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Investigations on carotenoids in *Embryophyta*. IV. The presence of apocarotenals in peatmosses

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Abstract

By means of column and thin-layer chromatography, the author investigated the presence of various carotenoids in stems of 3 species of the *Sphagnum* genus. Apocarotenals (β-apo-2′, β-apo-10′-carotenal and apo-12′-violaxanthal) and the following carotenoids were found: α-, β-, γ-carotene, β-cryptoxantin, lutein, lutein epoxide, zeaxanthin, adonixanthin, antheroxanthin, rhodoxanthin, rubixanthin, neoxanthin, violaxanthin and mutatoxanthin. The total content of carotenoids ranged from 11.954 to 41.579 $mg \cdot g^{-1}$ dry weight.

Key words: Sphagnum, peatmosses, carotenoids, apocarotenals

INTRODUCTION

The presence of carotenoids of the apocarotenals group was observed in some higher plants (Thommen 1961, 1962, Thommen and Wiss 1963, Yokoyama and White 1966, Egger and Kleinig-Voigt 1968). According to some investigators (Bauernfeind 1972, Simpson et al. 1976, Simpson and Chichester 1981), the carotenoids of this group arise in plant tissues as the result of β-carotene natural degradation. The process occurs mainly in the final stage of vegetation, that is in autumn in most cases.

If the process is connected with plant degradation, the carotenoids of the apocarotenals group should also occur in peatmosses as the result of their species growth. Like most plants, peatmosses are known to exhibit vertical growth with necrosis of the lower part of the stem at the same time. Thus, degradation processes occur independently of the season of the year.

We started to investigate carotenoids in several peatmoss species of the *Sphagnum* genus.

MATERIAL AND METHODS

The investigations were carried out in mid August, 1983, on three peatmose species — *Sphagnum palustre* L., *Sphagnum recurvum* P. Beauv. and *Sphagnum squarrosum* Crome. Moss stems with leaves were separated into three equal parts: upper, middle and lower.

The moss stems were cleaned of all organic debris, macerated, placed in dark glass bottles and covered with acetone. The air above the fluid in the bottle was changed for nitrogen to ensure an anaerobic atmosphere. The samples were kept in a refrigerator until used for chromatographic analysis of the carotenoid content.

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 a temperature of about 20°C for 24 hours in the dark in a nitrogen atmosphere.

Column and thin-layer chromatography, described in detail our previous paper (Czeczuga 1980) were used for separation of various carotenoids. A glass column (Quickfit-England) approximately 1 cm ∅ and 15-20 cm in legth, filled with Al_2O_3, was used in column chromatography. The extract was passed through the column and the different fractions were eluted with the solvent. Silica gel was used for thin-layer chromatography, with the appropriate solvent systems, the R_f values being determined for each spot. For identification of carotenoids co-chromatography was applied with the use of standard carotenoids (Hoffman — La Roche and Co. Ldt., Basle, Switzerland and Sigma Chemical Company — USA).

The pigments were identified by the following methods: a) behaviour in column chromatography, b) absorption spectra of the pigments in various solvents were recorded on a Beckman spectrophotometer model 2400 Du, c) partition characteristics of the carotenoid between hexane and 95% methanol, d) comparison of R_f values in thin-layer chromatography, e) the presence of allyllic hydroxyl groups was determined by the acid-chloroform test, and f) the epoxide test.

Quantitative determinations of the concentrations of carotenoid solutions were made from the absorption spectra. These determinations were based on the extinction coefficient E $1\% \cdot cm^{-1}$ at wavelengths of maximal absorbance in petroleum ether or hexane.

RESULTS

In the investigated parts of peatmosses the presence of 17 carotenoids was established (Table 1, Fig. 1). The finding of γ-carotene (*Sphagnum recurvum*), adonixanthin (*Sphagnum squarrosum*), rhodoxanthin (*Sphagnum *recurvum*), rubixanthin (*Sphagnum squarrosum*), and carotenoids of the apocarotenal group is worth noting. Particularly, the presence of apo-12′-violaxanthal in all investigated species should be emphasized. β-Carotene, β-cryptoxanthin, lutein, lutein epoxide, zeaxanthin, antheraxanthin and apo-12′-violaxanthal were found in all three peatmosses (Table 2).

