M-92]^+$ (11), 430 $[M-106]^+$ (3) Separation from *β,ε*-carotene on alkaline plates [16] was not attempted

3,4,7,8,3′,4′,7′,8′-Octadehydro-β,β-carotene (**6**) Total yield after purification 0·7 mg, R_f = 0·90; R_t (min) **6c** (9,9′-*dicis*) 6.5, **6b** (9-*cis*) 7·0 and **6a** (all-*trans*) 8·0, Vis λ_{max} (hexane) nm; **6a** 327, (445), 472, 501, % III/II = 14, **6b** 380 (438), 466, 492, % III/II = 26, **6c** 329, (382) 434, 458, 486, % III/II = 34; MS **6a,b,c** *m/z* (rel int.) 528 $[M]^+$ (100), 513 $[M-15]^+$ (9), 508 (9), 480 (6), 410 (41), 302 (35), 247 (67), after HPLC separation ^{1}H NMR ($CDCl_3$, 400 MHz), *cf* previous assignments [1,7] Compound **6a** (all-*trans*, 71 μg) δ 1·10 (12H, *s*, Me-16,17,16′,17′), 1.96 (6H, *s*, Me-20,20′), 1·98 (6H, *s*, Me-18,18′), 2.03 (6H, *s*, Me-19,19′), 2·12 (4H, *dd*, H-2,2′), 5·80-5.84 (2H, *m*, H-3,3′), 5.89 – 5·92 (2H, *m*, H-4,4′), 6.25 – 6.68 (10H, olefinic H); **6b** (9-*cis*, 91 μg) δ 1·10 (6H, *s*, Me-16′17′), 1·15 (6H, *s*, Me-16,17), 1.93 (3H, *s*, Me-20), 1.96 (3H, *s*, Me-20′), 1·98 (3H, *s*, Me-18′), 2.02 (3H, *s*, Me-19), 2,03 (6H, *s*, Me-18,19′), 2·12 (2H, *dd*, H-2′), 2·14 (2H, *dd* H-2) 5.80 – 5.87 (2H, *m*, H-3,3′), 5·89 – 5·95 (2H, *m*, H-4,4′), 6·25 – 6·90 (10H, olefinic H), **6c** (9,9′-*dicis*, 78 μg) δ 1·15 (12H, *s*, Me-16,17,16′,17′), 1·93 (6H, *s*, Me-20,20′), 2.02 (6H, *s*, Me-19,19′), 2·03 (6H, *s*, Me-18,18′), 2·14 (4H, *dd*, H-2,2′), 5·82 – 5·86 (2H, *m*, H-3,3′), 5.90 – 5.94 (2H, *m*, H-4,4′), 6·25 – 6.90 (10H, *m*, olefinic H)

Crystallization from Me_2CO – hexane of **6** provided a mixture of **6a** (54%), **6b** (10%) and **6c** (36%), determined by HPLC. The mother liquour contained **6a** (21%), **6b** (51%) and **6c** (23%). Identical I_2 catalysed stereoisomerization mixtures in benzene were obtained from **6** *ex* crystals and *ex* mother liquor The quasi-equilibrium mixture contained **6a** (10%), **6b** (38%) and **6c** (59%)

7,8,3′,4′,7′,8′-Hexadehydro-β,β-caroten-3-ol (**7**) Yield 0.1 mg from saponified extracts only, R_f = 0.70, Vis λ_{max} nm 457, MS *m/z* (rel.int.) 546 $[M]^+$ (2), 531 $[M-15]^+$ (0.5), 430 (1), 426 (1), 410 (34), 257 (100); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.10 (*s*, Me-16′,17′ in 9′-*trans*), 1.15 (*s*, Me-16′,17′ in 9′-*cis*; Me-16 in 9-*trans*), 1,20 *s* and 1·25 *s* (Me-17 in 9-*trans/cis*), 1·93 *s* and 1·94 *s* (Me-18 in 9-*trans/cis*), 1·94 *s* and 1.96 *s* (Me-20 in 9-*trans/cis*), 1·98 *s* and 2·03 *s* (Me-18′ in 9′-*trans/cis*), 1·96 *s* and 1·93 *s* (Me-20′ (in 9′-*trans/cis*), 2·01 *s* and 1·98 *s* (Me-19 in 9-*trans/cis*), 2.03 *s* (Me-19′), 2·12 – 2·14 *m* (H-2′), 5.83 *m* (H-3′), 5·92 *m* (H-4′), 6·28-6.90 *m* (olefinic H)

Compound **7** provided no new products upon treatment with 0·03 N HCl in Et_2O Acetylation gave a monoacetate, R_f = 0·90, Vis unchanged

