

**Yellow Pigments in the Wings of the Papilionid
Butterflies. VII. Consideration of the Nature
and Distribution of the Wing Pigments
of the Papilionid Butterflies**

By

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(Received January 16, 1962)

As is well known, the pigments of the wings of the Pieridae are pterin. However, strangely enough, the pigments of the Papilionidae, which is most closely allied to the Pieridae, are not pterin, and their nature has remained unknown until recently. Regarding the pigments of the Papilionidae, Ford (1941, 1944a, 1944b) investigated the nature and distribution of anthoxanthin and red pigments, from a biological, especially systematic, point of view. According to his report, it is evident that most of the yellow pigments which are distributed extensively in the Papilionidae are not anthoxanthin except in *Graphium* and *Parnassius*. In regard to this point, the author (Umebachi and Nakamura, 1954; Umebachi and Takahashi, 1956; Umebachi, 1958; Umebachi, 1959) found that the yellow scales of the wings of *Papilio*, *Chilasa*, *Zerynthia*, *Lühdorfia*, *Bhutanitis*, and *Sericinus* accumulate kynurenine. Since then, the author has examined the chemical nature of the yellow pigments, using mainly *P. xuthus*, and presumed that they are "kynurenine-pigments" (the pigments related to kynurenine) (the fifth paper of this series, Umebachi, 1961). So far as the author has examined up to the present, the presence of these yellow "kynurenine-pigments" seems to be characteristic only of the Papilionidae among butterflies. On the other hand, as Ford reported, anthoxanthin is said to be widespread in the Lepidoptera, although not very common. The author made some experiments concerning the relation between the distribution of the yellow kynurenine-pigment and that of anthoxanthin in the Papilionidae and reported the results in the fourth paper (Umebachi, 1960) of this series. In addition, the author has examined the type B red pigment characteristic of the Papilionidae, and supposed that it might also be derived from tryptophan.

The purpose of the present paper is to discuss the chemical nature of the yellow kynurenine-pigment of the Papilionidae in comparison with other pigments reported up to now, and to consider the distribution of yellow kynurenine-pigment in the Papilionidae and moreover in the Rhopalocera, on the basis of the results of the first - sixth papers of this series.

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Chemical properties of the yellow kynurenine-pigments of the Papilionidae

In the first place, a word must be said about the materials used for studying the yellow kynurenine-pigment. In this series, the nature of the yellow pigments of the Papilionidae has been examined mainly by using the male of *Papilio xuthus*, for in Japan the male of this species is obtained most easily. On the other hand, to the author's regret, the nature of the yellow pigments of other species has not been examined in such detail up to the present, for the number of butterflies available is small. So, the properties of the yellow kynurenine-pigments described in the present paper are not applicable to all the other species. Especially, the nature of the yellow pigments of *Papilio machaon* seems to be very different from that of *P. xuthus*, because when the yellow scales of *P. machaon* are extracted with 80 per cent ethanol or water, most of the yellow pigments remain undissolved. The pigments of the two species, however, are similar in that their hot water extracts contain a large quantity of kynurenine. On the other hand, the yellow pigments of *Papilio protenor* and *Papilio helenus* are similar to those of *P. xuthus*, not only in that the yellow scales accumulate kynurenine, but also in point of their solubilities. In the present paper, all of those yellow pigments are sometimes denoted as kynurenine-pigment. But here it should be kept in mind that the yellow pigment whose chemical properties have been examined in detail is only that of *P. xuthus*.

In the second place, the nature of the yellow pigments of *P. xuthus* will be discussed. Although these pigments were reported in the first paper of this series (Umebachi, 1958) to contain three components Y-I, Y-II, and Y-III, Y-I has later been proved to be either a degradation-product or a changed substance of Y-II and -III, and so the natural yellow pigments of this species have been presumed to be of two kind (Y-II and -III) and named "Papiliochrome - II and - III" respectively (Umebachi, 1961). That Papiliochrome - II and - III are not anthoxanthin can be presumed from Ford's report (1941) that anthoxanthin is not present in the wings of *Papilio*, and moreover, from the fact that Papiliochrome - II and - III are insoluble in ethyl acetate. On the other hand, it is clear from the experiment of tryptophan-C¹⁴ injection that Papiliochrome-II and -III are the pigments derived from tryptophan. But they seem to be different from ommochrome, the best known group of pigments derived from tryptophan. It is well known from the biochemical genetics of *Ephestia kuhniella*, *Drosophila melanogaster*, and *Bombyx mori* that ommochrome is formed from tryptophan through kynurenine and 3-hydroxykynurenine (Butenandt, 1952; Kikkawa, 1953). In respect to the pigment of the ommochrome group, detailed investigations have been made by Butenandt and his co-workers (Butenandt, 1957, 1959). Ommochrome is divided into ommatin, which has a lower molecular weight and is more labile to alkali, and ommin, which has a higher

