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## Investigations on Carotenoids in Lichens. XXV. Studies of Carotenoids in Lichens from Spitsbergen

by

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With 1 Figure

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### Summary

CZECZUGA B. & OLECH M. 1990. Investigations on carotenoids in lichens. XXV. Studies of carotenoids in lichens from Spitsbergen. – Phyton (Horn, Austria) 30 (2): 235–245, 1 figure. – English with German summary.

Carotenoids in 21 species of lichens from Spitsbergen have been investigated by means of column and thin-layer chromatography. The following carotenoids were found: neurosporene,  $\alpha$ - and  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, zeaxanthin,  $\beta$ -carotene epoxide,  $\beta$ -cryptoxanthin epoxide, lutein epoxide, antheraxanthin, canthaxanthin,  $\alpha$ -doradexanthin, astaxanthin, diadinoxanthin, fucoxanthin, fucoxanthinol, violaxanthin, neoxanthin, mutatochrome, flavochrome, mutatoxanthin, 3-hydroxy- $\beta$ -apo-10'-carotenal and paracentrone. The finding of diadinoxanthin, fucoxanthin, fucoxanthinol and paracentrone in the *Solorina crocea* thalli from Spitsbergen is the first report of this kind.

The total carotenoid content ranged from 13.6 (*Cetraria delisei*) to 79.8  $\mu\text{g/g dry weight}$  (*Solorina crocea*).

### Zusammenfassung

CZECZUGA B. & OLECH M. 1990. Untersuchungen über Carotinoide in Flechten. XXV. Carotine in Flechten von Spitzbergen. – Phyton (Horn, Austria) 30 (2): 235–245, 1 Abbildung. – Englisch mit deutscher Zusammenfassung.

An 21 Flechten – Species aus Spitzbergen wurden säulen- und dünnsschichtchromatographisch 23 Carotinoide identifiziert. Die Feststellung des Vorkommens von

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Diadinoxanthin, Fucoxanthin Fucoxanthinol und Paracentron in Thalli von *Solorina crocea* aus Spitzbergen stellt eine Erstbeobachtung dar. Der Gesamt-Carotingeinhalt lag in einem Bereich zwischen 13,6 (*Cetraria delisei*) und 79,8 µg/g TG (*Solorina crocea*).

### Introduction

The lichen is an important component of the vegetation of Spitsbergen. Of the five complexes of plant communities distinguished on these islands, two are lichen community complexes, and in the functioning of the other three communities, the lichen also plays an important role (DUBIEL & OLECH 1985). Since lichens are so prevalent in the plant communities of Spitsbergen, lichenologists from various countries have been studying them for a great many years, the first study being made in the latter half of the eighteenth century (FRIES 1867). The Spitsbergen lichen flora has therefore been comparatively well described (LYNGE 1924, 1938, MAGNUSSON 1935, HERTEL 1977) with a substantial contribution to our knowledge having been made by Polish lichenologists (NOWAK 1965, 1968, OLECH 1987).

While investigating carotenoids in lichens of various latitudes (see CZECHUGA 1988), we turned our attention to lichens from Spitsbergen which are characteristic for its specific climatic conditions. It is felt that the data obtained will, to a considerable extent, augment our knowledge of carotenoids in lichens.

### Material and Methods

The following 21 species of lichens have been investigated: *Sphaerophorus globosus* (HUDS.) VAIN., *Lobaria linita* (Ach.) Rab., *Nephroma arcticum* (L.) TORSS., *Peltigera leucophlebia* (NYL.) GYELN., *Peltigera malacea* (Ach.) FUNCK., *Solorina crocea* (L.) ACH., *Cladonia amaurocraea* (FLH.) SCHAER., *Cladonia coccifera* (L.) WILD., *Bryocaulon divergens* (Ach.) KAR., *Stereocaulon alpinum* LAUR., *Stereocaulon vesuvianum* PERS., *Umbilicaria arctica* (Ach.) N.YL., *Umbilicaria decussata* (VILL.) ZAHLBR., *Umbilicaria torrefacta* (LIGH.) SCHR., *Umbilicaria vellea* (L.) ACH., *Cetraria delisei* (BORY ex SCHAER.) N.YL., *Cetraria cucullata* (BELL.) ACH., *Cetraria nivalis* (L.) Ach., *Alectoria nigricans* (Ach.) N.YL., *Thamnolia vernicularis* (SW.) SCHAER and *Usnea sulphurea* (KONIG.) TH. FR. (Table 1).

