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CAROTENOIDS OF THE MARINE CHRYSOPHYTE *PELAGOCOCCUS SUBVIRIDIS**

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Key Word Index—*Pelagococcus subviridis*; Chrysophyceae; carotenoids; (6*S*,6′*S*)-ε,ε-carotene; fucoxanthin; 19′-butanoyloxyfucoxanthin; MS; CD; taxonomy.

Abstract—The identity of the chrysophyte *Pelagococcus subviridis* from the Norwegian Sea was established by light and electron microscopy and by detailed carotenoid analysis. The strikingly yellow colour of the carotene fraction was caused by the presence of (6*S*,6′*S*)-ε,ε-carotene, the chirality of which was confirmed by CD. The dihydroxy xanthophyll fraction consisted of the acetylenic diatoxanthin and diadinoxanthin, which only occasionally have been reported from the Chrysophyceae *sensu stricto*. The species possessed two main xanthophylls, identified as fucoxanthin and the *n*-butanoyl ester of 19′-hydroxyfucoxanthin. High precision mass spectrometry of 19′-butanoyloxyfucoxanthin confirmed a diagnostically important allenic fragment resulting from cleavage of the 12′,13′-single bond. A corresponding fragment is present in the mass spectra of fucoxanthin and structurally related allenic xanthophylls. CD properties, previously not reported for carotenoids *ex* Chrysophyceae, together with ^{1}H NMR data, suggested the same chirality as for the corresponding carotenoids from other biological sources. The taxonomic position of *P. subviridis* is discussed on the class level.

INTRODUCTION

Pelagococcus subviridis Norris is a species possibly of worldwide distribution. So far, however, the records have been very scattered and are restricted to the North Pacific Ocean [1] and the East Australian Current [2, 3]. This is probably mostly due to its inconspicuous size and appearance, the cells being yellowish brown spheres 3–5 μm in diameter.

In July–August 1982 a controlled oil pollution experiment was carried out at Haltenbanken in the Norwegian Sea [4, 5]. The phytoplankton was then dominated by a small coccoid alga ('Coc. Min. Haltenbanken', cf. [6]) of uncertain systematic position. Evidence from microscopy studies and detailed carotenoid pigment analysis of cultured material have now demonstrated that this species was identical with *P. subviridis*.

RESULTS AND DISCUSSION

The colour of the *Pelagococcus subviridis* cultures varied from golden brown to yellowish green and green. Cultured material for microscopical studies and carotenoid analysis was always obtained from golden brown cultures.

The light microscope revealed the presence of a single curved chloroplast of yellowish brown colour reminiscent of chrysophyceans studied under the same conditions. Further studies on whole mounts by scanning and transmission electron microscopy did not add much to the light microscope information except to verify the firm nature of the cell wall.

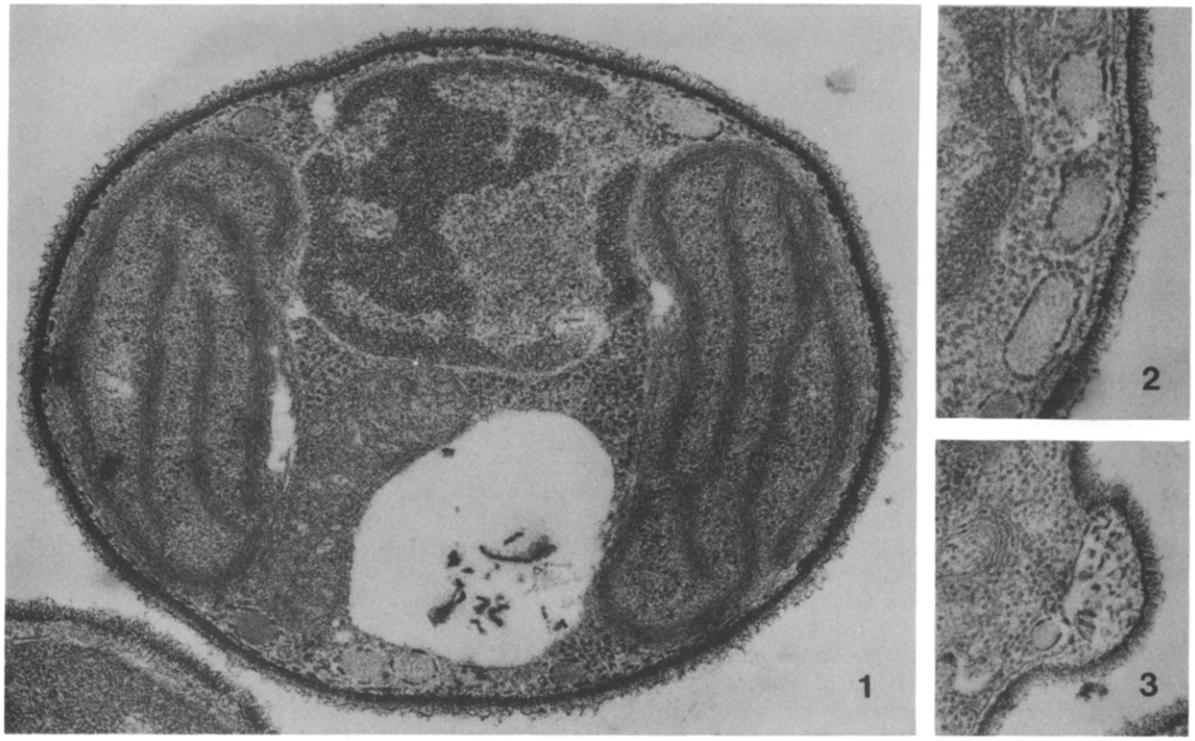
The identification of *P. subviridis* was further based on a direct comparison of sectioned resin embedded material with the original description [1] and the accompanying electron micrographs. Special emphasis was laid upon the similarity in certain unusual structures; the tomentum of the cell wall (Figs 1–3), the slightly flattened subsurface vacuoles (Fig. 2), as well as the protrusions from the otherwise spherical cell wall (Fig. 3). Less consistent was the tendency of cell chain formation reported by Lewin *et al.* [1] for their culture of *P. subviridis* from the North Pacific.

