ACTA ICHTHYOLOGICA ET PISCATORIA
Vol. XVIII. Fasc. 2 Szczecin 1988

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Biochemistry

CAROTENOIDS IN FISH. 44. *CYPRINIDAE: ASPIUS ASPIUS* (L.)

KAROTENOIDY U RYB- 44. *CYPRINIDAE: ASPIUS ASPIUS* (L.)

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The occurrence of different carotenoids in various body parts of *Aspius aspius* was determined by means of column and thin layer chromatography. The presence of 12 carotenoids was found. Low concentrations of $\alpha-$ carotene derivatives found are particularly worth stressing.

INTRODUCTION

Cyprinid species commonly and abundantly present in Polish waters may be divided into a few groups with respect to the type of their food. Some species, like roach, graze on phytoplankton; others, e.g. bleak, feed on zooplankton. There are also a few typical benthos-eaters preying upon invertebrates which inhabit bottom sediments of lakes and rivers. Typical representatives of that group are crucian carp and tench. As far as predacious cyprinids are concerned, *Aspius aspius* is the only – and rare – such species in Polish waters, its food consisting of other fish.

Having, for several years, studied carotenoids in fish, including cyprinids ingesting various types of food (see Czeczuga and Kziewicz, 1985), I became interested in the presence of these pigments in *A. aspius* as a typical cyprinid predator.

MATERIALS AND METHODS

The materials consisted of 3 adult *A. aspius* (L.) individuals (total length of 40–48 cm) of both sexes caught on 16 May 1980 in the river Narew near Wizna. The fish

Table 1

List of the carotenoid from *Aspius aspius*

Carotenoid	Structure (see Fig. 1)	Semi-systematic name
β-carotene	A–X–A	β,β-carotene
β-cryptoxanthin	A–X–B	β,β-caroten-3-ol
canthaxanthia	E–X–E	β,β-carotene-4,4'-dione
lutein	B–X–C	β,ε-carotene-3,3'-diol
e'-epilutein	B–X–C	β,ε-carotene-3,3'-diol (stereoisomeric)
lutein epoxide	C-X-G	5,6-epoxy-5,6-dihydro-β,ε-carotene-3,3'-diol
zeaxanthin	B–X–B	β,β-carotene-3,3'-diol
phoenicoxanthin	E–X–F	3-hydroxy-β,β-carotene-4,4'-dione
α-doradexanthin	C–X–F	3,3'-dihydroxy-β,ε-carotene-4-one
adonixanthin	B–X–F	3,3'-dihydroxy-β,β-caroten-4-one
idoxanthin	D–X–F	3,3'-trihydroxy-β,β-caroten-4-one
astaxanthin	F–X–F	3,3'-dihydroxy-β,β-carotene-4,4'-dione

Table 2

Carotenoid content in the body of the *Aspius aspius*

Carotenoids	Fins	Skin	Muscles	Liver	Intestive
β-carotene	12.3	4.7	17.3		
β-cryptoxanthin	15.5	trace	6.4	11.4	18.3
lutein	4.4	5.0			
lutein epoxide				4.4	
3'-epilutein	4.5				2.0
zeaxanthin	35.9	17.3	10.4	15.1	13.8
idoxanthin		21.1	35.8	trace	18.2
canthaxanthin	4.8	trace	4.6	18.8	9.3
α-doradexanthin		0.2			
adonixanthin		30.0	16.9	20.0	
phoenicoxanthin	12.0				
astaxanthin	10.6	21.7	8.6	30.3	38.4
Total content in μg/g wet weight	0.211	1.132	0.147	0.458	0.472

had just finished spawning. Carotenoids were determined in fins, skin, muscles, liver, and intestine.

Tissue samples of identical weight from each individual were blended together, homogenated, and covered with 95% acetone in a dark glass bottle kept in a refrigerator until analysed. Carotenoid pigments were separated by means of column and thin layer chromatography. Prior to the assays, the samples were hydrolysed for 24 h in 10% KOH in nitrogen at room temperature. The details of the column and thin layer chromatographic techniques were described in a previous paper (Czeczuga and Czerpak, 1976). The hydrolysed extract was transferred into an Al_2O_3-filled, 15 to 25 cm long, column (Quickfit, England). Different fractions were eluted using various solvent combinations (Czeczuga and Czerpak, 1976).

