Investigations on the Pigments of the *Apus cancriformis* (Bosc.)^{*} (Crustacea: Branchiopoda)

by

B. CZECZUGA & R. CZERPAK

The Chair of Biology, Białystok Medical Academy, Białystok, Poland

INTRODUCTION

Fox (1955) in his investigations on the quantitative relations of haemoglobin in several representatives of aquatic fauna, to the concentration of oxygen in the water inhabited by those species, mentioned the occurrence of carotenoid-protein complexes in specimens of the *Apus cancriformis*. It may, therefore, be assumed that a number of carotenoids are present in the body of the *Apus cancriformis* since it is of an intense brown colour. A review of the publications concerning carotenoids in water fauna did not reveal any papers in which the presence of carotenoids in the *Apus cancriformis* is discussed. This, added to the fact that the *Apus cancriformis* is rather a rare specimen usually found in the spring-summer period in ponds which dry up in winter, encouraged us to undertake investigations, the aim of which was to discover which carotenoids give the *Apus cancriformis* their brown colour.

MATERIAL AND METHODS

The *Apus cancriformis* specimens were collected from the fishery ponds of Łaki Jaktorowskie near Warsaw on June 21st, 1967. Water was also collected from the same ponds for chemical analysis, the

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results of which were given in a previous paper (CZECZUGA & CZERPAK, 1968e). From these specimens and from their carapaces, after being cleaned of peryphyton and dried on paper, an acetone extract was prepared. The method used for the separation of the acetone extract was based on those of SCHALTEGGER (1965), LEE (1966a) and CZECZUGA & CZERPAK (1968a). The details of the column chromatography methods are given in Tables I and II, and of the thin-layer chromatography method in Tables III and IV. The ester compounds of the carotenoids were hydrolyzed with 15 KOH in methanol at room temperature in a nitrogen atmosphere for 48 hours. The absorption maxima of the pigments were determined on a "Specol" spectrophotometer (Carl Zeiss, Jena).

RESULTS

The acetone extract of the *Apus cancriformis* specimen gave a light blueish-pink fluorescence and had the following absorption maxima: 326—327, 398, 570, 603, 624—625 and 663 m μ (Fig. 1). The extract in petroleum ether (b.p. 45—65°C) from the carapaces alone gave the following absorption maxima: 487—488, 515, 536—537, 557—558 and 597—598 m μ .

Fig. 1. The absorption spectra of the carotenoid-protein complex from *Apus cancriformis* (Bosc.) in acetone.

By means of column chromatography using different solvent systems, the acetone extract was divided into eight fractions (Fig. 2 A). The first seven fractions were of a yellow colour, sometimes a pale yellow, whereas the eight was orange. After eluting the various fractions, the absorption maxima were determined, the range of which varied between 409 and 488 $m\mu$ (Table I). The separation of the extract from the carapaces alone by means of column chromatography gave only four fractions and the absorption maxima ranged from 421 $m\mu$ to 521 $m\mu$ (Fig. 3 A, Table II).

Fig. 2. Column chromatogram (A) I. β -carotene; II. γ -carotene; III. unknown echinenone; IV. canthaxanthin; V. lutein ester; VI. lutein; VII. unknown xanthophyll; VIII. unknown and thin-layer chromatogram (B) 1. lutein unknow; 2. canthaxanthin; 3. echinenone; 4. γ -carotene; 5. β -carotene of carotenoids from *Apus cancriformis* (Bosc.).

By means of thin-layer chromatography with a solvent system of petroleum ether-benzene-methanol, the ether extract from the *Apus cancriformis* specimens was separated into five spots (Fig. 2 B), the colour of which varied from yellow to yellow-orange-red. In addition various spots, of different R_f values, had different absorption maxima. The first spot had an R_f value of 0.26, the last - 0.97. The absorption maxima of the various spots ranged from 443 to 521 $m\mu$ (Table III). The extract from the carapaces alone was separated into four spots by means of thin-layer chromatography. The spots were found to give different absorption maxima (Fig. 3 B, Table IV).

Fig. 3. Column chromatogram (A) I. canthaxanthin; II. lutein ester; III. lutein free; IV. unknown xanthophyll and thin-layer chromatogram (B) 1. unknown xanthophyll; 2. lutein free; 3. lutein ester; 4. canthaxanthin of carotenoids from carapace *Apus cancriformis* (Bosc.).

