Sci. Rep. Kanazawa Univ.
Vol. 23, No. 1, pp. 43—55
June 1978

The Presence or Absence of β-Alanine in the Wing-Scales of Butterflies

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(Received May 10, 1978)

Abstract (1) The wing-scales of twenty five species of butterflies belonging to the Pieridae, Papilionidae, Nymphalidae, and Satyridae were treated with 70% ethanol and 4% HCl-methanol. The residual scales were hydrolyzed in 1 N HCl and examined for the presence or absence of β-alanine with two-dimensional thin-layer chromatography.

(2) The scales containing Papiliochromes (Papilionidae) or ommatins (Nymphalidae) have proved to contain β-alanine, while the pteridine-containing scales of the Pieridae did not show β-alanine.

(3) Irrespective of family, black or pure white scales have proved to lack β-alanine. But some reddish brown, red and brown scales contain β-alanine.

(4) These results suggest that the presence or absence of β-alanine in the residual scales bears a close relationship to the pigments of scales.

(5) The presence or absence of kynurenine and an unidentified *o*-diphenolic substance is also described.

(6) In the three species, *Papilio xuthus, P. machaon,* and *Inachus io,* the β-alanine contents in soluble and insoluble fractions of scales were determined.

Introduction

Since 1954, Umebachi has investigated yellow pigments of the wings of butterflies belonging to the genus *Papilio* and found that the yellow pigments are neither pterin nor ommochrome but the pigments which are related to both kynurenine and N-(β-alanyl) DOPAmine derivative (Umebachi, 1975a; Umebachi and Yoshida, 1970; Umebachi and Yamashita, 1976, 1977). These yellow pigments are a new group of insect pigments and have been named Papiliochrome (Umebachi, 1961, 1962). Their chemical and physical properties were investigated in detail mainly using *Papilio xuthus.* A large portion of the Papiliochrome in the yellow scales of this species is extracted with 70% ethanol at 40°C. With 4% HCl-methanol, almost all the Papiliochrome is extracted from the scales, and the remaining scales are almost white, though they are not pure white. These remaining scales were called ghost scales in the previous paper (Umebachi,

1977a).

As already reported, Papiliochrome releases β-alanine on a mild hydrolysis, that is, in 1 N HCl at 100°C for 2hr (Umebachi, 1975a; Umebachi and Yamashita, 1977). In the course of these investigations, it was found that the scales remaining after the repeated extraction of Papiliochrome with 70% ethanol (at 40°C) and 4% HCl-methanol (at room temperature) still release β-alanine on a mild hydrolysis (Umebachi, 1975a, 1977a). This fact is interesting from the standpoints of the biochemistry of β-alanine in insects, the chemistry of insect cuticle, and the comparative biochemistry of nitrogen metabolism. The present paper deals with the presence or absence of β-alanine in the wing-scales of twenty five species of butterflies belonging to the Pieridae, Papilionidae, Nymphalidae, and Satyridae.

In the three species, *Papilio xuthus, Papilio machaon,* and *Inachus io,* furthermore, the β-alanine contents in soluble and insoluble fractions of scales were also determined.

Materials and Methods

Materials

Species names and their scales of the butterflies examined are as follows:

Pieridae (4 species)	
Pieris rapae	White scales
Colias erate	Yellow scales
Eurema hecabe	Yellow scales
Gonepterix mahaguru	Yellow scales
Papilionidae (13 species)	
Papilio xuthus	Pale Yellow scales
	Black scales
Papilio demoleus	Pale Yellow scales
	Reddish brown scales in the anal eye spot
Papllio protenor	Pale yellow scales
	Black scales
Papilio helenus	Pale yellow scales on the upperside of hind-wlngs
	White scales on the underside of hind-wings
Papilio castor	Pale yellow scales on the upperside of hind-wings
	White scales on the underside of hind-wings
Papilio polytes	Pale yellow scales on the upperside of hind-wings
Papilio dardanus	Pale yellow scales
Papilio machaon	Deep yellow scales
	Black scales
	Reddish brown scales in the anal eye spot
Lühdorfia japonica	Yellow scales
	Black scales
	Red scales of hind-wings
Sericinus telamon	Yellow scales

Iphiclides eurous	Yellowish white scales
Byasa alcinous	Red scales
	Black scales
Menelaides aristolochiae	Red scales
Nymphalidae (6 species)	
Vanessa indica	Red scales
	Dark brown scales on the upperside of fore-wings
Polygonia c-aureum	Orange scales
Inachus io	Brownish red scales
Hestina japonica	Yellowish white scales
Neptis aceris	White scales
Heliconius wallacei	Yellow scales
Satyridae (2 species)	
Ypthima motschulskyi	Dark brown scales
Mycalesis gotama	Brown scales

These wing-scales were scraped and stored. In all the species, except *L. japonica* and *H. japonica* which were used without distinction of sex, only male butterflies were used.

