Phytochemistry, Vol. 26, No. 1, pp. 113–119, 1987
Printed in Great Britain

0031-9422/87 $3.00 + 0.00
Pergamon Journals Ltd.

BIOGENETIC RELATIONSHIPS BETWEEN KETOCAROTENOIDS AND SPOROPOLLENINS IN GREEN ALGAE*

J. BURCZYK†‡

†Silesian Medical Academy, Department of Pharmacognosy and Phytochemistry, Katowice-Sosnowiec, ul. Jagiellońska 4, Poland; ‡Institute of Zootechnics, Cracov; Laboratory of Applied Biochemistry, PL-43400 Cieszyn/Gumna 43, Poland

(*Received* 18 *February* 1985)

Key Word Index Green algae; Chlorococcales; cell wall; ketocarotenoids; sporopollenins.

Abstract The IR characteristics are given of algal cell wall biopolymers which are highly resistant to non-oxidative degradation and which are called sporopollenins (SP). IR maxima of SP make it possible to confirm their presence in maternal cell walls (CWM) among other cell wall-components accompanying SP, among them proteins. Levels of SP in cell walls obtained from homogenates (CWH) of various strains are presented. An increase of SP in CWH of 3–12% occurred during the aging of cultures. The highest SP content of all hitherto described biological structures was observed in CWM (33–41% of dry wt). The deposition of SP in cell walls (CW) was accompanied by quantitative changes of CW-carotenoids. These are the first data concerning the dynamics of CW-carotenoid accumulation and SP-deposition in algal cell walls. It was shown, that SAN 9789, a known inhibitor of carotenogenesis, inhibits both the biosynthesis of CW-carotenoids and the forming of SP.

The hypothesis is presented that CW-carotenoids (mainly ketocarotenoids) found only in algae forming SP may be involved in the biosynthetic pathway to SP.

INTRODUCTION

Recently, attention has been focused on the dependency between three features of green algae: the formation of secondary carotenoids, the trilaminar outer cell wall layer (TLS) and sporopollenins (SP).

It has been confirmed that algae which are able to synthesize secondary carotenoids also produce cell walls which contain SP [1, 2]. Cell walls of these algae are pinkish-pigmented. This colouration arises from the presence of carotenoids, mainly ketocarotenoids (KC) [2, 3]. This finding is especially interesting because SP are considered as polyterpenes [1] which are formed by the oxidative polymerization of carotenoids and/or their esters.

SP can be synthesized *in vitro* from several carotenoids and their esters by oxidative copolymerization in the presence of BF_3 as catalyst. It is very probable that *in vivo* carotenoids and/or their esters could be polymerized to SP [1, 4, 5]. It was established that algal mutants defective in forming of KC contain neither SP nor TLS [2, 6, 7]. The co-occurrence of KC and SP in CW of algae from one side, as well as the absence of SP in several algal mutants and natural strains showing the absence of KC in CW, encourages a study of the biogenetic interdependence between KC and SP.

The present paper reports: the content of SP in CW of various algal strains of the Chlorococcales, the properties of SP isolated from algal CW, as well as the effects of an inhibitor of carotenogenesis (SAN 9789) on the level of carotenoids and SP in CW of these algae.

*Part 2 in series. For part 1 see ref. [12].

RESULTS AND DISCUSSION

The physico-chemical characterization of SP obtained from algal CW was based on the IR spectra. The IR spectra of two SP-samples from *Chlorella fusca*, strain 211-8p and *Scenedesmus obliquus*, strain 633 are shown in Fig. 1. The SP of *Chlorella* and *Scenedesmus* contain polymeric hydroxyls (broad band at 3100–3500 cm^{-1}). Two maxima between 2800 and 3000 cm^{-1} are typical for methyl and CH_2 stretching. The peaks in the range of 1616–1734 cm^{-1} can be due to the presence of C=C bonds (stretching) as well as of C=O groups; that at 1544 cm^{-1} is due to C=C bonds. The absorption maximum at 1466 cm^{-1} may be caused by methyl and $-CH_2-$ groups (asymmetric bending) or by C=O groups; at 1370 cm^{-1} by chiefly methyl symmetric bending; at 1163–1168 cm^{-1} by ether groups (C–O–C). The appearance of maxima between 1000 and 1100 cm^{-1} may originate from hydroxyl deformation and C–O stretching. However, the maxima in this range may also indicate the presence of C–C bonds of high cross-linking polymers as well as that of C–O–C groups. The occurrence of a peak at 960 cm^{-1} may be caused by *trans*-double bonds (C–H out of the plane deformation). The absorption maximum at 717–720 cm^{-1} may originate from *cis*-double bonds and also, possibly in part, from long methylenic chains $-(CH_2)_n-$, $n \geq 4$ (skeletal vibrations). This peak was also present in the SP-like polymer of *Botryococcus braunii* [8], but absent in synthetically obtained SP from β-carotene [4, 9]. Both absorption maxima at 720 cm^{-1} as well as that at 1466 cm^{-1} seem to be a characteristic features for previously investigated samples of SP and SP-like polymers of the green algae. Both mentioned absorption bands (Fig. 1) can serve as a simple test for the

