Pergamon

PII: S0031-9422(96)00650-4

*Phytochemistry*, Vol. 44, No. 6, pp. 1087–1097, 1997

0031-9422/97 $17.00+0.00

# ADDITIONAL CAROTENOID PROTOTYPE REPRESENTATIVES AND A GENERAL CHEMOSYSTEMATIC EVALUATION OF CAROTENOIDS IN PRASINOPHYCEAE (CHLOROPHYTA)*†

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(*Received* 8 *May* 1996)

**Key Word Index**—*Nephroselmis olivacea*; *Pyramimonas amylifera*; *Prasinococcus capsulatus*; Prasinophyceae; Chlorophyta; carotenoid composition; preprasinoxanthin; siphonaxanthin 19-(*trans*-Δ2-dodecenoate); 6′-hydroxysiphonaxanthin 19-(*trans*-Δ2-dodecenoate); general chemosystematic evaluation combined evidence; Prasinophyceae.

**Abstract**—Quantitative carotenoid analyses of 3 additional species representing each of three pigment types of Prasinophyte algae grown in pure culture are reported. The carotenoids were characterized by chromatographic (TLC, HPLC), spectroscopic (visible and mass spectroscopy, and, in part, $^1$H NMR, circular dichroism), and chemical methods. Preprasinoxanthin was isolated for the first time and the structure of 6′-hydroxysiphonaxanthin 19-(*trans*-Δ2-dodecenoate) was elucidated. The presence of siphonein, identified as siphonaxanthin 19-(*trans*-Δ2-dodecenoate) in Prasinophyceae was confirmed. Small amounts of the corresponding *trans*-Δ2-decenoates were present. Authentic siphonaxanthin 19-(*trans*-Δ2-dodecenoate) was isolated from *Codium fragile* for comparison. *Pyramimonas amylifera* belonged to the siphonein type. *Prasinococcus capsulatus* produced carotenoids of the prasinoxanthin/uriolide type, whereas *Nephroselmis olivacea* only contained common green algal carotenoids. A chemosystematic evaluation is made on the basis of 13 species examined by methods including mass spectroscopy and supplemented by less documented literature data on 26 species. Plausible biosynthetic routes are proposed from structural interrelationships, including chiralities. The Prasinophyceae displays a diversified carotenoid complement; 30 carotenoids have been identified, 14 of which are peculiar to this algal class. Three carotenoid prototypes emerge: type 1, producing only common green algal type carotenoids; type 2 with additional carotenoids of the siphonaxanthin series; and type 3 with additional carotenoids of the prasinoxanthin/uriolide series. Carotenoids with ε- and γ-end groups are abundant. The 7,8-dihydro feature of the unique uriolide series is compatible with unprecedented cyclization of the least unsaturated end group of neurosporene by an ε-cyclase. Copyright © 1997 Elsevier Science Ltd

## INTRODUCTION

The algal class Prasinophyceae (Chlorophyta) contains green, mainly scaly and flagellate microalgae [3]. The carotenoids of green flagellates of the Prasinophyceae were first examined by Ricketts [4–9] and more recently followed up by modern analyses in our laboratories [2, 10–14]. Structures have been assigned so far to 13 carotenoids not encountered outside the Prasinophyceae. Carotenoids with the rare γ-end group include prasinoxanthin (**1**) [10, 11], identical with xanthophyll K [7, 8], dihydroprasinoxanthin epoxide (**2**) [12] and anhydroprasinoxanthin (**3**) [12]; these are compiled in Scheme 1 together with preprasinoxanthin (**4**) [13]. Scheme 2 presents the lactones uriolide (**5**) [12], deepoxyuriolide (**6**), 3′-dehydrouriolide (**7**) and anhydrouriolide (**8**) [13], plus other C-19 in-chain substituted carotenoids, namely, the aldehydes micromonal (**9**) and anhydromicromonal (**10**) and the primary alcohols micromonol (**11**) and anhydromicromonol (**12**); also dihydrolutein (**13**), all of which possess a hydrogenated Δ7′ double bond [13]. Quantitative carotenoid analyses available

*Part 63 in the series 'Algal Carotenoids'. For part 62, see ref. [1].

†Part 7 in the series 'Carotenoids from Prasinophyceae'. For part 6, see ref. [2].

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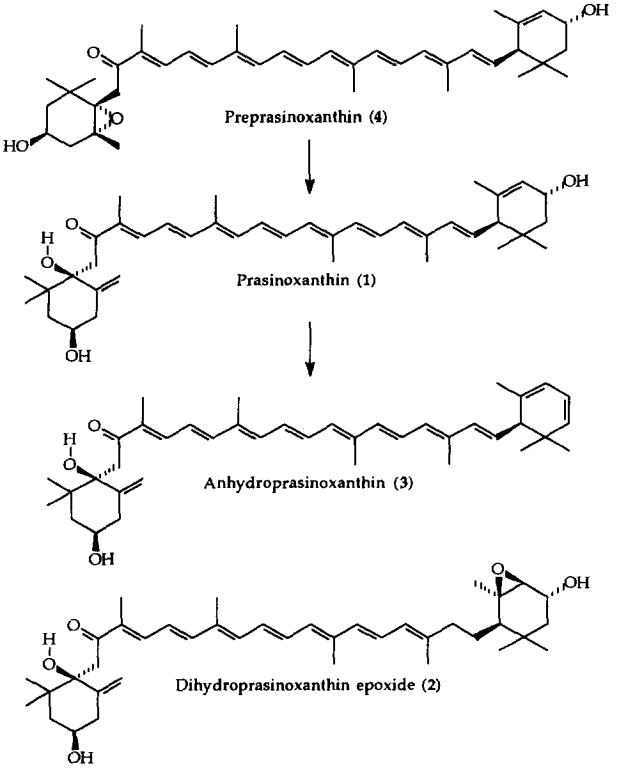
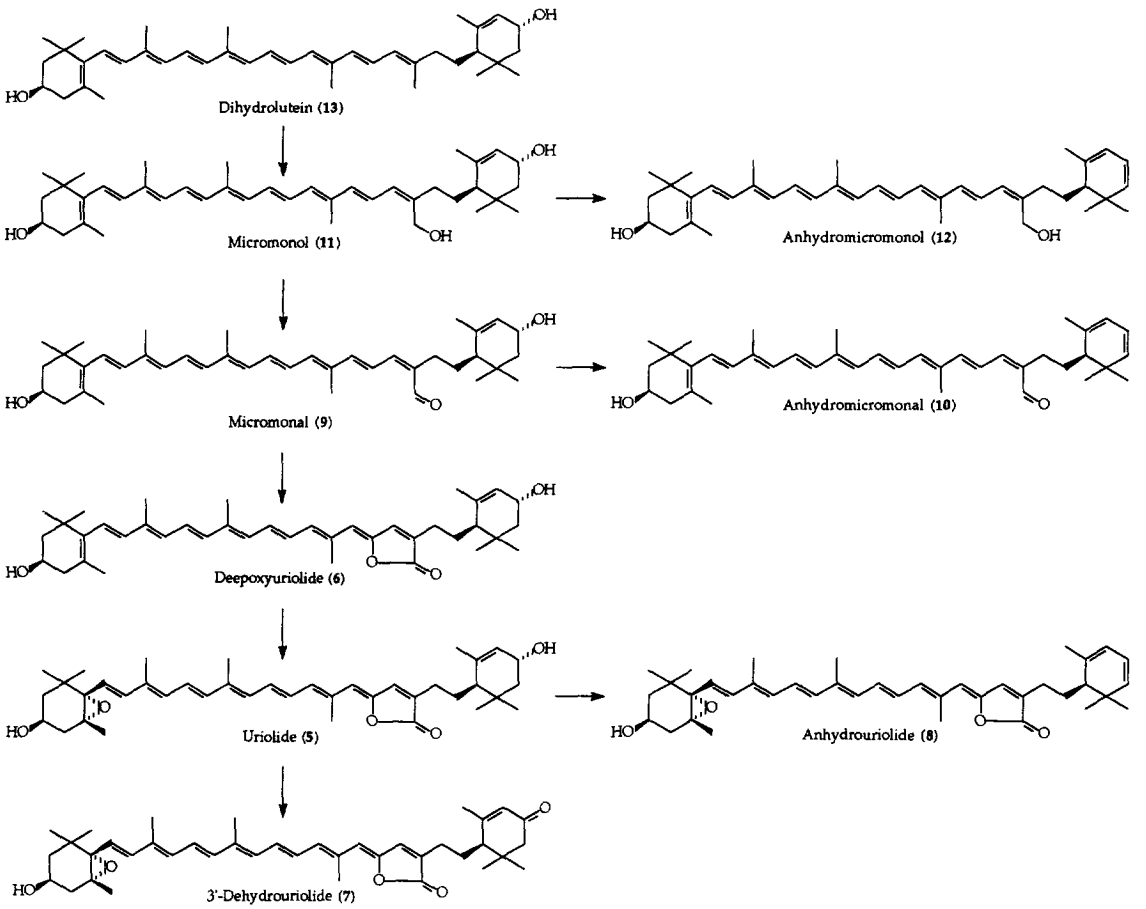