Preparation of the HCl-methanol residual scales

Scales were first treated with 70% ethanol at 40°C repeatedly until the extract became completely colorless. Next, the scales were treated with 4% HCl-methanol at room temperature repeatedly again until the extract became completely colorless.

In some scales, the remaining scales were almost white or pure white, but in other scales, they were yellow, brown, dark brown, or black. In other words, in some scales, the pigments were almost completely or completely extracted with the above treatments, but in other scales, the pigments were only partly extracted or remained insoluble in the scales. In the latter cases, the remaining scales do not fit the word, ghost scales. So, in the present paper, the remaining scales are all called not the ghost scales but the HCl-methanol residue of scales or the HCl-methanol residual scales.

Hydrolysis

The HCl-methanol residue of scales was hydrolyzed under reflux in 1 N HCl in a boiling water bath for 5hr. After centrifugation, the hydrolysate was evaporated to dryness under reduced pressure. The residue was dissolved in water and submitted to two-dimensional thin-layer chromatography.

Thin-layer chromatography

Cellulose thin-layer sheets (Merck No. 5552, 20×20cm) were used. The solvent for the first dimension was 70% methanol (MeOH) or a mixture of methanol, water, and pyridine (20 : 5 : 1) (MWP), and for the second dimension, a mixture of *n*-butanol-glacial acetic acid-water (120 : 30 : 50) (BAW). After development, the chromatogram was inspected under ultraviolet light, and then one of the ninhydrin, phosphomolybdic acid-NH_3, and sodium molybdate tests was performed (Umebachi and Yoshida, 1970). A typical example of the chromatogram developed with MWP and BAW is shown in Fig. 1. The chromatographic pattern with 70% MeOH and BAW was also essentially the same as Fig. 1.

The hydrolysates from the HCl-methanol residual scales all showed the presence of at least fifteen ninhydrin-positive substances including glycine (or glycine and serine), aspartic acid, glutamic acid, α-alanine, tyrosine, valine, leucine (and/or isoleucine), and phenylalanine. But, in the present paper, these

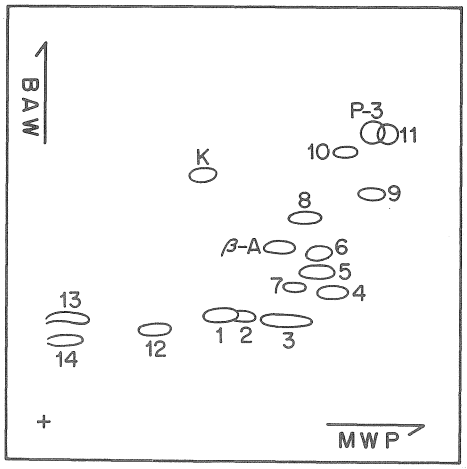

Fig. 1. Two-dimensional thin-layer chromatogram of the hydrolysate of the HCl-MeOH residue of scales.
Solvents: MWP and BAW
Spots 1-14 are glycine, serine, aspartic acid, glutamic acid, α-alanine, proline, threonine, tyrosine, valine, phenylalanine, leucine (or leucine and isoleucine), histidine, arginine, and lysine, respectively.
K, kynurenine; β-A, β-alanine; P-3, an *o*-diphenolic substance which is positive to both the phosphomolybdic acid-NH_3 test and the sodium molybdate test but is negative to the ninhydrin test.

amino acids will not be mentioned, because the purpose of the present paper is to describe the presence or absence of β-alanine. But, as kynurenine and the spot P-3 (Fig. 1) were dependent on the kind of scales, the presence or absence of these two substances will be described together with β-alanine. The P-3 substance seems to be an *o*-diphenolic substance because it was positive to both phosphomolybdic acid-NH_3 and sodium molybdate tests. This substance corresponds to the P-3 substance of the previous paper (Umebachi, 1977a).