The material was firstly dried at room temperature, then immersed in 95% acetone and kept in an nitrogen atmosphere in an refrigerator until the chromatographic analysis was carried out.

The carotenoid pigments were extracted by means of 95% acetone in a dark room. Saponification was carried out by means of 10% KOH in ethanol at temperature of about 20° for 24 hours in the dark in a nitrogen atmosphere.

Columnar and thin-layer chromatography, described in detail in our previous papers (CZECHUGA 1980c), were used for the separation of various carotenoids. A glass column approximately 1 cm Ø and 15–20 cm in length filled with  $\text{Al}_2\text{O}_3$ , was used in

column chromatography. Silica gel was used for thin-layer chromatography with the appropriate solvent systems, the  $R_f$  values have been determined for each spot. For identification of  $\beta$ -carotene, canthaxanthin, lutein, zeaxanthin and astaxanthin co-chromatography was applied using authentic carotenoids.

The pigments were identified by following methods (cf. CZECZUGA & al. 1989 a, b): a) behaviour on column chromatography, b) absorption spectra of the pigments in various solvents, recorded by a Beckman spectrophotometer model 2400 DU, c) the partition characteristics of the carotenoid between hexane and 95% methanol, d) comparision of  $R_f$  on thin layer chromatography, e) the presence of allylic hydroxyl groups, determined by the acid chloroform tests, f) the epoxide test and g) by infrared spectroscopy (VETTER & al. 1971).

Quantitative determinations of the concentrations of carotenoid solutions were made from the quantitative absorption spectra. These determinations were based of the extinction coefficient E % 1 cm at the wavelengths of maximal absorbance in petroleum ether or hexane (DAVIES 1976).

## Results

In the material investigated, 23 carotenoids were identified (Table 2, Fig. 1). Some of these carotenoids frequently have been noted in other lichen species formerly, whereas others such as neurosporene (*Peltigera leucophlebia*), flavochrome (*Usnea sulphurea*), and 3-hydroxy- $\beta$ -apo-10'-carotenal (*Stereocaulon vesuvianum*) only sporadically have been encountered.  $\beta$ -cryptoxanthin-5,6-epoxide (*Nephroma arcticum* and *Cladonia amauroceaea*), diadinoxanthin, fucoxanthin, fucoxanthinol and paracentrone (*Solorina crocea*) have been found in lichens for the first time (Table 3). The characteristics of fucoxanthin and its derivatives are presented in Table 4. The total carotenoid content of the material studied ranged from 13.6 (*Cetraria delisei*) to 79.8  $\mu\text{g/g}$  dry wt (*Solorina crocea*).

## Discussion

Among the lichens neurosporene to date has been found, besides other species, in the thalli of *Cetraria nivalis* from Greenland (CZECZUGA & ALSTRUP 1987) and in the thalli of European populations of *Peltigera polydactyla* and *Peltigera spuria* (CZECZUGA 1980a). Flavochrome has been found in a few species of the *Parmeliaceae* (CZECZUGA 1980b) and the apocarotenal, 3-hydroxy- $\beta$ -apo-10'-carotenal has been noted to date in *Teloschistes exilis* – thalli from the Argentine (CZECZUGA & FERRARO DE CORONA 1987).

In the plant world,  $\beta$ -cryptoxanthin-5,6-epoxide has to date been encountered in numerous algal species, representatives of the *Xanthophyceae*, *Chrysophyceae* and *Eustigmatophyceae*, families which, as we know, are of the *Chrysophyta* type. This carotenoid is found also quite frequently in flower petals and in ripe fruit of higher seminiferous plants (GOODWIN 1980).