molecular weight and is less labile to alkali. Of these two, the chemical structure has been examined in more detail in ommatin than in ommin. As to ommatins, up to the present, xanthommatin, rhodommatin, ommatin D, and ommatin C have been reported. Among these ommatins, the structure of xanthommatin was already determined. Xanthommatin is formed by condensation of two molecules of 3-hydroxykynurenine and by subsequent ring closure of one of the two molecules to quinoline ring. Rhodommatin is different from xanthommatin only in the point that a hydroxyl group is substituted for a amino group of the side chain*. Other ommatins also seem to be similar to xanthommatin in their main structure (3,4-pyridino-phenoaxzone)**. (But ommatin C has been reported to be an artificial substance produced from ommatin (Butenandt, Biekert, and Beckmann, 1957)). Xanthommatin was first isolated from "Schlupfsekret" of *Aglais urticae**** and since then xanthommatin, rhodommatin, and ommatin D have been reported to be present in the eye, excretion, wings, and epidermis of many species of insects (Butenandt and Neubert, 1955; Butenandt, Biekert, and Linzen, 1958b; Butenandt, 1959; Butenandt, Biekert, Kübler, and Linzen, 1960), the egg of *Urechis caupo* (Linzen, 1959), and the epidermis of *Asellus aquatics* (Needham and Brunet, 1957). On the other hand, the structure of ommin also has been recently determined. It has been reported to have the structure of triphen-oxazin-thiazin. The distribution of ommin is better known than that of ommatin. Ommin is present in the eye and epidermis of the Crustacea, the eye of insects, and the eye and epidermis of the Cephalopoda. In butterflies, ommin has been reported to be present in the eye of *Parnassius*, *Papilio*, *Colias*, *Aporia*, *Pyrameis*, *Vanessa*, *Argynnus*, and *Heliconius*, but absent in the wings. Butenandt has described in his report of the distribution of ommin that when the red, yellow or brown pigments of wings of butterflies are ommochrome, they are never ommin but ommatin. That is to say, in the wings of butterflies, ommatin is present but ommin is absent. Now, the author will compare Papiliochrome-II and -III of *P. xuthus* with the above-mentioned ommochrome. Ommochrome, including ommatin and ommin, produces xanthurenic acid by alkali hydrolysis, and produces 3-hydroxykynurenine by acid hydrolysis (Butenandt, Schiedt, and Biekert, 1954; Butenandt, Biekert, and Beckmann, 1957; Butenandt, Biekert, and Linzen, 1958a; Butenandt, 1959). This seems to be an important property of ommochrome and has been used for identifying ommochrome (Needham and Brunet, 1957). However, Papiliochrome-II and -III do not produce xanthurenic acid by alkali hydrolysis, and do not produce 3-hydroxykynurenine but a large quantity of kynurenine by acid hydrolysis. Moreover, according to Butenandt et al. (1957, 1958a), the oxidized form of ommatin is

* According to Butenandt's later report, rhodommatin has been supposed to be a glucoside of hydroxanthommatin. But its side chain has remained unsolved (Butenandt, 1960).

** Ommatin D has been reported to be a sulfate ester of hydroxanthommatin (Butenandt, Biekert, Koga, and Traub, 1960).

*** *Aglais urticae* is a butterfly belonging to the Nymphalidae, and the species name, *Vanessa urticae*, has been used in Butenandt's report.

yellowish or brownish, and has been reported to turn red by being reduced with sulfur dioxide or sodium hydrosulfite. But the yellow pigments of *P. xuthus* do not turn red when treated with the reducing agents. In these respects, Papiliochrome-II and -III are different from the ommochrome as reported so far. In addition, a conspicuous property of Papiliochrome-II and -III is to produce kynurenine and some phenolic compound easily. Such a property has not been reported for ommochrome. From such points, although Papiliochrome-II and -III are derived from tryptophan, they do not seem to belong to the ommochrome group reported so far. In consequence, Papiliochrome-II and -III are the pigments which produce kynurenine easily, and seem to be probably a combination of kynurenine with some other substance (probably a phenolic compound). For this reason, such pigments as Papiliochrome-II and -III have been denoted as "kynurenine-pigment" in this series. But the possibility that kynurenine is not contained as a component in the yellow pigments but is only adsorbed to the yellow pigments can not be also excluded, although the author supposes it to be improbable.

