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**Yale** PEABODY MUSEUM OF NATURAL HISTORY

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# Postilla

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## THE RED-YOLKED EGG OF THE TOURACO, *TAURACO CORYTHAIX*<sup>1</sup>

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The touracos are of biochemical interest because of the striking red and green colors imparted to their plumage by the copper porphyrins turacin and turacoverdin. It is therefore interesting to note that *Tauraco corythaix* lays an egg with a bright vermilion yolk. As might be expected, however, the color of the yolk is caused by carotenoids, a class of fat soluble pigments unrelated to turacin. Following is a brief description of the carotenoids of a touraco egg, in which it is shown that the principal member is either the red pigment astaxanthin (3, 3'-dihydroxy-4, 4'-diketo- $\beta$ -carotene) or astacene, the closely related tetraketo compound.

### MATERIALS AND METHODS

A single egg of *Tauraco corythaix* was made available by Dr. S. D. Ripley. The bird had been reared in captivity and was about a year old when it layed. The egg which we received had been accidentally broken. Several days elapsed before it was extracted, but for most of this period it was kept frozen ( $-15^{\circ}$  C).

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The bird had been fed fruit, but because it was kept in an open aviary, it could possibly also have eaten occasional insects and other animal matter.

The yolk was ground with anhydrous sodium sulfate until dry. The resulting powder was placed in a glass tube and extracted exhaustively by pouring acetone onto the top of the column. The red solution recovered from the bottom was diluted with water and the pigments transferred to petroleum ether in a separatory funnel. The petroleum ether solution was washed with water, dried over anhydrous sodium sulfate, filtered through a small plug of cotton, and evaporated to dryness under reduced pressure to remove the last traces of acetone.

The colored material was redissolved in fresh petroleum ether and chromatographed on a column of aluminum oxide (Merck reagent, "suitable for chromatographic adsorption") whose adsorptive strength had been weakened by the addition of 5 per cent (w/w) water. The column was developed with increasing concentrations of acetone in petroleum ether. The final fraction would not migrate in acetone or ethanol. It was recovered by extruding the top of the column and extracting the alumina with glacial acetic acid. This fraction was then transferred again to petroleum ether in a separatory funnel.

The eluted fractions were evaporated to dryness, dissolved in a known volume of fresh solvent, and their absorption spectra measured on a Cary recording spectrophotometer. Additional tests were performed in several cases; these will be described with the results.

#### RESULTS

The fractions recovered from the alumina column are listed in Table I. They are numbered in the order they were removed from the column but listed in order of abundance, which is simply the reverse of the sequence of elution. Each fraction will be described in turn, starting with No. 5.

No. 5 accounts for nearly two-thirds of the total pigment and shares a number of properties with free, i.e. unesterified, astaxanthin and astacene. (Astacene, tetraketo- $\beta$ -carotene, is readily formed by the oxidation of astaxanthin and is spectrally similar.) Like the red carotenoid of lobster shells, it was so tightly bound to the top of the column of aluminum oxide that it could not be

removed with acetone or ethanol; glacial acetic acid was required. It possessed a single broad absorption maximum in the visible, (Fig. 1) which is similar in shape and position to astaxanthin and astacene (Table II). Based on the molar extinction coefficient of astaxanthin, the yolk contained about  $2.4 \times 10^{-7}$  moles of this pigment.

TABLE I

Chromatographic fractions from the yolk of the touraco. The relative amount present (percentage of total) is approximate, as it is based on the assumption that the molar extinction coefficients of all the carotenoids are the same, which is only roughly true. The 1.3 per cent of the pigment which does not appear in the fourth column was eluted between bands.