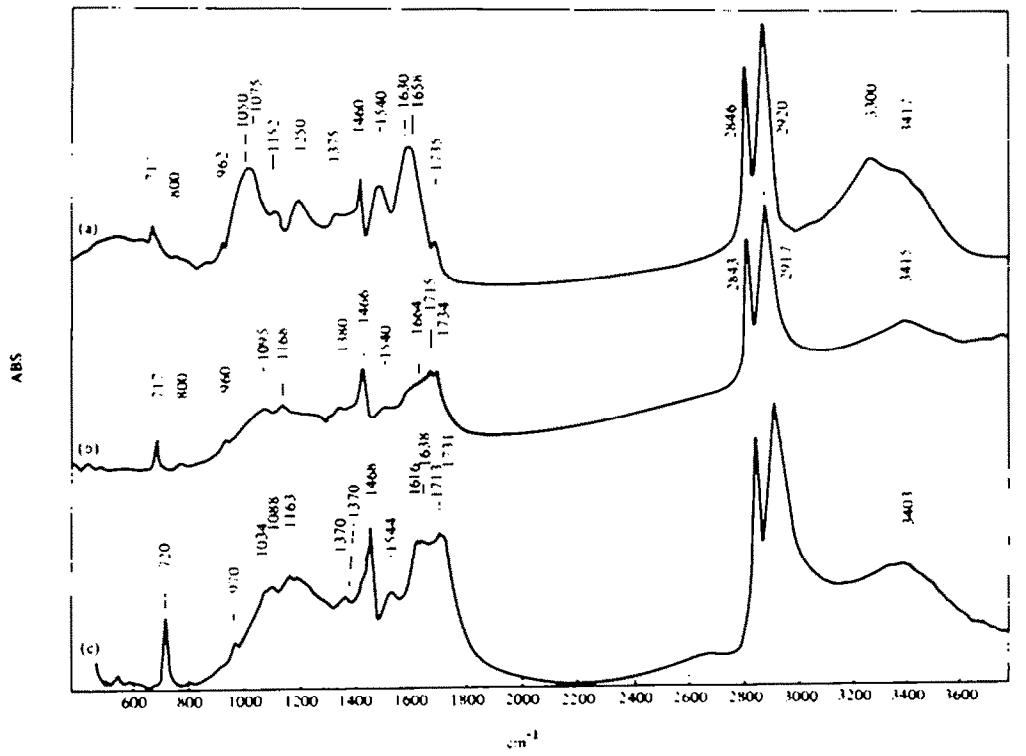


Fig. 1. IR spectrum of: (A) delipidized CWM of *Chlorella fusca*, strain 211-8p; (B) sporopollenins from the same cell walls; (C) sporopollenins from CWH of *Scenedesmus obliquus*, strain 633.

detection of the presence of SP in CWM without using acetolysis [2, 10].

Additionally absorption maxima present in spectrum A (Fig. 1) of CWM of *Chlorella fusca*, strain 211-8p indicate the presence of amide groups (1245–1250, 1535 and ~ 1650 cm^{-1}) [10] and they give evidence of the co-occurrence of SP with proteins in the trilaminar structure forming the outer CW layer of the described algae.

The IR data suggests that SP of *Chlorella* and *Scenedesmus* exhibits both the properties of SP synthetically obtained from β-carotene as well as of SP-like polymers of *Botryococcum braunii* described by Berkaloff *et al.* [8]. This may be caused by the heterogenic nature of SP and the prevelance of different chemical groups forming different SP types in various algae.

The CWM of *Chlorella* and *Scenedesmus* contain 33–41 % of SP in the dry wt of CWM (Table 1). This is the highest SP content of all hitherto described biological structures. It is 4–8 times higher than that of CWH of the same algae. This results from the fact that SP are mainly or exclusively located in the outer layer of the complete CW (CWH) in their trilaminar structure. The CWM preparations consist practically exclusively of trilaminar structures. Small quantities of cellulose-like acetolysable polysaccharide found in CWM may arise from the rest of the acetolysable inner 'cellulosic' layer of CWH. It consists of glucose and mannose [11]. The level of cellulose-like polysaccharide accounts for 3–5 % for *Scenedesmus* and 0.03–0.15 % for CWM of *Chlorella*. This content decreases with prolonged incubation time of CWM in the culture