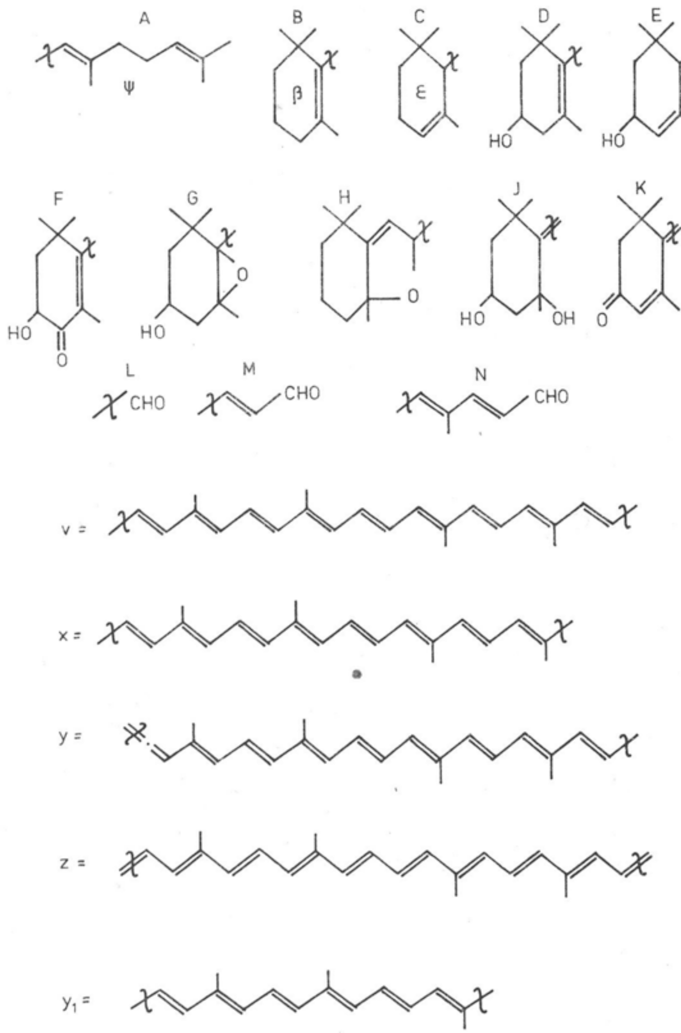

Fig. 1. Structural featurus of carotenoids from peatmosses

Total carotenoids content in the respective parts of the stem was different. In *Sphagnum palustre* and *Sphagnum squarrosum* the smallest amounts of carotenoids were found in the upper part of the stem, while the greatest quantity — in the lower one. In *Sphagnum recurvum*, on the

Table 1

List of the carotenoids from *Sphagnum* species

Carotenoid	Structure (see Fig. 1)	Semisystematic name
γ-Carotene	A-V-B	β,ψ-carotene
Rubixanthin	A-V-D	β,ψ-carotene-3-ol
α-Carotene	B-V-C	β,ε-carotene
β-Carotene	B-V-B	β,β-carotene
β-Cryptoxanthin	B-V-D	β,β-carotene-3-ol
Lutein	D-V-E	β,ε-carotene-3,3'-diol
Lutein epoxide	E-V-G	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
Zeaxanthin	D-V-D	β,β-carotene-3,3'-diol
Antheroxanthin	D-V-G	5,6-epoxy-5,6-dihydro-β,β-carotene-3,3'-diol
Adonixanthin	D-V-F	3,3'-dihydroxy-β,β-caroten-4-one
Neoxanthin	I-Y-G	5,6'-epoxy-6,7-didehydro-5,6,5',6'-tetrahydro-β,β-carotene-3,5,3'-triol
Violaxanthin	G-V-G	5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro-β,β-carotene-3,3'-diol
Mutatoxanthin	D-X-H	5,8-epoxy-5,8-dihydro-β,β-carotene-3,3'-diol
Rhodoxanthin	K-Z-K	4',5'-didehydro-4,5'-retro-β,β-carotene-3,3'-dione
β-Apo-2'-carotenal	B-V-N	3',4'-dihydro-2'-apo-β-caroten-2'-al
β-Apo-10'-carotenal	B-Y_1-M	10'-apo-β-caroten-10'-al
Apo-12'-violaxanthal	G-Y_1-L	5,6-epoxy-3-hydroxy-5,6-dihydro-12'-apo-β-caroten-12'-al