Regardless of the column chromatography, the acetone extract obtained was separated into thin layer chromatography fractions as well. Silica gel-covered glass plates and various solvent combinations were used for the purpose. Subsequently the R_f value was determined according to the generally accepted principles.

The carotenoids were identified from:

a) the appearance of column chromatograms,
b) pigment absorption peaks in various solvents, as determined in a Beckman 2400-DU spectrophotometer,
c) the epi- to hypophase ratio, as determined in hexane and 95% methanol,
d) comparison of R_f alues for thin layer chromatograms; to identify the carotenoids, co-chromatography with standards (Hoffman-La Roche and Co.Ltd, Basel, Switzerland, and Sigma Chemical Company, USA) was applied,
e) the presence of allylohydroxyl groups determined with acid chloroform,
f) epoxy test.

Quantitative assays of various carotenoids were performed from quantitative absorption spectra. The assays were based on the extinction coefficients E 1%/cm at relevant absorption peaks in petroleum benzine or hexane.

RESULTS

The presence of a total of 12 carotenoids was found in the fish organs examined (Table 1, Fig. 1). All the carotenoids, except for idoxanthin and 3′-epilutein, had already been, and rather frequently so, found in other fish species. Such carotenoids as β-cryptoxanthin, canthaxanthin, zeaxanthin, and astaxanthin were recorded in all the *A. aspius* body parts analysed (Table 2). The dominant carotenoids were: zeaxanthin (fins), adonixanthin (skin), idoxanthin (muscles), and astaxanthin (liver, intestine). The skin proved to be the carotenoid-richest organ (1.132 μg/g wet weight), while muscles showed the lowest concentrations of the pigments (0.147 μg/g wet weight).