<i>Band number</i>	<i>Color of Band</i>	<i>Eluant Required</i>	<i>Per-centage of total</i>	<i>Absorption maxima (and shoulders) in petroleum ether (<math>m\mu</math>)</i>
5	red	glacial acetic acid	62	464
4	yellow	25-30% acetone in petroleum ether	20.5	471, 443-444, (420)
3	diffuse pink	20% acetone in petroleum ether	7.4	470-471, (448)
2b	pale yellow	10% acetone in petroleum ether	5.6	438, (472)
2a	pale yellow	4% acetone in petroleum ether	2.1	439, (470)
1	pale yellow	petroleum ether	1.1	468, 426, 400

From the percentage of acetone required for elution, band No. 4 was clearly an unesterified xanthophyll with two hydroxyl groups. Precise identification, however, was not possible. The degree of fine structure in the absorption spectrum (Fig. 1) is intermediate between isomerates of lutein (3,3'-dihydroxy- $\alpha$ -carotene) and zeaxanthin (3,3'-dihydroxy- $\beta$ -carotene). In petroleum ether the pigment exhibited absorption maxima at 471 and 443-444  $m\mu$  and in ethanol at 473 and 447  $m\mu$ . These features suggest a mixture of zeaxanthin and lutein; however, the band appeared uniform in color on the column, and aliquots from the front and trailing portions were spectrally indistinguishable. There was no significant

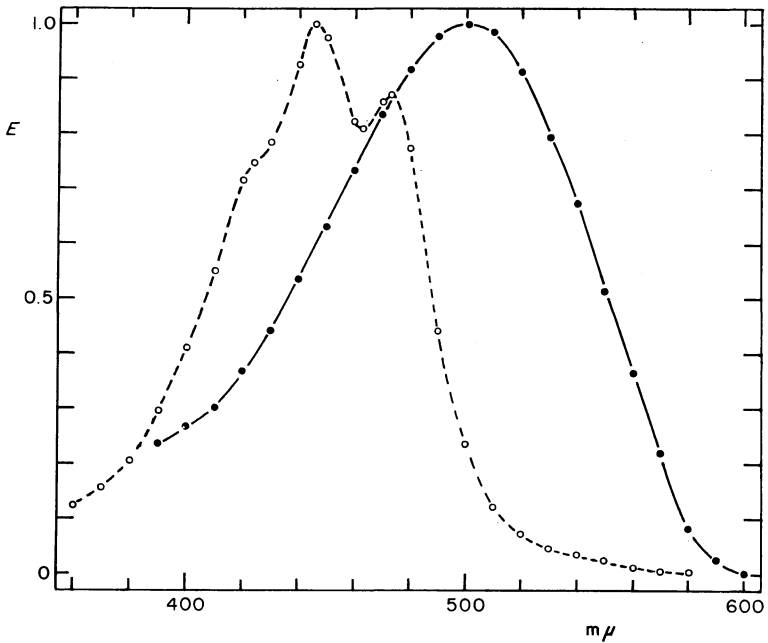


Fig. 1. Absorption spectra of touraco astaxanthin in carbon disulfide (filled circles, solid curve) and a xanthophyll (fraction No. 4) in ethanol (open circles, broken curve) from the yolk of *Tauraco corythaix*.

TABLE II

Comparison of the absorption properties of touraco pigment and lobster astaxanthin.

	Absorption maximum (m $\mu$ )			
	<i>petroleum ether</i>	<i>chloroform</i>	<i>carbon disulfide</i>	<i>pyridine</i>
Touraco pigment . . . . .	464	484	501	490
Astaxanthin . . . . . (from lobster shells)	469*	—	502**	491†

\* crystals, unpublished observations

\*\* Goodwin (1952)

† Karrer and Jucker (1950, pg. 354); also saponified material (astacene) after adsorption to alumina, unpublished observations of the author.

spectral shift in the presence of traces of HCl in ethanol, indicating the absence of 5:6 epoxide bridges.

No. 3, a diffuse pink band eluted with 20 per cent acetone in petroleum ether, possessed a rather unusual absorption spectrum (Fig. 2) with its principal maximum at 470-471  $m\mu$  in petroleum ether, 483  $m\mu$  in chloroform, and 484  $m\mu$  in benzene. The fraction appeared homogeneous when rechromatographed. It showed no change in absorption properties in the presence of potassium borohydride in ethanol. On partition between petroleum ether and aqueous methanol, the pigment distributed 33:67 (epiphase: hypophase) with 95 per cent methanol and 65:33 with 85 per cent methanol. This corresponds to an  $M_{50}$  coefficient (cf. Krinsky, 1963) of 90. The possibility that the pigment was present as an ester was not examined.