Table 2

Carotenoid pigments in three species of peatmosses (% of total pigments)

Carotenoid	*Sphagnum palustre*			*Sphagnum recurvum*			*Sphagnum squarrosum*		
	upper	middle	lower	upper	middle	lower	upper	middle	lower
α-Carotene	7.3				3.5				
β-Carotene	11.5	10.9	9.2	trace	16.8	4.3	10.1	trace	5.2
γ-Carotene					5.3				
β-Cryptoxanthin	10.3	7.4	32.8	34.4	14.2	19.5	trace	27.1	15.8
Lutein	17.9	13.9	29.2	13.8	16.9	17.9	trace	18.8	11.7
Lutein epoxide	10.5	9.8	trace	21.9	5.7	15.6	43.1	20.5	22.3
Zeaxanthin	27.0	5.5	3.0	12.5	8.3	39.6	17.3	trace	25.6
Adonixanthin							12.3		
Antheroxanthin		13.9		5.2	15.5	trace	trace	9.4	11.3
Rhodoxanthin				5.8					
Rubixanthin								19.0	2.4
Neoxanthin	trace	24.2	11.3						
Violaxanthin	15.5	6.7	9.0		9.5				
Mutatoxanthin							6.1	3.1	1.5
β-Apo-2'-carotenal			2.3						
β-Apo-10'-carotenal		2.6							
Apo-12'-violaxanthal		5.0	3.2	6.4	4.3	3.1	11.1	2.1	4.3
Total content in $mg \cdot g^{-1}$ dry weight	15.118	19.717	32.782	36.540	27.831	23.720	11.954	18.628	41.579

contrary, the amount of carotenoids was observed in the lower part of the stem, while the greatest quantity — in the upper one.

DISCUSSION

The majority of the carotenoids found in the investigated peatmosses have been many a time reported in other moss species (Suire 1975, Schmidt-Stohn 1977, Czeczuga 1980, Czeczuga et al. 1982). γ-Carotene, seldom found in mosses, has been reported to occur in few moss species, namely, in the thalli of *Marchantia polymorpha* and *Hypnum cupressiforme* among other (Czeczuga 1980). Rhodoxanthin is characteristic of some species of club-mosses, horsetails and ferns. It is often found in numerous species of conifers (Ida 1981), and some water plants of the *Potamogeton* genus in the first place (Neamtu and Illyes 1978). Rubixanthin, similarly, has been found many times in higher plants (Czeczuga 1978). The presence of β-apo-2′-carotenal and β-apo-10′-carotenal in *Sphagnum palustre* shoots as well the presence of apo-12′-violaxanthal reported in all three species of mosses investigated is worth noting. The carotenoids of the β-apocarotenal group have already been found in some higher plants (Goodwin 1980). As already mentioned, the most frequently accepted point of view in the pertinent literature is that carotenoids of the apocarotenals group arise as the result of β-carotene natural degradation (Yokoyama and White 1966). As for the occurrence of apo-12′-violaxanthal in plants, being a violaxanthin derivative, it has been studied so far by Curl (1965), who among other things established the absorption maxima in some solvents for this carotenoid. It is known (Goodwin 1980) that violaxanthin and lutein epoxide are the commonest carotenoids of the epoxy group in plants. It has been mentioned that peatmosses grow vertically with necrosis of the lower parts of the stem at the same time. According to this, apo-12′-violaxanthal should be considered as being the result of violaxanthin degradation, like the carotenoids of the β-carotenals group originating arising β-carotene degradation.