DISCUSSION

On comparing the absorption curve of the acetone extract (Fig. 1) with the results obtained by a number of authors (CECCALDI, 1965, CECCALDI & ALLEMAND, 1964, CECCALDI et al., 1966, LEE, 1966b, LEE & ZAGALSKY, 1966, CHEESMAN et al., 1967, ZAGALSKY et al., 1967), it may be assumed that carotenoid-protein or carotenoid-lipoprotein complexes are present in the *Apus cancriformis*. Similar complexes were found during our investigations on the *Limnadia lenticularis* (CZECZUGA & CZERPAK, 1968e). In addition, when the presence of haemoglobin in *Apus cancriformis* is taken into consideration (FOX, 1955, CZECZUGA, 1964) investigations into the probable presence of biliary pigments in this species should also be undertaken in the future. The possibility of such pigments being present is indicated by the comparison of our results with those of GILCHRIST & GREEN (1962) from their investigations on the *Chirocephalus diaphanus* PREVOST.

On the basis of their maximum absorption, it was possible to identify to a large extent the fractions obtained by column chromatography (Tables I and II) and the spots obtained by thin-layer chromatography (Tables III and IV).

β -carotene

The presence of this carotenoid in the *Apus cancriformis* specimens was indicated by the absorption maxima of the first fraction of

TABLE I
Column chromatogram of pigments from Apus cancriformis (Bosc.)

No. and colour of fraction	System of solvents	Maximum absorption in m μ	Solvent	Identification
I yellow	petroleum ether (b.p. 45–65°C)	427–428, 450, 475–476	hexane	β -carotene
II pale yellow	1% acetone in petroleum ether	409–410, 436–437 463–464, 473–475	hexane	γ -carotene
III yellow	5% acetone in petroleum ether	421–413, 422–423, 442–443 456–458	hexane	unknown echinenone
IV yellow-orange	10% acetone in petroleum ether	464–466	hexane	canthaxanthin
V yellow	25% acetone in petroleum ether	421–422, 453–455, 473–474 487–488	hexane	lutein ester
VI pale yellow	50% acetone in petroleum ether	421–422, 443–444, 470–471	hexane	lutein
VII yellow (traces)	100% acetone	427–428, 454–456, 477–478	hexane	unknown xanthophyll
VIII orange	5% cold acetic acid in ethyl ether	407–408, 419–421 447–448, 461–463	hexane	unknown

TABLE II
Column chromatogram of pigments from carapace Apus cancriformis (Bosc.)

No. and colour of fraction	System of solvents	Maximum absorption in m μ	Solvent	Identification
I orange	10% acetone in petroleum ether	464—465	hexane	canthaxanthin
II yellow	25% acetone in petroleum ether	421—422, 455, 474—475	hexane	lutein ester
III yellow	50% acetone in petroleum ether	421, 443—444, 471—473	hexane	lutein free
IV orange-yellow	5% cold acetic acid in ethyl ether	445, 505—506, 520—521	hexane	unknown xanthophyll

TABLE III
Thin-layer chromatograms of pigments from Apus cancriformis (Bosc.)

No. and colour of spots	R _f	Figure	System of solvents	i	Maximum absorption in mμ	Solvent	Identification
1. yellow-orange	0.30				443-445, 473-475, 498-499, 517-518	acetone	lutein unknown
2. orange-red	0.67	Fig. 2a	Petroleum ether		465-467, 495	acetone	canthaxanthin
3. orange-yellow	0.83		benzene-methanol		454-455	acetone	echinenone
4. yellow	0.91		40 : 15 : 3.6		461-463, 487-488	acetone	γ-carotene
5. yellow	0.97				450-451, 477-478	acetone	β-carotene
1. yellow-orange	0.26				447-448, 470-471	hexane	lutein
2. orange-red	0.57				477-478, 500-501, 520-521	hexane	unknown
3. yellow-orange	0.71	Fig. 2b	I. Benzene		464-466	hexane	canthaxanthin
4. yellow	0.86		II. Benzene-hexane 2 : 1		452-453	hexane	echinenone
5. yellow-orange	0.94				462-463, 477-478	hexane	γ-carotene
					450-451, 474-476	hexane	β-carotene

TABLE IV
Thin-layer chromatograms pigments from carapace Apus cancriformis (Bosc.)

