Pigments Occurring in *Hydrachna geografica* and *Piona nodata* (Hydracarina, Arachnoidea)

During recent years the Arachnoidea have become the subject of interest to scientists chiefly because of the various pigments of the carotenoid type found in them. METCALF and NEWELL[1] noticed the presence of these pigments in the land representatives of the Arachnoidea. The occurrence of carotenoids in the water species of the Eylais genus has been recently reported[2,3].

In the ponds found near Bialystok, the authors noted the presence of representatives of the Arachnoidea of a reddish-violet colour[4]. The preliminary examination of an acetone extract of these pigments showed that the maximum absorption occurs within the range of 474 to 476 nm, thus indicating the presence of carotenoids. Further investigations were then carried out by means of column and thin-layer chromatography. Specimens of both sexes with a body length of 1–4 mm were collected. After rinsing the specimens several times in distilled water and drying them on paper, an acetone extract of the pigments contained in the specimens was prepared. For separation of the pigments, column chromatography based on the method of LEE[5] and others[6,7] was used. Before the chromatographic analysis, hydrolysis of ester compounds was brought about by means of 15% KOH in methanol in nitrogen atmosphere. The absorption maxima of the pigments contained in the various fractions or the chromatograph spots were determined on a

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Table I. Column chromatogram of carotenoids from *Hydrachna geografica* and *Piona nodata*

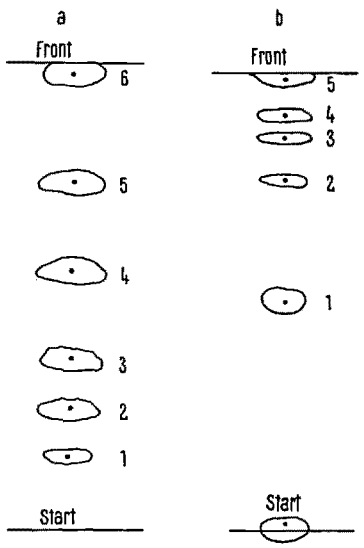
No. and colour of fraction	System of solvents	Maximum absorption in nm	Solvent	Identification
1 (yellow)	petroleum ether (45–65 °C)	425, 449–450, 477–478	petroleum ether	β-carotene
2 (yellowish)	1% acetone in petroleum ether	454–455	petroleum ether	echinenone
3 (yellow-orange)	2% acetone in petroleum ether	412–413, 440–441, 457–458	hexane	unknown
4 (orange)	5% acetone in petroleum ether	418–419, 449, 473–475	hexane	cryptoxanthin (?)
5 (orange-red)	10% acetone in petroleum ether	464–465	hexane	canthaxanthin
6 (yellow)	40–50% acetone in petroleum ether	422–423, 444–445, 472–473	hexane	lutein
7 (red-orange)	acetone	468–470, 474–476	hexane, acetone	astaxanthin free
8 (pink-violet)	5% cold acetic acid in ethyl ether	470–471, 478–480	hexane, acetone	astacene

Table II. Thin-layer chromatograms of carotenoids from *Hydrachna geografica* and *Piona nodata*

No. and colour of spots	Rf	System of solvents	Maximum absorption in nm	Solvent	Identification
Figure a					
1 (pink-violet)	0.20	A	477–479	acetone	astacene
2 (yellow)	0.28	I benzene	407–408, 442–443, 472–473	petroleum ether	lutein
3 (red-orange)	0.37	II benzene-hexane 2:1	476–477	acetone	astaxanthin free
4 (orange-red)	0.57		463–464	hexane	canthaxanthin
5 (red-orange)	0.74		452–453, 456, 467–468, 478	petroleum ether, hexane	astaxanthin ester and echinenone
6 (yellow-orange)	0.98		407, 425, 447–448, 457–458, 474–475	acetone	β-carotene and other unknown
Figure b					
1 (grey-yellow)	0.51	B			
2 (red-orange)	0.76	benzene-ethyl ether-methanol 17:2:1			
3 (pink)	0.84				
4 (orange-red)	0.89				
5 (yellow-orange)	0.99				

Specol spectrophotometer (Carl Zeiss, Jena). The results of the separation of the pigments by means of column and thin-layer chromatography in the specimens of Arachnoides mentioned above are given in Tables I and II and in the Figure. As the Rf values and the maximum absorption of the various fractions of the column and thin-layer chromatography show, the colour of the specimens of the Arachnoides investigated is due to the presence of a whole series of carotenoids.

From comparison of the absorption maxima of the fractions obtained by means of column and thin-layer chromatography with literature data[8–16], it is evident that such carotenoids as β-carotene, echinenone, cantaxanthin, lutein, astacene, and free astaxanthin are present. The absorption maxima of the fourth orange fraction from column chromatography indicate the presence of cryptoxanthin. A third yellowish-orange fraction of a carotenoid occurs which we have not been able to identify. Our results when compared with those obtained during investigations on other species of Hydracarina, show that[17] β-carotene, lutein, astaxanthin and keto-carotenoid are present in *Eylais extendens*. The authors of this paper have however noted in *Eylais hamata* the presence of the same carotenoids as those found in *Hydrachna geografica* and *Piona nodata*[18].



Thin-layer chromatograms of carotenoids from *Hydrachna geografica* and *Piona nodata* in systems of solvents A and B.

Résumé. Les auteurs à l'aide des méthodes de chromatographie sur colonne et sur couche fine, ont effectué la séparation des caroténoïdes chez *Hydrachna geografica* (Müller 1776) et *Piona nodata* (Müller 1776), qui appartiennent à Hydracarina (Arachnoidea). Ces recherches ont attesté la présence du β-carotène, de l'échinénone, de la cantaxanthine, de la lutéine, de l'astacène et de l'astaxanthine libre.

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Peroxidative Activity of Hemepeptides from Horse Heart Cytochrome *c*

Tuppy and Paléus[1] isolated hemepeptide by peptic digestion of cytochrome *c* and showed that it is an undecapeptide (HUP) with the sequence of

Val-Glu(NH_2)-Lys-Cys-Ala-Glu(NH_2)-Cys-His-Thr-Val-Glu
heme

By using pyrogallol as a hydrogen donor, Paléus et al.[2] reported that this compound exhibited a peroxidase-like activity 20 times higher than that of cytochrome *c*. Treatment of HUP with trypsin removed Val-Glu(NH_2)-Lys, giving a hemeoctapeptide (HOP). Although the peroxidative action of several hemepeptides has been reported, no extensive study has been made.

Horse heart cytochrome *c* (Type III, 99%) was purchased from Sigma Chemical Company and was further purified by means of an IRC-50 ion-exchange resin column. HUP was prepared by peptic digestion of cytochrome *c* according to the method of Tuppy and Paléus[1]. HOP was prepared by tryptic digestion of HUP following the method of Harbury and Loach[3]. However, a purification procedure involving Hyflo-super cel was repeated twice instead of once as in the procedure of Harbury and Loach. The HOP prepared by the Harbury and Loach method was further purified by using Sephadex G-25.

Peroxidative activities of the samples were measured spectrophotometrically at 460 nm in 0.01 *M* phosphate buffer pH 6.0, using *o*-dianisidine as a hydrogen donor[4,5]. The effect of substrate concentration on the rate of reaction was studied and all the enzyme activities were measured at the substrate concentration which gave the maximum velocity (Figure 1).

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