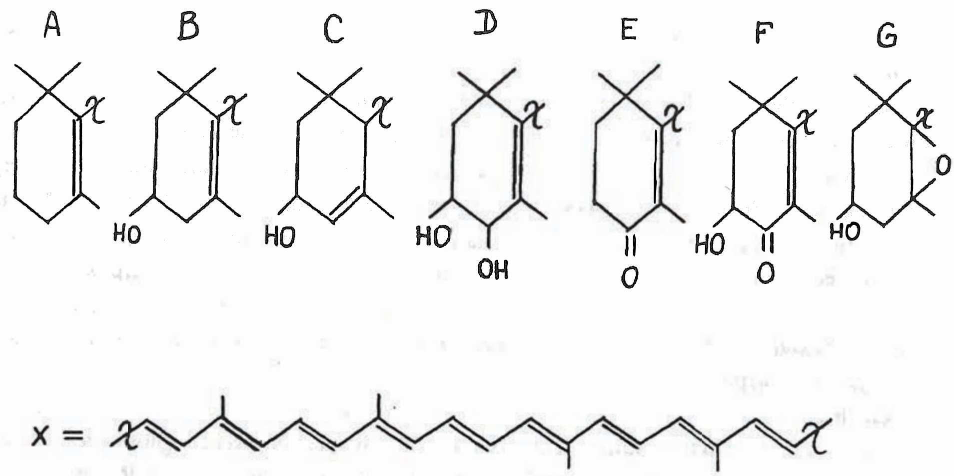

Fig. 1. Structural features of carotenoids from Aspius aspius specimens

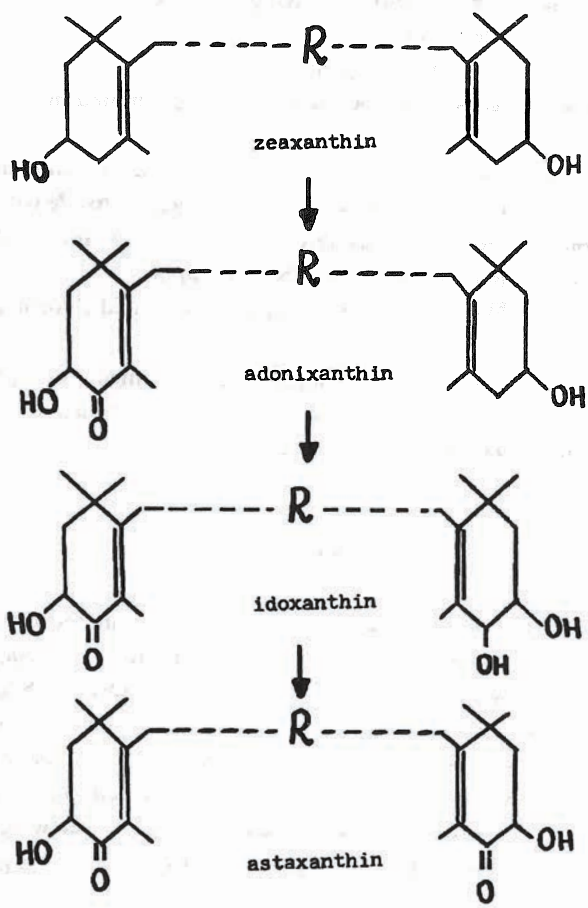

Fig. 2. Biosynthesis of idoxanthin in fish

DISCUSSION

As already mentioned, the carotenoids found in *A. aspius* individuals studied had previously been recorded in various fish species. Idoxanthin and 3′-epilutein were the exceptions, recorded previously in a few species only. Idoxanthin was first described by Herring (1969) from a marine crustacean *Idothea metallica*. Few years later the pigment was found in the fancy red carp (*Cyprinus carpio*) (Nagata and Matsuno, 1979). Subsequently the carotenoid was revealed in the lamprey, *Lampetra japonica* (Matsuno and Nagata, 1979) and in several Japanese species of the genus *Carassius* (Matsuno and Matsutaka, 1981). With respect to European fish species, the carotenoid was recorded in *Micropterus salmoides* (Czeczuga, 1981a), *Salmo trutta* (Czeczuga and Chełkowski, 1984), and *Thymallus thymallus* (Czeczuga et al., 1985). Idoxanthin is known to be involved in the zeaxanthin to astaxanthin transformation (Fig. 2).

3′-epilutein has so far been found in numerous Japanese fish species (Matsuno and Matsutaka, 1981); in Polish waters it was recorded, i.a., in trout (Czeczuga and Chełkowski, 1984) and in *Thymallus thymallus* (Czeczuga et al., 1985).

Noteworthy is the fact of a low content (of the order of a few per cent) of α-carotene derivatives; the commonest such derivatives in fish are lutein, 3′-epilutein, and predominantly lutein epoxide. In some fish these carotenoids are rather common and occur in high concentrations.

According to our previous studied, *Silurus glanis*, also a predator, showed α – carotene derivatives to make up 56.6–89.2% of all carotenoids (Czeczuga, 1977).

The available literature demonstrates *A. aspius* to feed on other fish species, bleak being the major food item of adult individuals in our lowland rivers (Szczerbowski, 1985).

Another α-carotene derivative is α-doradexanthin, usually present in small amounts in fish (Tanaka et al., 1976; Czeczuga 1981b) and formed as a result of lutein transformations into astaxanthin (Katayama et al., 1970). The *A. aspius* individuals examined contained α-doradexanthin in skin, the carotenoid making up as tittle as 0.2% of total carotenoids.

Usually, the liver and intestine are the carotenoid-richest organs in fish. In the present case the highest carotenoid contents in skin should be explained by the spawning period when the individuals were caught. Such phenomenon had already been observed in both sexes of trout (Czeczuga and Chełkowski, 1984) when, before spawning, carotenoids migrated from the internal organs to the external parts of the body, including the skin.

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STRESZCZENIE

Autor stosując chromatografię kolumnową i cienkowarstwową badał występowanie karotenoidów w poszczególnych częściach ciała bolenia.

W wyniku badań ustalono obecność takich karotenoidów, jak: β-karotenu, β-kryptoksantyny, luteiny, 3'-epiluteiny, epoksydową formę luteiny, zeaksantyny, idoksantyny, kantaksantyny, α-doradeksantyny, adoniksantyny, foenikoksantyny oraz astaksantyny. Podano również stosunki procentowe poszczególnych karotenoidów oraz ogólną ich zawartość w badanych częściach ciała boleni. Ponadto autor zwraca uwagę na małą ilość u badanych osobników boleni pochodnych α-karotenów.

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Received: 1986.08.12