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CELL WALL CAROTENOIDS IN GREEN ALGAE WHICH FORM SPOROPOLLENINS

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Key Word Index—Green algae; Chlorococcales; cell wall components; ketocarotenoids; sporopollenins; fritschiellaxanthin; 2,3-didehydro-fritschiellaxanthin; pigment mutants.

Abstract—Among the sixteen investigated algal strains (Chlorococcales) ten of them synthesize ketocarotenoids and sporopollenins which are located in the trilaminar structure of the cell wall. In contrast to the colourless cell walls of algae which are unable to form the above substances, the cell walls of strains synthesizing them are pinkish coloured due to the carotenoids which are mainly ketocarotenoids. Constant cell wall pigments are (3*S*,3′*S*)-astaxanthin, its oxidation products semiastacene and astacene, canthaxanthin, echinenone (only in cell walls obtained from homogenates), (3*S*,3′*R*)-fritschiellaxanthin, 2,3-didehydro-(6′*R*,3′*R*)-fritschiellaxanthin, (3*R*,3′*R*,6′*R*)-lutein and two unidentified carotenoids. All these cell wall-carotenoids are present in the free, unesterified form. This is the first discovery of the occurrence of fritschiellaxanthin and 2,3-didehydro-fritschiellaxanthin in the free form in plants. It is postulated that cell wall carotenoids of the described algae may be involved in sporopollenin formation.

INTRODUCTION

Natural algal strains belonging to the Chlorococcales (Chlorophyceae) show considerable differences in metabolism of carotenoids. The differences are attributed to unfavourable conditions of growth, especially deficiency of nitrogen and/or iron [1 5]. Such conditions occur in aging cultures of algae, in the stationary phase of growth. Two main algal types showing pigment changes can be distinguished. The cells of the first type are green, both in the log-phase as well as in the stationary phase, of growth. Only a short time before their decay the cells become successively pale and finally white. These strains are devoid of ketocarotenoids and are designated as '−' in the KC entry of Table 1. Cells of the other type change their colour from green during the log-phase of growth to yellow-green, orange or red in the stationary phase of growth. This change in colour is caused by accumulation of secondary carotenoids, mainly ketocarotenoids. Strains of this type are denoted as '+' in the KC entry of Table 1.

The increase in the KC level in cells of these algal strains is accompanied by the disappearance of chlorophyll and a decrease in primary carotenoids which are typical for chloroplasts, i.e. α- and β-carotene, lutein, lutein epoxide, violaxanthin, neoxanthin [3, 4] and in some strains loroxanthin [6].

Chemically, secondary carotenoids are derivatives of C_{40}-carotenoids, i.e. xanthophylls having hydroxyl groups and/or carbonyl groups in one or in both rings. The occurrence of the main representatives of this group, echinenone, canthaxanthin and astaxanthin, as well as of other related minor carotenoids, has been reported in various algae [1, 3–5, 7–10]. The ability to form secondary carotenoids is genus-specific in *Ankistrodesmus*, *Haematococcus* and *Scenedesmus* but species-specific in the genus *Chlorella*.

A characteristic feature of algae which produce KC is that they also form pink-coloured maternal cell walls (CWM) and eventually shed them into the culture medium. Such an example is *Scenedesmus obliquus*, strain 633, in which it has been confirmed that the pink colour comes from the presence of carotenoids, mainly KC [11]. Carotenoids have been reported also in complete algal cell walls isolated from homogenates of whole cells [11–14]. Cell wall carotenoids are located mainly or exclusively in the trilaminar structure, i.e. the outer layer of the cell wall of *Chlorella fusca* and *Scenedesmus obliquus* [11, 14–16]. This trilaminar structure is also the sporopollenin-containing cell wall structure [16]. Sporopollenins (SP) are assumed to be products of oxidative polymerization of carotenoids and/or their esters [17]. The co-occurrence of both kinds of substance in cell walls encourages a study of the biogenetic interrelationship between KC and SP.

The close relationship between three features of the cell walls, i.e. the occurrence of trilaminar structure, KC and SP, has been called by Atkinson *et al.* [16] 'three-way-correlation' and it is suggested that SP can be formed *in vivo* in a manner similar to that occuring *in vitro* [17, 18] from carotenoids and/or their esters by oxidative polymerization. The trilaminar structure was assumed to serve as a matrix for SP-polymerization and deposition [19].