Scheme 1. Structural interrelationship of prasinoxanthin type carotenoids.




Scheme 2. Structural interrelationship of uriolide type carotenoids.

include those of 11 species published from our laboratories [2, 10, 12, 14], of 22 species included in the early studies by Ricketts [4–9] and of nine species examined mainly by HPLC only [15–23].

It was desirable to confirm the identification of siphonaxanthin (xanthophyll KS1, **14**) and its ester siphonein (xanthophyll K1, **15**) [8, 9] in members of the Prasinophyceae. In the present paper is therefore presented a modern reinvestigation of *Pyramimonas amylifera* and of two additional species, shown to represent other carotenoid prototypes. The accumulated evidence serves as basis for a chemosystematic evaluation based on carotenoids in Prasinophyceae.

## RESULTS AND DISCUSSION

### *Individual algae*

*Pyramimonas amylifera*, grown in unialgal culture, had the carotenoid composition given in Table 1. Siphonein (**15**) was characterized as a 19-(*trans*-Δ2-dodecenoate) (**15a**) together with small (*ca* 10% of **15a**) amounts of a 19-(*trans*-Δ2-decenoate) (**15b**) of siphonaxanthin (**16**) in direct comparison with the siphonein (**15a**) isolated from *Codium fragile* (HPLC, MS and $^1$H NMR data). No free siphonaxanthin (**14**) was encountered, but a new carotenoid, shown to be a 19-(*trans*-Δ2-dococenoate) (**16a**) together with small (*ca* 10% of **16a**) amounts of a 19-(*trans*-Δ2-decenoate) (**16b**) of 6′-hydroxysiphonaxanthin (**16**) was characterized (TLC, HPLC, Vis, MS, 1D and 2D $^1$H NMR). $^1$H NMR-assignments of **15** and **16** are given in Fig. 1. After alkaline hydrolysis a more polar product was identified as 6′-hydroxysiphonaxanthin (**16A**). The presence of four hydroxy groups, including one tertiary, was confirmed by the preparation of the triacetyl monosilyl derivative (**16B**). Reaction of the bisallylic *t*-carotenol **16A** with dilute HCl gave, as expected, several products. As a tetrol monoester the new carotenoid **16**, presumably identical with xanthophyll K2 [7, 8], has a higher polarity than generally associated with carotenoid esters of higher fatty acids. Lutein (**17**) was a minor carotenoid and other typical green algal carotenoids were present, Schemes 3 and 4.

*Prasinococcus capsulatus*, grown in axenic culture, exhibited the carotenoid composition listed in Table 1. Preprasinoxanthin (**3**), Scheme 1, was encountered for the first time, and the structure elucidation has been reported elsewhere [13]. The carotenoid complement included carotenoids depicted in Scheme 1 (prasinoxanthin (**1**) and preprasinoxanthin (**3**)) and in Scheme 2 (uriolide (**5**), deepoxyuriolide (**6**) and dihydrolutein (**13**)) beside common green algal carotenoids such as lutein (**17**) and zeaxanthin (**21**), antheraxanthin (**22**), violaxanthin (**23**) and neoxanthin (**24**), shown in Scheme 4, as well as carotenes.

*Nephroselmis olivacea*, also grown in unialgal culture, had a simple carotenoid distribution pattern, Table 1, including zeaxanthin (**21**), antheraxanthin (**22**), violaxanthin (**23**) and neoxanthin (**24**), Scheme 4, as well as lutein (**17**), Scheme 3. The epoxidic carotenoids (**22**, **23**, **24**) were partly isolated as furanoid rearrangement products.

### *Chemosystematic evaluation*

Accumulated evidence supports that the Prasinophyceae contains microalgae of three different pigment types:

type 1, common green algal carotenoids (Scheme 4, lutein (**17**) and lutein epoxide (**20**));

type 2, common green algal carotenoids together with carotenoids of the siphonaxanthin (**14**) series (Scheme 3); and

type 3, common green algal carotenoids together with carotenoids of the prasinoxanthin (**1**) series (Scheme 1) and uriolide series (Scheme 2).

The three algae here analysed clearly belong to each of these types: *Nephroselmis olivacea* (type 1), *Pyramimonas amylifera* (type 2) and *Prasinococcus capsulatus* (type 3).

Prasinophytes previously analysed in our laboratory can also be divided into these categories (Table 1). Tentative assignments can also be made for Prasinophytes, including members recently reclassified as Pedinophyceae [3] with less documented carotenoid composition (Table 2).

In Schemes 1, 2, 3 and 4 the carotenoids encountered are arranged such that structural interrelationships are obvious, and where in many cases only a simple chemical step reaction would be required for the transformations indicated. The conversion of preprasinoxanthin (**4**) to prasinoxanthin (**1**), Scheme 1, has been rationalized [13].

In Scheme 5 the four structural schemes 1, 2, 3 and 4 are combined into a plausible biosynthetic scheme, illustrating the pathways required to obtain the three pigment types. The siphonaxanthin series (Scheme 3) is reached by an extension from the common pathway from lutein (**17**) and the prasinoxanthin series by another extension from **17**. The uriolide series may be reached via dihydrolutein (**13**), and a so far unprecendented cyclization of the most saturated end group of neurosporene (**27**) by an ε-cyclase is suggested. This scheme visualizes the biosynthetic capacity required by various Prasinophytes in order to obtain their characteristic carotenoid complement. Cyclization of lycopene (**28**) by β- or ε-cyclases is considered to be a general pathway in carotenogenesis. The high proportion of ε-type carotenoids in Prasinophyceae is consistent with an active ε-cyclase. The terminal methylene of the rare γ-end group is considered to be the result of a secondary biosynthetic event (ref. [13], Scheme 1).

