Yellow Pigments in the Wings of the Papilionid Butterflies. VI. Red Pigments of the Papilionid and Nymphalid Butterflies

By

Yoshishige UMEBACHI*

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Ford (1942, 1944) has examined the occurrence and properties of the red pigments of butterflies and divided them into six types, A, B, C, D, E, and the sixth, as are summarized in Table 1. Among these types, type A red pigment is widespread in the Lepidoptera, including the Papilionidae, while type B red pigment is present only in the three genera *Papilio, Chilasa,* and *Battus* of the Papilionidae. Type C red pigment is present in the Nymphalidae, type D and E red pigments are in the Pieridae, and the red pigment of the sixth type is in the Riodinidae. However, from a chemical point of view, it is still unknown to what kind of substance the red pigment of each type belongs respectively.

The author has investigated the nature and distribution of the yellow pigments of the wings of the Papilionidae (Umebachi and Nakamura, 1954; Umebachi and Takahashi, 1956; Umebachi, 1958; 1959a; 1960; 1961). As a part of the series, autoradiographs of the wings of *Papilio machaon, Papilio protenor, Papilio helenus,* and *Papilio bianor* injected with tryptophan-C^{14} were taken, and it was found that the C^{14} is incorporated into the reddish brown or brown scales of these butterflies (Umebachi, 1959b). As these reddish brown pigments are the type B red pigment reported by Ford, it is conceivable that the pigments of this type may be derived from tryptophan. So, in the present paper, the alkali hydrolysis method was used to see whether type B red pigment belonged to the ommochrome reported up to now. As a result, it has been supposed that the pigment of this type does not belong to the ommochrome as reported up to now. Moreover, whether another red pigment of the Papilionidae, type A red pigment, was also one of pigments derived from tryptophan or not was examined by the injection experiment of tryptophan-C^{14}, and it has been supposed that the red pigment of this type is not derived from tryptophan. For comparison, type C red pigment was also examined both by the injection experiment of tryptohan-C^{14} and by alkali hydrolysis, and has been presumed to be an ommochrome.

Although the present paper deals with the red pigments, it is included in this series for the sake of discussing the red pigment in connection with the yellow pigments.

* Department of Biology, Faculty of Science, Kanazawa University, Kanazawa, Japan.

Experimental Procedure and Results

Type B red pigment. —— The reddish brown or brown scales of the wings of *Papilio protenor* were used as materials. The butterflies used were either collected in Kanazawa at the adult stage or collected at the egg or larval stage and raised to adulthood in the laboratory. Ford (1944) reported that the wings of this species possess type B red pigment, and it was reported in the third paper (Umebachi, 1959b) of this series that the C^{14} of injected tryptophan-C^{14} is incorporated into the reddish brown or brown scales of this species. The reddish brown and brown scales of the hind wings of both sexes were gathered and washed with petroleum ether, ether, and 80 per cent ethanol. And then, the residual scales (reddish brown or brown) were hydrolysed with 1 N NaOH at 100°C for four hours. After that, the solution was acidified with HCl to pH 3, and, after centrifugation, the supernatant fluid was extracted repeatedly with butanol saturated with water. After the butanol layer was gathered and evaporated to dryness under reduced pressure, the residue was dissolved in 80 per cent ethanol and examined by two-dimensional chromatography. Ommochrome has been reported to produce xanthurenic acid by this procedure (Butenandt, Schiedt, and Biekert, 1954; Needham and Brunet, 1957). As solvents for two-dimensional chromatography, 80 per cent methanol and an upper layer of *n*-butanol-acetic acid-water (4 : 1 : 5) mixture were used. After development, the chromatogram was sprayed with Ehrlich's diazo reagent (Umebachi and Tsuchitani, 1955) to see whether xanthurenic acid was present or not. As a result, it has been proved that the reddish brown or brown scales of *P. protenor* do not produce xanthurenic acid by alkali hydrolysis.