Table 1. Sporopollenins and cellulose-like polysaccharide contents of CWM of *Chlorella fusca*, 211-8p and *Scenedesmus obliquus*, 633, isolated from cultures of different ages (conditions: 2000 lux)

Strain	Age of culture used for CWM isolation days	Sporopollenins % of dry wt of delipidized CWM	Cellulose-like polysaccharide % of dry wt of delipidized CWM
Chlorella fusca str. 211-8p	10	35.36 ± 2.10	15.02 ± 3.20
(medium I)	30	33.71 ± 3.56	0.03 ± 0.03
Scenedesmus	10	33.42 ± 0.33	5.14 ± 0.89
obliquus, str. 633	30	37.17 ± 0.63	4.30 ± 0.69
(medium IV)	60	40.48 ± 2.10	3.44 ± 1.53
	90	41.30 ± 0.99	3.53 ± 1.91

medium, i.e. the age of culture. In contrast to the decrease of cellulose-like material content in CWM an increase of SP concentration during the time of the experiment was observed (Table 1). From the increasing SP concentration and the decreasing cellulose-like material content it can be assumed that not only cellulose-like material but also other components disappear from CWM.

Table 2 presents the contents of SP in CW isolated from the homogenate (CWH) of mechanically disrupted algal cells at different times during the aging of the culture. The comparison of the data in Table 2 with the data given in a previous paper [12] proves that SP occurs only in algae belonging to Chlorococcales, which synthesize KC and show their presence in CW. Examples of this type of algae are *Ankistrodesmus braunii*, strain 202-7c, *Chlorella* sp. 113, *Chl. fusca*, strain 211-8p and 211-8b as well as the mutant 308 derived from the last strain, *Chl.* sp. strain 620 and *Chl. fusca* C.1.1.10. The presence of SP was confirmed in both strains of *Scenedesmus*. Other strains listed in Table 2 do not contain SP in cell walls, they are *Chlorella* mutants C.1.1.6, C.1.1.31, C.1.1.14, *Chl. vulgaris* 211-1e and 211-8k, and *Chl. saccharophila* 211-9a.

Table 2. Sporopollenins contents in CWH of some algal strains (percentages of dry weight of CWH)

Strains	Collection*	Age of culture used for cell walls isolation: 10 days	30 days	60 days	90 days
Ankistrodesmus braunii 202-7c	G	8.81 ± 0.10			
Chlorella sp. 113	L	4.14 ± 0.07	7.58 ± 0.07	8.25 ± 0.35	9.66 ± 0.12
Chl. fusca 211-8p	G	5.35 ± 0.12	8.49 ± 0.11	10.42 ± 0.22	11.35 ± 0.07
Chl. fusca 211-8b	G	3.08 ± 0.29	5.66 ± 0.15	8.44 ± 0.13	8.51 ± 0.04
Chl. mutant 308†	Cz		4.01 ± 0.16		
Chl. fusca 211-15	G		6.32 ± 0.15		
Chl. sp. 620	IZ			9.03 ± 0.12	
Chl. fusca C.1.1.10	Cz	3.74 ± 0.22	4.55 ± 0.12	6.34 ± 0.10	7.50 ± 0.06
Chl. mutant C.1.1.6†	Cz		0.00		
Chl. mutant C.1.1.31†	Cz		0.00		
Chl. mutant C.1.1.14†	Cz		0.00		
Chl. vulgaris 211-1e	G		0.00		
Chl. saccharophila 211-9a	G		0.00	0.00	
Chl. vulgaris 211-8k	G	0.00			
Scenedesmus obliquus 633‡	IZ	6.76 ± 0.53	10.26 ± 0.16	10.14 ± 0.18	10.00 ± 0.18
Scenedesmus quadricauda 449	IZ		10.57 ± 0.11	11.89 ± 0.21	

*Collection of: Cz– Prof. Dr. F.-C. Czygan, University of Würzurg, G—University of Göttingen, L University of Leningrad, IZ—Institute of Zootechny, Cracov.
†Medium III.
‡Medium IV [15].

Table 3. Changes in CWH-carotenoid composition during the aging of *Chlorella fusca*, strain 211-8p cultures*