The presence of KC together with SP in cell walls of all investigated algae belonging to the Chlorococcales suggests that KC can be precursors of SP, or metabolites formed parallel with it. For this reason it seemed worthwhile to check the 'three-way-correlation' hypothesis by studying the occurrence of cell wall carotenoids of several natural strains as well those of pigment mutants

Table 1. The carotenoid composition of maternal cell walls (CWM) and of complete cell walls obtained from the homogenate of disrupted algal cells (CWH) as a percentage of the total cell wall carotenoids of several algal strains*

Strain	*Ankistrodesmus braunii* 202-7c (G)		*Scenedesmus quadricauda* s. 449 (IZ)		*Scenedesmus obliquus* s. 633 (IZ)		*Chlorella fusca* 211-8p (G)		*Chlorella* sp. 113 (L)		*Chlorella fusca* 211 15 (G)		*Chlorella fusca* 211-8b (G)	
Culture age (days)	10		30		30		30		30		30		30	
Medium	I		I		I		I		I		I		I	
Cell wall Pigment	CWM	CWH	CWM	CWH	CWM	CWH	CWM	CWH	CWM	CWH	CWM	CWH	CWM	CWH
β-Carotene	–	trace	–	trace	–	trace	–	trace	–	trace			–	trace
Echinenone	–	–	–	trace	0–0.94	1.04	–	–	–	1.57	–		–	0.58
Canthaxanthin	–†	–†	+	48.94	20.66	27.93	4.22	6.62	+	9.84	5.62		+	9.78
Astacene	55.89	54.63	+	12.33	21.78	18.18	37.88	31.23	+	28.59	19.18		+	19.35
2,3-Didehydro fritschiellaxanthin	20.49	20.82	+	15.21	34.36	32.71	34.84	39.71	+	34.82	41.68		+	40.62
Lutein	23.59	24.32	+	23.15	22.89	20.13	23.06	22.38	+	25.17	35.51		+	29.66
Total carotenoids (μg/g CW dry wt)	123	39		129	166	128	167	68		83	84			85
KC‖	+	+		+	+	+	+	+	+	+	+		+	+
SP¶	+	+		+	+	+	+	+	+	+	+		+	+

Strain	*Chl. fusca mutant* 308 (Cz)		*Chlorella* sp. 620 (Iz)		*Chl. fusca* C.1.1.10 (Cz)		*Chl. fusca* mutant C.1.1.10.16 (Cz)		*Chl. fusca* mutant C.1.1.10.31 (Cz)		*Chl. fusca* mutant C.1.1.10.14 (Cz)		*Chl. vulgaris* 211-1e (G)		*Chl. saccharophila* 211-9a (G)		*Chl. vulgaris* 211-8k (G)	
Culture age	30		30		30		30		30		30		30		30		8	
Medium	I		I		I		I		I		I		I		I		I	
Pigment / Cell wall	CWM	CWH	CWM	CWH	CWM	CWH	CWM	CWH	CWM‡	CWH	CWM‡	CWH	CWM‡	CWH	CWM‡	CWH	CWM	CWH
β-Carotene	–	trace		trace	–	trace	–	trace	–		–		trace		trace		trace	
Echinenone	–	–		3.39	–	trace	–	–	–		–		–		–		–	
Canthaxanthin	+	8.39		23.68	10.97	11.67	–	–	–		–		–		–		–	
Carotenoid X§	–	–		13.47	–	–	–	–	–		–		–		–		–	
Carotenoid Y§	–	–		15.13	–	–	–	–	–		–		–		–		–	
Astacene	+	45.29		5.91	48.06	32.25	–	–	–		–		–		–		–	
2,3-Didehydro fritschiella xanthin	+	12.77		5.16	28.68	33.50	–	–	–		–		–		–			
Lutein	+	33.55		33.25	12.29	31.90	–	trace	trace		–		trace		trace		trace	
Total carotenoids (μg/g CW dry wt)		89		47	207	67		trace	trace		–		trace		trace		trace	
KC‖	+	+	+	+	+	+		–	–		–		–		–		–	
SP¶	+	+	+	+	+	+		–	–		–		–		–		–	

*Carotenoids are listed according to the sequence on TLC. Data in this Table concern the hydrolysed carotenoids.