In the third place, as some other pigments which are derived from tryptophan but which are not ommochrome also have been reported up to now, a few words will be said about them here. One of them is the aminoquinone pigment which is a combination of kynurenine with quinone derived from tyrosine (Glassman, 1957). Generally speaking, quinones are very active substances and are inclined to combine with many substances including amines and amino acids (Mason, 1955). These aminoquinone pigments also are supposed to be present in animal kingdom, and so the relation between such pigments and Papiliochrome-II and -III must be a subject for future research*. Moreover, it has been reported recently that a yellow pigment can be produced from kynurenic acid by a bacterium (Taniuchi et al., 1959), but such a yellow pigment does not seem to produce kynurenine easily. Although 3-hydroxykynurenine and 5-hydroxykynurenine also are yellow in color, Papiliochrome-II and -III have been confirmed not to correspond to them.

The above-mentioned discussion of the yellow pigments of the Papilionidae is based entirely on the yellow pigments of *P. xuthus* as mentioned above, and although the yellow pigments of other species also seem to be similar to them, it is not certain. Particularly, the yellow pigments of *P. machaon* insoluble in 80 per cent ethanol and water are different from the above-mentioned Papiliochrome-II and -III and remain to be investigated. *P. machaon* belongs to the machaon group of *Papilio* with *P. xuthus*, and as, according to Ae (1958), a hybrid can be obtained between the two species, the relation between Papiliochrome-II and -III and the yellow pigments of *P. machaon* is an interesting subject.

Finally, as it has been proved that Papiliochrome-II and -III are labile and produce kynurenine easily, the following question may arise here: whether a large

* The results of the experiments carried out up to the present suggest that Papiliochrome-II may be a combination of kynurenine and *o*-diphenol derived from dopa.

quantity of kynurenine found in the hot water extract of the yellow scales of wings of the Papilionid butterflies is present in free form in the scales or is produced by the decomposition of the yellow pigments during hot water extraction. When the extract obtained from the yellow scales without being heated are chromatographed, the quantity of kynurenine found is much smaller than when the extraction are made under heating, and so part of the kynurenine present in the hot water extract must be presumed to have come from the yellow pigments by heating. Moreover, it is also supposed that during the application of the extract to the chromatographic paper by means of drying repeatedly in the air at room temperature, a little kynurenine may be produced from the yellow pigments. On the other hand, the yellow pigments are not decomposed at all during the development with 80 per cent methanol. As mentioned above, it is only of *P. xuthus* that the chemical properties of yellow pigments have been examined in detail, and in other species the detection of kynurenine in hot water extract has been mainly performed. And so the above-mentioned point must be kept in mind.

Distribution of yellow kynurenine-pigments

In the first place, the presence or absence of kynurenine in the wings of the Papilionidae will be discussed. To be exact, as mentioned above, this means the presence or absence of kynurenine in the hot water extract of the wings. The family Papilionidae contains four subfamilies and seventeen genera (Shirozu, 1955). Among them, the genera which have been confirmed to possess kynurenine in the wings by the author up to the present, are *Papilio*, *Chilasa*, and four genera of the Zerynthiinae (*Zerynthia*, *Lühdorfia*, *Bhutanitis*, and *Sericinus*). In the yellow or pale yellow scales of these genera, kynurenine is accumulated. It is characteristic of yellow scales, and the butterflies which do not possess yellow scales in the wings do not accumulate kynurenine, even if they belong to *Papilio*. Now, it is interesting that the genera which accumulate kynurenine in the wings are included in those which have been reported by Ford (1941, 1944a) not to possess anthoxanthin in the wings. Moreover, in *Graphium*, the species which have been described to possess anthoxanthin by Ford do not seem to possess kynurenine in the wings, while those which have been reported not to possess anthoxanthin seem to accumulate kynurenine in the wings. Thus, it is of interest that, in the wings of the Papilionidae, kynurenine and anthoxanthin seem to be mutually exclusive. Although the species which possess both anthoxanthin and kynurenine (for example *Parnassius glacialis* and *Leptocircus curius*) are also found, the species which accumulate a large quantity of kynurenine in the wings are, in many cases, those which do not possess anthoxanthin. This problem has been discussed in the fourth paper (Umebachi, 1960) of this series.