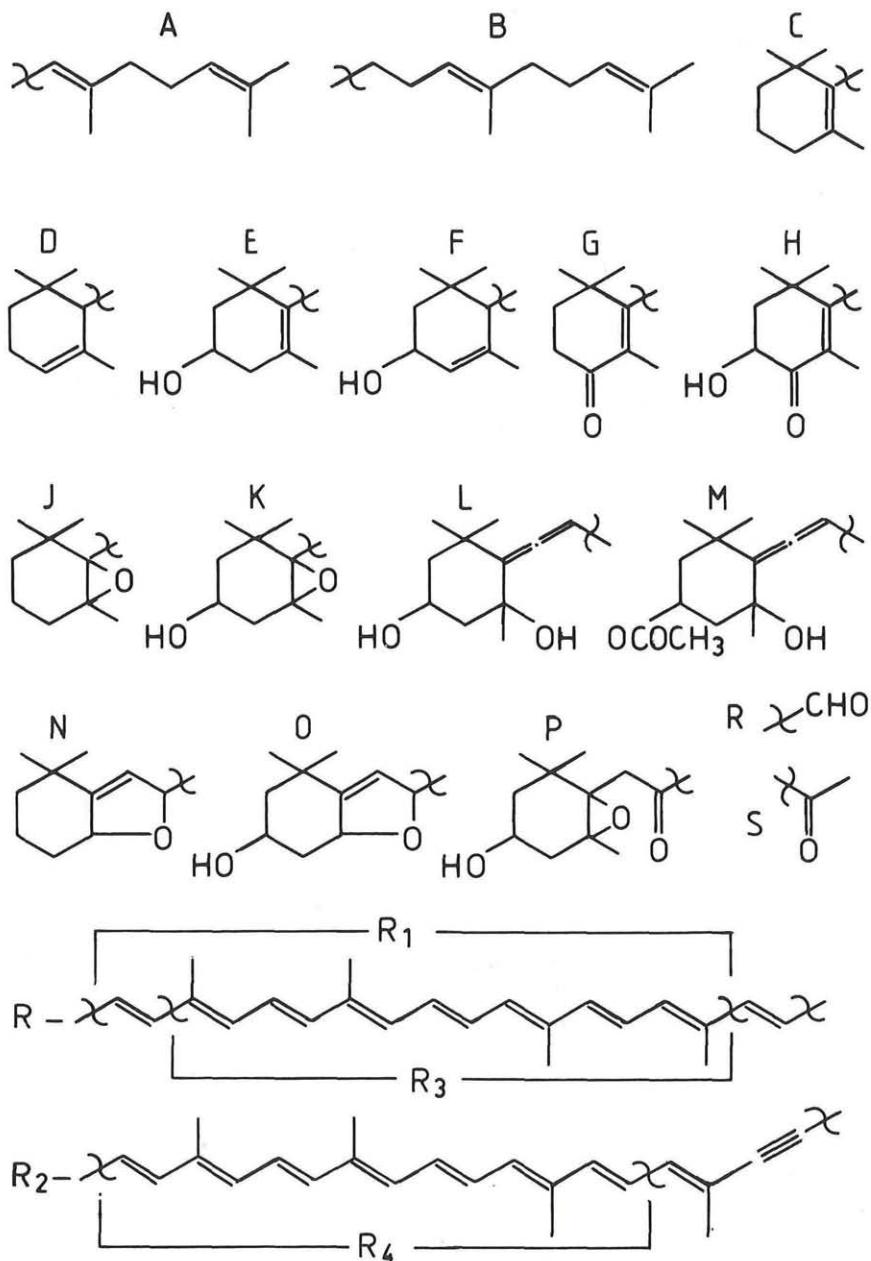


Fig. 1. Structural features of carotenoids from investigated materials.