Type A red pigment. —— *Graphium sarpedon* was used in order to see whether the red pigment of this type is derived from tryptophan or not. The butterflies used were all collected at the egg or larval stage and raised in the laboratory. It has already been reported by Ford (1944) that the wings of this species possess type A red pigment. The prepupa was injected with DL-tryptophan-2-C^{14}, which was obtained from the Daiichi Pure Chemical Co. Ltd.. The specific activity was 500 mc per mole. This was dissolved in hot water, and 0.03-0.06 ml of the solution were injected into each butterfly. After emergence of the butterflies, the wings were cut off, dried in a desiccator, and left in contact with X-ray film (Fuji) for 7-9 days in a dark room. The autoradiograph so obtained is shown in Figure 1, and the ordinary photograph of the same wings is illustrated in Figure 2. It will be seen from these figures that the C^{14} of tryptophan-C^{14} is not incorporated into the type A red pigment of *G. sarpedon*. Although Figure 1 is the autoradiograph of the male, the female also gave the same result.

Type C red pigment. —— As a material for examining type C red pigment, *Vanessa indica* was used. The buttrflies were either collected in Kanazawa at the egg or larval stage and raised in the laboratory or collected at the adult stage.

The autoradiograph of the wings was taken in the same way as in *G. sarpedon*. The result is shown in Figure 3, and the ordinary photograph of the same wings is given in Figure 4. From these figures, it is evident that the C^{14} of tryptophan-C^{14} was incorporated into the red scales of this species. This suggests that type C red pigment is the pigment derived from tryptophan. Although Figure 3 is the autoradiograph of the male, the female also gave the same one. The red scales of both sexes of this species were gathered and washed with petroleum ether, ether, and 80 per cent ethanol,* and then the residual scales (red) were hydrolysed with 1 N NaOH in the same way as in *P. protenor*. The hydrolysate was examined by paper chromatography, and the presence of xanthurenic acid was confirmed.

Discussion

As shown in Table 1, Ford (1942, 1944) divided the red pigments of the wings of butterflies into six types A, B, C, D, E, and the sixth. Among these types, type D and E red pigments seem to be pterin or its allied substance, for they are

Table 1. The occurrence and reactions of some red pigments in the Lepidoptera as reported by Ford.

Type	Occurrence	Reactions to acid and alkali	Solubility	Murexide test
A	Widespread in the Lepidoptera. Arctiidae (moth) Zygaenidae (moth) Papilionidae *Graphium, Parnassius, Menelaides*** Others	Ghanged to bright yellow by HCl, and reconverted to red again by alkali. Both reactions take place with great rapidity.	Soluble only to a very slight degree in hot water or dilute alkali. Insoluble in fat solvents.	Negative
B	Present only in the Papilionidae, and within this family only in *Papilio, Chilasa****, and *Battus*.	Almost unaffected by HCl, and also unaffected by ammonia.	Insoluble in hot water and dilute alkali. Insoluble in fat solvents.	Negative
C	Vanessidi	Caused to assume a duller tone by acids, and not restored by alkali.	Rapidly soluble in hot water and dilute alkali.	Negative
D	Pieridae *Delias* (except the Pasithoe group)	Converted to a bright yellow shade by strong acid, and reconverted to red by alkali.	Rapidly soluble in hot water and dilute alkali.	Positive
E	Pieridae *Delias* (the Pasithoe group)	Almost unaffected by acid.	Rapidly soluble in hot water and dilute alkali.	Positive
6th.	Riodinidae	—	—	—

* As the red pigment of *V. indica* is soluble in 80 per cent ethanol to some extent, the wash with 80 per cent ethanol was made briefly.

