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The Presence of 2-Hydroxy-3′,4′-dihydroxyacetophenone in Hydrolysates of the Wing-Scales of Butterfly

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Abstract Yellow scales of the pierid butterfly, *Eurema hecabe* were washed with 70% ethanol and 4% HCl-methanol repeatedly, and the residual scales was hydrolyzed in 1 N HCl. From the hydrolysate, the phenolic substance, P-3, was isolated with thin-layer chromatography. By its chromatographic behavior, chemical properties, and absorption spectra, the P-3 substance has been identified as 2-hydroxy-3′,4′-dihydroxyacetophenone. This compound is present also in the hydrolysate of wing-scales of three other species in addition to the species reported in the previous paper.

Introduction

Since 1954, Umebachi (1975a, b) have investigated the pale yellow pigments in the wings of *Papilio xuthus* (a swallowtail butterfly) in detail and reported that these pale yellow pigments readily decompose to kynurenine and a N-(β-alanyl) DOPAmine derivative. The latter compound have recently proved to be N-(β-alanyl) noradrenaline (Rembold et al., 1978). These yellow pigments form a new group of insect pigments and have been named Papiliochrome.

In the course of those investigations on Papiliochrome, it was found that, after the repeated extraction of Papiliochrome from the pale yellow scales with 70% ethanol and 4% HCl-methanol, the residual scales still released β-alanine on acid hydrolysis. On the basis of this finding, the residual scales from various species of butterflies were examined for the presence or absence of β-alanine (Umebachi and Aburano, 1978). It was found that (1) the presence or absence of β-alanine in the residual scales is closely related to the color or pigment of scales, (2) the Papiliochrome-containing scales and the red scales in the Papilionidae contain β-alanine, (3) the ommochrome-containing scales in the Nymphalidae, too, contain β-alanine, (4) the pterin-containing scales in the Pieridae do not contain β-alanine, and (5) irrespective of family, black scales and pure white scales do not contain β-alanine. Interestingly, in the scales which did not contain

β-alanine or contained only a small quantity of β-alanine in their residual scales, a phenolic substance (P-3) was found in their hydrolysate, though black scales showed neither β-alanine nor the P-3 substance. On the other hand, the scales which contained a large quantity of β-alanine did not show the P-3 substance. Therefore, with the exception of black scales, the quantity of β-alanine seemed to be inversely related to that of the P-3 substance.

In the present paper, the P-3 substance was isolated from the residual scales of the yellow scales of *Eurema hecabe* and has been identified as 2-hydroxy-3′,4′-dihydroxyacetophenone.

Materials and Methods

Materials

For the identification of the P-3 substance, yellow scales of the wings of the pierid butterfly, *Eurema hecabe* were used, because, as reported in the previous paper (Umebachi and Aburano, 1978), the pterin-containing scales of pierid butterflies do not contain β-alanine in their residual scales and release a large quantity of the P-3 substance on acid hydrolysis and because yellow pteridine pigments of the yellow scales of this species are all soluble in 70% ethanol and their residual scales are white.

Besides the identification of the P-3 substance, the scales of the following three species were examined for the presence or absence of the P-3 substance: yellow and pale yellow scales of *Catopsilia crocale* (the Pieridae), white scales of *Appias indra* (the Pieridae), and metallic blue scales of *Morpho rhetenor* (the Morphidae).

All these butterflies were obtained from either the Okura Biological Institute or some other commercial sources.

Residual scales

Scales were washed first with 70% ethanol at 40°C, five to seven times and then with 4% HCl-methanol at room temperature, five times. After that, the scales were washed with 99.5% ethanol and ethyl ether, and dried. The scales thus obtained are called the residual scales throughout the present paper.

Hydrolysis

The residual scales were hydrolyzed under reflux in 1 N HCl at 100°C for five hr, and the hydrolysate was evaporated to dryness under reduced pressure. The residue was dissolved in water and submitted to thin-layer chromatography.