The TLC-chromatograms (cf. Table 1) of the total acetone–methanol extract possessed three remarkable properties. First, the carotene fraction was strikingly yellow, demonstrating that components other than β,β-carotene (**1**, Fig. 4) had to be present. Secondly, the chromatograms revealed two main xanthophylls (R_f 0.45 and 0.40, cf. Table 1), of which the less polar co-chromatographed with authentic fucoxanthin (**2**). Thirdly, there were large amounts of polar chlorophylls relative to chlorophyll *a*. Similar observations were reported for *P. subviridis* from the Pacific Ocean [1].

The yellow carotene fraction was resolved on special alkaline TLC-plates [7] into five components, of which the two major ones have been characterized and identified in the present work. Contrary to the assumption made for the North Pacific isolate [1], β,ε-carotene (**3**) could not be detected. The main component was β,β-carotene (**1**) (identified by co-chromatography, UV-Vis, MS and ^{1}H NMR), while the yellow colour of the fraction could be mainly attributed to a component less polar than both β,β-carotene (**1**) and β,ε-carotene (**3**). The positions of the absorption maxima and the pronounced

To the memory of Professor Edgar Lederer.

*Part 41 in the series 'Algal Carotenoids'. For part 40 see (1988) *Biochem. Syst. Ecol.* **16**, 589.



Figs 1–3. *Pelagococcus subviridis* EM sections. Fig. 1, whole cell ×40 000. Fig. 2, subsurface vesicles ×60 000. Fig. 3, protuberance of the cell wall ×40 000.

Table 1. Carotenoids of *P. subviridis* from the Norwegian Sea

Carotenoid	Amount		R_f‡
	mg/g dry wt*	% of total	
(6*S*,6′*S*)-ε,ε-Carotene (**4**)	0.02	0.2	1.00
β,β-Carotene (**1**)	0.03	0.3	1.00
(3*R*,3′*R*)-Diatoxanthin (**6**)	0.07	0.7	0.52
(3*S*,5*R*,6*S*,3′*R*)-Diadinoxanthin (**5**)	1.2	12†	0.48
(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-Fucoxanthin (**2**)	4.0	39	0.45
(3*S*,5*R*,6*S*,3′*S*,5′*R*,6′*R*)-19′-Butanoyloxyfucoxanthin (**8**)	4.4	42	0.40
Minor unknowns	0.64	6	>0.48

*Base: lipid-extracted cells.
†Includes 2% diadinochrome (**7**).
‡TLC: silica gel–$CaCO_3$ (2:1), *n*-C_6H_{14}–Me_2CO–*i*-PrOH (35:14:1).

spectral fine structure (III/II (%) [8] = 101) of the UV-Vis spectrum were consistent with an aliphatic nonaene chromophore. The 400 MHz ^{1}H NMR spectrum revealed a carotene with two ε (**b** or **c**, Fig. 4) end-groups. Decoupling experiments by irradiation at δ2.02 and 2.18 confirmed the expected coupling pattern [9] for the ring protons and H-7 (7′).

The Cotton effect of (6′*R*)-*all trans*-β,ε-carotene (**3**) is positive in the wavelength region 200–400 nm, with a maximum value of $\Delta\varepsilon = 5$ at *ca* 238 nm [10]. The Cotton effect of our sample of ε,ε-carotene (**4**) was, however, negative within the UV-region 200–300 nm with a minimum value of $\Delta\varepsilon = -13.5$ at 265 nm (Fig. 5). We therefore conclude that ε,ε-carotene (**4**) from *P. subviridis* has (6*S*,6′*S*)-chirality. Our results are therefore consistent with the few previous reports on the chirality of ε,ε-carotene, including samples from avian retinas [11] and the marine green alga *Ulva lactuca* L. [12]. The absolute configuration of this carotene thereby deviates from the one established for other algal carotenoids with ε end-groups [13–16].

The xanthophyll fraction of intermediate polarity on calcium carbonate containing TLC-plates (R_f 0.48–0.52, cf. Table 1) was rechromatographed on special alkaline plates [7]. These plates resolved the fraction into the three acetylenic xanthophylls diadinoxanthin (**5**), diatoxanthin (**6**) and diadinochrome (**7**), mentioned in order of increasing polarity. Each xanthophyll was identified by

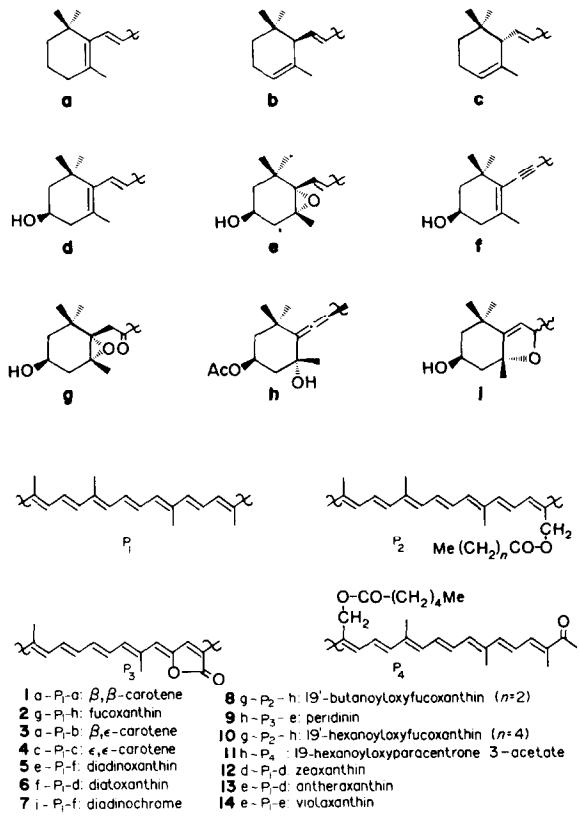


Fig. 4. Carotenoid structures.