No. and colour of spots	R _f	Figure	System of solvents	Maximum absorption in mμ	Solvent	Identification
1. brown-yellow	0			402-403, 427-428, 447-448, 490-491	acetone	unknown xanthophyll
2. yellow	0.28	Fig. 3	Petroleum ether- benzene-methanol	420-421, 444-445, 473-474	acetone	lutein free unknown
3. yellow	0.43		40 : 15 : 3.6	505-506	acetone	lutein ester
4. yellow-orange	0.62			421-423, 442-443, 470-471 465-466	acetone	canthaxanthin

the column chromatogram and the fifth spot of the thin-layer chromatogram (GREEN, 1957, LENEL, 1961, GOODWIN, 1965, LEE, 1966b, GILCHRIST, 1968, CZEZUGA & CZERPAK, 1966, 1968b, d).

γ -carotene

On comparing the absorption maxima of the second fraction and the fourth spot with data given in literature (IWATA et al., 1961, JEFFREY, 1961, GOODWIN, 1962) the presence of this carotenoid in the *Apus cancriformis* was confirmed.

Echinenone

The presence of this carotenoid in the *Apus cancriformis* is indicated by the absorption maxima of the third fraction and third spots of the thin-layer chromatograms. Similar maxima were obtained from extracts of other aquatic fauna (CZEZUGA & CZERPAK, 1968, a, b, c; GILCHRIST, 1968).

Canthaxanthin

Both the fourth fraction of the column chromatogram and the second spot of the thin-layer chromatogram gave absorption maxima in hexane within a range of 464—466 m μ , characteristic as we know (THOMMEN & WACKERNAGEL, 1963, 1964; GOODWIN, 1952, KRINSKY, 1965, LEE, 1966a; GILCHRIST, 1968) of canthaxanthin. The presence of this carotenoid in the carapace of this crustacean is indicated by the absorption maxima of the first fraction and the fourth spot.

Lutein

The absorption maxima of the fifth and sixth fraction of the column chromatogram and the first spots of the thin-layer chromatograms indicate the presence of lutein in the *Apus cancriformis* specimen (GILCHRIST & GREEN, 1960, IWATA et al., 1961, LEE, 1966a). The second and third fraction of the extract from the *Apus cancriformis* carapace and the second and third spots indicate that this carotenoid is also present in the carapace.

It was not possible to identify exactly the seventh and eighth fraction. The absorption maxima of the seventh fraction seems to indicate some type of xanthophyll.

On comparing the results of the investigations on the *Apus cancriformis* with those of the investigations on *Limnadia lenticularis* (CZEZUGA & CZERPAK, 1968e) also Branchiopoda (Table V), it should be noted that there are fewer carotenoids in the *Limnadia lenticularis* (Table V).

Attention should also be drawn to the presence of the carotenoids in the carapace of the *Apus cancriformis* (Table VI) alone. Canthaxan-

TABLE V
Carotenoids in two species of Branchiopoda

No.	Pigment	<i>Apus cancriformis</i> (Bosc.)	<i>Limnadia lenticularis</i> L.
1	β -carotene	+	+
2	γ -carotene	+	+
3	echinenone	+	—
4	canthaxanthin	+	—
5	lutein	+	+
6	xanthophyll	+	—
7	zeaxanthin	—	+

TABLE VI
Carotenoids in carapace of three species of Crustacea

No.	Pigment	<i>Apus cancriformis</i> (Bosc.)	<i>Astacus astacus</i> (L.)	<i>Orconectes limosus</i> (Raf.)
1	β -carotene	—	+	+
2	echinenone	—	+	—
3	lutein	+	+	+
4	astaxanthin	—	+	+
5	astacene	—	+	—
6	canthaxanthin	+	+	—
7	xanthophyll	+	—	+

thin was found in the carapace of *Astacus astacus* (CZECZUGA & CZERPAK, 1969). Lutein was found in the carapace of the *Astacus astacus* and in that of the *Orconectes limosus* (CZECZUGA & CZERPAK, 1968f). Xanthophyll was found by the authors in the carapace of the *Orconectes limosus*.

SUMMARY

1. The presence of pigments in *Branchiopoda* of an intense brown colour — *Apus cancriformis* (Bosc.) was studied.
2. In extracts separated by means of column and thin-layer chromatography, the following carotenoids were identified: β -carotene, γ -carotene, echinenone, canthaxanthin, lutein and xanthophyll.

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