** *Menelaides* corresponds to *Atrophaneura* in Ford's report (Shirozu, 1955).

*** Ford included *Chilasa* in *Papilio*, but Shirozu (1955) separated them.

positive to the murexide test. The chemical nature of the red pigment of the sixth type is unknown. Type B and C red pigments are supposed to be the pigments derived from tryptophan as presented in the previous (Umebachi, 1959b) and present papers. Regarding the pigments derived from tryptophan, the only detailed investigation made up to now is that of Butenandt (1957, 1959) about ommochrome. According to him, ommochrome, including ommatin and ommin, produces xanthurenic acid by alkali hydrolysis (Butenandt, Schiedt, and Biekert 1954; Butenandt, Biekert, and Linzen, 1958a), and this property has been used for identifying ommochrome (Needham and Brunet, 1957). From the results of the present experiments, type B red pigment does not appear to be the ommochrome as reported up to now, while type C red pigment is presumed to be an ommochrome. On the other hand, type A red pigment does not seem to be derived from tryptophan. But, in the present experiments, the possibility remains that a part other than the alanine side chain of tryptophan-2-C^{14} may be incorporated into type A red pigment.

It is interesting that type A red pigment, which is widespread in the Lepidoptera including the Papilionidae, is not a pigment derived from tryptophan, while type B red pigment which is present only in the Papilionidae seems to be derived from tryptophan but does not seem to belong to the ommochrome as reported up to now. As already reported by the author, the yellow pigments of the Papilionidae are derived from tryptophan. However, it is not ommochrome but "kynurenine-pigment" (the fifth paper of this series, Umebachi, 1961). As far as the auhtor has examined up to the present, these kynurenine-pigments are not present among butterflies other than the Papilionidae. Therefore, the Papilionidae may be said to be in a special position in regard to the wing pigment derived from tryptophan. At any rate, it is interesting that the Papilionidae, which is most closely allied to the Pieridae possessing pterin, possesses the pigments derived from tryptophan.

Type C red pigment, which has been presumed to be an ommochrome, does not seem to be ommin but rather ommatin, for Butenandt, Biekert, and Linzen (1958b) have reported that ommin is not present in the wings of butterfies, and also reported that when the red, yellow or brown pigments of the wings of butterflies are ommochrome, they are not ommin but ommatin. According to Butenandt (Butenandt, 1959; Butenandt, Biekert, Kübler, and Linzen, 1960) who has reported the distribution of ommochrome in animal kingdom, ommatins are present in the wings of the Nymphalidae (*Pyrameis atalanta, Pyrameis cardui, Aglais urticae**, *Nymphalis io**, *Argynnis paphia,* and *Heliconius* spec.). Probably the red pigment of *V. indica* also seems to be ommatin.

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* In Butenandt's report, the genus name, *Vanessa*, was adopted for these species.

Summary

(1) As type B red pigment of the Papilionidae appeared to be derived from tryptophan in the previous study, experiments were made to see whether the red pigment belonged to the ommochrome as reported up to the present. The reddish brown or brown scales of *Papilio protenor* were hydrolysed with alkali, and the occurrence of xanthurenic acid was tested. As a result, xanthurenic acid was not detected in the hydrolysate, and accordingly it has been supposed that type B red pigment may not belong to the ommochrome as reported up to now.

(2) In order to see whether another red pigment of the Papilionidae (type A red pigment) is derived from tryptophan, the prepupa of *Graphium sarpedon* was injected with tryptophan-C^{14}, and after emergence of the butterflies, the autoradiograph of the wings was taken. The C^{14} was not incorporated into type A red pigment, and it has been presumed that the pigment of this type is not derived from tryptophan.

(3) In comparison with types A and B, type C red pigment of *Vanessa indica* was examined by the injection experiment of tryptophan-C^{14} and by alkali hydrolysis. It has been presumed that the pigment of this type is ommochrome.

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Explanation of Plate

The autoradiographs of the wings of butterflies injected with tryptophan-C^{14} are shown above, and the ordinary photographs of the same wings below. The right wings of each figure show the upperside, and the left wings, the underside.

In the ordinary photographs, the colors are indicated by the following abbreviations : r, red or orange ; g, green, yellowish green, bluish green or greenish blue ; w, white, yellowish white or bluish white ; o, pale orange (a mixture of red and white scales) ; b, brown; d, dark brown.

1 and 2. *G. sarpedon*

3 anb 4. *V. indica*

Plate