Pigments	10 days	30 days	60 days	90 days
β-Carotene	trace	0–trace	trace	trace
Echinenone	0.00	0.00	0.00	0.00
Canthaxanthin	2.21 ± 0.1	6.52 ± 1.0	10.3 ± 0.4	13.77 ± 1.7
Astacene	31.10 ± 0.3	31.23 ± 1.1	22.89 ± 2.3	22.60 ± 2.1
2,3-Didehydrofritschiellaxanthin	35.04 ± 3.2	42.20 ± 1.6	39.71 ± 1.2	30.50 ± 2.6
Lutein	25.09 ± 2.3	22.38 ± 2.2	36.31 ± 4.4	35.09 ± 3.4
Violaxanthin	0–trace	0–trace	trace	trace
Neoxanthin	0–trace	0–trace	0–trace	0–trace
Total KC in hydrolysate in μg/g dry wt of CWH	15.94 ± 1.8	24.33 ± 1.7	43.68 ± 2.4	52.54 ± 0.7
Total carotenoids in hydrolysate in μg/g dry wt of CWH	23.32 ± 2.8	30.43 ± 2.1	59.9 ± 7.8	78.57 ± 5.5
Chlorophyll *a* in μg/g dry wt of CWH	20.0 ± 2.6	27.11 ± 4.0	83.4 ± 12.8	73.9 ± 6.4
Chlorophyll *b* in μg/g dry wt of CWH	7.9 ± 1.1	9.62 ± 1.9	59.65 ± 9.2	41.5 ± 9.1

*Culture conditions: medium I; illumination 2000 lux. The carotenoid compositions are given as percentages of total carotenoids in the hydrolysate.

The content of SP in CW undergoes changes between 3 and 12% on a dry wt basis. These changes seem to result not only in part from the nature of the strains but also mainly from the age of culture. In all cases the level of SP in CW shows an increase. In *Chl. fusca*, strain 211-8b and *Scenedesmus obliquus*, strain 633 a tendency to achieve a plateau was observed. The increase of SP-content in CWH during the cultivation time means that during this time synthesis of SP and transport of SP-precursors takes place.

Quantitative studies on the accumulation of carotenoids in CWH were carried out on *Chlorella fusca* strain 211-8p and *Scenedesmus obliquus*, strain 633 using 10, 30, 60 and 90-day-old cultures. The results are presented in Tables 3 and 4 as well as Figs 2–4 where some additional growth parameters accompanying the SP formation are given. In CWH of both algae an increase of the total KC was observed simultaneously with the aging of the culture. In both algae, canthaxanthin, and in *Scenedesmus* also echinenone, show an increased concentration in total CW-carotenoids in contrast to astacene and 2,3-didehydro-fritschiellaxanthin (present in hydrolysed pigment extracts).

The participation of lutein as the moiety of CW-carotenoids is notable. Nevertheless various CWH samples show remarkable variability of lutein concentration even at the same age of algal culture. The high content of lutein in algal protoplasts might result in absorption of this carotenoid on the CWH-surface during the preparation of CW from the homogenate. In order to

Table 4. Changes in CWH-carotenoid composition during the aging of cultures of *Scenedesmus obliquus*, strain 633*

Pigments	10 days	30 days	60 days	90 days
β-Carotene	0–trace	trace	0–trace	0–trace
Echinenone	0.00	1.04 ± 0.08	1.8 ± 0.19	2.17 ± 0.41
Canthaxanthin	7.50 ± 0.45	27.93 ± 4.42	47.96 ± 1.01	54.98 ± 3.38
Astacene	41.00 ± 1.38	18.18 ± 1.59	12.6 ± 1.84	9.38 ± 0.43
2,3-Didehydrofritschiellaxanthin	37.00 ± 0.69	32.71 ± 2.30	18.65 ± 1.29	18.02 ± 2.05
Lutein	14.50 ± 1.09	20.13 ± 2.5	19.0 ± 2.69	15.44 ± 1.81
Violaxanthin	0–trace	0–trace	0–trace	0–trace
Neoxanthin	0–trace	0–trace	0–trace	0–trace
Total KC in hydrolysate in μg/g dry wt of CWH	64.70 ± 8.8	79.1 ± 5.6	99.3 ± 6.4	106.9 ± 10.1
Total carotenoids in hydrolysate in μg/g dry wt of CWH	75.7 ± 11.0	99.0 ± 16.0	122.2 ± 28	125.3 ± 21
Chlorophyll *a* in μg/g dry wt of CWH	19.9 ± 2.6	47.5 ± 4.51	68.7 ± 15	35.01 ± 10
Chlorophyll *b* in μg/g dry wt of CWH	9.8 ± 2.5	30.1 ± 3.5	40.6 ± 5.9	20.38 ± 5.9

*Culture conditions: medium IV, illumination 2000 lux. The carotenoid composition is given in percentages of total carotenoids in the hydrolysate.