†Small quantities of canthaxanthin were found in whole cells of this strain [3].

‡CWM quantities were insufficient to carry out analyses.

§Additionally two carotenoids X: λ_{max}^{EtOH} 421, 445, 473 and Y: λ_{max}^{EtOH} 421, 445, 473 nm located on TLC between canthaxanthin and astacene were found. Their position on chromatogram is similar to that of cryptoxanthin.

‖KC '+' indicates the presence of ketocarotenoids (pinkish coloured cell walls); KC '–' indicates the absence of ketocarotenoids (colourless cell walls); + carotenoids confirmed qualitatively; – absence of carotenoid.

¶SP '+' presence; '–' absence of sporopollenins in cell walls.

with altered or blocked metabolism of KC and SP.

Cell wall carotenoids have been isolated and estimated in the strains listed in Table 1. Special attention has been given to elucidation of the absolute configuration of cell wall carotenoids.

RESULTS AND DISCUSSION

Sixteen algal strains were tested (Table 1). Among them were three *Chlorella* mutants which are derivatives of *Chl. fusca*, C.1.1.10. defective in synthesis of KC. These mutants were induced from the wild strain which was able to form all three markers (trilaminar structure, KC and SP). It was shown that they were not forming SP and KC. They are the first described SP-deficient mutants [20]. The orange-coloured mutant 308 of *Chl. fusca*, 211-8b studied so far has been shown to be identical with its parent type with regard to KC, SP and trilaminar structure [3, 15, 16, 20, 21]. All tested strains confirm the hypothesis of the existence of a biogenetic connection between KC and SP in these algae (Table 1). It was shown that all aforementioned algal strains forming these three markers released CWM into the culture medium as a result of cell division (autospore liberation). They were pinkish-red coloured due to the presence of carotenoids (mainly KC). These strains have been described as '+' in Table 1. In some cases, e.g. *Ankistrodesmus braunii* 202-7c or some *Chlorella* strains, a lower intensity of CWM-colouration was observed. The colour of such CWM preparations should be observed in a layer of adequate thickness obtained by centrifugation. It should be emphasized that CWM of all strains forming SP are pinkish in colour. This is in contrast to the colourless CWM of strains unable to form KC and SP designed as KC '−' in Table 1. CWM of strains unable to form SP were obtained in much smaller quantities than previously described pigmented CWM. This seems to be caused by their disappearance (autolysis) from culture medium.

CWH of SP-forming algae are characterized by a similar but less intense (more beige) colour caused by traces of chlorophylls. Aqueous suspensions of both kinds of cell walls show a broad band of absorption at 450–500 nm. Petrol extracts show more distinct absorption maxima at 447–449 nm and 468–470 nm, similar to those presented previously [11]. Extracts from clean CWM did not show any measurable maximum in the red range of the spectrum, whereas extracts from CWH showed small maxima which resulted from traces of chlorophylls.

From the CW-extracts individual carotenoids were obtained by TLC and in some cases additionally by HPLC (Table 2). The use of HPLC allowed the TLC separations to be optimized. The employment of TLC plates partially impregnated with citric acid resulted in clear separations. The majority of CW-carotenoids were identified previously [11, 15]. The following carotenoids have been discovered as integral CWM-components of the genus *Chlorella* and *Scenedesmus*: astaxanthin, canthaxanthin, lutein and two KC identified as 2,3-didehydrofritschiellaxanthin (**3**) and fritschiellaxanthin (**2**). In CWH of *Scenedesmus* as well as in some *Chlorella* strains, small quantities of echinenone were found in addition.

Structural elucidations of the carotenoids present in the CWM were carried out on μg quantities by absorption, ^{1}H NMR, CD and mass spectrometry studies, as well as using the HPLC data.