Thin-layer chromatography

Cellulose thin-layer sheet (Merck No. 5552) was used. The first solvent for two-dimensional chromatography was 70% methanol or a mixture of methanol, water, and pyridine (20 : 5 : 1) (MWP), and the second solvent, a mixture of *n*-butanol, glacial acetic acid, and water (12 : 3 : 5) (BAW). The solvent for one-dimensional chromatography was BAW, MWP, a mixture of benzene, glacial acetic acid, and water (125 : 72 : 3) (BeAW), or a mixture of ethyl acetate, 90% formic acid, and water (3 : 1 : 3) (EFW). After development, the chromatogram was inspected under ultraviolet light, and the phosphomolybdic acid-NH_3 test (Riley, 1950) was performed (Umebachi and Yoshida, 1970). After that,

the chromatogram was again inspected under ultraviolet light. In some cases, the ninhydrin test, the ethylenediamine-NH_3 test for *o*-dipenols (Sourkes et al., 1963), the Evans test for *o*-diphenols (Coulson and Evans, 1958), or Ehrlich's test for indole structure and aromatic amino compounds (Dalgliesh, 1952) was performed (Umebachi and Yoshida, 1970).

Isolation of the P-3 substance

The hydrolysate of the residual scales from the yellow scales of *E. hecabe* was dissolved in water, applied as a streak on a cellulose thin-layer sheet, and developed one-dimensionally with MWP. After development, the areas 1cm from both sides of the chromatogram were cut off as a guide strip, and the phosphomolybdic acid-NH_3 test was made. There were two substances which were positive to the test. One was the P-3 substance. Another was a substance which was found just above the P-3 substance and was named P-4. The P-4 substance was too small in quantity to be examined in detail. In some cases, the P-4 substance was absent.

Anyway, using the above-mentioned guide strips, the area of the P-3 substance was scraped and extracted with water. After centrifugation, the supernatant was evaporated to dryness under reduced pressure.

The residue was dissolved in mater, again applied as a streak on a thin-layer sheet, and developed one-dimensionally with BAW this time. After development, the area of the P-3 substance was scraped and extracted with water in the same way as mentioned above. The extract was again evaporated to dryness under reduced pressure. The P-3 substance thus obtained was thin-layer chromatographically pure. So, the last residue will be referred to below as the purified P-3 substance.

Absorption spectra

Absorption spectra of the P-3 substance were taken in the range from 210 to 400 nm with a Hitachi 240 spectrophotometer. The solvent was distilled water or 0.2N acetic acid.

Synthesis of 2-hydroxy-3′,4′-dihydroxyacetophenone

This compound was synthesized from 2-acetoxy-3′,4′-diacetoxyacetophenone by Voswinckel's method (1909).

Results

Chromatographic behavior and some chemical properties of the P-3 substance

The purified P-3 substance was dissolved in water and submitted to one-dimensional thin-layer chromatography with the following four kinds of solvents: MWP, BAW, BeAW, and EFW. In all these solvents, the P-3 substance moved to the same position as did synthetic 2-hydroxy-3′,4′-dihydroxyacetophenone. Co-chromatography gave completely a single spot. In two-dimensional chromatography, both substances showed the same position.

The P-3 substance and synthetic 2-hydroxy-3′,4′-dihydroxyacetophenone gave the same color reactions on the chromatogram. Both substances were blue to the phosphomolybdic acid-NH_3 test and negative to both the ninhydrin test and Ehrlich's test. In the ethylenediamine-NH_3 test, both substances gave a sky-bluish yellow fluorescence, which turned greenish yellow after several hours. For the Evans test, both

substances became orangish yellow after the first reagent (sodium molybdate in HCl) but did not turn pink after the third reagent (0.5N NaOH). Both substances showed a dull fluorescence (a very weak fluorescence) without any reagent. After the phosphomolybdic acid-NH_3 test, both substances absorbed ultraviolet light and showed a dark spot on the chromatogram under ultraviolet light.

Absorption spectra

The purified P-3 substance was dissolved in water and, after centrifugation, the absorption spectrum of the supernatant was taken. The reference solution was prepared from a blank thin-layer sheet in the same way as mentioned above. The absorption spectrum of synthetic 2-hydroxy-3′,4′-dihydroxyacetophenone was taken by dissolving the compound directly in water without submitting to chromatography. As shown in Fig.1, both the P-3 substance and synthetic 2-hydroxy-3′,4′-dihydroxyacetophenone gave