In the algal context, the class Prasinophyceae displays a large diversity of carotenoid structures, having 30 identified carotenoids of which some 14 have special structural features peculiar to this class. Relative

Table 1. Prasinophytes examined by modern methods including mass spectrometry in our laboratories

| Present classification [3] | Carotenes* | | | | | Type 3 carotenoids | | | | | | | | | | | | | Type 2 carotenoids | | Common green algal carotenoids | | | | | | Unknowns (%) | Ref. | Other ref.† |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | β,β | β,ε | ε,ε | β,ψ | **28** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **15** | **16** | **17** | **21** | **22** | **23** | **24** | **26** | | | |
| MAMIELLALES | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mamiellaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Bathycoccus prasinos* | 0.8‡ | 0.4 | – | | | 49 | – | | | 7 | | 0.8 | | 16 | – | | | 0.08 | | – | | – | | 0.8 | 14 | | 12 | [14] | – |
| *Mantoniella squamata* | 1–17 | 0.3–5 | | | | 23–41 | | 0–1 | | 4–19 | | | 0–2 | 5–16 | 0–3 | 0–2 | 0–1 | 0–7 | | | 0–10 | ,? | 0–1 | 0–16 | 15–20 | | 0–6 | [2] | [6, 15–17] |
| *Micromonas pusilla* | 3 | 5 | | | | 24 | | | | 11 | | | 4 | 27 | – | 10 | | | | | | | | | 14 | – | 3 | [2] | [4, 15, 18, 19] |
| Mamiellaceae??? | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Arousa 2 | 7 | 0.8 | | | | 37 | | | | 18 | | | | 10 | | | | | | | | | | 2 | 15 | | 8 | [2] | |
| Pycnococcaceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Prasinococcus capsulatus*§ | 3–7 | 2–6 | 0–0.6 | | | 36–50 | 0–4 | | 0–10 | 8–14 | 0–0.1 | | | | | | | 0–3 | | | 0–2 | 0–3 | 0–2 | 0–6 | 15–25 | | 0–1 | ‖, [12] | [15, 17] |
| *Pycnococcus provasolii* | 3–9 | 1 | | | | 58–69 | | | | | | | | | | | | | | | | 2–6 | | | 13–21 | 0–17 | 0–4 | [10, 12] | [15, 18, 19] |
| CHLORODENDRALES | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Chlorodendraceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Nephroselmis olivacea* | 13 | | | | | | | | | | | | | | | | | | | | 44 | 13 | 9 | 6 | 9 | | 7 | ‖ | |
| *Nephroselmis pyriformis* | 9 | 25 | | | | | | | | | | | | | | | | | | | | 47 | | 10 | 8 | | 2 | ¶ | [7] |
| *Nephroselmis rotunda* | 48 | | | 4 | 4 | | | | | | | | | | | | | | | | 24 | 0.8 | | 6 | 7 | | 5 | [2] | [8] |
| *Pseudoscourfieldia marina* | 5 | 2 | | | | 53 | | | | 3 | | | ? | 6 | – | | | 4 | | | – | ? | – | | 21 | | 6 | [2] | [15] |
| *Tetraselmis wettsteinii* | 28 | 12 | | | | | | | | | | | | | | | | | | | 24 | | | | 28 | | 7 | [2] | |
| *Tetraselmis sp.* | 6 | 2 | – | | | | | | – | | | | | | – | – | | – | | | 35 | 1 | | 25 | 16 | | 14 | [2] | – |
| Halosphaeraceae | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *Pyramimonas amylifera* | 22 | | – | | | | | | – | | | | | | – | – | | | 26 | 5 | 1 | 12 | 4 | 10 | 12 | | 8 | ‖ | [7] |

*For carotenoid name and structure, see Schemes 1–5.

†Other examinations, partly of other strains.

‡% of total carotenoids.

§Clone IIA2 and Clone URI 266G. These clones and clone IV E5G [15] are now considered to represent the same species (J. McN. Sieburth *et al.*, pers. comm.).

‖This work.

¶B. Bjerkeng and S. Liaaen-Jensen, unpublished.

