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Moss Pigments

8. The Carotenoids of *Fontinalis antipyretica* L. ex Hedw.

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From *Fontinalis antipyretica* ten carotenoids have been isolated. Of these eight have been tentatively identified as α- and β-carotene, neo-β-carotene U, lutein, 5,6-epoxy-lutein, violaxanthin, neoxanthin, and auroxanthin or auroxanthin-like pigment.

Although the presence of carotenoids in bryophytes was established by Kohl [1] as early as 1902 still little is known about the quantitative and qualitative occurrence of carotenoids in this plant group. Since the bryophytes are comparatively advanced photosynthesizing plants one has to expect them to show a carotenoid production which in its general features resembles that of ordinary plants. This has also been confirmed by recent investigations.

By means of paper chromatography Douin [2] examined the carotenoid composition of 40 species of *Bryales* and some species of *Sphagnales* and *Andreaeales* as well as 20 species of *Marchantiales* and *Jungmanniales*. By the method used he identified mainly the major carotenoids and found that the composition of these was the same in all the species investigated *viz.* α- and β-carotene, lutein, and 5,6-epoxy-lutein. From these results he drew the conclusion that the carotenoid distribution seems to be fairly uniform in bryophytes, the difference being mainly of a quantitative nature.

However, not only the quantitative but also the qualitative composition of the carotenoid contents in bryophytes ought to show, as in ordinary plants, some variations and these might be due to many facts. For instance the physiological conditions (nutrification and habitat conditions in general, season *etc.*) might be of some importance. Also intraspecific and interspecific differences of genetical origin cannot be excluded.

Some qualitative variations have also been found by Freeland [3] when investigating five species of *Musci*. By conventional methods of differential solubility, column chromatography, and spectrophotometry he found that, in addition to the obviously ubiquitously distributed α- and β-carotene and lutein, the five species also contained violaxanthin and zeaxanthin. Furthermore,

in two of the species neoxanthin was found and in one of them also cryptoxanthin. There was no qualitative difference in the major pigments of the sporophytes and the gametophytes of the mosses.

Our preliminary investigations of six species of *Musci* with regard to their carotenoid contents have confirmed the results obtained by Freeland. They all contained the three ordinary carotenoids as well as two or several more. It seems as if the carotenoid pattern varies to a certain degree in different species.

From one of the species investigated, *Fontinalis antipyretica*, also investigated by Douin,[2] the carotenoids were isolated by chromatography on alumina before further study. They were purified on magnesium oxide or zinc carbonate and tested for homogeneity by TLC. Since the investigation was carried out in a qualitative manner involving no direct comparison with authentic carotenoids the identifications are only tentative. They are based on the adsorption affinities of the different pigments on alumina, on their visible absorption spectra in different solvents and on their partition coefficients between petroleum ether and 90 % aqueous methanol.[4] All pigments were also subjected to the hydrochloric acid-ether test used for identification of epoxides and furanoid carotenoids.[5] The presence of one or two epoxy groups in the epoxy-carotenoids was established by measuring the hypsochromic shift occurring upon addition of a trace of hydrochloric acid to their chloroform solutions.[6]

The pigments 2b and 3 (Table 1) were further purified by TLC and obtained in very small amounts. Hence it was not possible to get sufficient data

Table 1. Chromatographic separation and purification of the carotenoids.

Zone in order of increasing adsorption	Colour of the zone	Required eluent	Adsorbant used for purification	Colour of the zone	No. of pigment
1	orange	petroleum ether	MgO-celite	yellow orange	1 a 1 b
2	yellow	petroleum ether-benzene (1:2)	MgO-celite silica gel G	lemon-yellow yellow	2 a 2 b
3	orange	benzene-ether (4:1)	MgO-celite silica gel G	orange	3
4	light yellow	benzene-ether (2:1)	silica gel G	light yellow	4
5	orange	benzene-ether (1:2)	$ZnCO_3$-celite	yellow-orange yellow-orange	5 a 5 b
6	yellow	ether-ethanol (4:1)	$ZnCO_3$-celite	yellow yellow-orange yellow lemon-yellow	6 a 6 b 6 c 6 d
7	lemon-yellow	ethyl acetate-methanol (1:2)	$ZnCO_3$-celite	lemon-yellow	7

Table 2. Properties of the isolated pigments.