Estimation of β-alanine in soluble and insoluble fractions of scales

The β-alanine content of scales was determined in the following three kinds of scales: pale yellow scales of *P. xuthus,* deep yellow scales of *P. machaon,* and brownish red scales of *I. io.*

Scales were first treated with 70% ethanol at 40—50°C repeatedly until the extract became completely colorless. The combined extract is called the EtOH fraction. Next, the remaining scales were treated with 4% HCl-methanol at room temperature repeatedly until the extract became again colorless. The combined extract is named the HCl-MeOH fraction. The remaining scales were further treated with 19 M formic acid repeatedly at room temperature. The combined extract is called the HCOOH fraction. The remaining scales were finally treated with 1 N NaOH repeatedly at room temperature. The combined extract is called the NaOH fraction. The last remaining scales are referred to as the residue or insoluble part of scales.

The EtOH, HCl-MeOH, and HCOOH fractions were evaporated in a rotary evaporator at 40°C, respectively. The residue was dissolved in 5−7ml of 1 N HCl and hydrolyzed under reflux at 100°C for 5hr. The hydrolysate was evaporated in a rotary evaporator at 60°C, dissolved in water, applied to a Dowex 50W X4 column (1×11cm), and after being washed with water, eluted with 2 N ammonia water. The amino acid fraction (the front part of ammonia water) was combined, evaporated to dryness in a rotary evaporator at 40°C, and dissolved in water.

The NaOH fraction was, without any pre-treatment, hydrolyzed at 100°C for 5hr. The hydrolysate was filtered through the Centriflow CF-25 (Amicon) in order to remove high molecular substances. The filtrate was applied to the Dowex 50W X4 column and, after that, treated in the same way as above.

The residue of scales (insoluble part of scales) were hydrolyzed in 5−7ml of 1 N HCl under reflux at 100°C for 5hr. The hydrolysate was treated in the same way as described above.

The β-alanine contents in the hydrolysates of the above five fractions (EtOH, HCl-MeOH, HCOOH,

NaOH, and residue) were determined by a modification of the Shinoda and Satake method (1961) which had been reported for the estimation of amino acids on paper chromatogram. The above each hydrolysate was quantitatively applied on a Cellulose thin-layer sheet (Merck No. 5552) and two-dimensionally developed with MWP (or 70% MeOH) and BAW. The chromatogram was sprayed with 0.3% 2,4,6-trinitrobenzene-1-sulfonic acid (TNBS) in 80% methanol and then with borate--phosphate buffer (1 vol. of 1/80 M $Na_2B_4O_7$ and and 2 vols. of 1/40 M KH_2PO_4 in 70% methanol, pH 8.5). After being kept in the dark overnight, the area of β-alanine was scraped. To the cellulose powder thus obtained, 1 ml of 0.1% TNBS and 2ml of borate buffer (7.25 vols. of 1/2.5 M boric acid-1/10 M NaCl and 2.75 vols. of 1/10 M $Na_2B_4O_7$, pH 8.0) were added, and the suspension was kept at 40°C. After 2hr, 1ml of 2 N HCl was added, and after centrifugation, the absorbance of the supernatant was measured at 340nm. The calibration curve which was obtained with authentic β-alanine was used.

The β-alanine content of each fraction is expressed as percent of the total β-alanine.

Apart from these five fractions, untreated (original) scales were also hydrolyzed in 1 N HCl, and the β-alanine was determined in the same way as mentioned above. The results are expressed as μg β-alanine per mg dry weight of scales.

Results

Pieridae

The HCl-methanol residues from the yellow scales of *C. erate* and *E. hecabe* were white, especially in the latter species, pure white. Those of *G. mahaguru* were also white but not pure white, rather creamy. In the white scales of *P. rapae,* almost no white pigment was extracted with 70% ethanol and 4% HCl-methanol as was expected, because the white pterin of this species is almost insoluble in these solvents.

In all these residual scales, β-alanine was not found (Table 2). Kynurenine was not found, either. On the other hand, a large quantity of P-3 substance was present.

Papilionidae (Papilio)

The HCl-methanol residues from the pale yellow scales of *P. demoleus, P. protenor, P. helenus, P. castor, P. polytes,* and *P. dardanus* were white. Those from *P. xuthus* were almost white but not pure white. On the other hand, in the deep yellow scales of *P. machaon,* the HCl-methanol residue was pale brown. The solubilities of these pale yellow and deep yellow pigments were already reported in the previous papers (Umebachi, 1977a,c).