Table 1.  
Investigated species of lichens from Spitsbergen

Family and species	Date of Collection	Locality	Altitude m
<i>Sphaerophoraceae</i>			
<i>Sphaerophorus globosus</i>	2. 8. 1982	"Sørkappland, Hohenlohefjellet	180
<i>Stictaceae</i>			
<i>Lobaria linita</i>	8. 8. 1985	"Sørkappland, Lidfjellet	50
<i>Peltigeraceae</i>			
<i>Nephroma arcticum</i>	14. 7. 1985	"Sørkappland, Sergejevfjellet	10
<i>Peltigera leucophlebia</i>	17. 7. 1985	"Sørkappland, Sergejevfjellet	50
<i>Peltigera malacea</i>	9. 8. 1981	"Sørkappland, Sore Sergejevfjellet	150
<i>Solorina crocea</i>	18. 7. 1985	"Sørkappland, Lidfjellet	380
<i>Cladoniaceae</i>			
<i>Cladonia amaurocraea</i>	29. 7. 1985	"Sørkappland, Wiederfjellet	120
<i>Cladonia coccifera</i>	2. 8. 1982	"Sørkappland, Hohenlohefjellet	180
<i>Stereocaulaceae</i>			
<i>Bryocaulon divergens</i>	25. 7. 1985	"Sørkappland, Sergejevfjellet	350
<i>Stereocaulon alpinum</i>	17. 8. 1985	"Sørkappland, Sore Sergejevfjellet	360
<i>Stereocaulon vesuvianum</i>	17. 8. 1985	"Sørkappland, Sore Sergejevfjellet	430
<i>Umbilicariaceae</i>			
<i>Umbilicaria arctica</i>	24. 6. 1985	Hornsund, Ariekammen	30
<i>Umbilicaria decussata</i>	22. 8. 1985	Hornsund, Gulliksenfjellet	400
<i>Umbilicaria torrefacta</i>	17. 7. 1985	"Sørkappland, Sergejevfjellet	50
<i>Umbilicaria vellea</i>	30. 9. 1985	Hornsund, Gulliksenfjellet	400
<i>Parmeliaceae</i>			
<i>Cetraria delisei</i>	25. 8. 1982	"Sørkappland, Rahsodden	5
<i>Cetraria cucullata</i>	22. 7. 1982	"Sørkappland, Hohenlohefjellet	110
<i>Cetraria nivalis</i>	22. 7. 1982	"Sørkappland, Hohenlohefjellet	110
<i>Usneaceae</i>			
<i>Alectoria nigricans</i>	22. 7. 1982	"Sørkappland, Hohenlohefjellet	110
<i>Thamnolia vernicularis</i>	4. 8. 1982	"Sørkappland, Hohenlohesnordet	30
<i>Usnea sulphurea</i>	22. 8. 1985	Hornsund, Treshelen	100