With respect to the position of the principal peak and the

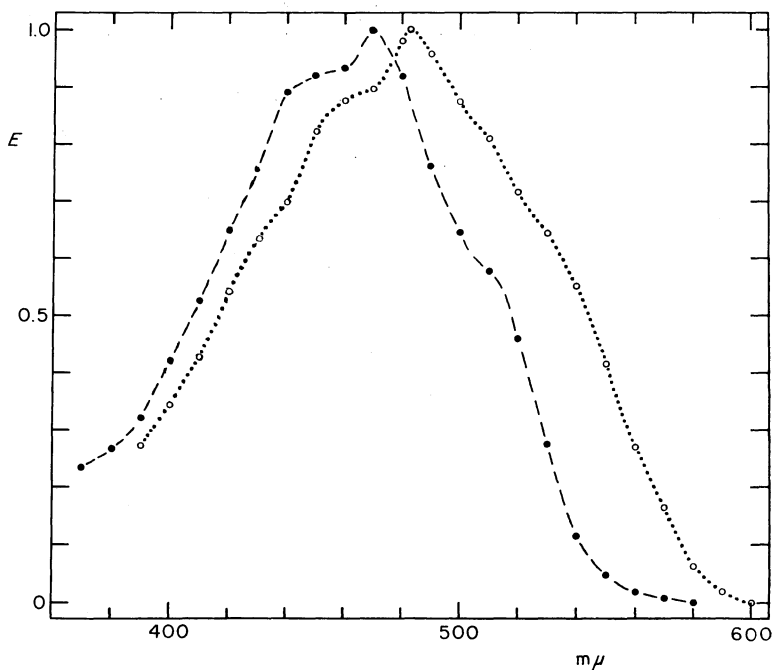


Fig. 2. Absorption spectrum of pigment No. 3 from the egg of *Tauraco corythaix* in chloroform (open circles, dotted curve) and petroleum ether (filled circles, dashed curve).

relative degree of fine structure in the absorption spectrum, this red pigment is suggestive of capxanthin; however, it differs in other spectral details and in its chromatographic properties.

No. 2a and 2b were spectrally indistinguishable, with  $\lambda_{\max}$  at 438-439  $m\mu$  and a shoulder at 470-472  $m\mu$  in petroleum ether. They differed only in the extent to which they were adsorbed on aluminum oxide (Table I). No. 1 showed sharp maxima at about 468, 426 and 400  $m\mu$ , but there was much end absorption which interfered with precise measurement. The small amounts of these three pigments precluded further work.

#### DISCUSSION

Birds tend to accumulate xanthophylls in preference to carotenes, and in this respect the egg of the touraco is no exception (for reviews see Fox, 1953; Goodwin, 1952). Astaxanthin, although not found in higher plants, is frequently encountered in animals. In birds, it has previously been reported from the eggs of a gull (*Larus ridibundus*) and a stork (*Ciconia ciconia*), as well as the wattles of pheasants (Brockmann and Völker, 1934), the cone oil drops of the chicken retina (Wald and Sussman, 1938), and occasionally in the feathers (Völker, 1950). It seems to be made from plant carotenoids by the chicken (Wald and Zussman, 1938) and probably also the flamingo (Fox, 1960), but if the diet of the bird contains sources of astaxanthin—for example, when planktonic crustaceans occur in the food chain—the ability to synthesize astaxanthin is possibly not present. The present results with the touraco are interesting, for it is doubtful that the parent bird received more than traces of astaxanthin in its food. The large amount of astaxanthin in the yolk therefore indicates that the touraco is able to form this pigment by oxidizing other carotenoids.

There is evidence that one or both the red pigments of the egg occur elsewhere in the bird, for the red color of the bill and skin about the eye are reported to be carotenoid (L. Auber, cited in Moreau, 1958).

#### SUMMARY

The yolk of *Tauraco corythaix* is bright vermilion. The carotenoids responsible have been separated by chromatography and

their absorption spectra recorded. About three-fifths of the color is astaxanthin (or astacene) and one-fifth a xanthophyll similar to lutein and zeaxanthin. Both pigments were found unesterified. Several other carotenoids are present in minor amounts, but these could not be identified from the sample available.

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