In all of the HCl-methanol residues from these pale yellow and deep yellow scales, β-alanine was found (Table 1). The quantity was larger in the deep yellow scales of *P. machaon* than in the pale yellow scales of other seven *Papilio* species. In both *P. xuthus* and *P. machaon,* the above HCl-methanol residual scales were further treated with 8.5%formic acid-methanol and then with 2.8% ammonia-methanol. The residual scales thus obtained were almost white but not pure white in *P. xuthus* and were grey in *P. machaon.* These residual scales were hydrolyzed in the same way as mentioned above.

In both scales, the hydrolysate still showed the presence of β-alanine.

The pale yellow scales of *P. xuthus, P. demoleus, P. protenor, P. helenus, P. castor, P. polytes,* and *P. dardanus* showed the distinct spot of P-3. On the other hand, in the deep yellow scales of *P. machaon,* the P-3 substance was not found or, if any present at all, in a trace amount. Kynurenine was absent in the HCl-methanol residues of the pale yellow scales of *P. xuthus, P. demoleus, P. protenor, P. helenus, P. castor, P. polytes,* and *P. dardanus,* while, in those from the deep yellow scales of *P. machaon,* kynurenine was faintly found.

The reddish brown pigments of the reddish brown scales of *P. demoleus* and *P. machaon* were insoluble in 70% ethanol and 4% HCl-methanol, and the HCl-methanol residues of scales were brown. In these scales, β-alanine was found (Table 1). Kynurenine was also present. On the other hand, the P-3 substance was not found.

The HCl-methanol residues from the white scales of *P. helenus* and *P. castor* were white, and neither β-alanine nor kynurenine was found (Table 2). But the P-3 substance

Table 1. The HCl-MeOH residual scales possessing β-alanine

Species	Original scales *		Residual scales			
	Color	Pigment	Color	β-Ala*	Kyn*	P-3
Papilionidae						
P. xuthus	Pale yellow	Papiliochrome group	Almost white	±	−	+
P. demoleus	Pale yellow		White	±	−	+
P. protenor	Pale yellow		White	±	−	+
P. helenus	Pale yellow		White	±	−	+
P. castor	Pale yellow		White	±	−	+
P. polytes	Pale yellow		White	±	−	+
P. dardanus	Pale yellow		White	±	−	+
P. machaon	Deep yellow		Pale brown	+	Faint	− or Trace
L. japonica	Yellow		Yellowish brown	+	+	+
S. telamon	Yellow		Brown	+	+	+
P. demoleus	Reddish brown	R_1red pigment	Brown	+	+	−
P. machaon	Reddish brown		Brown	+	+	−
L. japonica	Red	P_2red pigment	Orange or yellowish	+	+	−
B. alcinous	Red		Orange	+	−	−
M. aristolochiae	Red		Orange	+	−	−
Nymphalidae						
V. indica	Red	Ommochrome group	Brown	+	−	−
I. io	Red		Brown	+	−	−
P. c-aureum	Orange		Brown	+	−	−
Satyridae						
Y. motschulsky	Dark brown		Dark brown	±	−	−
M. gotama	Brown		Brown	− or faint	−	−

* β-Ala, β-alanine; Kyn, kynurenine

Table 2. The HCl-MeOH residual scales lacking β-alanine

Species	Original scales		Residual scales			
	Color	Pigment	Color	β-Ala	Kyn	P-3
Papilionidae						
I. eurous	Yellowish white	Antho-xanthin	White	−	−	+
Nymphalidae						
H. japonica	Yellowish white		White	−	−	+
H. wallacei	Yellow	3-Hydro-xykynu-renine	Pure white	−	−	+
Pieridae						
P. rapae	White	Pteridine group	White	−	−	+
C. erate	Yellow		White	−	−	+
E. hecabe	Yellow		Pure white	−	−	+
G. mahaguru	Yellow		White (Creamy)	−	−	+
Papilionidae						
P. xuthus	Black	Melanin	Black	−	−	−
P. protenor	Black		Black	−	−	−
P. machaon	Black		Black	−	−	−
L. japonica	Black		Black	− or ?	?	−
B. alcinous	Black		Black	−	−	−
P. helenus	White		White	−	−	+
P. castor	White		White	−	−	+
Nymphalidae						
V. indica	Dark brown		Dark brown	− or ?	−	−
N. aceris	White		Almost white	−	−	−

was present.

