Yellow Pigments in the Wings of the Papilionid Butterfly, Luhdorfia japonica

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Yellow Pigments in the Wings of the Papilionid Butterfly, *Lühdorfia japonica*

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Abstract Yellow scales of the wings of *Lühdorfia japonica* were treated with 70% ethanol and then with 4% HCl-methanol, and the extracts were submitted to thin-layer chromatography.

The scales have proved to contain Papiliochrome IIa, IIb, IIIa, IIIb, M_1, and M_2. The scales remaining after the extraction were still yellowish brown and released kynurenine, β-alanine, and phenolic substances on hydrolysis.

The prepupae were injected with ^{14}C-tryptophan, and, after emergence of butterfly, autoradiograph of the wings was taken. It has been proved that yellow and red scales incorporate the ^{14}C.

For the purpose of comparison, the red scales were also examined in the same way. The scales has proved to contain small quantities of Papiliochrome IIa and IIb in addition to the red pigment.

Introduction

Yellow pigments of the wings of papilionid butterflies are neither pterin nor ommochrome. Since 1954, chemical and physical properties of the yellow pigments of *Papilio xuthus* have been investigated in detail (Umebachi, 1958, 1961, 1962b, 1975a and b, Umebachi and Yoshida, 1970), and the pigments were named Papiliochrome. The main yellow pigment of this species, Papiliochrome II, is formed from both kynurenine and a N-(β-alanyl)DOPAmine derivative (Umebachi, 1975a and b; Umebachi and Yamashita, 1976, 1977). In addition to Papiliochrome II, Papiliochrome III is a minor yellow pigment, which shows chemical properties similar to those of the II. The exract of such yellow pigments readily releases kynurenine and the N-(β-alanyl)DOPAmine derivative, SN-1, by being heated. Both Papiriochrome II and III are respectively separated into two components a and b, that is, IIa and IIb or IIIa and IIIb. The IIa and IIb seem to be optically isomeric with each other. The pale yellow scales of some other *Papilio* species also contain the yellow pigments of the same kind.

On the other hand, the deep yellow scales of *Papilio machaon* contain the brownish yellow pigments M_1 and M_2 in addition to Papiliochrome II and III (Umebachi, 1977). The M_1 and M_2 have also been presumed to belong to the Papiliochrome group, because they are related to kynurenine, an *o*-diphenolic substance, and β-alanine.

These *Papilio* species belong to the subfamly Papilioninae of the Papilionidae, while *L. japonica* belongs to the subfamily Zerynthiinae of the same family. Under this subfamily, four genera (*Zerynthia, Lühdorfia, Bhutanitis,* and *Sericinus*) are included. Umebachi (1959) already reported that the hot water extracts of the wings of six species (*Zerynthia polyxena, Z. rumina, L. japonica, L. puziloi, Bhutanitis lidderdalei,* and *Sericinus telamon*) contain kynurenine.

The present paper deals with the yellow pigments of the wings of *L. japonica* and with the autoradiograph of wings of the butterfly which was injected with ^{14}C-tryptophan. For the purpose of comparison, pigments of the red scales have also been examined.

Materials and Methods

Materials

The butterflies, *L. japonica,* were collected in the suburbs of Kanazawa and used without distinction of sex.

For the injection experiment of ^{14}C-tryptophan, the larvae were collected and raised in laboratory with the food plant, *Asarum* sp..

Extraction and separation of yellow pigments

The yellow scales, which had been scraped from the wings and pooled, were treated with 70% ethanol at room temperature. The extract (A) was, without any treatment, submitted to two-dimensional thin-layer chromatography.

The remaining scales were further treated with 70% ethanol at 40°C and then with 4% HCl-methanol at room temperature. The extract with 4% HCl-methanol was evaporated to dryness under reduced pressure. The residue was dissolved in water (Extract B) and submitted to thin-layer chromatography.

The red scales of the hind-wings were also treated and examined in the same way as described above.