Carotenoids **2** and **3** had R_f and R_t values distinct from all authentic carotenoids. The 'rounded' double peak absorption spectrum of these substances showed the presence of a carbonyl group. The typical three-peak fine structure was restored after reduction with sodium borohydride which caused a hypsochromic shift (Fig. 1). Carotenoid **3** showed on HPLC a higher polarity then β-doradecin. The mass specturm with *m/z* 580 was consistent with $C_{40}H_{52}O_3$. The ^{1}H NMR ($CDCl_3$) spectrum allowed the identification of the (3',6')-*trans*, i.e. (3'*R*,6'*R*)-configuration. The similarity of the CD spectra of pigment **3** and lutein **1** support the (3'*R*,6'*R*)-chirality. The data presented confirmed the structure of 2,3-didehydro-(6'*R*,3'*R*)-fritschiellaxanthin (**3**). This is the first discovery of this pigment in algae or plants. It was previously reported in an animal *Sesarma haematocheir* (crab) and probably originated from algae. It has also been obtained semisynthetically by oxidation of fritschiellaxanthin ester found in *Fritschiella tuberosa* [22]. The mass spectrum of carotenoid **2** with *m/z* 582 was consistent with $C_{40}H_{52}O_3$ as required for adonixanthin. However, this pigment (**2**) did not show an identical R_t value to β-adonixanthin. Its higher polarity, suggested the presence of α-adonixanthin. The CD spectrum (Fig. 2) confirmed the presence of the *S*-

Table 2. Native (not hydrolysed) CWM-carotenoids of *Scenedesmus obliquus*, strain 633 (30-day-old culture, medium IV, illumination 2000 lux)

Peak No. from start	Pigment	Absorption maxima measured in (nm): Effluent of HPLC1*	Solvent
1.	Astacene	472, 473	486 (CH_2Cl_2)
2.	Canthaxanthin	467	
3.	Semiastacene	474	
4.	2,3-Didehydrofritschiellaxanthin	454 (sh), 470	
5.	Astaxanthin	473	
6.	Unidentified carotenoid	373, 443, 460	
7.	Unidentified carotenoid	469	
8.	Fritschiellaxanthin	452, 469	452, 470 (EtOH)
9.	Lutein	420, 443, 473	

*Solvent system: *n*-hexane–10% CH_2Cl_2–0.75% *iso*-PrOH.

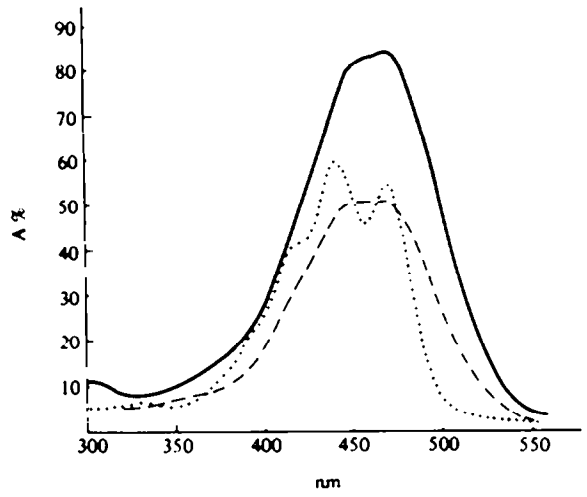

Fig. 1. Absorption spectrum of: - - - fritschiellaxanthin (2); . . . . reduced 2 with $NaBH_4$; —·— 2,3-didehydro-fritschiellaxanthin (3), all spectra in conc. EtOH.

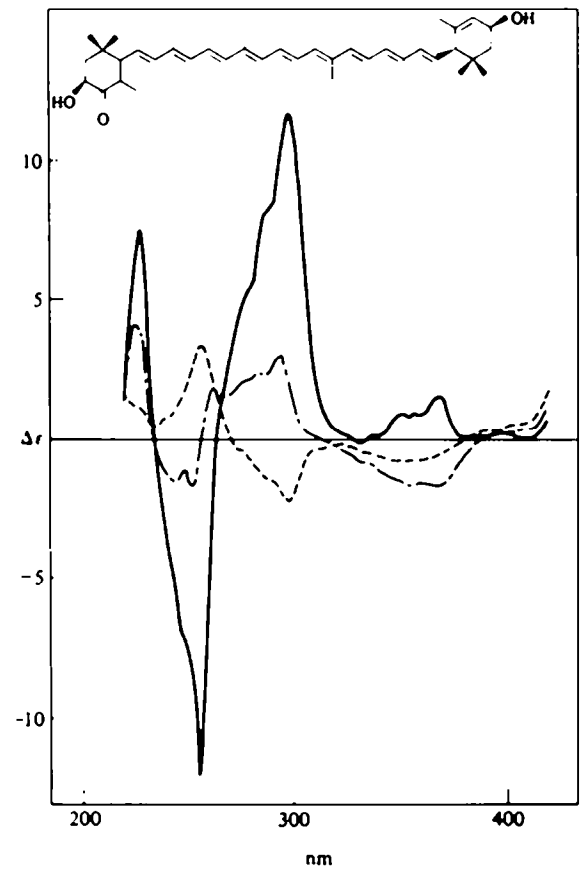

Fig. 2. CD spectrum of fritschiellaxanthin, from CWM-*Scenedesmus obliquus*, strain 633. - - at + 20°; - · - · - at − 120°; —— at − 180°.