Siphonein (**11**). From unsaponified extracts only, R_f = 0.41, R_f = 0·50 on alkaline plates [17], inseparable from authentic **11** *ex E gymnastica* [1]. Separation from diatoxanthin (**2**) was achieved only on special plates [17] Siphonein (**11**) had Vis λ_{max} nm.448 (471), MS *m/z* (rel int.) 780 $[M]^+$ (1), 762 $[M-18]^+$ (2), 688 $[M-92]^+$ (1), 674 $[M-106]^+$ (2), 658 $[M-16-106]^+$ (1), 656 $[M-18-106]^{+\cdot}$ (1), 640 $[M-16-18-106]^+$ (3), 625 $[M-15-16-18-106]^+$ (3), 522 $[M-158]^+$ (3), 603 $[M-177]^+$ (7), 600 $[M-180]^+$ (6), 582 $[M-198]^+$ (11), 577 $[M-203]^+$ (10), 568 $[M-212]^+$ (28), 553 $[M-15-212]^+$ (42), 522 $[M-258]^+$ (20), 507 $[M-15-258]^+$ (29), 490 (10), 475 (18), 440 (18), 410(39), 237 (100), 213 (73), 211 (69), 181 (72). Compound **11** was stable towards standard acid treatment and provided siphonaxanthin (**12**) upon alkaline hydrolysis

Diatoxanthin (**2**) R_f = 0.40, R_f = 0·20 on alkaline plates [17] (40% AH), inseparable from authentic **2** in both systems, Vis λ_{max} nm, (425), 448, 478 nm, MS *m/z* (rel int.): 566 $[M]^+$ (100), 564 $[M-2]^+$ (14), 548 $[M-18]^+$ (6), 474 $[M-92]^+$ (14) Compound **2** was stable towards treatment with 0·03 N HCl in Et_2O *Diadinochrome* (**3**) R_f = 0·40, separated from **2** only on alkaline plates [17], R_f = 0.20 (40% AH); Vis λ_{max} nm (402), 425, 452; MS *m/z* (rel int.): 582 $[M]^+$ (13), 490 $[M-92]^+$ (11), 221 (80), 181 (100) Compound **3** was stable towards treatment with 0·03 N HCl in Et_2O

Diadinoxanthin (**4**) R_f = 0.10 (40% AH) on alkaline plates [17], Vis λ_{max} nm (424), 447, 472, MS *m/z* (rel int.) 582 $[M]^+$ (10), 564 $[M-18]^+$ (4), 181 (100) Treatment with 0·03 N HCl in Et_2O gave a rearranged product with Vis λ_{max} nm (400), 425, 445, *cf* **3** above

Neochrome (**9**). R_f = 0.35, R_f = 0·30 (40% AH) on special plates [17], inseparable from an authentic standard Vis λ_{max} nm400, 421, 448 Compound **9** was stable towards standard acid treatment

Neoxanthin (**8**) R_f = 0·32, R_f = 0.15 (40% AH) on special plates [17], inseparable from authentic **8**. Separation of all-*trans* and a mono-*cis* isomer was achieved on special plates [16], MS *m/z* (rel int.) 600 $[M]^+$ (12), 582 $[M-18]^+$ (23), 564 $[M-18-18]^+$ (16), 502 $[M-92]$ (30), 221 (90), 181 (100) Compound **8** provided neochrome (**9**) upon standard acid treatment

Siphonaxanthin (**12**) Isolated from saponified extracts only, R_f = 0·20, inseparable from an authentic standard, Vis λ_{max} nm 442, 462, MS *m/z* (rel int.) 600 $[M]^+$ (23), 582 $[M-18]^+$ (15), 564 $[M-18-18]^+$ (4), 426 (72), 410 (71), 408 (54), 406 (37), 328 (100), ^{1}H NMR ($CDCl_3$ 400 MHz): δ 0·85 (3H, *s*, Me-17′), 1·00 (3H, *s*, Me-16′), 0·95 (3H, *s*) and 1.00 (3H, *s*) together Me-16,17, 1.50 (3H, *s*, Me-18), 1.62 (3H, *s*, Me-18′), 1·92 (3H, *s*, Me-19′), 2.00 (6H, *s*, Me-20,20′), 3·50 (2H, *s*, H-7), 4.22 (1H, *m*, H-3′),4.49 (2H, *s*, H-19), 5·43 (1H, *d*, H-7′), 5.55 (1H, *d*, H-4′), 6·10 – 6·80 (*ca* 11H, olefinic)

Heteroxanthin (**5**) R_f = 0·10, Vis λ_{max} nm: (418), 442, 420, MS *m/z* (rel.int.): 600 $[M]^+$ (35), 582 $[M-18]^+$ (19), 580 $[M-2-18]^+$ (18), 564 $[M-18-18]^+$ (15), 508 $[M-92]^+$ (17), 502 $[M-18-80]^+$ (8), 410 (43), 378 (68), 313 (52), 256 (100), 239 (65) 221 (49), 181 (75)

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