Table 2.  
List of carotenoids from investigated materials

Carotenoid	Structure (see Fig. 1)	Semisystematic name
1. Neurosporene	A - R <sub>1</sub> - B	7,8-dihydro- $\psi,\psi$ -carotene
2. $\alpha$ -Carotene	C - R - D	$\beta,\epsilon$ -carotene
3. $\beta$ -Carotene	C - R - C	$\beta,\beta$ -carotene
4. $\beta$ -Cryptoxanthin	C - R - E	$\beta,\beta$ -carotene-3-ol
5. Lutein	E - R - F	$\beta,\epsilon$ -carotene-3,3'-diol
6. Zeaxanthin	E - R - E	$\beta,\beta$ -carotene-3,3'-diol
7. $\beta$ -Carotene epoxide	C - R - I	5,6-epoxy-5,6-dihydro- $\beta,\beta$ -carotene
8. $\beta$ -Cryptoxanthin epoxide	C - R - K	5,6-epoxy-5,6-dihydro- $\beta,\beta$ -carotene-3-ol
9. Lutein epoxide	F - R - K	5,6-epoxy-5,6-dihydro- $\beta,\epsilon$ -carotene-3,3'-diol
10. Antheraxanthin	E - R - K	5,6-epoxy-5,6-dihydro- $\beta,\beta$ -carotene-3,3'-diol
11. Canthaxanthin	G - R - G	$\beta,\beta$ -carotene-4,4'-dione
12. $\alpha$ -Doradexanthin	F - R - H	3,3'-dihydroxy- $\beta,\epsilon$ -carotene-4-one
13. Astaxanthin	H - R - H	3,3'-dihydroxy- $\beta,\beta$ -carotene-4,4'-dione
14. Diadinoxanthin	E - R <sub>2</sub> - K	5,6-epoxy-7',8'-didehydro-5,6-dihydro- $\beta,\beta$ -carotene-3,3'-diol
15. Fucoxanthinol	L - R <sub>3</sub> - P	5,6-epoxy-3,3',5'-trihydroxy-6',7'-dihydro-5,6,7,8,5',6'-hexahydro- $\beta,\beta$ -carotene-8-one
16. Fucoxanthin	M - R <sub>3</sub> - P	5,6-epoxy-3,3',5'-trihydroxy-6',7'-dihydro-5,6,7,8,5',6'-hexahydro- $\beta,\beta$ -carotene-8-one-3'-acetate
17. Violaxanthin	K - R - K	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- $\beta,\beta$ -carotene-3,3'-diol
18. Neoxanthin	K - R <sub>1</sub> - L	5',6'-epoxy-6,7-didehydro-5,6,5'6'-tetrahydro- $\beta,\beta$ -carotene-3,5,3'-triol
19. Mutatochrome	C - R <sub>1</sub> - N	5,8-epoxy-5,8-dihydro- $\beta,\beta$ -carotene
20. Flavochrome	D - R <sub>1</sub> - N	5,8-epoxy-5,8-dihydro- $\beta,\epsilon$ -carotene
21. Mutatoxanthin	E - R <sub>1</sub> - O	5,8-epoxy-5,8-dihydro- $\beta,\beta$ -carotene-3,3'-diol
22. 3-Hydroxy- $\beta$ -apo-10'-carotenal	E - R <sub>4</sub> - R <sub>0</sub>	3-hydroxy-10'-apo- $\beta$ -carotene-10'-al
23. Paracentrone	L - R <sub>3</sub> - S	3,5-dihydroxy-6,7-didehydro-5,6,7',8'-tetrahydro-7'-apo- $\beta$ -carotene-8'-one