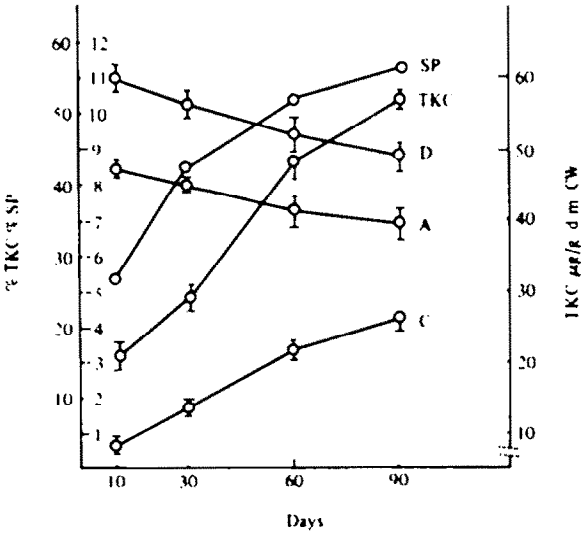


Fig. 2. Changes in KC-composition and level of SP in CWH of *Chlorella fusca* 211-8p at different ages of the culture. TKC, Total ketocarotenoids; A, astacene; C, canthaxanthin; D, 2,3-didehydro-fritschiellaxanthin (as a percentage of TKC); SP, sporopollenins (as a percentage of dry wt of CW).

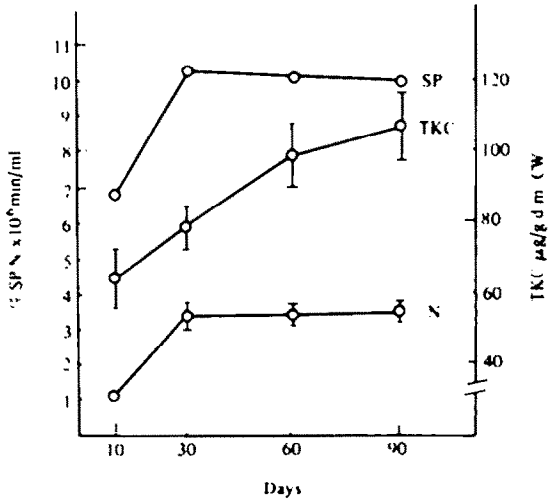


Fig. 3. Changes in the level of total KC and SP in CWH of *Scenedesmus obliquus*, strain 633 obtained from cultures of different ages. TKC, Total ketocarotenoids; SP, sporopollenins (as a percentage of dry wt of CW); N, number of cells (× 10^6/ml).

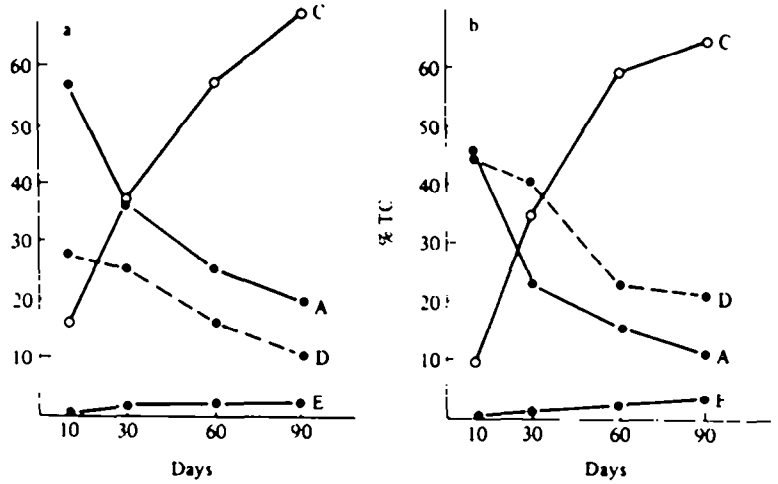


Fig. 4. Changes in the CWH carotenoid pattern of *Scenedesmus obliquus*, strain 633 isolated from cultures of different ages. A, Astacene; C, canthaxanthin; D, 2,3-didehydro-fritschiellaxanthin; E, echinenone; (a) medium I; (b) medium IV.

eliminate the interference of lutein concentration variability on the composition of other CW-carotenoids the lutein was omitted in Fig. 4. Similar dynamics of accumulation of particular KC in CWH of *Scenedesmus obliquus*, strain 633, as presented in Fig. 4a, was shown when medium IV of different composition was used (Fig. 4b). This indicates the more general character of the described changes. The studies on the dynamics of the accumulation of CW-carotenoids support the idea that they may be involved in the synthesis of SP.