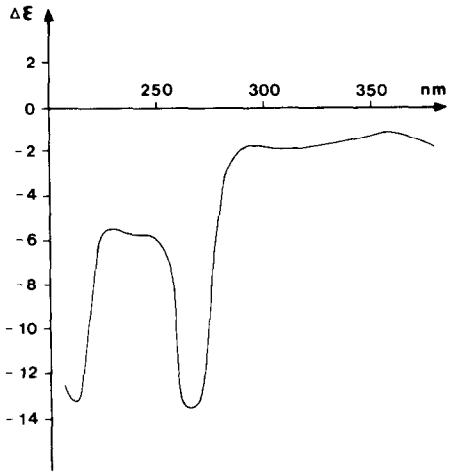


Fig. 5. CD spectrum of (6*S*,6′*S*)-ε,ε-carotene (**4**) recorded in EPA (Et_2O–*i*-pentane–EtOH; 5:5:2).

UV-Vis, MS and 400 MHz ^{1}H NMR as well as by co-chromatography with authentic samples. Diadinochrome (**7**), considered as a rearrangement artifact of native diadinoxanthin (**5**), was characterized as a mixture of its (8*R*)- and (8*S*)-isomers, cf. [17]. The CD data for diadinoxanthin (**5**) and diatoxanthin (**6**) confirmed the (3*S*,5*R*,6*S*,3′*R*)- and (3*R*,3′*R*)-chirality, respectively, as would be expected from previous results for **5** and **6** from other biological sources [18].

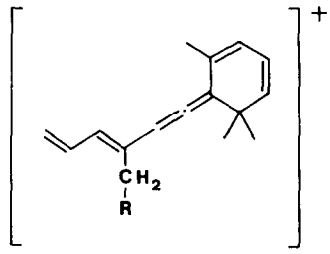
Pelagococcus subviridis contained two main xanthophylls (Table 1). The less polar was identical with fucoxanthin (**2**), based on UV-Vis, CD, IR, MS, 400 MHz ^{1}H NMR and co-chromatography with an authentic sample from *Fucus serratus* L. The more polar xanthophyll has been shown here to be the new *n*-butanoyl ester of 19′-hydroxyfucoxanthin, i.e. **8**.

With petrol and acetone as solvents, the main UV-Vis maximum of 19′-butanoyloxyfucoxanthin (**8**) was shifted 1–2 nm towards lower wavelength and the spectrum had more fine structure than fucoxanthin (**2**). This difference was most pronounced when the spectra were recorded in acetone. The fucoxanthin (**2**) spectrum then possessed an inflection only at the long wavelength absorption maximum whereas 19′-butanoyloxyfucoxanthin (**8**) possessed a III/II (%)-value of 37.

The 400 MHz ^{1}H NMR spectrum of the new xanthophyll **8** supported a structural relationship with fucoxanthin (**2**). The Me-signals of the epoxidic end-group **g** of fucoxanthin (**2**) at δ0.96 (Me-16), 1.03 (Me-17) and 1.22 (Me-18) were encountered in the ^{1}H NMR spectrum of **8**, as well as the signals of the neighbouring methylene protons at C-7 (δ2.60 *d* and 3.67 *d*, J_{gem} = 20 Hz) and H-10 (δ7.15*d*, J = 11 Hz), with characteristic downfield chemical shifts due to the proximity of the C-8 keto-group. Signals for the acetylated end-group **h** of fucoxanthin (**2**), however, had slightly different chemical shifts in **8** [δ1.07 (Me-16′), 1.35 (Me-18′) and 1.38 (Me-17′) for fucoxanthin (**2**) (cf. assignments for peridinin (**9**) in [19]), compared with δ1.08 (Me-16′) and 1.37 and 1.38 (Me-17′,18′) for **8**]. IR absorption at 1960 cm^{-1} demonstrated that the new xanthophyll **8** was allenic. This was further supported by the characteristic singlet at δ6.06 for the allenic proton (H-8′). However, the expected signal at δ1.81 for the adjacent Me-group (Me-19′) was lacking, leaving only nine intact Me-groups in the molecule. Contrary to fucoxanthin (**2**), two doublets (J = 11 Hz) in an AB system centred at δ4.75 and 4.81 were present. Together with triplets at δ0.94 and 2.28 (J = 7 Hz; terminal-Me and α-CH_2, respectively, of an unbranched fatty acid ester), these data suggested a 19′-acyloxyfucoxanthin structure. The identity of the ester moiety in the 19′-position was established by MS (including high precision MS of the *m/z* 726 and 298 fragments) and subsequent GC studies. MS of a crystalline sample gave a molecular ion at *m/z* 744. In addition to single and combined losses of 16, 18 and 60 mass units, also observed for fucoxanthin (**2**), fragments resulting from losses of 88 mass units, compatible with butanoic acid, were present in the spectrum. Together with the ^{1}H NMR triplets at δ0.94 and 2.28, these data established the identity of the ester moiety as *n*-butanoyl. This was confirmed by GC of methyl *n*-butanoate obtained after alkaline hydrolysis of the natural ester and subsequent methylation.

Attention should be drawn to the mass spectral *m/z* 298.1940 ($C_{20}H_{26}O_2$) fragment of 19′-butanoyloxyfucoxanthin (**8**). By analogy with the fragmentation pattern of peridinin (**9**) [20], this fragment may arise from the allenic end of the molecule by cleavage of the 12′,13′-single bond with hydrogen transfer to the charged fragment and elimination of water and acetic acid.