In the second place, discussion will be given about the point that such yellow

kynurenine-pigments as mentioned above are characteristic of the Papilionidae among butterflies. So far as the author has examined about the Pieridae, Papilionidae, Satyridae, Nymphalidae, Lycaenidae, and Hesperiidae up to the present, yellow kynurenine-pigments have not been found except in the Papilionidae. The pigments of the Pieridae, which is most closely allied to the Papilionidae as mentioned previously, are pterin and not kynurenine-pigments. On the other hand, in the Papilionidae, the yellow kynurenine-pigments are present, but pterin is not present as a wing pigment. Thus, in the wings of butterflies, the yellow kynurenine-pigment seems to be a characteristic of the Papilionidae, just as it is a characteristic of the Pieridae to accumulate a large quantity of pterin as the wing pigment. In respect to the wing pigment of butterflies, it is sometimes said "There are some cases where the wing pigment indicates systematics." (Yagi, 1957). That is to say, in butterflies, the wing pigment becomes sometimes a characteristic of a systematic group. In some cases, the group is family or subfamily, and in other cases, genus. At any rate, there are some cases where such groups possess their own characteristic pigments. The most conspicuous example is the pterin of the Pieridae and the yellow kynurenine-pigment of the Papilionidae. The same matter can be said also about the red pigments of the wings. As mentioned in the sixth paper of this series, type B red pigment, which has been reported to be present only in the Papilionidae by Ford, is supposed to be the pigment which is derived from tryptophan but has the property different from that of ommochrome reported so far. On the other hand, type C red pigment present in the Vanessidi of Nymphalidae is presumed to be ommochrome. Thus, as to the red pigments also, there are some cases where the systematic groups have their own characteristic pigments. Hereupon, an interesting matter is that pterin, kynurenine-pigment, and ommochrome, which are characteristic of each systematic group, are the pigments synthesized by the animal. It is clear that kynurenine-pigment and ommochrome are synthesized by the animal, and moreover, it is also presumed from the experiment of Weygand and Waldschmidt (1955) that pterin of the Pieridae is synthesized by the butterflies. On the other hand, the above-mentioned anthoxanthin is clearly plant pigment derived from the food plant of the larva (Thomson, 1926). The anthoxanthin is widely distributed in the Lepidoptera, although in a small number of species, and is said to be more primitive as the wing pigment of butterflies. Especially, in the Pieridae and the Papilionidae, the common ancestor has been supposed to have possessed anthoxanthin in the wings by Ford (1944b).

Finally, the biological significance of studying the distribution of the wing pigment will be described here. It seems to contain the following two problems: (1) What kind of substance is more primitive as the wing pigment of butterflies? In other words, what kind of substance did the primitive butterflies possess as the wing pigment? Is the supposition correct that anthoxanthin is at a lower order as the wing pigment of butterflies? (2) As butterflies have evolved and

branched into various systematic groups, how has the pigment characteristic of each group developed? "In butterflies, there are some cases where the wing pigment indicates the systematics." What biological meaning has this? The above two problems seem to be important. Of course, for the investigation of these problems, not only a biochemical study of the nature and metabolism of pigments but also its interaction to the taxonomy, evolution, genetics, embryology, and physiology of butterflies are necessary.

Conclusion

Although most of the yellow pigments of the Papilionidae are derived from tryptophan except in cases where they are anthoxanthin, they are generally supposed not to be ommochrome but to be "kynurenine-pigment". The presence of a large quantity of kynurenine in the hot water extract of the yellow scales is characteristic of these yellow kynurenine-pigments.

In the wings of many species of the Papilionidae, kynurenine and anthoxanthin seem to be mutually exclusive.

Among butterflies, the yellow kynurenine-pigment has not been found except in the Papilionidae up to the present, and so it may be said to be characteristic of the family, together with type B red pigment.

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