Hydrolysis of residual scales

After the yellow scales were treated repeatedly with 70% ethanol and 4% HCl -methanol as mentioned above, the scales were still pale brown or yellowish blown. These scales, which are called residual scales in the present paper, were hydrolyzed in 1N HCl under reflux in a boiling water bath. After 5 hours, the hydrolysate was evaporated to dryness under reduced pressure, dissolved in water, and submitted to two-dimensional thin-layer chromatography.

Thin-layer chromatography

Cellulose thin-layer sheet (Merck No. 5552, 20×20 cm) was used. The first solvent for two-dimensional chromatography was 70% methanol (MeOH) and the second, a mixture of *n*-butanol, acetic acid, and water (120:30:50) (BAW). After development, the chromatogram was inspected under ultraviolet light, and then either the ninhydrin test or phosphomolybdic acid-ammonia test (Umebachi and Yoshida, 1970) was performed. The latter is a test for phenolic substances.

Autoradiograph of wings

The prepupae were injected with 0.03 to 0.05 ml of the saturated solution of DL-tryptophan-2-^{14}C (Daiichi Pure Chemicals Co. Ltd., specific activity, 0.5 mc/mM) in water. After emergence of butterflies, the wings were cut off, dried in a vacuum desiccator, and brought into contact with x-ray film (Fuji, Medical KX). The time of exposure was nineteen days.

Results

Thin-layer chromatography of the extract A

Two-dimensional thin-layer chromatogram of the extract A of yellow scales is given in Fig. 1a, which shows the presence of kynurenine and Papiliochrome IIa, IIb, IIIa, and IIIb. Papiliochrome II and III are pale yellow in visible light and show yellow fluorescence under ultraviolet light. To the ninhydrin test, they were yellowish brown

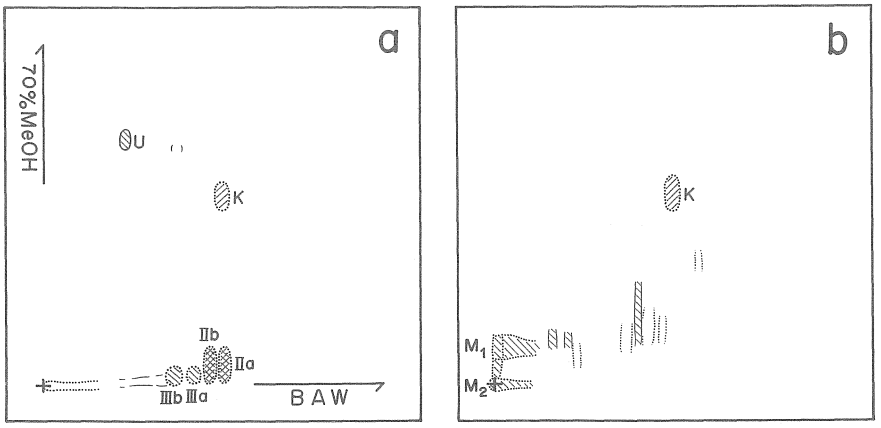

Fig. 1. Two-dimensional thin-layer chromatograms of (a) the 70% ethanol extract A and (b) the 4% HCl-methanol extract B from yellow scales.

Dotted circle, fluorescent substance; ////, positive to the ninhydrin test; ≡, positive to the phosphomolybdic acid-NH_3 test.

K, kynurenine; IIa, IIb, IIIa, and IIIb, Papiliochrome; M_1 and M_2, brownish yellow pigments.

U corresponds to the spot U of the previous paper (Umebachi and Yoshida, 1970; Umebachi, 1977).

and to the phosphomolybdic acid-ammonia test, blue. These IIa, IIb, IIIa, and IIIb were the same as those of *Papilio xuthus* and *P. machaon* (Umebachi, 1977).

Besides, the spot U was found, which was positive to the phosphomolybdic acid-ammonia test.

Thin-layer chromatography of the extract B

Two-dimensional thin-layer chromatogram of the 4% HCl-methanol extract B of yellow scales is given in Fig. 1b, which shows the presence of kynurenine, M_1, and M_2. The M_1 and M_2 were brownish yellow pigments and corresponded to the deep yellow pigments M_1 and M_2 of *P. machaon* (Umebachi, 1977).