The effect of SAN 9789, a strong inhibitor of carotenogenesis [13], on the level of CW-carotenoids and SP is shown in Table 5. The presented data confirm that CW-carotenoids are synthesized in darkness both in the control without inhibitor as well as in cultures containing SAN 9789. Cultures of *Scenedesmus obliquus*, strain 633 containing the inhibitor show nearly eight times less KC in the CWM than does the control. At the same time the level of SP in CWM decreases by about 50%. This value is lower than expected if carotenoids were assumed as the only SP-precursors. The described effect of SAN 9789 may suggest that SAN 9789 inhibits directly carotenogenesis and indirectly the forming of SP. This seems to indicate that SP is not a homogenous, but rather a heterogenous group of chemically different substances, all of them being resistant to acetolysis. This may mean that SAN 9789 is an effective inhibitor of the SP-fraction originating by involvement of, carotenoids (KC). However, it is difficult to exclude the alternative that SAN 9789 lowers the level of other acetolysis resistant

Table 5. Levels of carotenoids, sporopollenins and cellulose-like polymers in cells walls of 30-day-old cultures of *Scenedesmus obliquus*, strain 633 treated with SAN 9789*

	Cell walls			
	Control		+ SAN 5×10^{-6} M	
Cell wall constituents	Maternal (a)	From homogenate	Maternal (b)	From homogenate
Phytoene	—	—	-	768
Phytofluene in μg/g of CW dry wt	—	—	—	33
Echinenone		trace	—	
Canthaxanthin	41.78 (%)	33.36 (%)	51.72 (%)	47.75 (%)
Astacene + astaxanthin	15.09	10.23	10.00	4.96
2,3-Didehydrofritschiellaxanthin	21.00	23.61	18.91	12.18
Fritschiellaxanthin	16.57	20.00	10.05	18.44
Lutein	5.56	12.80	9.32	16.67
Total KC in native extracts μg/g of CW dry wt	149.6	112.1	19.2	11.5
% content of sporopollenins in CW dry wt	37.23 ± 0.16	13.80 ± 0.04	18.64 ± 0.23	18.32 ± 0.15
% content of cellulose-like polymer in CW dry wt	03.07 ± 0.19	48.64 ± 0.19	1.89 ± 0.23	43.90 ± 0.79

*Culture conditions: darkness, medium IV.

polymers which are constituents of the trilaminar structure of the CW. Differences in IR spectra of SP samples of various origin can serve as an argument supporting such a point of view [1, 2, 8].

It is very probable that carotenoids which accumulate in CW represent an excess of metabolites at these slowest stages of the biosynthetic pathway. The decrease of carotenoid concentration in CWM of chlorococcalean algae with increasing concentration of SAN 9789 [2] supports this idea. The CWM obtained from cultures of *Scenedesmus obliquus*, strain 633 grown in the presence of SAN 9789 are colourless. This is in contrast with the pinkish colour of CWM of the control.

It is interesting that simultaneously with the lowering of the carotenoid concentration an increase of UV absorbing compounds in CWM was observed. The HPLC of colourless polyenes obtained from CWM of *Sc. obliquus*, strain 633 treated with SAN 9789 showed three main peaks when recorded at $\lambda_{285\,nm}$ in *n*-hexane containing 0.1% diisopropyl ether. The spectrum of peak 1 showed absorption maxima at 257, 273, 284 and 295 nm; peak 2 at 239, 260, 271, 283, sh 295, 317 and 332 nm and peak 3 at 234, 260, 275, 285, 306, 321, 340 and 357 nm. Although the R_f value and absorption maxima of peak 1 corresponded with phytoene [14], the absorption maxima of peak 2 with *cis*-phytoene and 3 with *cis*-phytofluene it is not possible solely on this basis to identify the substances present in these peaks.

The occurrence of KC with SP in CW of all investigated strains belonging to the Chlorococcales is intriguing and may indicate a biogenetic connection between both groups of substances. The nearly similar carotenoid pattern of CW of all chlorococcalean algae forming SP raises the question of the sequence in KC biosynthesis and transformation. Carotenoids found as CW-components of chlorococcalean algae forming SP are presented in Fig. 5. If all CW-carotenoids are constituents of one metabolic pathway it seems probable that its role consists