Table 3.  
The carotenoid composition of lichens

Family and species	Carotenoids (see Table 2)	Major caro- tenoid (%)	Total content ( $\mu\text{g g}^{-1}$ dry wt)
<i>Sphaerophoraceae</i>			
<i>Sphaerophorus globosus</i>	3, 4, 6, 7, 9, 11, 13	6 (31.2)	19.9
<i>Strictaceae</i>			
<i>Lobaria linita</i>	2, 3, 4, 5, 9, 13, 17	13 (31.4.)	32.2
<i>Peltigeraceae</i>			
<i>Nephroma arcticum</i>	3, 4, 5, 7, 8, 9, 10, 13	13 (33.6)	20.2
<i>Peltigera leucophlebia</i>	1, 3, 5, 6, 9, 13, 17	9 (35.0)	21.7
<i>Peltigera malacea</i>	2, 3, 9, 11, 12, 13, 17	12 (43.1)	31.7
<i>Solorina crocea</i>	3, 7, 10, 11, 14, 15, 16, 18, 23	15 (49.8)	79.8
<i>Cladoniaceae</i>			
<i>Cladonia amauro-</i> <i>craea</i>	2, 3, 4, 5, 6, 8, 9, 12, 13, 17	13 (27.8)	23.9
<i>Cladonia coccifera</i>	2, 3, 4, 5, 9, 10, 11, 12, 13, 17, 21	12 (33.7)	24.3
<i>Stereocaulaceae</i>			
<i>Bryocaulon divergens</i>	2, 3, 4, 6, 9, 12, 13, 17	13 (42.8)	32.1
<i>Stereocaulon alpinum</i>	2, 3, 5, 6, 9, 12, 17, 18	9 (27.9)	26.0
<i>Stereocaulon ves-</i> <i>vianum</i>	2, 3, 4, 6, 9, 10, 13, 18, 22	13 (43.8)	18.2
<i>Umbilicariaceae</i>			
<i>Umbilicaria arctica</i>	2, 3, 4, 5, 9, 10, 12, 13, 17, 19	12 (26.6)	26.3
<i>Umbilicaria decussata</i>	2, 3, 5, 9, 10, 17, 21	17 (42.1)	15.1
<i>Umbilicaria torrefacta</i>	5, 9, 10, 17, 21	17 (42.0)	18.7
<i>Umbilicaria vellea</i>	2, 3, 5, 9, 10, 13, 17, 21	21 (31.9)	18.5
<i>Parmeliaceae</i>			
<i>Cetraria delisei</i>	2, 3, 5, 6, 9, 10, 13, 17, 21	21 (38.3)	13.6
<i>Cetraria cucullata</i>	2, 3, 4, 5, 9, 10, 13	10 (37.6)	17.6
<i>Cetraria nivalis</i>	2, 3, 4, 5, 9, 10, 13, 17	10 (30.2)	19.2
<i>Usneaceae</i>			
<i>Aleoria nigricans</i>	2, 3, 5, 6, 9, 13, 17, 24	13 (27.9)	19.7
<i>Thamnolia verni-</i> <i>cularis</i>	3, 4, 7, 9, 10, 13, 17, 21	21 (32.8)	17.6
<i>Usnea sulphurea</i>	2, 3, 4, 5, 7, 9, 10, 13, 17, 20	20 (23.7)	15.2

Table 4.  
Characteristics of fucoxanthin and its derivatives

Parameters	Fucoxanthin $C_{42}H_{58}O_6$ nm	Fucoxanthinol $C_{40}H_{56}O_5$ nm	Paracentrone $C_{31}H_{42}O_2$ nm
Visible light absorption in:			
petroleum ether	425, 446, 475	425, 448, 476	
hexane	427, 450, 476		417, 439, 468
ethanol	426, 449, 465	452	
benzene	443, 461, 485	436, 460, 487	440, 456, 480
carbon disulphide	450, 478, 508		
methanol			444
Partition coefficients	hypophasic	hypophasic	hypophasic
Infrared light absorption in $CCl_4$			
	3615, 1927,	3580, 3450,	3608, 1926,
	1740, 1660,	1920, 1655,	1660, 1608,
	957	1608, 978	1045, 964

The carotenoid composition of the *Solorina crocea* thallus is particularly worthy of note. In addition to the carotenoids commonly occurring in lichens, this species contained diadinoxanthin, fucoxanthin, fucoxanthinol and paracentrone. As is known, diadinoxanthin is formed from diatoxanthin; both these carotenoids are quite frequently found in various alga species of different systematic groups. As regards lichens, only diatoxanthin has been noted and that rarely, so it seems that the finding of diadinoxanthin in *Solorina crocea* thalli from Spitsbergen is the first report of this kind. The occurrence of another carotenoids, fucoxanthin and ist two derivatives, fucoxanthinol and paracentrone, ist restricted in nature, the first two appearing only in certain groups of algae whereas the third, paracentrone, to date has only been noted in an animal (sea-urchin, GALASKO & al. 1969). Fucoxanthin is a carotenoid characteristic of the *Phaeophyta*, *Bacillariophyceae* and three families of the *Chrysophyta*, the *Chrosophyceae*, *Haptophyceae* (GOODWIN 1980) and *Prymnesiaceae* (BJØRNLAND & al. 1988). It has also been found sporadically in some algal species of the *Gymnodiniaceae* and *Peridiniaceae* families of the *Pyrrophyta* type. Fucoxanthinol, on the other hand, to date has been found in plants only in certain algal species of the *Haptophyceae/Chrysophyta/* whereas fucoxanthin and its derivatives have not been previously encountered in the representatives of the *Chlorophyta* type. The phycobiont of the *Solorina crocea* is the alga