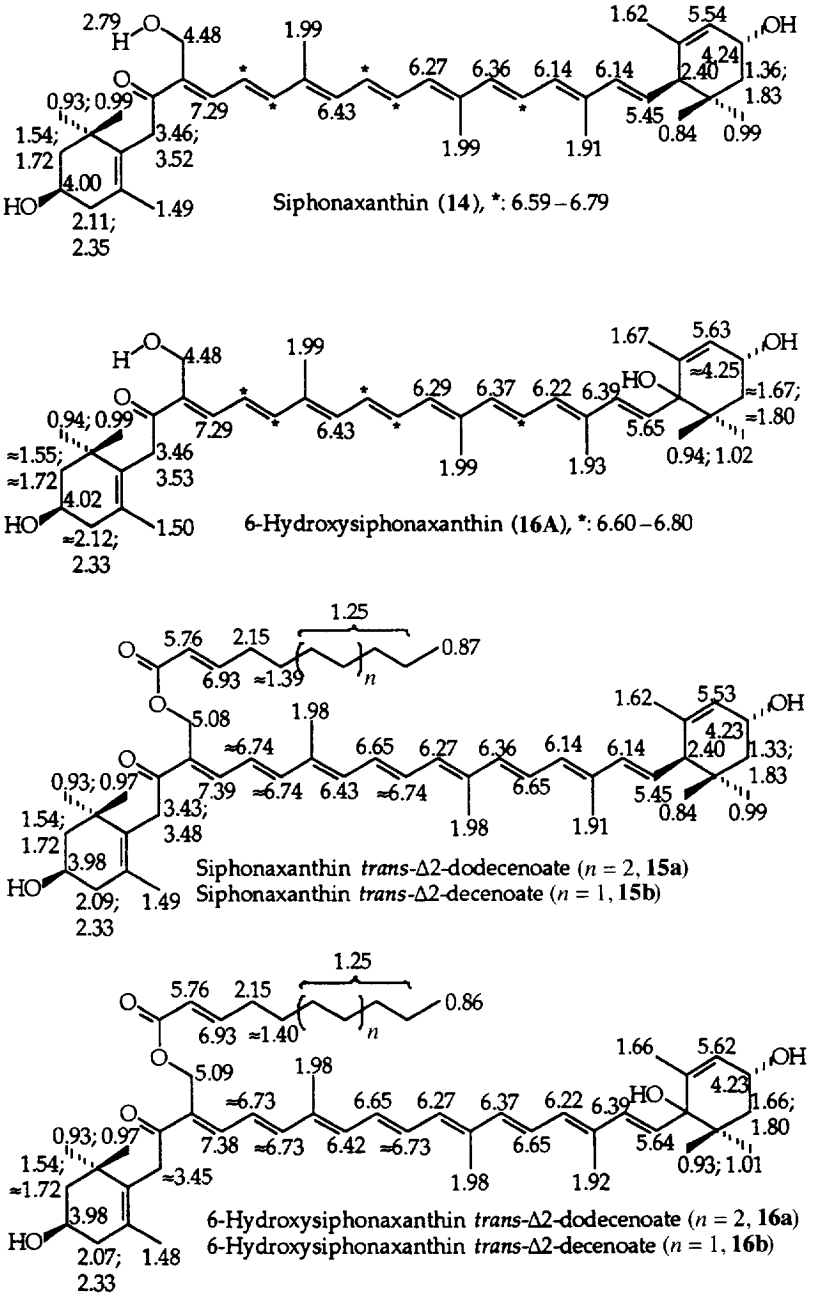


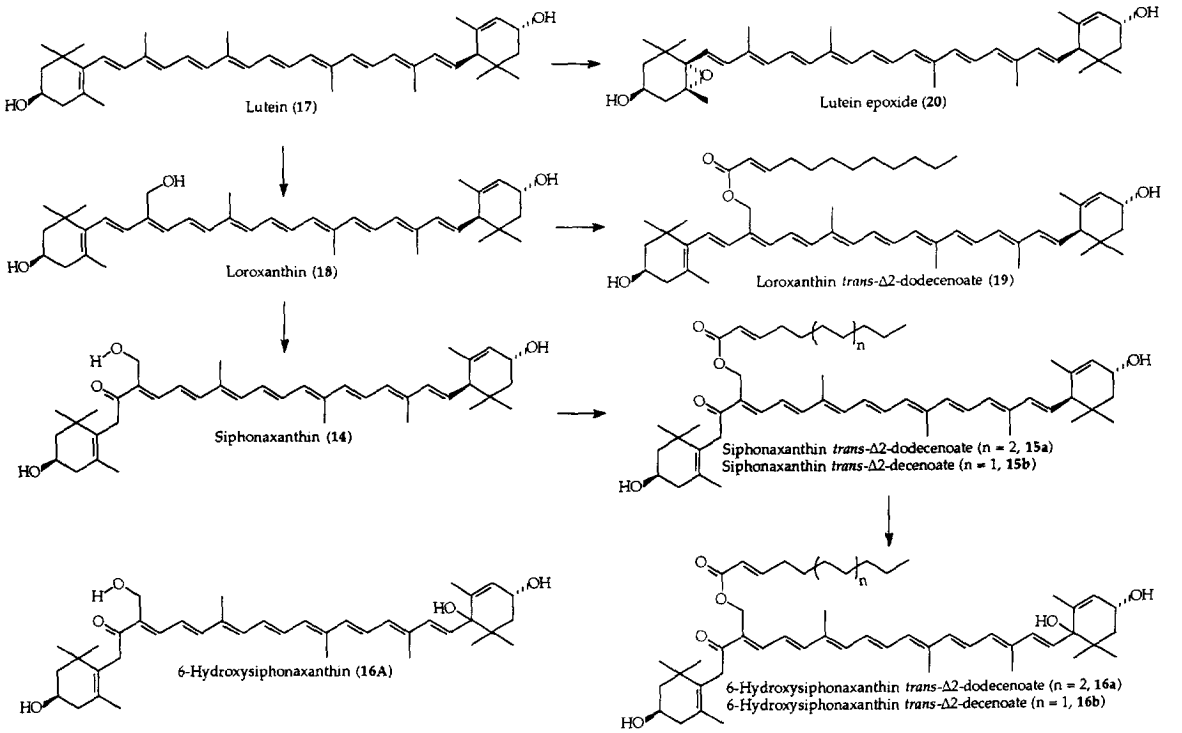

Fig. 1. $^1$H NMR assignments of siphonaxanthin derivatives.

to other microalgae, Prasinophytes of pigment type 3 can be identified readily because of their unique carotenoids; algae of pigment types 2 and 1 require additional identification criteria.

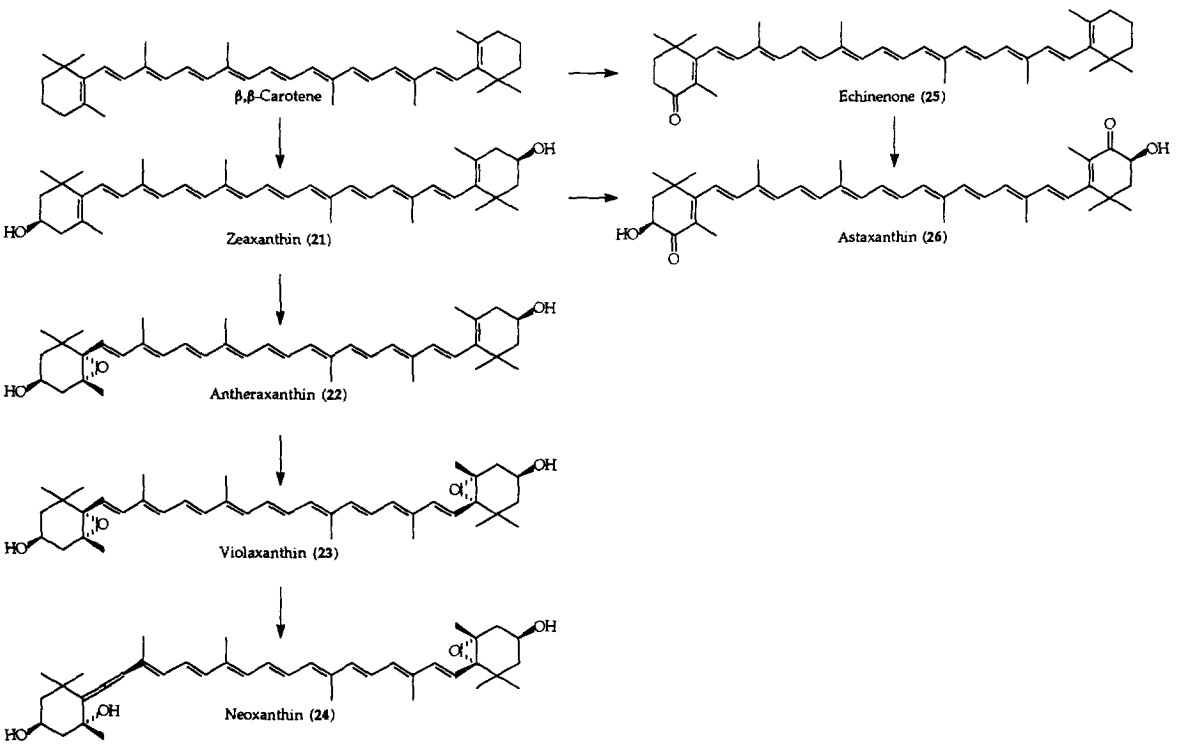
It is suggested that the carotenoid composition may also be a useful criterium for classification within the Prasinophyceae at the ordinal level. *Pseudoscourfieldia marina* [2] contains type 3 carotenoids, and is at present [3] classified together with type 1/type 2 carotenoid-producing algae (*Tetraselmis* [2], *Nephroselmis*) in the family Chlorodendraceae, order Chlorodendrales. Transfer of this genus to the order Mamiellales, family Mamiellaceae, should be considered on the basis of carotenoid biosynthesis.

The new species *Prasinoderma coloniale* Hasegawa et Chihara was described recently [28] and the family Pycnococcaceae amended by Miyashita and Chihara [28] to include *Prasinoderma* and *Prasinococcus* as well as *Pycnococcus* [29].

Two features described for *Prasinoderma coloniale*




Scheme 3. Structural interrelationship of siphonaxanthin type carotenoids.