type configuration at C-3 by analogy to astaxanthin with this configuration [23]. The CD spectrum of this KC recorded at − 120° and − 180° showed a clear conversion point of the values with increasing cooling, similar to that observed for astaxanthin (Fig. 2). The ^{1}H NMR spectrum of **2** was similar to those of astaxanthin and lactucaxanthin. Pigment **2** showed chemical shifts typical for an end group of the 3',6'-ε (0.85 ppm) type and for astaxanthin [24]. Differentiation between the (3'*R*,6'*R*) and (3'*S*,6'*S*) configurations was made on the basis of the CD spectrum of this pigment after reduction with sodium borohydride to yield the trihydroxy derivative of pigment **2**. This spectrum was very similar to that of (3*R*,3'*R*,6'*R*)-*trans*-lutein but different from the CD spectrum of 3'-epilutein, the 3',6'-*cis*-epimer with (3*R*,3'*S*,6'*S*)-chirality. The ^{1}H NMR spectrum of pigment **2** excluded the configuration of 3'-epilutein and confirmed the configuration of all-*trans*-fritschiellaxanthin (**2**). The CD spectrum (Fig. 2) indicates that a part of compound **2** may have the *cis*-configuration in the polyene chain (peak at 350 nm). This is the first report of the presence of free (unesterified) fritschiellaxanthin in the cell wall of an alga.

HPLC showed the presence of astacene and semi-astacene as well that of all-*trans*-lutein in CW of the algae. It also showed the presence of a yellow dihydroxy carotenoid characterized by the same R_f value as that of lutein. This gives unequivocal evidence for the presence of the (3',6')-chirality, i.e. all-*trans*-lutein, in contrast to 3'-lutein, which was not found in cell walls. Additionally, small quantities of unidentified carotenoids were found in CWM extract of *Scenedesmus obliquus* (peaks **6** and **7**, Table 2). The quantities of these compounds only allowed a recording of the absorption spectrum. The 'rounded' maxima of both these minor carotenoids seem to indicate KC. The small peak at 373 nm of pigment **6** indicates that

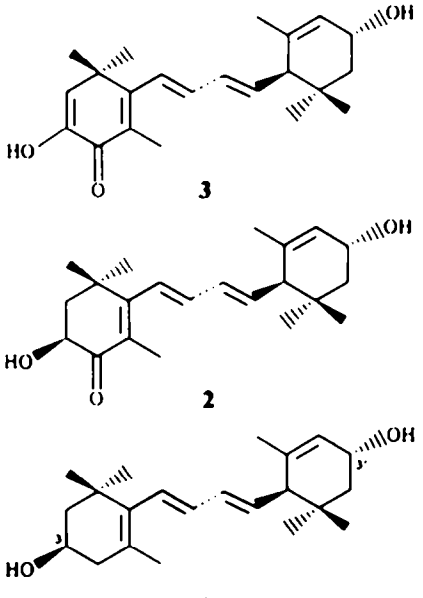

Fig. 3. Chirality of lutein (**1**), fritschiellaxanthin (**2**) and 2,3-didehydro-fritschiellaxanthin (**3**).

a part of this pigment is present as a *cis*-isomer in the polyene chain. Sporadic increases in the level of carotenoid **6** to values similar to those of fritschiellaxanthin were observed in CWM of *Scenedesmus obliquus*, 633. However, factors producing this increase were not elucidated.

Traces of other carotenoids, such as β-carotene, violaxanthin and neoxanthin were not observed in clean CWM. They were found only in CWH. These minor pigments, as well as chlorophylls, were due to contamination derived from extra cell wall components, which were adsorbed on the cell wall surface. Their disappearance from CWH preparations after successive cleaning, and their concentration changes independent of KC, support the conclusion that these minor pigments are merely contaminants. In contrast, KC (especially of CWM) are integral cell wall components. The high level of KC in cell walls and their low level in homogenates of the algae, exclude the possibility of an artifact or contamination from other cell components [14].