The finding of apo-12′-violaxanthal in peatmosses increases the list of carotenoids so far reported in mosses in general and supports the view on the origin of the carotenoids of the apocarotenals group, being the result of their precoursor's degradation

REFERENCES

Bauernfeind J. C., 1972. Carotenoid vitamin A precursors and analogues in foods and feeds. J. Agr. Food Chem. 20: 456-473.

Curl A. L., 1965. The occurrence of beta-citraurin and of beta-apo-8′-carotenal in the peels of California tangerines and organes. J. Fd. Sci. 30: 13-18.

Czeczuga B., 1978. The carotenoid content in certain plants from Abisko National Park (Swedish Lapland). Acta Soc. Bot. Pol. 47: 205-209.

Czeczuga B., 1980. Investigations on carotenoids in *Embryophyta*. I. *Bryophyta*. The Bryologist 83: 21-28.

Czeczuga B., Gutkowski R., Czerpak R., 1982. Investigations of carotenoids in *Embryophyta*. II. *Musci* from the Antarctic. Nova Hedwigia 36: 695-701.

Egger K., Kleinig-Voigt H., 1968. Carotenoids der *Commelinaceae Palisota barteri* — eine neues β-Citraurin-Vorkommen. Z. Naturforsch. 23b. 1105-1108.

Goodwin T. W., 1980. The Biochemistry of the Carotenoids. Volume I. Plants. Chapman and Hall, London-New York.

Ida K., 1981. Eco-physiological studies on the response of Taxodiaceous conifers to shading, with special references to the behaviour of leaf pigments. I. Distribution of carotenoids in green and autumnal reddish brown leaves of Gymnosperms. Bot. Mag. Takyo 94: 41-54.

Neamtu G., Illyes G., 1978. Chemotaxonomic researches in higher plants. X. Carotenoid and chlorophyll pigments in aquatic higher plants. St. Cerc. Biochim. 21: 63-67.

Schmidt-Stohn G., 1977. Anderungen der plastidenpigmente bei *Sphagman magnellanicum* Brid. in abhangigkeit von standort, verfarbungsgrad und alter. Z. Pflanzenphysiol. 81: 289-303.

Simpson K. L., Chichester C. O., 1981. Metabolism and nutritional significance of carotenoids. Ann. Rev. Nutr. 1: 351-374.

Simpson K. L., Tung-Ching Lee, Rodrigeuz D. S., Chichester C. O., 1976. Metabolism in senescent and stored tissues. In: Chemistry and Biochemistry of Plant-Pigments. Vol. 1. T. W. Goodwin (ed.) Academic Press, London.

Suire C., 1975. Chimie des Bryophytes. Rev. Bryol. Lichenol. 41: 105-256.

Thommen H., 1961. Studies on the metabolism of β-apo-8′ carotenal. Chimia 15: 433-434.

Thommen H., 1962. Uber das Vorkommen von β-Apo-8′--carotinal im Saft und der Schale von frischen Orangen. Naturwissensch. 22: 517-518.

Thommen H., Wiss O., 1963. Die Isolierung von Apo-carotinalen aus Luzernemehl. Z. Ernahr. 3: 18-23.

Yokoyama H., White M. J., 1966. Citrus carotenoids. VI. Carotenoid pigments in the flavedo of sinton citrangequat. Phytochem. 5: 1159-1173.

Badania karotenoidów u Embryophyta. IV. Obecność karotenoidów z grupy apokarotenalów u torfowców

Streszczenie

Stosując chromatografię kolumnową i cienkowarstwową badano występowanie poszczególnych karotenoidów w łodyżkach trzech gatunków mchów torfowców. W wyniku badań ustalno występowanie następujących karotenoidów: 3 z grupy apokarotenalów (β-apo-2′-, β-apo-10′-karotenal, apo-12′-wiolaksantal) oraz α-, β-, γ-karoten, β-kryptoksantyna, luteina, epoksyd luteiny, zeaksantyna, adoniksantyna, anteroksantyna, rodoksantyna, rubiksantyna, neoksantyna, wiolaksantyna i mutatoksantyna. Ogólna zawartość karotenoidów wahała się od 11,954 do 41,579 $mg \cdot g^{-1}$ suchej masy.