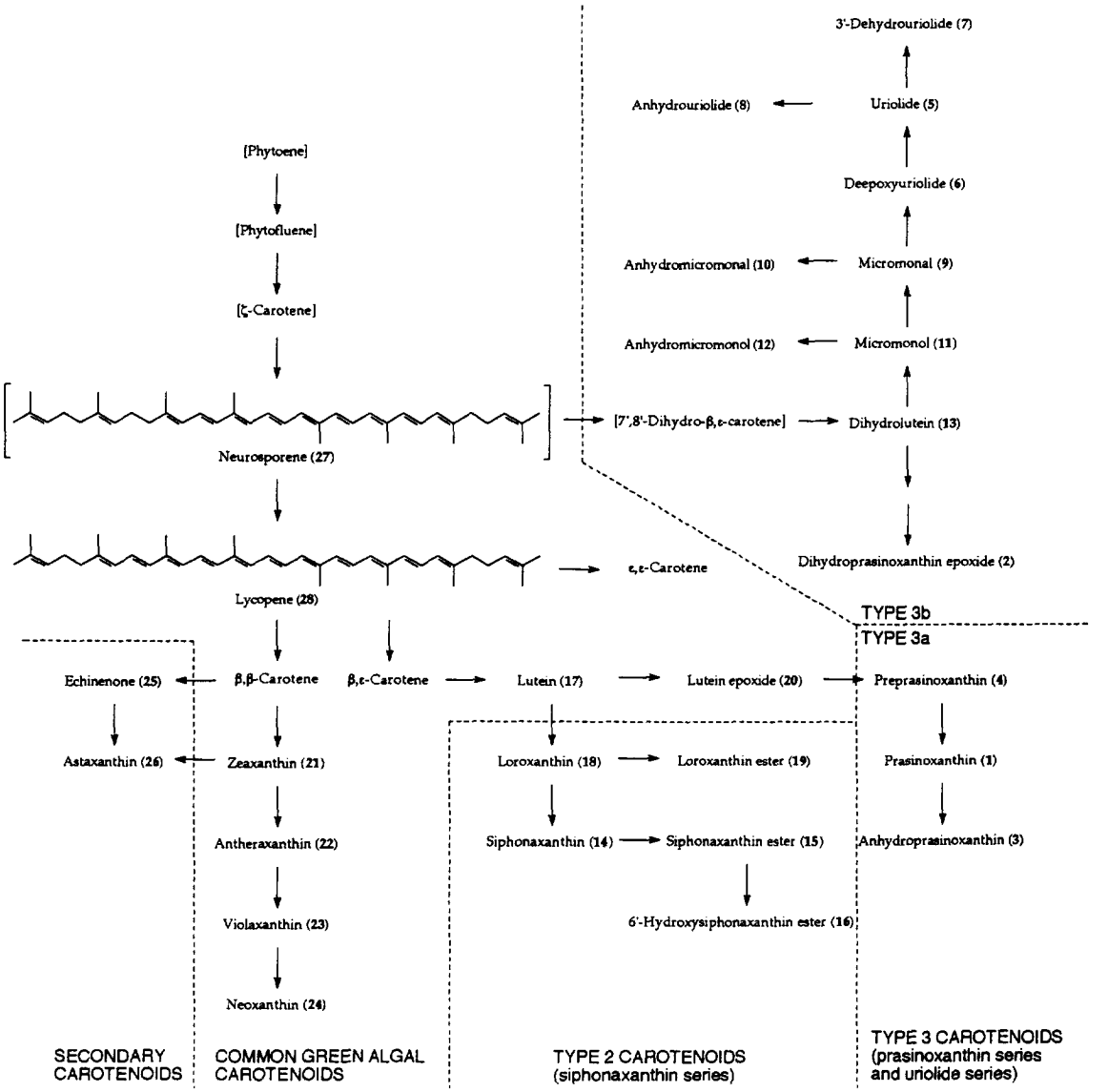

Scheme 4. Structural interrelationship of common, green algal carotenoids.

are compatible with our observations on strain IIA2, which we assigned tentatively to *Prasinococcus* in this paper. First, two unidentified pigments reported for *Prasinoderma* [28], namely 'unknown 1' and another unnamed pigment (eluting after neoxanthin, Fig. 27 [28]) may be, respectively, dihydrolutein (**13**) and prasinoxanthin (**4**) as found in strain IIA2. Second, the cell wall structure described for *Prasinoderma* is

Table 2. Algae belonging to Prasinophyceae or Pedinophyceae examined for carotenoids in other laboratories

| Present classification [1] | Ref. | Carotenes | Carotenes* | | | | Type 3 carotenoids | | | | Type 2 carotenoids | | | | | Common green algal carotenoids | | | | | | | Unknowns (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | β,β | β,ε | β,ψ | 28† | 1 | 5 | 9 | 13 | 14 | 15 | 16A‡ | 18 | 19 | 17 | 20 | 21 | 22 | 23 | 24 | 25 | |
| **PRASINOPHYCEAE** | | | | | | | | | | | | | | | | | | | | | | | |
| **MAMIELLALES** | | | | | | | | | | | | | | | | | | | | | | | |
| Mamiellaceae | | | | | | | | | | | | | | | | | | | | | | | |
| '*Nephroselmis*' *gilva* | [7] | | 15§ | 0.7 | | | 38 | | | | | | | | | 1 | 3 | | | 6 | 21 | | 15 |
| *Mamiella gilva* | [15] | 25 | – | – | – | – | 18 | 8 | 9 | 5 | | | | | | | | 0–3 | | 13 | 14 | – | 6 |
| *Micromonas* sp. (P265)‖ | [8] | 20–29¶ | | | | | 30–48 | | | | | | | | | | | | | 10–19 | 10–19 | | 22–46 |
| Mamiellaceae??? | | | | | | | | | | | | | | | | | | | | | | | |
| *Ostreococcus tauri* | [20] | – | 4 | 1 | | | – | | | | 10** | 5** | | | | 3?†† | | 3? | 5 | 64 | 7 | – | |
| Pycnococcaceae??? | | | | | | | | | | | | | | | | | | | | | | | |
| CS-126 | [19] | | + | | | | + | | | | | | | | | + | | + | | + | + | | + |
| **CHLORODENDRALES** | | | | | | | | | | | | | | | | | | | | | | | |
| Mesostigmataceae | | | | | | | | | | | | | | | | | | | | | | | |
| *Mesostigma viride* | [8] | 20–29 | – | – | – | – | | | – | | – | | | | | 20–29 | | 10–19 | – | 10–19 | 10–19 | – | 4–13 |
| *Mesostigma viride* | [23] | | 5 | 5 | | | | | | | 8 | 16 | | | | | | | | 32 | 10 | 9 | 17 |
| Chlorodendraceae | | | | | | | | | | | | | | | | | | | | | | | |
| '*Heteromastix*' sp. (B148)‡‡ | [8] | 20–29 | | | | | | | | | – | – | – | – | – | 20–29 | – | 10–19 | – | 10–19 | 10–19 | – | 8–18 |
| '*Heteromastix*' sp. (P198) | [8] | 20–29 | | | | | | | | | 20–29‡ | | 10–19 | – | | | | 20–29 | | 4–9 | 10–19 | | 0–4 |
| '*Heteromastix*' sp. (P397) | [8] | 30–39 | | | | | | | | | | | | | | 20–29 | | 10–19 | – | 10–19 | 10–19 | – | 8–22 |
| '*Platymonas*' *chuii* | [6] | | 12 | 0.7 | 4 | 0.9 | | | | | | | | | | 32 | 4 | 13 | – | 12 | 9 | | 14 |
| *Tetraselmis chui* | [19] | – | + | | – | – | – | | – | | | | | | | + | | + | + | + | + | | + |
| '*Platymonas*' *striata* | [8] | 30–39 | | | | | | | | | | | | | | 20–29 | | 4–9 | – | 10–19 | 10–19 | – | 0–8 |
| '*Platymonas*' *subcordiformis* | [8] | 30–39 | | | | | | | | | | | | | | 20–29 | | 10–19 | | 20–29 | 10–19 | | 0–8 |
| '*Platymonas*' *tetrathele* | [8] | 30–39 | | | | | | | | | | | | | | 20–29 | | 4–9 | | 10–19 | 20–29 | | 4–13 |
| *Prasinocladus* sp. (P371) | [6] | | 15 | 3 | 3 | 2 | | | | | | | | | | 27 | 6 | 3 | | 20 | 16 | – | 6 |
| *Prasinocladus lubricus* | [8] | 20–29 | | | | | | | | | | | | | | 10–19 | | 4–9 | | 10–19 | 10–19 | | 18–37 |
| *Prasinocladus marinus* | [8] | 20–29 | | | | | | | | | | | | | | 20–29 | | 10–19 | | 10–19 | 10–19 | | 8–18 |
| Halosphaeraceae | | | | | | | | | | | | | | | | | | | | | | | |
| '*Pachysphaera*' sp. (P339) | [8] | 10–19 | | | | | | | | | 30–39‡ | | 30–39 | | | | | 4–9 | | 4–9 | 4–9 | | 4–9 |
| *Pterosperma* sp. (P302) | [8] | 10–19 | | | | | | | | | 30–39‡ | | 20–29 | | | | | 10–19 | | 4–9 | 10–19 | | 0–4 |
| *Halosphaera rusellii* (motile) | [8] | 20–29 | | | | | | | | | | | | | | 4–9 | | 4–9 | | 10–19 | 10–19 | | 38–57 |
| *Pyramimonas cordata* | [19] | – | + | – | + | – | – | – | – | – | – | – | | | | + | – | + | + | + | + | | + |
| *Pyramimonas grossii* | [8] | 30–39 | | | | | | | | | | | | | | 10–19 | | 10–19 | | 10–19 | 10–19 | | 14–28 |
| *Pyramimonas obovata* | [7] | | 28 | 1 | 4 | | | | | | – | | | – | – | 16 | – | 18 | – | 10 | 11 | – | 11 |
| *Pyramimonas parkeae* | [21] | | + | + | | | | | | | | | | + | + | + | | + | + | + | + | | + |
| *Pyramimonas parkeae* | [22] | – | + | – | – | – | – | – | – | – | – | – | | | + | + | | + | + | + | + | | + |
| *Pyramimonas urceolata* | [8] | 30–39 | | | | | | | | | – | | | – | – | 20–29 | – | 10–19 | – | 10–19 | 10–19 | – | 0–4 |
| **PEDINOPHYCEAE** | | | | | | | | | | | | | | | | | | | | | | | |
| **PEDINOMONADALES** | | | | | | | | | | | | | | | | | | | | | | | |
| Pedinomonadaceae | | | | | | | | | | | | | | | | | | | | | | | |
| *Pedinomonas minor* | [5] | | 22 | 5 | 22 | | | | | | | | – | – | | 34 | 3 | 1 | | 4 | 7 | | 3 |
| *Pedinomonas tuberculata* | [5] | | 15 | 0.9 | 6 | 3 | | | | | | | | | | 33 | | 3 | | 23 | 15 | | 2 |
| ??? | | | | | | | | | | | | | | | | | | | | | | | |
| ??? | | | | | | | | | | | | | | | | | | | | | | | |
| Monomastigceae | | | | | | | | | | | | | | | | | | | | | | | |
| *Monomastix minuta* | [8] | 20–29 | – | – | – | – | – | – | – | – | – | – | | | | 20–29 | | 20–29 | | 20–29 | 4–9 | | 0–8 |