A great similarity in the carotenoid patterns of CWH and CWM of *Scenedesmus obliquus* is obvious [14]. KC amount to 1.1 % of the total cell carotenoids. Among cell wall-pigments KC clearly prevail: 85 % for CWH and 94.1 % in CWM.

It is a characteristic feature of algal CW-carotenoids that they all exist in free form [15]. This contrasts with the accumulation of esterified carotenoids in other cell structures of algal cells during ageing.

Experiments with Triton X-100 (1 % aqueous solution) showed the possibility of removing a considerable part of the pigments originating from the homogenate and adsorbed on the CWH surface, i.e. β-carotene, violaxanthin, neoxanthin and chlorophylls. A considerable decrease in lutein content in comparison with that of KC was observed (Table 3). Carotenoids typical for CW occur probably as complexes formed with other CW-components. Their stability causes failure when attempts have been made to isolate carotenoids as soluble and native complexes by using various solutions of salts, solubilization mixtures [25], detergents and 8 M urea. Actually there are no reported successful reagents and procedures for the solubilization of the trilaminar structure of the cell wall with the carotenoid- and SP-containing complexes.

The average content of total carotenoids in algal CW shows a considerable variation within the range of 40–460 μg/g of dry wt. The content is in all cases higher for CWM than for CWH.

The carotenoid patterns of the CW obtained from 10 algal strains show considerable similarities (Table 1). In all cases canthaxanthin, astacene and 2,3-didehydrofritschiellaxanthin were found in hydrolysates of CW-carotenoid extracts. The two last mentioned carotenoids arose as oxidation products of astaxanthin, semiastacene and fritschiellaxanthin, respectively. Other minor KC of the CWM were not taken into account in routine analyses made by TLC. Echinenone was found only in some strains (*Ankistrodesmus*, *Scenedesmus* and some *Chlorella*) and then only in small quantities. Therefore it is impossible to exclude their presence in other strains but in quantities too small to be detected by the analytical methods employed. Similarly, canthaxanthin was not confirmed in CW of *Ankistrodesmus braunii* 202-7c, known as a strain capable of forming this carotenoid [3]. This may have resulted from too small a quantity of canthaxanthin being present or to its rapid disappearance.

Carotenoids exist in algal CW mainly as all-*trans* isomers. The presence of various isomers of CW-astaxanthin were shown in the HPLC studies. The following isomers were separated as (−)-dicamphanates from CWH-extracts of *Chlorella* mutant 308: (3*S*,3′*S*)-*trans*-astaxanthin (main component), (3*S*,3′*S*)-*cis*-astaxanthin (small quantities) and (3*S*,3′*R*)-meso-*trans*-astaxanthin (traces). It is difficult to say with absolute certainty whether this latter minor component is an artifact of the work-up procedure [26].

The discovery of several chemically very closely related carotenoids in the CW of many different algal strains (all forming SP) may suggest that these pigments represent stages of a distinct metabolic chain. The significance of this presumed pathway would be in the transformation of the ε-ring of lutein into derivatives of β-carotene similar to the biosynthetic pathway postulated in animals [22, 27, 28]. However, the involvement of CW-KC in the biogenesis of SP seems to be very probable in algae

Table 3. Influence of Triton X-100 on the CW-carotenoid pattern of *Scenedesmus obliquus*, strain 633 (30-day-old cultures medium IV, illumination 2000 lux)

	CWM		CWH (control)		CWH + Triton X-100	
Pigments	μg/g dry wt	%	μg/g dry wt	%	μg/g dry wt	%
β-Carotene			trace	trace		—
Echinenone			trace	trace	trace	trace
Canthaxanthin	35.16	20.66	20.51	25.52	15.27	26.71
Astaxanthin + astacene	39.82	23.38	16.76	19.22	12.96	22.67
2,3-Didehydrofritschiellaxanthin	43.32	25.44	22.82	26.17	16.12	28.20
Lutein	22.45	13.18	17.56	20.14	6.65	11.63
Violaxanthin	—		2.15		—	
Neoxanthin	—		1.71			
Total carotenoid (μg/g CW dry wt)	174.12		87.18		57.16	
Chlorophyll *a*			28.46		—	
Chlorophyll *b* (μg/g CW dry wt)	—		10.97		—	

belonging to the Chlorococcales, but the presented results do not give final arguments confirming this fact. These results also do not explain the sequence of the KC pathway as well as the previous hypothesis [2] that echinenone can be the immediate precursor of SP in algae [17, 18]. Currently, nothing is known about the site of formation of KC, their transport into the CW, or about plasmalemma or CW-bound enzyme activities which would be able to transform carotenoids and polymerize them.