Similar fragments, formally represented in Fig. 6, may also be rationalized for other allenic carotenoids. The structurally related 19′-hexanoyloxyfucoxanthin (**10**) from the coccolithophore *Emiliania* (*Coccolithus*) *huxleyi* (Lohm.) Hay *et* Mohler has been characterized previously



for peridinin (**9**), R=H; *m/z* 212 (43% rel int.)
fucoxanthin (**2**), R=H; *m/z* 212 (30%)
19'-butanoyloxyfucoxanthin (**8**), R=O-CO-$(CH_2)_2$-Me; *m/z* 298 (33%)
19'-hexanoyloxyfucoxanthin (**10**), R=O-CO-$(CH_2)_4$-Me; *m/z* 326 (24%)
19-hexanoyloxyparacentrone 3-acetate (**11**), R=O-CO-$(CH_2)_4$-Me; *m/z* 326 (18%)

Fig. 6. Formal structure of a diagnostically important mass spectral ion for 19'-acyloxyfucoxanthins and structurally related xanthophylls.

[21, 22]. In the mass spectrum of **10**, the *m/z* 298 fragment has been replaced by a *m/z* 326 fragment [23], also present in the mass spectrum of the allenic apocarotenoid 19-hexanoyloxyparacentrone 3-acetate (**11**) [23, 24] (Fig. 6). A corresponding fragment ion is observed at *m/z* 212 for fucoxanthin (**2**) in the present study. Consequently these fragments are diagnostically important for the mass spectral identification of 19'-acyloxyfucoxanthins and structurally related xanthophylls, cf. [23, 24].

With six chiral centres and weak Cotton effects the CD data for fucoxanthin (**2**) and 19'-butanoyloxyfucoxanthin (**8**) must be used with caution in stereochemical interpretations. Both the ^{1}H NMR and CD data of **2** and **8** support, however, the same chirality as reported previously for fucoxanthin (**2**) from other biological sources [18] and using 19'-hexanoyloxyfucoxanthin (**10**) as a model for **8** [22].

With reference to our first report [6] on 19'-butanoyloxyfucoxanthin (**8**), a xanthophyll with similar HPLC and UV-Vis properties has now been reported from both cultured plankton algae as well as from mixed phytoplankton samples (see [25] for a compilation). In ecological work with marine plankton both from temperate and tropical waters, 19'-butanoyloxyfucoxanthin (**8**) has locally been reported among the major carotenoids [26, 27]. It may be an ecologically important xanthophyll, taking part in the light harvesting process as an accessory pigment in photosynthesis as has already been shown for the structurally related 19'-hexanoyloxyfucoxanthin (**10**) [28]. As a marker for taxonomic groups in the plankton community, however, 19'-butanoyloxyfucoxanthin (**8**) is of more limited value, being distributed among the four algal classes Bacillariophyceae, Chrysophyceae, Dinophyceae and Prymnesiophyceae (see [25]). Furthermore, **8** has only a scattered distribution pattern within each of these classes, being at present reported from only one (Bacillariophyceae [29], Chrysophyceae [3,6, present work]) and two (Dinophyceae [25], Prymnesiophyceae [23, 30]) species of each class, in addition to the undescribed microflagellate MC-1 of uncertain systematic position (cf. [31] and below). Considering the recent frequent reports of 19'-butanoyloxyfucoxanthin (**8**) in natural mixed phytoplankton samples [26, 27, 30], one may expect that further studies on monoalgal cultures will reveal a wider systematic distribution pattern of **8** than recognized hitherto. Additional support for this view is provided by the early analysis of the undescribed marine flagellate Plymouth 133 [32] and the small undescribed marine coccoid Plymouth 407 [33], tentatively assigned the Prymnesiophyceae and Chrysophyceae, respectively. Both species contained fucoxanthin (**2**) in addition to another major xanthophyll, possessing both chromatographic and UV-Vis spectral properties which may now be expected for a 19'-butanoyloxyfucoxanthin (**8**) structure. The similarity in the carotenoid complement with that obtained for MC-1 and *P. subviridis* should encourage both electron microscopical ultrastructural studies of Plymouth 133 and 407 as well as a reinvestigation of their pigmentation by modern chromatographic and spectrometric methods.

19'-Butanoyloxyfucoxanthin (**8**) is slightly more polar than fucoxanthin (**2**) in the normal-phase systems (TLC and HPLC) tested, and also exhibits a hypsochromic shift of 1–2 nm of its main UV-Vis absorption maximum relative to fucoxanthin (**2**). 19'-Butanoyloxyfucoxanthin (**8**) thereby possesses properties encountered for neofucoxanthin, a term applied for various *cis*-isomers of fucoxanthin (**2**) irrespective of the exact position of the *cis*-bond(s). Besides an unambiguous distinction by MS, these pigments may also be distinguished by their UV-Vis properties, **8** possessing considerably more UV-Vis spectral fine structure than neofucoxanthin.

Both the general fine structural features and the carotenoid pigmentation of our isolate from the Norwegian Sea are consistent with those of *P. subviridis* from the North Pacific. Systematically *P. subviridis* has been placed within the algal class Chrysophyceae, but it is realized [1, 3] that the species possesses several ultrastructural and biochemical features which make an assignment to existing algal classes difficult. This is also evident from its chlorophyll (not investigated in the present work) and carotenoid pigmentation.

In the first comprehensive investigation on the systematic distribution pattern of Chl. ph. c_1 and c_2 [34], members of the Chrysophyceae were found to possess both Chl. ph. *c* components. This general view now needs a critical reconsideration. Firstly, members of *Mallomonas* and *Synura* possess only Chl. ph. c_1 as accessory chlorophyll [35]. Secondly, the combined use of several TLC-systems, including RP-8 HPTLC, has now revealed three spectrally distinct Chl. ph. *c* components in algae [36, 37]. Applied on the North Pacific and Australian isolates of *P. subviridis* these methods supported the presence of Chl. ph. c_2 and c_3 while Chl. ph. c_1 was absent. So far this Chl. ph. *c* complement is unique within the Chrysophyceae, but is found in five (out of nine) species of the Prymnesiophyceae and eight (out of 51) species of the Bacillariophyceae [29].

