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 sent 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 roport (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-C^{14} 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 kühniella, 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, datailed 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-phenoxazone)**. (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*, *Argynnis*, 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 intesting 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.

References

Ae, S., 1958. A study of hybrids in *Papilio machaon* group and *P. xuthus*. A lecture at the 30th. annual meeting of the Genetics Society of Japan, (Nagoya).

Butenandt, A., 1952. The mode of action of hereditary factors. Endevour, **11** : 188–192.

Butenandt, A., 1957. Über Ommochrome, eine Klasse natürlicher Phenoxazon-Farbstoffe. Angew. Chem., **69** : 16–23.

Butenandt, A., 1959. Wirkstoffe des Insektenreiches. Naturwissenschaften, **46** : 461–471.

Butenandt, A., 1960. Über neue Naturfarbstoffe, ihre Biogenese und physiologische Bedeutung. XVII Internat. Kongr. f. Reine und Angew. Chemie, München 1959, Verlag Chemie 1960, S. 11–31.

Butenandt, A., E. Biekert, and R. Beckmann, 1957. Über Ommochrome, XII. Reindarstellung von Rhodommatin und Ommatin D zur Struktur des Rhodommatins. Liebigs Ann. Chem. **607**: 207–215.

Butenandt, A., E. Biekert, N. Koga, and P. Traub, 1960. Über Ommochrome, XXI. Konstitution und Synthese des Ommatins D. Z. physiol. Chem., **321** : 258–275.

Butenandt, A., E. Biekert, H. Kübler, and B. Linzen, 1960. Über Ommochrome, XX. Zur Verbreitung der Ommatine im Tierreich, neue Methoden zu ihrer Identifizierung und quantitativen Bestimmung. Z. physiol. Chem., **319** : 238–256.

Butenandt, A., E. Biekert, and B. Linzen. 1958a. Über Ommochrome, XIII. Isolierung und Charakterisierung von Omminen. Z. physiol. Chem., **312** : 227–236.

Butenandt, A., E. Biekert, and B. Linzen, 1958b. Über Ommochrome, XIV. Zur Verbreitung der Ommine im Tierreich. Z. physiol. Chem., **313** : 251–258.

Butenandt, A., and G. Neubert, 1955. Über Ommochrome, V. Xanthommatin, ein Augenfarbstoff der Schmeissfliege *Calliphora erythrocephala*. Z. physiol. Chem., **301** : 109–114.

Butenandt, A., and G. Neubert, 1959. Über Ommochrome, XVII. Zur Konstitution der Ommine, I. Liebigs Ann. Chem., **618** : 167–173.

Butenandt, A., G. Neubrt, and U. Baumann, 1959. Über Ommochrome, XVl. Versuche zur Reindarstellung eines Ommins. Z. physiol. Chem., **314** : 15–21.

Butenandt, A., U. Schiedt, and E. Biekert, 1954. Über Ommochrome, II. Alkalischer und fermentativer Abbau von Xanthommatin und Rhodommatin. Alkalischer Abbau der Kynurenin-Seitenkette. Liebigs Ann. Chem., **586** : 229–239.

Ford, E. B., 1941. Studies on the chemistry of pigments in the Lepidoptera, with reference to their bearing on systematics. 1. The anthoxanthin. Proc. R. Ent. Soc. Lond. (A), **16** : 65–90.

Ford, E. B., 1944a. Studies on the chemistry of pigments in the Lepidoptera, with reference to their bearing on systematics. 3. The red pigments of the Papilionidae. Proc. R. Ent. Soc. Lond. (A), **19** : 92–106.

Ford, E, B., 1944b. Studies on the chemistry of pigments in the Lepidoptera, with reference to their bearing on systematics. 4. The classification of the Papilionidae. Trans. Roy. Ent. Soc. Lond., **94** : Part 2, 201–223.

Glassman, E., 1957. Tyrosinase-produced quinones and the disappearance of kynurenine in larvar extracts of *Drosophila melanogaster*. Arch. Biochem. Biophys., **67** : 74–89.

Taniuchi, K., M. Tashiro, H. Yamada, S. Hisano, and O. Hayaishi, 1959. [Enzymatic degradation of kynurenic acid]. J. Jap. Biochem. Soc., **31** : 335.

Kikkawa, H., 1953. Biochemical genetics of *Bombyx mori* (Silkworm). Advances in Genetics, **5** : 107–140.

Linzen, B., 1959. Über Ommochrome, XV. Über die Identifizierung der "Urechochromes" als Xanthommatin. Z. physiol. Chem., **314** : 12–14.

Mason. H. S., 1955. Comparative biochemistry of the phenolase complex. Advances in enzymology, **16** : 105–184.

Needham, A. E., and P. C. J. Brunet, 1957. The integumental pigment of *Asellus*. Experientia, **13** : 207–209.

Shirozu, T., 1955. [A note on butterflies(3)]. Shin Konchu, **8** : No. 4, 38–45.

Thomson, D. L., 1926. The pigments of butterflies' wings. II. Occurrence of the pigment of *Melanargia galatea* in *Dactylis glomerata*. Biochem. J., **20** : 1026–1027.

Umebachi, Y., 1958. Yellow pigments in the wings of the Papilionid butterflies. I. The relation between kynurenine and the yellow pigments of *Papilio xuthus*. Sci. Rep. Kanazawa Univ., **6** : 45–55.

Umebachi, Y., 1959. Yellow pigments in the wings of the Papilionid butterflies. II. The presence of a pale blue fluorescent substance supposed to be kynurenine in the wings of the Zerynthiinae. Sci. Rep. Kanazawa Univ., **6** : 69-75.

Umebachi, Y., 1960. Yellow pigments in the wings of the Papilionid butterflies. IV. The presence or absence of kynurenine in the wings of the genus *Graphium*. Sci. Rep. Kanazawa Univ., **7** : 107–112.

Umebachi, Y., 1961. Yellow pigments in the wings of the Papilionid butterflies. V. Some chemical properties of the yellow pigments of *Papilio xuthus*. Sci. Rep. Kanazawa Univ., **7** : 139–150.

Umebachi, Y., and A. Nakamura, 1954. The presence of kynurenine in the wings of the Papilionid butterflies. Zool. Mag., **63** : 57–61.

Umebachi, Y., and H. Takahashi, 1956. Kynurenine in the wings of the Papilionid butterflies. J. Biochem. (Tokyo), **43** : 73–81.

Weygand, F., and M. Waldschmidt, 1955. Über der Biosynthese des Leucopterins, untersucht mit ^{14}C-markierten Verbindungen am Kohlweissling. Angew. Chem., **67** : 328.

Yagi, N., 1957. [Textbook of Entomology] (Konchu Gaku Honorn). P 206 ; Tokyo ; Yoken Do,