The fact that SP can be formed *in vitro* from vitamin A palmitate [17] means that not only carotenoids but also smaller molecules of a terpenoid nature can be polymerized to SP. It seems very probable that similar substances may serve as SP precursors also *in vivo*. Although in all reported green algae belonging to the Chlorococcales the formation of SP seems to be closely related with the presence of KC in CW, this co-occurrence cannot serve as a final argument for the existence of a biogenetic relationship between both groups of substances. For these reasons specific SP-precursors and appropriate polymerases may exist in other systematic groups of algae and other plant taxa.

EXPERIMENTAL

Biological material. Axenic strains, listed in Table 1, were used. Origin of strains: Cz—Prof. F.-C. Czygan, University of Würzburg, G- Collection of Pringsheim, University of Göttingen; IZ—Institute of Zootechnics (Cracov); L—University of Leningrad. All strains were grown mixotrophically under sterile conditions in Erlenmayer flasks closed with cotton wool plugs.

Most of the strains were cultivated on medium I of Kessler *et al.* [30] enriched with 5 g of glucose/l. Medium II of Kessler *et al.* [30] was used for testing the presence of secondary carotenoids in algal cells. Medium III was as medium I [30] but plus 0.2 % glucose and 0.2 % casamino acids ('Difco'). *Scenedesmus obliquus*, strain 633 was cultivated on medium I, as well as on medium IV [15, 31]. Growth conditions were the same as described previously [11]. Generally, 30-day-old cultures were used for isolation of maternal cell walls (CWM) as well as for complete cell walls from homogenates (CWH) of mechanically disrupted cells. The isolation and purification procedures were the same as previously described [14].

Methods. For analytical studies 0.4–1.0 g dry wt of CWH and 0.060–0.23 g dry wt of CWM were used. The cell walls were harvested by centrifugation at 4500 *g* and quantitatively extracted with 10–15 ml aliquots of Me_2CO precooled to 0°. Pigments were transferred to petrol, dried with Na_2SO_4 and concd under N_2. TLC was carried out on [1] silica gel G, solvent system: petrol (93°)–*iso*-PrOH–$CHCl_3$ (9:1:7) [3]. TLC plates [2] impregnated to 10 cm by immersion in a 2.5 % methanolic soln of citric acid were used to eliminate 'tail'-forming zones [3]. Carotenoids were eluted from TLC by EtOH, concd and used in part in the native form, especially for structure elucidation. In most cases for the quantitative comparison of carotenoid patterns of various strains the extracts were hydrolysed with an equal vol. of a 10 % methanolic soln of KOH, under N_2, for 2 hr at room temp. Quantitative pigment analyses were the same as previously described [11, 14, 15].

The HPLC was carried out using an Altex 100 (Perkin–Elmer) with a UV-VIS detector LCD-726 (Kontron) [1], stationary phase: Nucleosil 100-5 H_3PO_4 (250 × 4 mm); system 1: *n*-hexane, 10 % CH_2Cl_2, 0.75 % of *iso*-PrOH. The separation of lutein and 3'-epilutein was carried out [2] on the stationary phase Spherisorb S 5-W-Silica using system 2: *n*-hexane–CH_2Cl_2–*iso*-PrOH–*N*-ethyldiisopropylamine (91.9:6.5:2.5:0.1).

Lutein (1). From CWM of *Scenedesmus obliquus* 633, CD spectrum in E.P.A. *M*, 569.0 (MS).