The two classes Chrysophyceae and Prymnesiophyceae (Haptophyceae, cf. [38, 39]) generally have fucoxanthin (**2**) as their characteristic and main xanthophyll [40]. *P. subviridis*, however, also contains the fucoxanthin-derived 19'-butanoyloxyfucoxanthin (**8**), while HPLC of the main xanthophyll fraction gave no peak for 19'-hexanoyloxyfucoxanthin (**10**), cf. [30]. 19'-Acyloxyfucoxanthins have so far not been reported for other chrysophytes, but are major xanthophylls in six members of the Prymnesiophyceae and three members of the Dinophyceae (see the compilation in [25]) and **8** is recently also reported from the diatom *Thalassiothrix heteromorpha* Karsten [29]. Also remarkable is the possession of the

acetylenic xanthophylls diatoxanthin (**6**) and diadinoxanthin (**5**). Concerning dihydroxy xanthophylls, the two classes Prymnesiophyceae and Chrysophyceae generally have a different distribution pattern: the Prymnesiophyceae have the acetylenic xanthophylls diatoxanthin (**6**) and diadinoxanthin (**5**) [41, 42], while the Chrysophyceae have the non-acetylenic zeaxanthin (**12**), antheraxanthin (**13**) and violaxanthin (**14**) [43]. Regarding dihydroxy xanthophylls, however, exceptions have been reported. Among the chrysophytes both *Sarcinochrysis marina* Geitler [42] and *Rhizochromulina marina* Chretiennot-Dinet *et* Hibberd [44] contain the acetylenic diatoxanthin (**6**) and diadinoxanthin (**5**).

Based on the chlorophyll and carotenoid pigmentation it seems justified to conclude that *P. subviridis* shows a greater affinity with the Prymnesiophyceae (to a lesser degree also with the Bacillariophyceae) than the Chrysophyceae. Its assignment to the latter class must therefore mainly rely on fine structural features, of which the structure of the chloroplast (including girdle lamellae) and the position of the dictyosome in a nuclear depression are most prominent [1, 3] (cf. [39]).

Our results on the carotenoid pigmentation and fine structure of *P. subviridis* from the Norwegian Sea support the recent conclusion [3] based on pigmentation [1, 3, 30], fine structure [1, 3] and detailed study of the mitotic process [3] that *P. subviridis* fits the general class characteristics of neither the Chrysophyceae nor the Prymnesiophyceae. So far, however, *P. subviridis* is only known in its coccoid stage, implying that no data related to the fine structure of the flagellar apparatus have been available for consideration. In Hibberd's comparative study on the ultrastructure of these two classes [39] features associated with the flagella were among the major distinctive features between the classes. A preliminary study of the carotenoid pigmentation of the marine microflagellate MC-1 of uncertain systematic position, revealed a carotenoid complement similar to the one in *P. subviridis*, including the two main xanthophylls fucoxanthin (**2**) and 19′-butanoyloxyfucoxanthin (**8**), the acetylenic dihydroxy xanthophylls diatoxanthin (**6**) and diadinoxanthin (**5**) as well as ε,ε-carotene (**4**) [31]. This similarity suggests that *P. subviridis* and MC-1 may be taxonomically related. An ultrastructural study of MC-1 and its flagellar apparatus, now being undertaken by Dr C. J. O'Kelly at the Massey University in New Zealand, may therefore also give further clues regarding the taxonomic position of *P. subviridis*.

EXPERIMENTAL

Biological material. Pelagococcus subviridis Norris [1] was brought into monoalgal culture by a serial dilution culture method applied to water samples from Haltenbanken, the Norwegian Sea, in July/August 1982 [4]. The specimen culture established from dilution series C was used in the present investigation.

Electron microscopy. Cultured cells were fixed with glutaraldehyde and osmic acid, dehydrated, embedded and sectioned for electron microscopy at the EM Laboratory for Biosciences at the University of Oslo. The sections were stained with uranyl acetate and lead citrate and studied in a JEOL transmission electron microscope.

Culture methods. The alga was grown in 32 aerated 5 l Erlenmeyer flasks, each with 4 l of the culture medium IMR [45]. The culture medium was based on filtered (Whatman GF/C) 34‰ natural seawater diluted to 25‰ before autoclaving and enrichment. The flasks were continuously illuminated from above with Philips fluorescent tubes (TL/32). The light intensity was 35 $\mu E/m^2$ 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 25–27 days. The dry wt of the lipid extracted cells was 3.48 g.

Isolation of carotenoids. The harvested algal cells were extracted with Me_2CO, Me_2CO–MeOH (7:3) and MeOH. The cells were faint green by the end of the extraction. The total extract was separated into 3 fractions (carotenes, di-OH xanthophylls, fucoxanthin (**2**)/19′-butanoyloxyfucoxanthin (**8**)) by TLC on silica–$CaCO_3$ (1:1) (TLP-I). Each fraction was further resolved and purified by TLC on special alkaline plates with silica G–Kieselguhr–$Ca(OH)_2$–MgO (14:16:9:9) (TLP-II) as an adsorbent [7]. The eluent was appropriate mixtures of n-C_6H_{14}, Me_2CO and i-PrOH.

HPLC. R_t-values for fucoxanthin (**2**), 19′-hexanoyloxyfucoxanthin (**10**) and 19′-butanoyloxyfucoxanthin (**8**) were obtained on analytical silica columns (Si-5, 4.6 × 250 mm) using 20% Me_2CO in n-C_6H_{14}–0.1% MeOH as an eluent. The flow rate was 1.5 ml/min and the detector set at 445 nm.