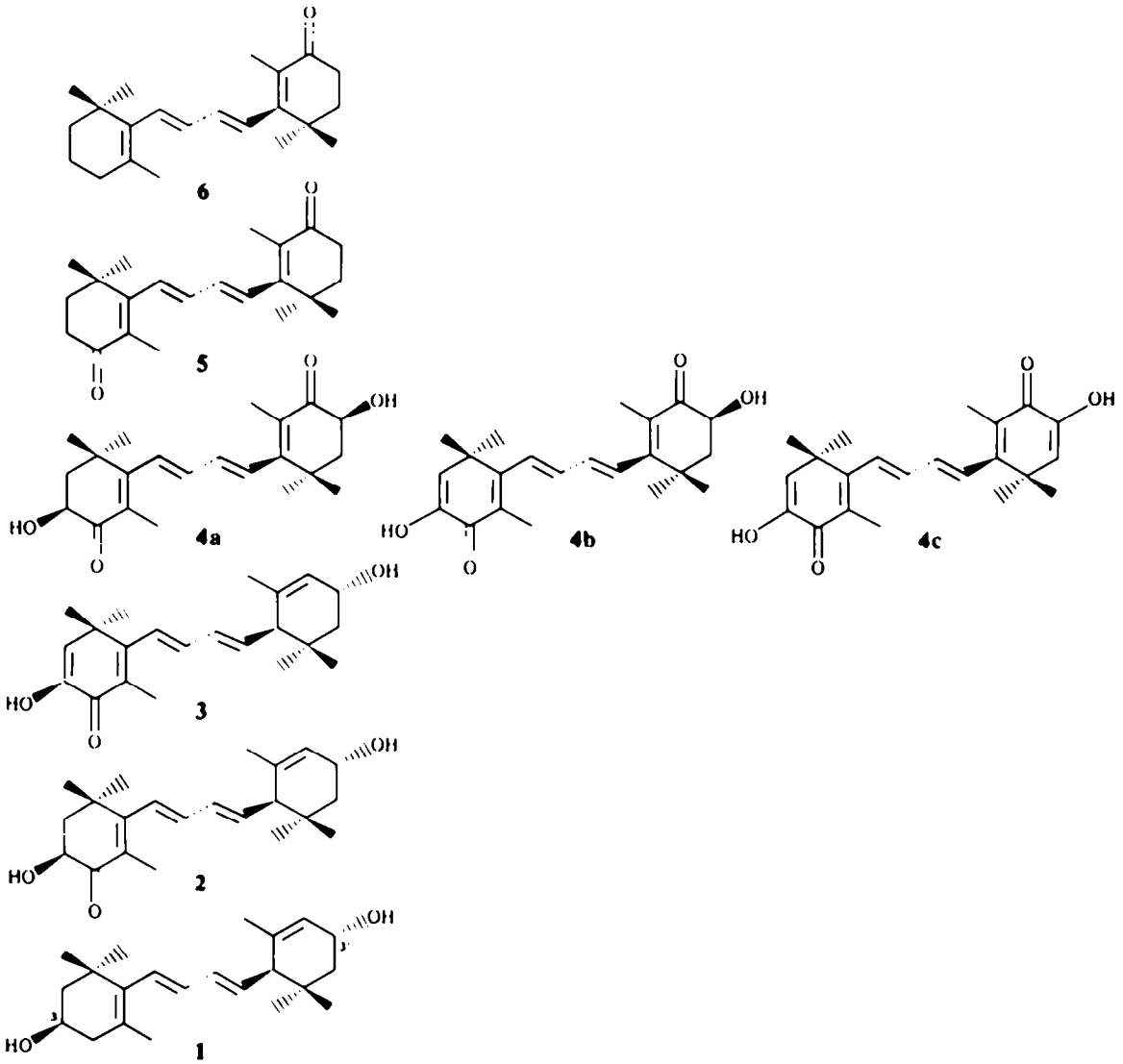
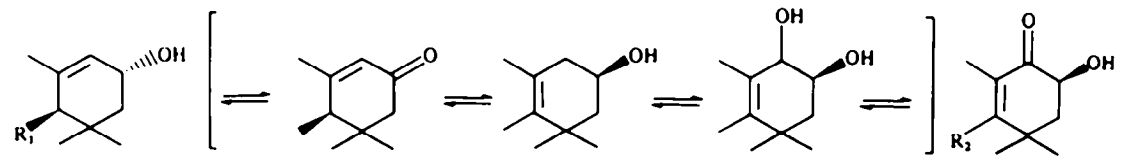


Fig. 5. Carotenoids found as cell wall components of green algae (Chlorococcales) forming sporopollenins: 1, lutein (1); 2, fritschiellaxanthin (2); 3, 2,3-didehydrofritschiellaxanthin (3); 4a, (3S,3'S)-astaxanthin; 4b, semiastacene; 4c, astacene (oxidation products of 4a); 5, canthaxanthin; 6, echinenone.



ε - ring of lutein

β'- ring of astaxanthin

Fig. 6. Presumed transformation steps of ε-ring for CW carotenoids of green algae (Chlorococcales) forming SP.

of the transformation of the ε-ring of α-carotenoids into the β-ring of the β-carotenoids (Fig. 6). Echinenone is present only in CWH and absent in CWM but in the latter the synthesis of SP has been completed. It has been established that echinenone 4-keto-β-carotene can, in contrast to other 4,4'-diketo-β-carotenoids of algal CW, polymerize *in vitro* to yield SP. It is an open question if echinenone can serve also *in vivo* (in green algae) as immediate precursor of SP.

The following alternative explanations of the role of KC in CW can be presented. (a) KC may be an immediate or intermediate precursor of SP; (b) KC may be involved not as precursors but only as co-factors in SP-biogenesis allowing the polymerization of substances of another kind than tetraterpenoids; (c) the co-occurrence of KC and SP in all algae so far investigated has no especial significance. However, the latter point would be difficult to explain on the basis of data presented in this paper.