*The carotenes were not separated or identified. †For carotenoid name and structure, see Schemes 1–5. ‡Results from hydrolysed extracts. §% of total carotenoids. ‖The Plymouth Laboratory collection number.
¶The carotenoid content is given by intervals only. **'Siphonaxanthin-like'. ††Lutein (**17**) and zeaxanthin (**21**) were not separated. ‡‡The Windermere collection number.




Scheme 5. Proposed biosynthetic pathways of carotenoids in Prasinophyceae.

compatible with our finding that the pigment extraction for IIA2 was difficult. Other techniques must be used to determine if IIA2 should be assigned to *Prasinoderma* rather than to *Prasinococcus*.

## EXPERIMENTAL

*Biological material. Pyramimonas amylifera* (Plymouth strain 246, CCMP 720, unialgal) was obtained from the Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences. It was grown in seawater (32–34‰ salinity) enriched as medium L1 [23]. For carboy sized cultures (8–14 l) the nutrients were added aseptically after autoclaving. Cultures were grown at 15° under 14 hr/day of 'cool white' fluorescent light measuring $2 \times 10^{16}$ to $4 \times 10^{16}$ photons/s cm$^2$ at the carboy surface; cultures were started at lower intensity. Continuous aeration was begun when the population was moderately dense and $CO_2$ pulses were added daily later. The yield by Sharples continuous centrifugation from 96 l of culture was 11.6 g dry weight. The population harvested consisted of $1.4 \times 10^{10}$ cells (counted) plus some that had sedimented. Strain IIA2 (CCMP 1192, axenic) is tentatively assigned to the species *Prasinococcus capsulatus* Miyashita et Chihara [17] on the basis of pigment content and of ultrastructure (J. McN. Sieburth *et al.*, personal communication). It was grown essentially as described for *Pyramimonas amylifera* except at 20° and 50 $\mu$M $NH_4Cl$ was added to the L1 enrichments. The centrifuged yield from 99.5 l of culture was 17.53 g dry weight, representing about $6.4 \times 10^{12}$ cells.

The freshwater *Nephroselmis olivacea*, strain S. A. G.

No. 40.89, was received in unialgal culture from the Sammlung von Algenkulturen, Pflanzenphysiologisches Institut, Universität Göttingen. It was transferred to and cultured in the basal medium WC [25] with 50 $\mu$M boric acid and with the L1 trace metal solution [24] rather than with the F/2 trace metals originally specified. Trace metals and phosphate were added aseptically after autoclaving when carboy lots of medium were prepared. Strain 40.89 required the vitamins called for in WC medium, and grew well without added $NH_4Cl$. Initial pH values from 7.5 to 8.2 were satisfactory; a wider range was not tested. Carboy cultures were grown at 20–21° under 14 hr/day of 'cool white' illumination of about $1.5 \times 10^{16}$ photons/s cm$^2$ at the surface of the carboy at the start, supplemented with about the same level of illumination from bulbs adjacent to the carboys as cultures became dense. The centrifuged yield from 84.5 l was 4.675 g dry weight, representing about $7.4 \times 10^{10}$ cells.

*Instruments and spectroscopy*. These were as recently specified [26]. Spectral fine-structure in the Vis absorption spectra is defined as %III/II and $D_V$ [13]. Diagnostically useful peaks only are reported for the mass spectra. $^1$H NMR coupling constants $J$ are given in Hz.

*Extraction*. For general precautions and procedures, cf. [27]. *Pyramimonas amylifera* (2.1 g lyophilized cells) was treated with phosphate buffer (Merck 9879) prior to solvent extraction with acetone. *Nephroselmis olivacea* (0.51 g lyophilized cells) was extracted with acetone:methanol 7:3. *Prasinococcus capsulatus* (5.2 g lyophilized cells) was first treated with phosphate buffer:acetone 1:2, then extracted several times with acetone:methanol 7:3 until colourless.

*Chromatography*. Systems for analytical TLC and HPLC and preparative separation by TLC were as specified elsewhere [14].

*Pyramimonas amylifera*

Total amount of carotenoids available: 7.4 mg. The carotenoid distribution is given in Table 1.

*β,β-Carotene*. Vis $\lambda_{max}^{acetone}$ nm: (422), 448, 473; %III/II = 7, $D_V = 0.86$; for $R_F$, $t_R$-values, and mass spectrum cf. [2, 14].

*Lutein* (**17**). Vis $\lambda_{max}^{acetone}$ nm: (421), 445, 472; %III/II = 47, $D_V = 0.76$; for $R_F$, $t_R$-values, and mass spectrum cf. [2].

*Zeaxanthin* (**21**). Vis $\lambda_{max}^{acetone}$ nm: (425), 450, 474; %III/II = 3, $D_V = 0.80$; for $R_F$, $t_R$-values, and mass spectrum cf. [2].

*Antheraxanthin* (**22**). Isolated as its furanoxides: Vis $\lambda_{max}^{acetone}$ nm: (402), 426, 451; %III/II = 56, $D_V = 0.80$; for $R_F$-value and mass spectrum cf. [2].

*Violaxanthin* (**23**). Isolated as its furanoxides luteoxanthin and auroxanthin: Vis $\lambda_{max}^{acetone}$ nm: (378), 398, 421, 446; %III/II = 64, $D_V = 0.62$; for $R_F$, $t_R$-values, and mass spectrum cf. [2, 14].

*Neoxanthin* (**24**). Isolated partly as its furanoxides neochrome: Vis $\lambda_{max}^{acetone}$ nm: 415, 437, 465; %III/II = 67, $D_V = 0.65$; for $R_F$, $t_R$-values, and mass spectrum cf. [2, 14].