Physical and chemical methods. UV-Vis spectra were recorded in Me_2CO or/and n-C_6H_{14}; $E^{1\%}_{1cm}$-values applied at λ_{max} for quantitative calculations (solvent: Me_2CO): fucoxanthin (**2**): 1650 [46]; 19′-butanoyloxyfucoxanthin (**8**): 1550 (calculated value, assuming the same molar extinction coefficient as for fucoxanthin (**2**)); other carotenoids: 2500. IR spectra were recorded in KBr pellets, ^{1}H NMR at 400 MHz in $CDCl_3$ with TMS as internal standard and mass spectra at 70 eV and 200–230°. Perfluorokerosene was used as an internal standard for high precision MS of 19′-butanoyloxyfucoxanthin (**8**).

Carotenoid data. Amount and TLC-property of each carotenoid is included in Table 1.

(6*S*,6′*S*)-*ε,ε-Carotene* (**4**). Semicryst. from Me_2CO; UV-Vis $\lambda^{n-C_6H_{14}}_{max}$ nm: 415, 439 and 469, III/II (%) [8] = 101 (semi-cryst.); MS *m/z* (rel. int.): 536 $[M]^+$ (100), 480 $[M-56]^+$(1) and 444 $[M-92]^+$(6); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ0.82*s* (6H, Me-16, 16′), 0.90 *s* (6H, Me-17,17′), *ca* 1.18 *m* (*ca* 2H, H-2,2′), *ca* 1.45 *m* (*ca* 2H, H-2,2′), 1.58 *d* (J = 1.4 Hz, 6H, Me-18,18′), 1.91 *s* (6H, Me-19,19′), 1.96 *s* (6H, Me-20,20′), 2.02 *m* (*ca* 4H, H-3,3′), 2.18 *d* ($J_{6,7}/J_{6',7'}$ = 9 Hz, 2H, H-6,6′), 5.41 *m* (2H, H-4,4′), 5.53 *dd* ($J_{6,7}/J_{6',7'}$ = 9 Hz, $J_{7,8}/J_{7',8'}$ = 15 Hz, 2H, H-7,7′), 6.11 *d* ($J_{7,8}/J_{7',8'}$ = 15 Hz, 2H, H-8,8′), 6.12 *d* ($J_{10,11}/J_{10',11'}$ = 12 Hz, 2H, H-10,10′), 6.2–6.7 *m* (*ca* 8H, conj. olefinic); decoupling expts (first figure denotes point of irradiation, next figure(s) signal(s) affected) (δ-values) 2.02(*m*)/1.18 *m*→*d* (J_{gem} = 12 Hz), *ca* 1.45 *m*→*d* (J_{gem} = 12 Hz), 5.41 *m*→*s*; 2.18(*d*)/5.53 *dd*→*d* ($J_{7,8}/J_{7',8'}$ 15 Hz); CD (EPA) nm (Δε): 211 (−13.2), 229 (−5.5), 245 (−5.8), 265 (−13.5), 294 (−1.8), *ca* 310 (−2.0) and 360 (−1.1).

β,β-Carotene (**1**). Semicryst. from Me_2CO; UV-Vis $\lambda^{n-C_6H_{14}}_{max}$ nm: (425), 449 and 476, III/II (%) = 33; MS *m/z* (rel. int.): 536 $[M]^+$ (100), 444 $[M-92]^+$ (11) and 268 $[M]^{2+}$ (11); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ1.03 *s* (12H, Me-16,17,16′,17′), 1.47 *m* (4H, H-2,2′), 1.61 *m* (4H, H-3,3′), 1.72 *s* (6H, Me-18,18′), 1.97 *s* (12H, Me-19,20,19′,20′), 2.02 *m* (4H, H-4,4′) and 6.1–6.7 *m* (*ca* 14H, conj. olefinic); co-chromatographed (TLP-I) with β,β-carotene (**1**) from *Daucus carota* L.

(3*R*,3′*R*)-*Diatoxanthin* (**6**). Crystallized from MeOH; UV-Vis $\lambda^{n-C_6H_{14}}_{max}$ nm: (428), 449 and 478, III/II (%) = 39; $\lambda^{Me_2CO}_{max}$ nm: (431), 452 and 481, III/II (%) = 36; MS *m/z* (rel. int.): 566 $[M]^+$ (100), 474 $[M-92]^+$ (4) and 283 $[M]^{2+}$ (12); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ1.07 *s* (6H, Me-16′,17′), 1.15 *s* (3H, Me-16), 1.20 *s* (3H, Me-17), 1.74 *s* (3H, Me-18′), 1.92 *s* (3H, Me-18), 1.95 *s* (3H,

Me-19'), 1.97 *s* (3H, Me-20'), 1.98 *s* (3H, Me-20), 2.01 *s* (3H, Me-19), 4.01 *m* (2H, H-3,3') and 6.1–6.7 *m* (*ca* 12H, conj. olefinic); CD (EPA) nm ($\Delta\varepsilon$): 222 (−3.3), 235 (0), 252 (+3.5), 264 (0), 289 (−6.8), 317 (0), 346 (+2.1) and *ca* 385–410 (0); co-chromatographed (TLP-I and TLP-II) with diatoxanthin (**6**) from *Eutreptiella gymnastica* Throndsen [47].