The HCl-methanol residues from the black scales of *P. xuthus*, *P. protenor,* and *P. machaon* were, of course, black, because these black pigments are probably melenin and are insoluble to the solvents tried. These black scales did not show β-alanine, kynurenine, or the P-3 substance (Table 2).

Papilionidae (*except Papilio*)

The HCl-methanol residues from the yellow scales of *L. japonica* and *S. telamon* were yellowish brown and brown, respectively. In these scales, β-alanine, kynurenine, and the P-3 substance were found (Table 1). The HCl-methanol residues of the red scales of *L. japonica* was yellowish or orange. In this case, β-alanine and kynurenine were found (Table 1), but the P-3 substance was absent. In the black scales, β-alanine was absent or uncertain (Table 2). Kynurenine was faintly found, but the P-3 substance was absent.

The HCl-methanol residues from the red scales of *B.alcinous* and *M. aristolochiae*

were orange. Beta-alanine was found, but neither kynurenine nor P-3 was found (Table 1). In the black scales of *B. alcinous,* β-alanine, kynurenine, and the P-3 substance were absent (Table 2).

The yellowish white scales of *I. eurous* showed neither β-alanine nor kynurenine, but the P-3 substance was present (Table 2).

Nymphalidae

The HCl-methanol residues from the red scales of *V. indica* and *I. io* and those from the orange scales of *P. c-aureum* were brown. All these residual scales showed β-alanine, but kynurenine and the P-3 substance were absent (Table 1). The HCl-methanol residue from the dark brown scales of *V. indica* was dark brown, because the dark brown pigment was insoluble in the solvents used. Beta-alanine was absent or uncertain, and neither kynurenine nor the P-3 substance was found (Table 2).

The HCl-methanol residue from the white scales of *N. aceris* was almost white. Beta-alanine, kynurenine, or the P-3 substance was not found (Table 2).

In the yellowish white scales of *H. japonica,* the white pigment was insoluble in the solvents tried, and the HCl-methanol residual scales were white. Neither β-alanine nor kynurenine was found, but a large quantity of the P-3 substance was present (Table 2).

The HCl-methanol residue from the yellow scales of *H. wallacei* was pure white. In this case, neither β-alanine nor kynurenine was present, but the P-3 substance was found (Table 2).

Satyridae

The HCl-methanol residue from the dark brown scales of *Y. motschulsky* was dark brown, and that from the brown scales of *M. gotama* was brown. In these cases, β-alanine was found, though it was faint in the latter species (Table 1). The P-3 substance and kynurenine were absent in both scales.

Estimation of β-alanine contents

In the pale yellow scales of *P. xuthus*, the deep yellow scales of *P. machaon*, and the brownish red scales of *I. io,* the β-alanine contents of untreated (original) scales were 59.7, 39.8, and 5.8 μg per mg dry weight of scales, respectively. In the hydrolysates of the yellow scales of *P. xuthus* and *P. machaon*, a large quantity of kynurenine was found. On the other hand, in those from the brownish red scales of *I. io,* a large quantity of 3-hydroxykynurenine was found.

The β-alanine contents in the five fractions (EtOH, HCl-MeOH, HCOOH, NaOH, and residue) of scales are given, in percentage, in Table 3. In the pale yellow scales of *P. xuthus,* a large portion of β-alanine is removed in the EtOH fraction. In the deep yellow scales of *P. machaon,* on the other hand, a considerable part of β-alanine is removed in the HCl-MeOH fraction. In addition, β-alanine in the NaOH fraction is not so little as in *P. xuthus.* In the brownish red scales of *I. io,* a small quantity of β-alanine

was found in all the five fractions.

Table 3. Beta-alanine contents in untreated scales and its distribution in five fractions

	Pale yellow scales of *P. xuthus*	Deep yellow scales of *P. machaon*	Brownish red scales of *I. io*
μg β-Alanine per mg dry wt. scales	59.7	39.8	5.8
Distribution	%	%	%
EtOH fraction	81.5	54.1	22.5
HCl-MeOH fraction	15.6	32.9	30.5
HCOOH fraction	1.3	2.3	26.3
NaOH fraction	1.3	8.9	16.4
Residue	0.3	1.8	<4.3

Discussion

Pieridae

It is well known that the pigments of the wing-scales of pierid butterflies such as *P. rapae, C. erate, E. hecabe,* and *G. mahaguru* are pteridine derivatives. And this is a characteristic of this family (Ford, 1947). Now, from the results of the present paper, the absence of β-alanine in the HCl-methanol residual scales seems to be a property common to these butterflies. In this respect, the scales of pierid butterflies are entirely different from the pale yellow and deep yellow scales of *Papilio* species (Papilionidae) and from the red and orange scales of *Vanessa, Polygonia,* and *Inachus* (Nymphalidae). And this is one of the conclulsions drawn in the present paper.