*Siphonein* (**15**). Siphonaxanthin 19-(*trans*-Δ2-dodecenoate) (**15a**, *ca* 90% of **15**), and siphonaxanthin 19-(*trans*-Δ2-decenoate) (**15b**, *ca* 10% of **15**): Vis $\lambda_{max}^{acetone}$ nm: 448, (463); $R_F = 0.31$ (TLC plate 3 [14], 30% acetone in heptane), $t_R = 25.8$ (**15a**, 89%), 23.7 (**15b**, 11%) (system [13], flow 1.25 ml/min); EIMS 70 eV, 210°, *m/z* (rel. int.): 780 [M = **15a**]$^+$ (59), 762 [M − 18]$^+$ (29), 752 [M′ = **15b**]$^+$ (13), 734 [M′ − 18]$^+$ (8), 688 [M − 92]$^+$ (5), 582 [M − 180 − 18]$^+$ (42), 568 [M − 180 − 18 − 18]$^+$ (56), 180 (100); $^1$H NMR (400 MHz, $CDCl_3$, including $^1$H–$^1$H COSY), see Fig. 1. Double primed numbers refer to the fatty acid side chain: $\delta$ 0.84 (3H, *s*, H-17′), 0.87 (3H, *t*, H-12″), 0.93 (3H, *s*, H-16 or H-17), 0.97 (3H, *s*, H-16, or H-17), 0.99 (3H, *s*, H-16′), 1.25 (*ca* 12 H, *s*, $CH_2$ in ester), ≈1.33 (1H, *m*, H-2′ax), ≈1.39 (2H, *m*, H-5″), 1.49 (3H, *s*, H-18), 1.54 (1H, *m*, H-2ax), 1.62 (3H, *s*, H-18′), 1.72 (1H, *m*, H-2eq), 1.83 (1H, *dd*, $J = 6$, 13, H-2′eq), 1.91 (3H, *s*, H-19′), 1.98 (6H, *s*, H-20, H20′), 2.09 (1H, *dd*, $J = 10$, 16, H-4ax), 2.15 (2H, *q*, $J = 7.5$, H-4″), 2.33 (1H, *dd*, H-4eq), 2.40 (1H, *d*, $J = 10$, H-6′), 3.43 (1H, *d*, $J = 20$, H-7A), 3.48 (1H, *d*, $J = 20$, H-7B), 3.98 (1H, *m*, H-3), 4.23 (1H, *m*, H-3′), 5.08 (2H, *s*, H-19), 5.45 (1H, *dd*, $J = 10.8$, 16.0, H-7′), 5.53 (1H, *s*, H-4′), 5.76 (1H, *d*, $J = 15.6$, H-2″), 6.14 (2H, *d*, $J = 15$, H-8′, H-10′), 6.27 (1H, *d*, $J = 11.2$, H-14′), 6.36 (1H, *d*, $J = 14.8$, H-12′) 6.43 (1H, *d*, $J = 12.0$, H-14), 6.65 (2H, *dd*?, H-15, H-11′), ≈6.74 (3H, *m*, H-11, H-12, H-15′), 6.93 (1H, *dt*, $J = 6.8$, 16.0, H-3″), 7.39 (1H, *dd*, $J = 2$, 8.4, H-10).

*6′-Hydroxysiphonein* (**16**). (6′-Hydroxysiphonaxanthin 19-(*trans*-Δ2-dodecenoate) (**16a**, *ca* 90% of **16**) and 6′-hydroxysiphonaxanthin 19-(*trans*-Δ2-decenoate) (**16b**, *ca* 10% of 16): Vis $\lambda_{max}^{acetone}$ nm: 449, (466); $\lambda_{max}^{MeOH}$ nm: 452, (465); $\lambda_{max}^{heptane}$ nm: 452, (478); $\lambda_{max}^{chloroform}$ nm: 463; $R_F = 0.42$ (TLC plate 2 [2], 40% acetone in heptane), $t_R = 22.7$ (**16a**, 90%), 20.4 (**16b**, 10%) (system [13], flow 1,25 ml/min); EIMS 70 eV, 220°, *m/z* (rel. int.): 796 [M = **16a**]$^+$ (7), 778 [M − 18]$^+$ (18), 768 [M′ = **16b**]$^+$ (2), 760 [M − 18 − 18]$^+$ (9), 750 [M′ − 18]$^+$ (5), 722 (10), 694 (4), 616 [M − 180]$^+$ (8), 598 [M − 180 − 18]$^+$ (27), 580 [M − 180 − 18 − 18]$^+$ (51), 180 (100); $^1$H NMR (400 MHz, $CDCl_3$, including $^1$H–$^1$H COSY): $\delta$ 0.86 (3H, *t*, H-12″), 0.93 (6H, *s*, H-16 or H-17, and H-16′ or H-17′), 0.97 (3H, *s*, H-16 or H-17), 1.01 (3H, *s*, H-16′ or H-17′), 1.25 (*ca* 12 H, *s*, $CH_2$ in ester), ≈1.40 (2H, *m*, H-5″), 1.48 (3H, *s*, H-18), 1.54 (1H, *m*, H-2ax), 1.66 (3H, *s*, H-18′), 1.66 (1H, *m*, H-2′ax), ≈1.72 (1H, *m*, H-2eq), 1.80 (1H, *m*, H-2′eq), 1.92 (3H, *s*, H-19′), 1.98 (6H, *s*, H-20, H20′), 2.07 (1H, *dd*, H-4ax), 2.15 (2H, *q*, $J = 7.0$, H-4″), 2.33 (1H, *dd*, H-4eq), ≈3.45 (2H, *s*, H-7 and imp.), 3.98 (1H, *m*, H-3), ≈4.23 (1H, *m*, H-3′), 5.09 (2H, *s*, H-19), 5.62 (1H, *s*, H-4′), 5.64 (1H, *d*, $J = 15.6$, H-7′), 5.76 (1H, *dt*, $J = 15.6$, H-2″), 6.22 (1H, *d*, $J = 11.2$, H-10′), 6.27 (1H, *d*, $J = 12.0$, H-14′), 6.37 (1H, *d*, $J = 15.2$, H-12′), 6.39

(1H, *d*, $J$ = 16.0, H-8′), 6.42 (1H, *d*, $J$ = 12.8, H-14), 6.65 (2H, *dd*?, H-15, H-11′), ≈6.73 (3H, *m*, H-11, H-12, H-15′), 6.93 (1H, *dt*, $J$ = 7.0, 15.6, H-3″), 7.38 (1H, *dd*, $J$ = 4.0, 6.8, H-10). Acetylation gave two products with lower polarity (TLC). Alkaline hydrolysis provided:

*6′-Hydroxysiphonaxanthin* (**16A**). Vis $\lambda_{max}^{acetone}$ nm: 442, (463); $\lambda_{max}^{chloroform}$ nm: 461; $t_R$ = 10.2 (system as in [13], flow 1.25 ml/min); EIMS 70 eV, 220°, *m/z* (rel. int.): 616 $[M]^+$ (40), 598 $[M-18]^+$ (57), 582 $[M-18-16]^+$ (64), 580 $[M-18-18]^+$ (100), 566 $[M-18-16-16]^+$ (43), 564 $[M-18-18-16]^+$ (67), 562 $[M-18-18-18]^+$ (51); $^1$H NMR (400 MHz, $CDCl_3$ including $^1$H–$^1$H COSY): $\delta$ 0.94 (6H, *s*, H-16 or H-17, and H-16′ or H-17′), 0.99 (3H, *s*, H-16 or H-17), 1.02 (3H, *s*, H-16′ or H-17′), 1.50 (3H, *s*, H-18), ≈1.55 (1H, *m*, H-2ax), 1.67 (3H, *s*, H-18′), 1.67 (1H, *m*, H-2′ax), ≈1.74 (1H, *m*, H-2eq), 1.81 (1H, *dd*, $J$ = 6.4, 13.7, H-2′eq), 1.93 (3H, *s*, H-19′), 1.99 (6H, *s*, H-20, H-20′), ≈2.12 (1H, *m*, H-4ax), 2.33 (1H, *m*, H-4eq), 3.46 (H, *d*, $J$ = 18.1, H-7A), 3.53 (H, *d*, $J$ = 17.6, H-7B), 4.02 (1H, *m*, H-3), ≈4.25 (1H, *m*, H-3′), 4.48 (2H, broad *s*, H-19), 5.63 (1H, *s*, H-4′), 5.65 (1H, *d*, $J$ = 15.6, H-7′), 6.22 (1H, *d*, $J$ = 11.2, H-10′), 6.29 (1H, *d*, $J$ = 11.2, H-14′), 6.37 (1H, *d*, $J$ = 15.1, H-12′), 6.39 (1H, *d*, $J$ = 16.1, H-8′), 6.43 (1H, *d*, $J$ = 12.7, H-14), 6.60–6.80 (5H, *m*, H-11, H-12, H-15, H-11′, H-15′), 7.29 (1H, *m*, H-10). Acetylation gave the less polar mono-, di- and triacetylated derivatives. CD of the diacetate showed a positive Cotton effect between 220–280 nm.