(3*S*,5*R*,6*S*,3'*R*)-*Diadinoxanthin* (**5**). Crystallized from MeOH; UV-Vis $\lambda_{max}^{n\text{-}C_6H_{14}}$ nm: 442, 445 and 476, III/II (%) = 70; $\lambda_{max}^{Me_2CO}$ nm: (426), 447 and 477, III/II (%) = 69; MS *m/z* (rel. int.): 582 $[M]^+$ (100), 566 $[M-16]^+$ (7), 564 $[M-18]^+$ (7), 502 $[M-80]^+$ (7), 490 $[M-92]^+$ (2), 291 $[M]^{2+}$ (15), 221 [homopyryllium]$^+$ (44) and 181 [furyllium]$^+$ (44); ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ0.98 *s* (3H, Me-16), 1.15 *s* (6H, Me-17,16'), 1.19 *s* and 1.20 s (3H + 3H, Me-18,17'), 1.93 *s* (6H, Me-19,18'), 1.96 *s* and 1.97 *s* (3H + 3H, Me-20,20'), 2.01 *s* (3H, Me-19'), 3.92 *m* (1H, H-3), 4.01 *m* (1H, H-3'), 5.88 *d* ($J_{7,8}$ = 15.5 Hz, 1H, H-7), 6.29 *d* ($J_{7,8}$ = 15.5 Hz, 1H, H-8) and 6.1–6.7 *m* (*ca* 11H, conj. olefinic); decoupling expts (points of irradiation, δ-values): 3.95 and 5.88; CD (*i*-PrOH) nm ($\Delta\varepsilon$): 215 (−10.8), 226 (0), 241 (+8.5), 249 (0), 279 (−20.9), 302 (0), 341 (+7.5) and *ca* 375–400 (0); co-chromatographed (TLP-I and TLP-II) with diadinoxanthin (**5**) from *E. gymnastica* [47].

Diadinochrome (**7**). Precipitated from *n*-C_6H_{14}; UV-Vis $\lambda_{max}^{n\text{-}C_6H_{14}}$ nm: (406), 426 and 453, III/II (%) = 64; $\lambda_{max}^{Me_2CO}$ nm: (408), 429 and 456, III/II (%) = 58; MS *m/z* (rel. int.): 582 $[M]^+$ (77), 580 $[M-2]^+$ (23), 567 $[M-15]^+$ (4), 566 $[M-16]^+$ (2), 564 $[M-18]^+$ (2), 562 $[M-2-18]^+$ (1), 502 $[M-80]^+$ (9), 490 $[M-92]^+$ (13), 475 $[M-15-92]^+$ (4), 436 $[M-146]^+$ (6), 402 $[M-180]^+$ (5), 352 (19), 291 $[M]^{2+}$ (7), 221 [homopyryllium]$^+$ (100) and 181 [furyllium]$^+$ (30); ^{1}H NMR (400 MHz, $CDCl_3$, TMS) signals assigned to (8*R*)-diadinochrome (cf. [17]): δ1.17 *s* (Me-16), 1.33 *s* (Me-17), 1.62 *s* (Me-18), 1.72 *s* (Me-19), 5.17 *s* (H-8) and 5.25 *s* (H-7); signals assigned to (8*S*)-diadinochrome: δ1.19 *s* (Me-16), 1.34 *s* (Me-17), 1.68 *s* (Me-18), 1.81 *s* (Me-19), 5.08 *d* ($J_{7,8}$ = *ca* 1.7 Hz, H-8) and 5.31 *d* ($J_{7,8}$ = 1.7 Hz, H-7); signals common to (8*R*)- and (8*S*)-diadinochrome: 1.15 *s* (Me-16'), 1.20 *s* (Me-17'), 1.92 *s* (Me-18'), 1.95 *s* (Me-20, Me-20'), 2.00 *s* (Me-19'), 3.99 *m* (H-3'), 4.25 *m* (H-3) and 6.1–6.7 *m* (conj. olefinic); signal integrals indicated a 8*R*/8*S*-ratio of 3:1; co-chromatographed (TLP-I and TLP-II) with partially synthetic diadinochrome (**7**) prepared from diadinoxanthin (**5**) *ex E. gymnastica* [47].

(3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*R*)-*Fucoxanthin* (**2**). UV-Vis $\lambda_{max}^{n\text{-}C_6H_{14}}$ nm: (429), 448 and 477, III/II (%) = 30; $\lambda_{max}^{Me_2CO}$ nm: 447 and (466); $\lambda_{max}^{CHCl_3}$ nm: 460; IR ν_{max}^{KBr} cm^{-1}: 3420 *s* (OH); 3040 *w*, 2970 *s*, 2930 *s* and 2870 *m* (CH); 1930 *w* (C=C=C); 1730 *s* (C=O, ester); 1660 *s* (conj. C=O); 1610 *m*; 1580 *w* (C=C); 1530 *m*; 1455 *w* (CH_2); 1385 *s* and 1370 *s* (CH_3); 1255 *m* (CO–O–C); 1200 *w* and 1170 *w* (*tert.* OH); 1080 *w*, 1055 *m* and 1035 *s* (*sec.* OH) and 975 *s* (*trans* CH=CH); MS *m/z* (rel. int): 658 $[M]^+$ (0.5), 642 $[M-16]^+$ (2), 640 $[M-18]^+$ (8), 622 $[M-18-18]^+$ (21), 580 $[M-18-60]^+$ (8), 578 $[M-80]^+$ (5), 562 $[M-18-18-60]^+$ (16), 560 $[M-18-80]^+$ (8), 221 [homopyryllium]$^+$ (68), 212 (30) and 197 (100); ^{1}H NMR (400 MHz, $CDCl_3$ D-locked): δ0.96 *s* and 1.03 *s* (3H + 3H, Me-16,17), 1.07 *s* (3H, Me-16'), 1.22 *s* (3H, Me-18), 1.35 *s* (3H, Me-18'), 1.38 *s* (3H, Me-17') (cf. [9]), 1.81 *s* (3H, Me-19'), 1.94 *s* (3H, Me-19), 1.99 *s* (6H, Me-20,20'), 2.04 *s* (3H, Me in acetyl at C-3'), 2.60 *d* (J_{gem} = 20 Hz, 1H, H-7), 3.67 *d* (J_{gem} = 20 Hz, 1H, H-7), 3.84 *m* (1H, H-3), 5.39 *m* (1H, H-3'), 6.05 *s* (1H, H-8'), 6.13 *d* ($J_{10',11'}$ = 10 Hz, 1H, H-10'), 7.15 *d* ($J_{10,11}$ = 11 Hz) and 6.2–6.8 *m* (*ca* 8H, conj. olefinic); CD (EPA) nm ($\Delta\varepsilon$): 216 (+0.2), 230 (+0.6), 247 (0), 257 (−0.3), 290 (0), 309 (+0.2), 339 (0), 354 (−0.1) and 381 (0); HPLC (Si-5, 20% Me_2CO in *n*-C_6H_{14}–0.1% MeOH): R_t = 15.7 min, 19'-hexanoyloxyfucoxanthin (**10**) (reference sample from *Emiliania huxleyi* [21, 22], R_t = 16.6 min) was not present; co-chromatographed (TLP-I and TLP-II; HPLC) with fucoxanthin (**2**) from *Fucus serratus* L.

