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Yellow Pigments in the Wings of the Papilionid Butterflies. IV. The Presence or Absence of Kynurenine in the Wings of the Genus *Graphium*

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Ford (1941, 1944a, 1944b) examined the presence or absence of anthoxanthin in the wings of all the genera of Papilionidae and used the data as a criterion for considering the systematics of the family. According to his report, most of the species of *Graphium* and all the species of *Parnassius* possess anthoxanthin in their wings, while *Papilio*, *Chilasa***, *Zerynthia*, *Lühdorfia*, *Bhutanitis*, and *Sericinus* do not. The author reported, in previous papers (Umebachi and Nakamura, 1954; Umebachi and Takahashi, 1956; Umebachi, 1959), that kynurenine was accumulated in large quantities in the yellow scales of these six genera which do not possess anthoxanthin. Moreover, he has presumed, using *Papilio xuthus*, that the yellow pigments may be "kynurenine-pigment" (Umebachi, 1958; Umebachi, the fifth paper of this series). On the other hand, kynurenine was not detected in the wings of some species of *Graphium*. So, the author has supposed that, in the wings of the Papilionidae, some relation may exist between the presence of kynurenine and that of anthoxanthin, and is examining the wings of all the genera of Papilionidae for kynurenine. The present paper deals with the results obtained with the genus *Graphium*.

According to Ford's report, he examined the presence or absence of anthoxanthin in the wings of 111 out of 136 known species of *Graphium*, and found that all the species examined except fifteen possessed anthoxanthin. The author has examined the presence or absence of kynurenine in the wings of eighteen species available up to now and found that four species possess kynurenine, while fourteen species do not. These four species which possess kynurenine belong to the fifteen species which Ford reported as not possessing anthoxanthin. On the other hand, the fourteen species which do not possess kynurenine are exactly those which possess anthoxanthin. It is interesting that all the species which possess kynurenine in the wings have yellow pigments.

Materials and Methods

Eighteen species of *Graphium* listed in Table 1 were examined. In all the cases, only the male was used, for the male can be secured more easily than the female.

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** Ford included *Chilasa* in *Papilio*, while Shirozu (1955) has reported that they are to be separated.

In all the species examined, the wings of one to four individuals were cut off, put in a beaker with a little distilled water in it, and warmed a little on a boiling water bath. During the process of warming, the wings were steeped well with a glass rod. Kynurenine is extracted from the wings very easily by this procedure.

The hot water extracts so obtained were subjected to one- or two-dimensional chromatography. In one-dimensional chromatography, 80 per cent methanol was used as the solvent. In two-dimensional chromatography, 80 per cent methanol and an organic layer of *n*-butanol-acetic acid-water mixture, 4 : 1 : 5 (BAW) were used as solvents. After development, the chromatogram was examined under ultraviolet light (Mazda UV-D1 filter) for kynurenine, and then the following color tests were made.

(1) Ninhydrin reaction

(2) Ehrlich's aldehyde reaction

(3) Reaction for aromatic amines by Tsuda's reagent (Umebachi and Tsuchitani, 1955)

The position of kynurenine on the chromatogram was confirmed to correspond to that of L-kynurenine by comparison with synthetic DL-kynurenine. The Rf values were the same as those reported in the previous paper (Umebachi and Takahashi, 1956; Umebachi, 1959).

In the species where kynurenine was detected on the chromatogram and where the number of individuals available was not small, the ultraviolet absorption curve of the eluate of kynurenine was taken between 230 and 380 mμ in 1/45 M phosphate buffer (pH 7.39) in the same manner as described in the second paper of this series.

The species in which Ford did not confirm the presence or absence of anthoxanthin in the wings were examined by the author, by means of the following two methods previously used by Ford : (1) In the species where the number of individuals available was small, the wings were fumed with strong ammonia and examined for the formation of a yellow color. (2) Anthoxanthin of the wings was extracted with ethyl acetate, and after being shaken with an aqueous solution of sodium carbonate, the upper layer of the extract was examined for the formation of a yellow color.

Results

The results are summarized in Table 1. In the chromatograms of *G. podalirius*, *G. gyas*, *G. payeni*, and *G. illyris*, a pale blue fluorescent substance, which gave a red-purple color with ninhydrin, a purple color with Tsuda's reagent, and an orange color with aldehyde reagent in the same manner as synthetic kynurenine, was detected. As to *G. podalirius*, the ultraviolet absorption curve of the eluate of the pale blue fluorescent substance was taken. The wave lengths of the absorption maxima were 275 and about 360 mμ, which agreed with the data of the eluate of synthetic kynurenine. In the chromatograms of *G. agamemnon* and *G. xenocles*, a spot which gave a slightly purple color with Tsuda's reagent was found near the position of kynurenine, but the spot was negative to ninhydrin and aldehyde reagents. Also in some of the species other than the above-mentioned, fluorescent substances

were present near the position of kynurenine, but these spots were negative to ninhydrin, Tsuda's reagent, and aldehyde reagent.

Table 1. The presence or absence of kynurenine and anthoxanthin in the wings of *Graphium*. All the species were examined by two-dimensional chromatography, except *eurous*, *cloanthus*, and *sarpedon*, which were examined by one-dimensional chromatography. +, present; −, absent.