EXPERIMENTAL

Biological material and analyses of carotenoids. All strains listed in Table 2 used in these experiments were axenic. Culture conditions, methods of isolation of cell walls from the medium (CWM) and from the homogenate of mechanically disrupted cells (CWH), extraction of cell wall-polyenes, TLC and HPLC separations and estimation of carotenoids were the same as previously described [2, 15].

Experiments with inhibitor of carotenogenesis. SAN 9789 ('Nonflurazon' a product of Sandoz, Basel) was used as an inhibitor of carotenogenesis. Cultures of *Scenedesmus obliquus*, strain 633 were grown in the dark in the presence of 5×10^{-6} M SAN 9789 in 10 l. medium IV, previously described [2]. Cultures containing SAN 9789 were aerated and stirred by air. Cell walls of both kind were obtained as earlier described [15].

Assay of sporopollenins. Weighed amounts of the dry cell walls (50–1000 mg) were successively extracted with 10% aq. KOH soln (5 hr at 100°), 5% ethanolic KOH (3 hr, 75°), H_2O (10 min, 100° and thoroughly washed with hot water to pH 7.0), then with EtOH and Et_2O. The residue of cell walls was suspended in 85% orthophosphoric acid at 28° for 10 days (acetolysis) [16], then collected, washed with hot water to pH 7.0, then with EtOH and Et_2O. The residues (sporopollenins) were dried for 24 hr at 105° and then show to IR spectra determination *in vacuo* over P_2O_5.

IR spectra. CWM (2.6 mg) of *Chlorella fusca*, strain 211-8p, from 10-day-old culture on medium I, after extraction with Me_2CO (Fig. 1A), 2.6 mg sporopollenins from CWM of the same strain (Fig. 1B) and 2.0 mg of sporopollenins isolated from CWH of *Scenedesmus obliquus*, strain 633 (Fig. 1C) were incorporated of 600 mg of KBr. A Fourier transform spectrophotometer, Digilab FTS-14v, was used for spectra recording.

Chromatography of colourless polyenes of CWM treated with SAN 9789. HPLC separation of colourless polyenes from CWM treated with SAN 9789 was performed on Lichrosorb Si 60 5 μm coloumn (50 cm × 3.1 mm). Eluent: *n*-hexane–0.1% (iso-Pr)$_2$O.

Acknowledgements—The author is most grateful to M.Sc. K. Schiedt, Dr. G. Englert, Dr. H. Mayer, Dr. K. Noack, Dr. M. Vecchi (Hoffmann-La Roche, Basel) for help with the structure elucidation of some CW carotenoids. The author thanks Dr. Eder F., Sandoz, Basel for a generous supply of the SAN 9789 sample.

REFERENCES

1. Atkinson, A. W., Gunning, B. E. S. and John, P. C. L. (1972) *Planta* **107**, 1.
2. Burczyk, J. (1982) *Badania nad karotenoidami i sporopollenina w ścianie komórkowej glonów*. Instytut Zootechniki, Cracov.
3. Burczyk, J. (1979) *Bull. Acad. Polon. Sci. Biol.* **27**, 13.
4. Brooks, J. (1970) Ph.D. Thesis, University of Bradford, U.K.
5. Shaw, G. (1971) in *Sporopollenin* (Brooks, J., Grant, P., Muir, M. D., Shaw, G. and van Gijzel, P., eds) pp. 303–350. Academic Press, London.
6. Burczyk, J. and Hesse, M. (1981) *Plant Syst. Evol.* **138**, 121.
7. Burczyk, J. and Czygan, F.-Ch. (1983) *Z. Pflanzenphysiol.* **111**, 169.
8. Berkaloff, C., Casadeval, E., Largeau, C., Metzger, P., Peracca, S. and Virlet, J. (1983) *Phytochemistry* **22**, 389.
9. Furch, B. and Gooday, G. W. (1978) *Trans. Br. Mycol. Soc.* **70**, 303.
10. Tabb, D. L. and Koenig, J. L. (1974) Infrared spectra of globular proteins in aqueous solutions, Dept. of Macromolecular Science, Case Western Reserve University, Cleveland, OH 44106.
11. Burczyk, J. (1973) *Folia Histochem. Cytochem.* **11**, 135.
12. Burczyk, J. (1987) *Phytochemistry* **26**, 121.
13. Eder, F. A. (1979) *Z. Naturforsch.* **34c**, 1052.
14. Foppen, F. H. (1971) *Chromatogr. Rev.* **14**, 1257.
15. Burczyk, J., Szkawran, H., Zontek, I. and Czygan, F.-Ch. (1981) *Planta* **151**, 247.
16. Kwiatkowski, A. and Lubliner-Mianowska, K. (1957) *Acta Soc. Bot. Pol.* **26**, 501.