(3*S*,5*R*,6*S*,3'*S*,5'*R*,6'*R*)-19'-*Butanoyloxyfucoxanthin* (**8**). Crystallized from *i*-butylmethylketone–*n*-C_6H_{14}; UV-Vis $\lambda_{max}^{n\text{-}C_6H_{14}}$ nm: (426), 446 and 473, III/II (%) = 57; $\lambda_{max}^{Me_2CO}$ nm: (423), 446 and 472, III/II (%) = 37; $\lambda_{max}^{CHCl_3}$ nm: 456 and 481, III/II (%) = 10; IR ν_{max}^{KBr} cm^{-1}: 3430 *s* (OH); 2960 *s*, 2930 *s* and 2860 *s* (CH); 1930 *w* (C=C=C); 1730 *s* (C=O, ester); 1660 *m* (conj. C=O); 1610 *m*; 1580 *w* (C=C); 1530 *m*; 1465 *s* (CH_2); 1380 *m* and 1365 *m* (CH_3); 1250 *m* (CO–O–C); 1190 *w* and 1175 *m* (*tert.* OH); 1080 *w*, 1055 *w* and 1030 *m* (*sec.* OH) and 970 *s* (*trans* CH=CH); MS *m/z* (rel. int.): 744 $[M]^+$ (0.8), 726.4501 (calculated for $C_{46}H_{62}O_7$: 726.4496) $[M-18]^+$ (6), 710 $[M-16-18]^+$ (2), 708 $[M-18-18]^+$ (3), 666 $[M-18-60]^+$ (0.9), 664 $[M-80]^+$ (0.4), 656 $[M-88]^+$ (0.5), 650 $[M-16-18-60]^+$ (0.6), 648 $[M-18-18-60]^+$ (0.7), 640 $[M-16-88]^+$ (0.3), 638 $[M-18-88]^+$ (0.2), 630 (1), 622 $[M-16-18-88]^+$ (1), 620 $[M-18-18-88]^+$ (0.6), 580 $[M-16-60-88]^+$ (0.6), 578 $[M-18-60-88]^+$ (0.6), 562 $[M-16-18-60-88]^+$ (0.7), 560 $[M-18-18-60-88]^+$ (0.8), 536 (1), 298.1940 (calculated for $C_{20}H_{26}O_2$: 298.1933) (33), 221 [homopyryllium]$^+$ (20) and 195 (100); ^{1}H NMR (400 MHz, $CDCl_3$, D-locked): δ0.94 *t* (*J* = 7.7 Hz, 3H, end-Me of butanoyl), 0.96 *s* (3H, Me-16), 1.03 *s* (3H, Me-17), 1.08 *s* (3H, Me-16'), 1.22 *s* (*ca* 3H, Me-18), 1.37 *s* and 1.38 *s* (3H + 3H, Me-16',18'), 1.94 *s* (3H, Me-19), 2.00 *s* (6H, Me-20,20'), 2.04 *s* (3H, Me of acetyl at C-3'), 2.28 *t* (*J* = 7.6 Hz, 2H, α-CH_2 of butanoyl), 2.60 *d* (J_{gem} = *ca* 19 Hz, 1H, H-7), 3.66 *d* (J_{gem} = *ca* 19 Hz, 1H, H-7), 3.83 *m* (1H, H-3), 4.62 *s* (CH_2-19',9'-*cis*, *ca* 27% in mixture with 9'-*trans*), 4.75 *d* (J_{gem} = 11 Hz, *ca* 1H, CH_2-19',9'-*all-trans*), 4.81 *d* (J_{gem} = 11 Hz, *ca* 1H, CH_2-19',9'-*all-trans*), 5.38 *m* (1H, H-3'), 6.06 *s* (1H, H-8'), no 6.13 *d*, 6.2–6.8 *m* (*ca* 9H, conj. olefinic) and 7.15 *d* ($J_{10,11}$ = 11 Hz, 1H, H-10); CD (EPA) nm ($\Delta\varepsilon$): 218 (−0.4), 227 (0), 233 (+0.3), 243 (0), 271 (−0.5), 291 (0), 332 (+0.5) and *ca* 355–410 (0); HPLC (Si-5, 20% Me_2CO in *n*-C_6H_{14}–0.1% MeOH): R_t = 19.2 min.

GC of methyl n-*butanoate*. 19'-Butanoyloxyfucoxanthin (**8**, 0.8 mg pptd from Et_2O–*n*-C_6H_{14}) was saponified with 5% KOH in MeOH–Et_2O (1:1) overnight, and neutral lipids removed by addition of H_2O and extraction with Et_2O. Butanoic acid was transferred to Et_2O after acidification with 1 M HCl, and evapd to dryness under slightly alkaline conditions (2 drops of 5% KOH). Butanoic acid was esterified by treatment with 1.5 M HCl in MeOH at 60° for 1 hr, and identified by isothermal GC (Pye Unicam 104 Chromatograph) at 50° on a 5% TCEP column [22]. Co-chromatography with a reference similarly prepared from butanoic acid was positive: R_t 7.5 min (R_t for the references methyl *n*-pentanoate and methyl *n*-hexanoate were 17 and 38 min, respectively).

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