Species	Kynurenine	Anthoxanthin	Remarks on the color of wing
G. eurous	−	+*	Yellowish white pigment, +.
G. podalirius	+	−*	Yellow pigment, +.
G. agetes	−	+*	Whitish yellow pigment, +.
G. gyas	+	−*	Yellow pigment, +.
G. payeni	+	−*	ditto
G. cloanthus	−	+***	Yellow pigment, −; Green pigment, +.
G. sarpedon	−	+***	ditto
G. doson	−	+*	ditto
G. bathycles	−	+*	ditto
G. agamemnon	−	+*	ditto
G. xenocles	−	+*	Yellow pigment, −; White part, +.
G. indicus	−	+**	Yellow pigment, −; Yellowish white part, +.
G. endochus	−	+*	Yellow pigment, −; White pigment, +.
G. leonidas	−	+*	Yellow pigment, −; Green pigment, +.
G. antheus	−	+*	ditto
G. policenes	−	+*	ditto
G. illyris	+	−*	Yellow pigment, +.
G. serville	−	+*	Yellowish white pigment, +.

* The data reported by Ford.
** Examined by the first method described in this paper.
*** Examined by the second method described in this paper.

The three species, *G. cloanthus*, *G. sarpedon*, and *G. indicus*, were examined for anthoxanthin, as they had not been examined by Ford. The wings of *G. cloanthus* and *G. sarpedon* were examined by the second method mentioned above, from three to four individuals being used, and were found to be positive to the test, although the yellow color was slight. *G. indicus* was examined by the first method mentioned above, for only a few samples were available. In this case, although the detection of anthoxanthin was not easy, some parts of the hind wings gave a yellow color and were clearly positive to the test. On the other hand, when *G. eurous*, *G. agetes*, *G. endochus*, and *G. serville* were examined by this method, the white or yellowish white parts of the wings became extensively yellow.

Discussion

It is well known that the white and yellow pigments of the wings of the Pieridae are pterin. However, strangely enough, the yellow pigments of the Papilionidae, which is most

closely allied to the Pieridae, are not pterin, and their nature has remained unknown until recently. Regarding this problem, the author reported that kynurenine accumulates in the yellow scales of wings of *Papilio, Chilasa, Zerynthia, Lühdorfia, Bhutanitis,* and *Sericinus*. At that time, it was a matter of interest that these six genera belong to those reported by Ford as not possessing anthoxanthin. In the present paper, morever, it has been proved in *Graphium* that the species which possess kynurenine in the wings are those which do not possess anthoxanthin. So it seems that in the wings of the Papilionidae, kynurenine and anthoxanthin are mutually exclusive. Ford reported already that the species of *Graphium* which did not possess anthoxanthin fluoresced under ultraviolet light. It is conceivable from the results of the present paper that the fluorescence may have been due to kynurenine or some yellow pigments related to kynurenine. At any rate, no species so far examined which accumulate a large quantity of kynurenine in the wings possess anthoxanthin in large quantities. However, species which possess both anthoxanthin and kynurenine are also found. For example, *Parnassius* has been reported to possess anthoxanthin in the wings like *Graphium*. The author examined *Parnassius glacialis* and found that the wings possess a little kynurenine. So this species seems to possess both kynurenine and anthoxanthin. In this case, however, the quantity of kynurenine is much smaller than in *Papilio, Chilasa, Zerynthia, Lühdorfia, Bhutanitis,* and *Sericinus*. Among *Lamproptera* which was also reported by Ford to possess anthoxanthin in the wings, the author has examined *Lamproptera curius* and found that the wings possess kynurenine. However, in the wings of this species, the yellow scales are rather few. Generally speaking, it seems that in the Papilionidae no wings in which kynurenine accumulates in large quantities possess a large quantity of anthoxanthin as mentioned above. The biochemical significance of this fact is still unknown, and must be the subject of further research.

A matter which is noteworthy here is that all the species which accumulate kynurenine in the wings possess the yellow or pale yellow pigments. Probably these are "kynurenine-pigments". Further research must be made to find out whether the kynurenine-pigments and the anthoxanthin pigments are related to each other directly through the enzyme system of their metabolism or accumulation, or indirectly through some other factors. Another noteworthy matter is that anthoxanthin pigments are said to have been possibly present in the common ancestor of the Pieridae and the Papilionidae (Ford, 1944b; Shirozu, 1955). It is conceivable that, as the Pieridae and the Papilionidae branched from a common ancestor, the former family may have acquired the nature to accumulate pterin in the wings and the latter the nature to accumulate kynurenine-pigments. And in the Papilionidae, the ancestral nature to possess anthoxanthin in the wings is found here and there as if preserved in some genera (Ford, 1944b). Thus, in *Graphium*, the species which possess anthoxanthin in the wings may be said to be more primitive than those which possess kynurenine in the wings, so far as the wing pigment is concerned. In this respect, it is interesting that *Lamproptera* possesses kynurenine in the wings as mentioned above. As *Lamproptera* is said to have been derived from *Graphium* according to Ford's report, the fact that the former genus

possesses kynurenine in the wings will cast an interesting light on considering the evolution of pigment in the Papilionidae.

Among the four species (*G. podalirius*, *G. gyas*, *G. payeni*, and *G. illyris*) which have been proved to possess kynurenine in the wings, *G. podalirius* is different from the other species of *Graphium* in the nature of the red pigment also. According to Ford, the red pigments of *Graphium* are generally of type A, and only *G. podalirius* possesses type B red pigment like *Papilio*. So, this species can be said to be similar to *Papilio* regarding both kynurenine and the red pigment.

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Summary

(1) The presence or absence of kynurenine in the wings of eighteen species belonging to the genus *Graphium* of the Papilionidae were examined by means of paper chromatography.

(2) Among the eighteen species, only four species, *G. podalirius*, *G. gyas*, *G. payeni*, and *G. illyris*, possessed kynurenine in the wings, while the other fourteen species did not. The four species which possessed kynurenine all had the yellow pigments in their wings.

(3) These four species which possessed kynurenine were those reported by Ford as not possessing anthoxanthin. On the other hand, the other fourteen species all possessed anthoxanthin in the wings. The relation between kynurenine and anthoxanthin in the wings of the Papilionidae was discussed.

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