Papilionidae (*Papilio*)

In the genus *Papilio*, the following conclusion can be drawn. Almost all of the scales which have proved to contain β-alanine are the scales which were reported to possess Papiliochrome in the previous paper (Umebachi, 1977c). It is probable that all the wing-scales possessing Papiliochrome contain β-alanine in their HCl-methanol residue.

As already reported, pigments of the pale yellow scales of *P. xuthus, P. demoleus, P. protenor, P. helenus, P. castor, P. polytes,* and *P. dardanus* are Papiliochrome IIa, IIb, IIIa, and IIIb (Umebachi, 1977c). These Papiliochromes are soluble in water and 70% ethanol. Therefore, the HCl-methanol residues of these scales were pure white or almost white. But the possibilities can not be ruled out that a trace of Papiliochrome remains insoluble in the HCl-methanol residual scales and that a small quantity of β-alanine found in the residual scales may have come from these remaining Papiliochromes. In this respect, it is worth nothing that, even after the HCl-methanol residue from the

pale yellow scales of *P. xuthus* was further treated with 8.5% formic acid-methanol and 2.8% ammonia-methanol, β-alanine was found in the hydrolysate and, furthermore, that the HCl-methanol residues from the pale yellow scales of *P. xuthus, P. demoleus, P. protenor, P. helenus, P. castor, P. polytes,* and *P. dardanus* did not show kynurenine. So, even if some Papiliochrome remains insoluble in these residual scales, it must be only a trace.

On the other hand, in the HCl-methanol residue of the deep yellow scales of *P. machaon*, the quantity of β-alanine was larger than in the above-mentioned pale yellow scales. In the HCl-methanol resldue of these deep yellow scales also, the possibility can not be ruled out that a part of the yellowish brown Papiliochrome remains insoluble. But anyway, in both the pale yellow scales and the deep yellow scales, it is sure that a part of β-alanine is tightly bound to the scale itself.

It is interesting that the reddish brown scales of *P. machaon* and *P. demoleus* also showed β-alanine. These reddish brown pigments are named R_1 red pigment and are now being investigated.

Papilionidae (*except Papilio*)

As already reported, the yellow scales of *L. japonica* and *S. telamon* contain Papiliochrome (Umebachi, 1977c). The present paper has shown that the HCl-methanol residues from these yellow scales contain β-alanine. Therefore, it can be said that the yellow scales of the Zerynthiinae are similar to those of *Papilio* species.

It was also reported in the previous paper (Umebachi, 1977c) that the red scales of *L. japonica* contain a small quantity of Papiliochrome in addition to the red pigment. The red pigment of the same kind is also present in *B. alcinous* and *M. aristolochiae* and is named R_2 red pigment. The pigment of the latter species is now being investigated.

The yellowish white scales of I. *eurous* contain anthoxanthin but not Papiliochrome (Ford, 1941 ; Umebachi, 1960, 1977c).

Nymphalidae

The red pigments of *V. indica* and *I. io* and the orange pigment of *P. c-aureum* are ommochrome and are soluble in 70% ethanol and 4% HCl-methanol. But the HCl-methanol residual scales were still brown. It is interesting whether or not these remaining pigments bear any relationship to the presence of β-alanine.

The present paper has shown that both the Papiliochrome-containing scales of Papilionidae and the ommochrome-containing scales of Nymphalidae have a point common to each other in that, after the extraction of Papiliochrome or ommochrome with 70% ethanol and 4% HCl-methanol, the residual scales still showed the presence of β-alanine. This is also one of the conclusions drawn in the present paper.

The HCl-methanol residue from the yellowish white scales of *H. japonica* showed neither β-alanine nor kynurenine, but a considerable quantity of P-3 was present. The nature of the yellowish white pigment remains unknown.