Hydrolysate of the residual scales

Two-dimensional thin-layer chromatogram of the hydrolysate of the residual scales from yellow scales is shown in Flg. 2. More than thirteen ninhydrin-positive substances were found. Among them, glycine, aspartic acid, glutamic acid, α-alanine, and leucune (or leucine and isoleucine) were distinct. Tyrosine, phenylalanine, and valine were also present, though they were faint. Interestingly, kynurenine and β-alanine were found. Besides, phenolic substances (P and P-3) were found.

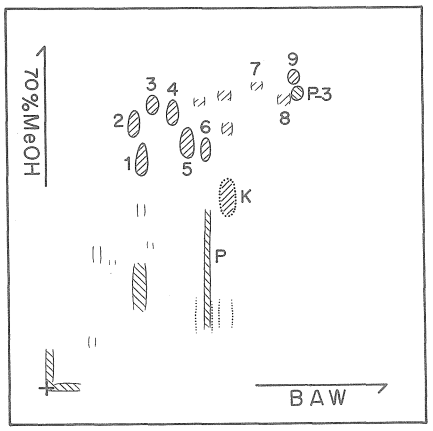

Fig. 2. Two-dimensional thin-layer chromatogram of the hydrolysate of the residual scales from yellow scales.

All the symbols are the same as in Fig. 1.

Spots 1, 2, 3, 4, 5, 6, 7, 8, and 9 are glycine, aspartic acid, glutamic acid, α-alanine, β-alanine, tyrosine, valine, phenylalanine, and leucine (or leucine and isoleucine), respectively.

P and P-3 are probably phenolic subsatnces. The latter corresponds to the P-3 of the previous paper (Umebachi, 1977).

For the purpose of comparison, the black scales were also treated in the same way, and the residual scales were hydrolyzed. As the result, the black scales have proved to lack β-alanine. or, if any present, it was a trace or uncertain. Kynurenine was also absent or, if any present, faint. The pattern of amino acids other than kynurenine and β-alanine was similar to that in the yellow scales.

Autoradioraph of wings

The autoradiograph of wings of the butterfly, which was injected with tryptophan-2-^{14}C at the prepupal stage, is given in Fig. 3, which shows that the yellow scales incorporated the ^{14}C. This is consistent with the above-mentioned results that the yellow

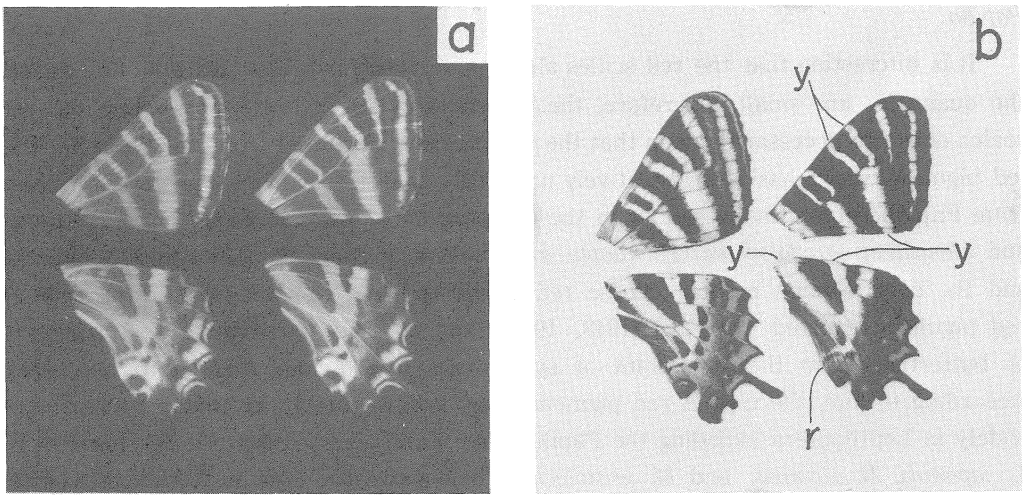

Fig. 3. (a) The autoradiograph of wings of the butterfly which was injected with ^{14}C-tryptophan at the prepupal stage. (b) The ordinary photograph of the same wings. The letters, y and r indicate yellow and red scales, respectively.

scales contain Papiliochrome II and III and that the residual scales also contain kynurenine. Interestingly enough, as seen in Fig 3, the red scales were also radioactive.