Fritschiellaxanthin (2). From CWM of *Scenedesmus obliquus* 633. The pigment had λ_{max}^{EtOH} nm: 452, 469 (Fig. 1); MS *m/z* (rel. int.): 582 $[M]^+$ (3) $[C_{40}H_{52}O_3]$, 564 $[M-H_2O]^+$ (22), 546 $[M-2H_2O]^+$ (3), 472 $[M-110]^+$ (4), 458 $[M-124]^+$ (6), 442 $[M-110]^+$, (3); 410 $[M-172]^+$ (7), 233 $[C_{15}H_{21}O_2]^+$ (5), 219 $[C_{14}H_{21}O_2]^+$ (4); this latter corresponds to fragment Z described by Enzell *et al.* [32]. ^1H NMR ($CDCl_3$, 400 MHz): δ0.81 (*s*, H_3-1', ax) 1.01 (*s*, H_3-1', eq), 1.211 (*s*, H_3-1, eq), 1.322 (*s*, H_3-1, ax), 1.364 (*m*, H-2, ax), 1.626 (*s*, H_3-5'), 1.810 (*m*, H-2, ax), 1.84 (*m*, H-2, eq), 1.54 (*m*, H-2, eq), 1.913 (*s*, H_3-9'), 1.946 (*s*, H_3-5), 1.974 (*s*, H_3) and 1.981 (*s*, H_3 from C-13 or C-13'), 1.997 (*s*, H_3-9), 2.405 (*d*, H-6'), 3.677 (*d*, H-3, OH), 5.549 (*m*, H-4'), 4.320 (*m*, H-3, ax), 5.440 (*m*, H-7), 5.547 (*s*, H-4'), 6.137 (*d*, H-10'), 6.139 (*d*, H-8'), 6.206 (*d*, H-7), 6.257 (*d*, H-10), 6.305 (*d*, H-14), 6.358 (*d*, H-12'), 6.432 (*d*, H-8), 6.444 (*d*, H-12), 6.628 (*m*, H-11 + H-11'), 6.635 (*m*, H-15 + H-15').

Reduction of 2 with $NaBH_4$ in concd. EtOH for 30 min restored the typical carotenoid three-peak fine structure λ_{max}^{EtOH} nm: 421, 443, 471 (Fig. 1); $E\,|_{cm}^{1\%} = 2500$; CD spectrum (Fig. 2).

2,3-*Didehydro-fritschiellaxanthin* (3). The absorption spectrum shows λ_{max}^{EtOH} nm: 454, 470 (Fig. 2). MS *m/z* (rel. int.): 580 $[M]^+$ (1), $[C_{40}H_{52}O_3]$, 562 $[M-H_2O]^+$ (8), 470 $[M-H_2O$ – toluene or β-end group$]^+$ (1), 410 $[M-170$ (dimethylnaphtalene$)]^+$ (8), 267 $[M-313]^+$, (11), 203 $[C_{13}H_{15}O_2]^+$, (70), 91 $[C_7H_7]^+$ (100); the latter corresponds to tropylic ion. ^1H NMR ($CDCl_3$, 400 MHz): δ0.851 (*s*, H_3-1', ax), 0.825 (*s*, H_3-1', eq), 0.892 (*s*, H_3-1, eq), 0.912 (*s*, H_3-1, ax), 0.921 (*s*, H_3-5'), 0.939 (*s*, H_3-5), 1.001 (*s*, H_3-13' or 13), 1.254 (*s*, H_3-9), 1.298 (*s*, H_3-9'), 1.626 (*s*, H-18'), 1.845 (*q*, H-2'), 1.913 and 2.029 (*d*, H-19 and H-19'), 1.975 and 1.987 (*d*, H-20 and H-20'), 2.096 (*s*, H-18), 2.40 (*d*, H-6'), 5.45 (*q*, H-7'), 5.55 (*as*, H-4'), 6.04 (*s*, H-2), 6.09 (*s*, H-1), 6.12 and 6.12 (*d*, H-10'), 6.24 and 6.27 (H-8'), 6.31 and 6.37 (*d*, H-7), 6.27 6.39 (*m*, H-14) and (H-14'), 6.34 and 6.39 (*d*, H-12'), 6.399 (*s*, OH), 6.49 and 6.55 (*d*, H-8'), 6.6 6.7 (*m*, H-11) and (H-11'), 6.6 6.7 (*m*, H-15) and (H-15'). CD spectra were recorded on modified Dichrograph 185 (Jobin-Yvon). All CD spectra ($\Delta\varepsilon$) were recorded in E.P.A. (Et_2O isopentane–EtOH, 5:5:2).

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