The HCl-methanol residue from the yellow scales of *H. wallacei* lacks both β-alanine and kynurenine but possesses the P-3 substance. Tokuyama et al. (1967) and Brown and Domingues (1970) reported that yellow pigment of the genus *Heliconius* is 3-hydroxykynurenine itself. We also examined the yellow pigment of *H. wellacei* and confirmed that the pigment is 3-hydroxykynurenine itself and that the yellow scales contain neither kynurenine nor Papiliochrome.

It is interesting that the white scales of *N. aceris* lack β-alanine as in those of *P. helenus* and *P. castor* As far as examined until now, all the pure white scales lack β-alanine.

Satyridae

It is also interesting that, in spite of the fact that the black scales lack β-alanine, the dark brown scales of *Y. motschulsky* and the brown scales of *M. gotama* possess β-alanine.

Black scales

Irrespective of genus, all the black scales examined lack β-alanine. This is also one of the conclusions reached in the present paper and is interesting in connection with several reports that pupal cases of black mutants of some insects lack β-alanine, whereas this amino acid is present in the pupal cases of wild type strain (Seki, 1962; Fukushi and Seki, 1965; Jacods and Brubaker, 1963).

It is interesting that both pure white and coal black scales lack β-alanine. It is also known that β-alanine is incorporated into cuticle at the time of sclerotization (Bodnaryk and Levenbook, 1969; Bodnaryk, 1971). All the results of the present paper suggest that the presence or absence of β-alanine in scales bears a close relationship to the pigments of those scales. In both scales and cuticles, to what constituent or to what position, is the β-alanine attached? In this connection, the presence of N-(β-alanyl) DOPAmine derivative as a constituent of Papiliochrome is very interesting.

P-3 substance

The P-3 substance is an *o*-diphenolic substance but has remained unidentified. Interestingly enough, the present paper, with the exceptions of the white scales of *N. aceris,* the dark brown scales of *V. indica,* and all the black scales, shows a general tendency that the quantity of P-3 substance in the HCl-methanol residue is larger in the scales where β-alanine is not found at all or found only in a small quantity than in the scales which contain a large quantity of β-alanine. The meaning of this fact has been left to be investigated.

Kynurenine

The presence of kynurenine in the HCl-methanol residual scales is limited to the Papilionidae. This is in accord with the fact that the distribution of Papiliochrome is

limited to the Papilionidae. But, a trace or slight amount of kynurenine is found also in the HCl-methanol residual scales of some other families.

Beta-alanine contents

It is natural that the acid hydrolysates of both the pale yellow scales of *P. xuthus* and the deep yellow scales of *P. machaon* contain much more β-alanine and kynurenine than those of the brownish red scales of *I. io* and that the acid hydrolysate of the brownish red scales of the last species showed a large quantity of 3-hydroxykynurenine. Because the pigments of the yellow scales of the former two species are Papiliochromes, while those of the brownish red scales of *I. io* are ommatins.

In *P. xuthus,* a large portion of β-alanine is removed in the EtOH fraction (Table 3). In the deep yellow scales of *P. machaon*, however, a considerable part of β-alanine is extracted in the HCl-MeOH fraction. This coincides with the facts that the major yellow pigments of the pale yellow scales of *P. xuthus* are Papiliochrome IIa and IIb which are extracted with water and 70% ethanol and that the deep yellow scales of *P. machaon* contain, in addition to Papiliochrome II and III, the deep yellow pigments M_1 and M_2 which are extracted with 4% HCl-methanol (Umebachi, 1977a). Part of the M_1 and M_2 seem to be extracted with 70% ethanol at 50°C.

The NaOH fraction of the deep yellow scales of *P. machaon* contains more β-alanine than that of the pale yellow scales of *P. xuthus* (Table 3). This may bear a close relationship to the fact that the HCl-methanol residue from the pale yellow scales of *P. xuthus* is white or almost white, while the residue from the deep yellow scales of *P. machaon* is still brown. Anyway, the presence of β-alanine in the NaOH fraction and in the insoluble part of scales is interesting to the biochemistry of cuticle.

In the brownish red scales of *I. io,* the β-alanine content is low, but this amino acid is distributed in all the five fractions. After ommochrmes were removed with 70% ethanol and 4% HCl-methanol, the scales were still dark brown. There seems to be a general tendency that, when the HCl-methanol residue of scales is still brownish, they contain β-alanine.

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