Thin-layer chromatography of the extract from red scales

The red pigment was partly extractable with 70% ethanol, and the extrac A was red. Two-dimensional thin-layer chromatogram of the extract showed the presence of kynurenine and Papiriochrome IIa and IIb, though their quantities were small.

The red pigment was not completely extracted with 70% ethanol at 40°C, and after being treated with the ethanol, the scales were still red.

When the remaining red scales were treated with 4% HCl-methanol, the scales immediately turned yellow. The color changed to orange after one day. These results show that the red scales contain both an unidentified red pigment and small quantities of the yellow pigments (Papiliochrome IIa and IIb).

Discussion

Until now, six kinds of Papiliochrome (IIa, IIb, IIIa, IIIb, M_1, and M_2) have been reported. Papiliochrome IIa and IIb are the main yellow pigments of the wings of *P. xuthus*. These two pigments are also present in the yellow scales of *P. machaon*. But the main yellow pigments of the latter species are the brownish yellow pigments M_1 and M_2, which have also been presumed to belong to the Papiliochrome group (Umebachi, 1977). The present paper clearly shows that the yellow scales of *L. japonica* contain all of these six kinds of yellow pigments (IIa, IIb, IIIa, IIIb, M_1, and M_2). As far as the yellow pigments are concerned, it can be said that the Zerynthiinae is similar to the genus,

Papilio.

It is interesting that the red scales also contain Papiliochrome IIa and IIb, though the quantities are small. Therefore, the incorporation of ^{14}C-tryptophan into the red scales does not necessarily mean that the red pigment is derived from tryptophan. The red pigment, which has been tentatively named R_2, is different in its chemical properties from Papiliochrome and is similar to the red pigment of *Byasa alcinous* (*Parides alcinous*) and *Menelaides aristolochiae* (*Pachliopta aristolochiae*) in their solubility in 70% ethanol and the color change by acid. These red pigments seem to correspond to the type A red pigment reported by Ford (1942, 1944a and b). He investigated red pigments of butterflies from the standpoint of systematics and divided them into six types. According to him, the type A red pigment turns yellow quickly by acid and distributes widely in Lepidoptera including the Papilionidae. Ford reported that the red pigment of *L. japonica, B. alcinous,* and *M. aristolochiae* belongs to the type A. Umebachi (1962a) reported that the type A red pigment of *Graphiun sarpedon* did not incorporate ^{14}C-tryptophan. On the other hand, in *Papilio* species, there is the reddish brown pigment, which has been tentatively named R_1 and corresponds to the type B red pigment reported by Ford. As far as the red pigment is concerned, therefore, it can be said that *L. japonica* is different from *Papilio* species. Chemical properties of the red pigment of *B. alcinous* and *M. aristolochiae* and the reddish brown pigment of *P. machaon* are being investigated in detail.

After the yellow scales of *L. japonica* were treated repeatedly with 70% ethanol at 40°C and then with 4% HCl-methanol at room temperature, the remaining scales were still yellowish brown, and the hydrolysate has proved to contain kynurenine, phenolic substances, and β-alanine. This may mean that small quantities of the Papiliochrome (for example M_1 and M_2) still remain insoluble in the residual scales. Moreover, there is also the possibility that, apart from the yellow or yellowish brown pigments, the residual scale itself may contain β-alanine.

The fact that the black scales lack β-alanine or contain only a trace of it, is interesting, for it has been reported that, in some insects, the cuticle of black mutants lacks β-alanine (Seki, 1962;Fukushi, 1967;Fukushi and Seki, 1965; Jacods and Brubaker, 1963). The possibility is now suggested that the formation of N-(β-alanyl)DOPAmine derivative may make the melanin formation from DOPAmine impossible.

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