*Coccomyxa solorinae* var. *croceae* CHODAT (BUBRICK 1988) which is classified as a member of the *Chlorophyta*. According to AHMADJIAN (1967) only species of one genus, the *Heterococcus*, are phycobionts of lichens belonging to the *Chrysophyceae*. The presence or absence of fucoxanthin is one of several systematic features distinguishing the *Chrysophyceae* from the *Chlorophyta* (KADŁUBOWSKA 1975). In the light of the above data, the presence of fucoxanthin and its derivatives in the *Solorina crocea* indicates that the phycobiont of this lichen should be classified as a *Chrysophyceae* and not a *Chlorophyta* as it has been to date, unless we consider the presence of fucoxanthin and its derivatives in the phycobiont of the *Chlorophyceae* *Coccomyxa solorinae* var. *croceae* to be an exception among the species belonging to the *Chlorophyta* type. It is possible that this is a case in which the chemism of the soil has a decisive effect on the presence of a given carotenoid as was observed in the *Chlorella pyrenoidosa* in which it was noted that the cells contained large amounts of canthaxanthin when this alga grew on nitrogen-poor soil (KESSLER & CZYGAN 1967).

Fucoxanthin and its derivatives (fucoxanthinol and paracentrone) belong to the group of allenic carotenoids (BONNET & al. 1969). The two derivatives of fucoxanthin have been isolated from the coelomic epithelium of the sea-urchin, *Paracentrotus lividus*, which was also found to contain traces of fucoxanthin (HORA & al. 1970). This led these authors to suggest that they are formed in these animals from the fucoxanthin in their food (thallophytic algae) as products of fucoxanthin during digestion. It should also be mentioned that paracentrone belongs to the apocarotenal group which, as we know, is formed in nature as a result of the end process of metabolism in plants or of the natural degradation of precursors in the decadent period of vegetation. In view of the perennial vegetation period of lichens and the specific conditions prevalent of Spitsbergen, it can be concluded that paracentrone is formed as a result of the natural degradation of fucoxanthin of its derivative fucoxanthin or its derivative fucoxanthinol (HORA et al. 1970).

The total carotenoid content of the material from Spitsbergen is slightly higher than that noted in the lichens of Lapland (CZECZUGA 1986), Greenland (CZECZUGA & ALSTRUP 1987), and Kamchatka (CZECZUGA & al. 1989). A similar value of total carotenoid content was, however, observed in lichens from the Taimyr region of North Siberia (CZECZUGA & SHEHELKUNOVA 1986).

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## Recensiones

**FRANKE Wolfgang 1989. Nutzpflanzenkunde.** Nutzbare Gewächse der gemäßigten Breiten, Subtropen und Tropen. 4 neubearbeitete und erweiterte Auflage. – 8°, XI + 490 Seiten, 153 Abbildungen; brosch. – Georg Thieme Verlag Stuttgart, New York. – DM 44,–. – ISBN 3-15-530-404-3.

Die 2. Auflage dieses, die botanische Seite, Nutzungsmöglichkeiten, Kultur und Produktion wirtschaftlich wichtiger Pflanzen berücksichtigenden Taschenbuches wurde in Phyton 23 (1): 164–165 ausführlich besprochen.

Die 3. Auflage (1985) war eine unveränderte Ausgabe der 2. Auflage (1981); die nun vorliegende 4. Auflage ist wieder eine überarbeitete. Der Band ist um 20 Seiten umfangreicher, hat einen etwas größeren Satzspiegel, enthält 3 Abbildungen mehr (u. a. kam ein Photo von *Vigna subterranea*, p. 148, dazu) und lt. Vorwort sind 125 Arten und Unterarten zusätzlich aufgenommen. Die Tabellen mit den Produktionszahlen und den Inhaltsstoffen sind auf einen neueren Stand gebracht worden und auch der Text zeigt an vielen Stellen die Verbesserung früherer Unzulänglichkeiten und Fehler.

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