*6′-Hydroxysiphonaxanthin triacetate* (**16C**). Vis as for **16**; $R_F$ = 0.25 (TLC plate 1 [2], 30% acetone in heptane); EIMS 70 eV, 220°C, *m/z* (rel. int.): 742 $[M]^+$ (25), 682 $[M-60]^+$ (59), 622 $[M-60-60]^+$ (100). Silylation of the triacetyl derivative gave the less polar corresponding monotrimethylsilyl triacetylderivative.

*6′-Monotrimethylsilyloxysiphonaxanthin triacetate* (**16B**): Vis as for **16**; $R_F$ = 0.42 (TLC plate 1 [2], 30% acetone in heptane); EIMS 70 eV, 220°, *m/z* (rel. int.): 814 $[M]^+$ (44), 682 $[M-72-60]^+$ (100).

## Codium fragile

*Codium fragile* was harvested at the coast of Western Norway in the summer of 1990. The material (1.036 kg frozen, wet weight) was homogenized and extracted with acetone followed by acetone/methanol 7:3. The extract was separated by CC (silica, increasing amounts of acetone in heptane) and TLC (silica/calcium carbonate plates), and authentic siphonein (**15**) and siphonaxanthin (**14**) were isolated. Total amount of carotenoids available: 7.4 mg.

*Siphonein* (**15**). Siphonaxanthin 19-(*trans*-Δ2-dodecenoate) (**15a**, 25% of total carotenoids). No siphonaxanthin 19-(*trans*-Δ2-decenoate) (**15b**) could be detected by HPLC and MS. CD $\lambda_{nm}^{hexane}$: 237 (Δε10), 249 (Δε ≈ 4), 273 (Δε18), 292 (Δε0), 340 (Δε2) (high Δε-values ascribed to instrument calibration.)

*Siphonaxanthin* (**14**, 30% of total carotenoids). Vis $\lambda_{max}^{acetone}$ nm: 441, (461); $R_F$ = 0.71 (TLC plate 2 [2], 11% EtOH in $CHCl_3$), $t_R$ = 13.5 (system [13], flow 1.25 ml/min); CD as for siphonein (**15**), see above; EIMS 70 eV, 210°, *m/z* (rel. int.): 600 $[M]^+$ (100), 582 $[M-18]^+$ (95), 566 $[M-18-18]^+$ (30); $^1$H NMR (400 MHz, $CDCl_3$, including $^1$H–$^1$H COSY), see Fig. 1: $\delta$ 0.84 (3H, *s*, H-17′), 0.93 (3H, *s*, H-16 or H-17), 0.99 (6H, *s*, H-16 or H-17, and H-16′), 1.36 (1H, *m*, H-2′ax), 1.49 (3H, *s*, H-18), ≈1.54 (1H, *m*, H-2ax), 1.62 (3H, *s*, H-18′), 1.72 (1H, *m*, H-2eq), 1.83 (1H, *dd*, $J$ = 5.8, 13.2, H-2′eq), 1.91 (3H, *s*, H-19′), 1.99 (6H, *s*, H-20, H20′), 2.11 (1H, *dd*, $J$ = 9.6, 16.5, H-4ax), 2.35 (1H, *m*, H-4eq), 2.40 (1H, *d*, $J$ = 9.9, H-6′), 2.79 (1H, broad *s*, 19-OH), 3.46 (1H, *d*, $J$ = 17.8, H-7A), 3.52 (1H, *d*, $J$ = 17.9, H-7B), 4.00 (1H, *m*, H-3), 4.24 (1H, *m*, H-3′), 4.48 (2H, *s*, H-19), 5.45 (1H, *dd*, $J$ = 10.0, 15.4, H-7′), 5.54 (1H, *s*, H-4′), 6.14 (2H, *d*, $J$ = 14.9, H-8′, H-10′), 6.27 (1H, *d*, $J$ = 11.4, H-14′), 6.36 (1H, *d*, $J$ = 14.9, H-12′), 6.43 (1H, *d*, $J$ = 11.6, H-14), 6.59–6.79 (5H, *m*, H-11, H-12, H-15, H-11′, H-15′), 7.29 (1H, *d*, $J$ = 10.7, H-10).

## Prasinococcus capsulatus

Total amount of carotenoids available: 18.5 mg. The carotenoid distribution is given in Table 1.

For characterization of lutein (**17**), zeaxanthin (**21**), antheraxanthin (**22**), violaxanthin (**23**), and neoxanthin (**24**), see *Pyramimonas amylifera* above.

*β,β-Carotene*. Vis $\lambda_{max}^{HPLC\text{-}eluent}$ nm: (421), 448, 472; for $R_F$, $t_R$-values cf. [2, 14].

*β,ε-Carotene*. Vis $\lambda_{max}^{HPLC\text{-}eluent}$ nm: (418), 443, 470; for $R_F$, $t_R$-values cf. [2, 14].

*ε,ε-Carotene*. Vis $\lambda_{max}^{HPLC\text{-}eluent}$ nm: 417, 440, 469; $t_R$ = 36.9 min (system [13], flow 1.25 ml/min).

*7′,8′-Dihydrolutein* (**12**). Vis $\lambda_{max}^{acetone}$ nm: (402), 426, 452; %III/II = 47, $D_V$ = 0.78; $R_F$, $t_R$-values, and mass spectrum [13, 14].

*Uriolide* (**5**). Vis $\lambda_{max}^{acetone}$ nm: 447, 469: %III/II = 5, $D_V$ = 0.91; for $R_F$, $t_R$-values, and mass spectrum cf. [14].

*Deepoxyuriolide* (**6**). Vis $\lambda_{max}^{acetone}$ nm: 450, (476); $R_F$ = 0.42 (plate 1) [2, 14], 6% EtOH in $CHCl_3$, $t_R$ = 22.8 (system as in [11], flow 1.25 ml/min); mass spectrum [11].

*Prasinoxanthin* (**1**). Vis $\lambda_{max}^{HPLC\text{-}eluent}$ nm: 455 (round shaped); $t_r$ = 17.6 (system [13], flow 1.25 ml/min); $R_F$-values and mass spectrum [2, 13, 14].

*Preprasinoxanthin* (**4**). Vis $\lambda_{max}^{HPLC\text{-}eluent}$ nm: 448, (466); $t_R$ = 17.0 (system as in [13], flow 1.25 ml/min); mass spectrum [13].

## *Nephroselmis olivacea*

The carotenoid distribution are given in Table 1. For characterization of *β,β*-carotene, lutein (**17**), zeaxanthin (**21**), antheraxanthin (**22**), violaxanthin (**23**) and neoxanthin (**24**), see *Pyramimonas amylifera* above.

*Acknowledgements*—We thank Dr Uve Schlösser for a starter culture of *Nephroselmis olivacea*. E. S. E. was supported by a research grant from Hoffmann-La Roche, Basel, to S. L.-J. R. R. L. G. was partially supported by Grant NA56RG0159 from NOAA to the University of Maine/University of New Hampshire. See Grant College Program.

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