Pigment No.	λ_{max} nm in: hexane	chloroform	carbon disulphide	Reaction with HCl/ether	Tentative identification
Epiphasic					
1 a	422	—	—	negative	α-carotene
	445	455	478		
	474	486	508		
1 b	426	—	450	negative	β-carotene
	451	465	484		
	482	496	519		
2 a	449	461	479	negative	neo-β-carotene U
	479	490	512		
Hypophasic					
2 b	403		—	green-yellow	unidentified
	427		453		
	451		478		
3	445		475	negative	unidentified
	472		502		
4	275				unknown
	342				
5 a	421	430	447	negative	lutein
	447	456	475		
	477	487	506		
5 b, 6a	(422)		—	green	5,6-epoxy-lutein
	441		471		
	471		501		
6 b	422		440	blue	violaxanthin
	445		469		
	478		501		
6 c	419		—	weak-blue	neoxanthin
	438		465		
	466		495		
6 d, 7			394	blue	auroxanthin-like
			420		
			449		

for a tentative identification of them, especially as the purification methods used increased the danger of isomerisation. This can perhaps account for the fact that their absorption spectra do not agree very well with any known carotenoids with the same adsorption powers. In the two solvents used their spectral properties in the visible region show some resemblance of flavochrome [7] and physoxanthin (*cis*-3-hydroxy-β-carotene) [8] which also have similar adsorption properties. The absorption maxima of pigment 7 (Table 1) indicate that this carotenoid does not have more than seven spectroscopically active,

conjugated double bonds in an aliphatic system. This as well as it adsorption power and hypophasic behaviour, suggests that it might be auroxanthin or an auroxanthin like pigment. Since the same pigment (6d, Table 1) was also obtained when violaxanthin was further purified on zinc carbonate it seems plausible that it is a rearrangement product of violaxanthin.[9] Judging from the absorption spectrum in visible light the pigment 4 is not a carotenoid.

A fuller report will appear later.

EXPERIMENTAL

Reagents and solvents, except petroleum ether, ether and acetone, were of analytical grade. *Fontinalis antipyretica* was collected in the parish of Transtrand in Dalarna, dried, ground and extracted at room temperature with successive portions of ether-methanol (1:1). The extract was concentrated to 1/5th of its volume and hydrolysed under nitrogen with KOH (6 %) for 3 h at room temperature. After addition of an equal volume water the unsaponifiable matter was extracted with ether, the ethereal solution was washed free from alkali, dried and concentrated to a small volume.

The carotenoids were chromatographed on columns of Woelm neutral alumina activity grade 4. They were further purified by chromatography on columns of:

1. magnesium oxide-celite (1:1), the eluents being: petroleum ether (1a—2a) and petroleum ether-benzene (3:1 and 1:1, v/v) (2b and 3).

2. zinc carbonate-celite (3:1), the eluents being: petroleum ether-ether (1:1, v/v) (5a and 5b), ether (6a—6c), and ether-methanol (4:1, v/v) (6d and 7).

The purity of the different pigments was tested by TLC on silica gel G; the solvent used was petroleum ether-benzene-acetone (80:20:1, by vol.).[10] The partition ratio test was performed with petroleum ether and 90 % aq. methanol (by vol.) and the HCl-ether test according to Curl and Bailey.[5] Absorption spectra were recorded on a Beckman DU spectrophotometer. Particulars of the deactivated alumina chromatogram and the further purification of the different pigments are recorded in Table 1. The absorption maxima of the different pigments in various solvents and their colour reaction in the HCl-ether test are given in Table 2.

All solvents were kept in the dark under nitrogen and all isolation and purification work was performed as quickly as possible in order to avoid isomerisation.

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