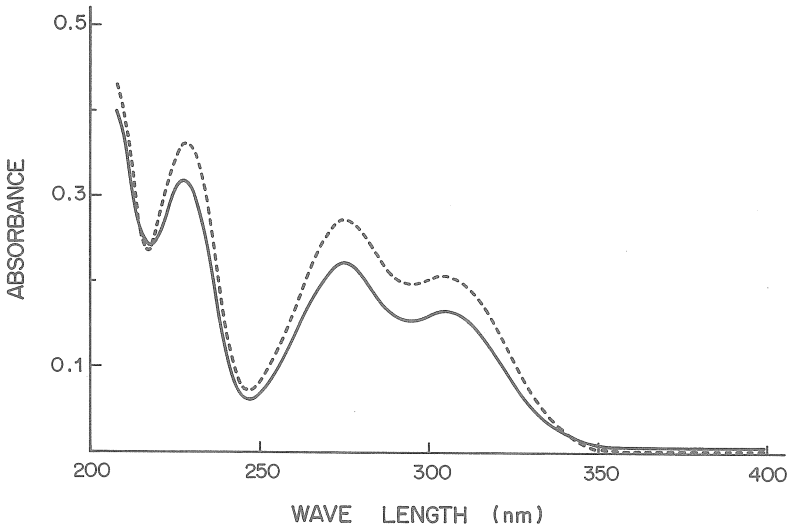

Fig. 1. Absorption spectra of the P-3 substance (solid line) and synthetic 2-hydroxy-3′,4′-dihydroxyacetophenone (broken line) in water.

the same absorption spectrum, which was typical of ketocatechols. The wave lengths of absorption maxima were 228, 274.5—275, and 305nm. The absorption spectra in 0.2N acetic acid were essentially the same as in Fig. 1.

Distribution of the P-3 substance

The residual scales were prepared from the wing-scales of the following three species: *C. crocale, A. indra*, and *M. rhetenor.* After these residual scales were hydrolyzed in 1N HCl, the hydrolysate was submitted to two-dimensional chromatography and examined for the presence or absence of the P-3 substance and β-alanine.

In all these scales, the P-3 substance was found, whereas β-alanine was absent or, if any present, it was only a trace or uncertain.

Discussion

In the previous paper (Umebachi and Aburano, 1978), the residual scales of thirty six kinds of scales from twenty five species of butterflies were hydrolyzed in 1N HCl and examined for the presence or absence of β-alanine. It was found that the presence or absence of β-alanine was closely related to the color or pigment of scales. At that time, in addition to β-alanine, the presence or absence of the phenolic compound, P-3, was also found to depend on the kind of scale. Interestingly, there seemed to be a general tendency that the residual scales which contained no β-alanine or only a trace of it gave, after acid hydrolysis, a clear and dense spot of the P-3 substance. The residual scales which contained a large quantity of β-alanine did not show the P-3 substance. But black scales were exceptional, because they showed neither β-alanine nor the P-3 substance.

In the present paper, there seems to be no doubt that the P-3 substance is 2-hydroxy-3′,4′-dihydroxyacetophenone. Both substances showed the same absorption spectra, chromatographic behaviors, color reactions, and fluorescence. Among color reactions, it is important that, in the ethylenediamine-NH_3 test, the P-3 substance and 2-hydroxy-3′,4′-dihydroxyacetophenone both showed the same sky-bluish yellow fluorescence. Because the ethylenediamine-NH_3 test is known to give different fluorescences depending on *o*-diphenols. Furthermore, the result of the Evans test is also important. Most *o*-diphenolic substances show a yellow color after the first reagent and turn pink after the third reagent. But neither the P-3 substance nor 2-hydroxy-3′,4′-dihydroxyacetophenone did not become pink after the third reagent.

Andersen (1970, 1971) and Andersen and Barrett (1971) reported that several kinds of ketocatechols were released by acid hydrolysis of insect cuticle. Among them, are included 2-hydroxy-3′,4′-dihydroxyacetophenone, 3,4-dihydroxyphenylglyoxal, 2-amino-3′,4′-dihydroxyacetophenone (arterenone), and N-acetylarterenone. The kind of ketocatechol released depends on the condition of hydrolysis. On the ground of these findings, Andersen proposed a new mechanism of the sclerotization of cuticle and named it β-sclerotization (Andersen, 1976, 1977). In both β-sclerotization and the well-known quinone tanning, the sclerotizing agent seems to be N-acetylDOPAmine. In this respect, both mechanisms are similar to each other. But, in the quinone tanning, it is assumed that, after N-acetylDOPAmine is oxidized to *o*-quinone, protein is linked to the benzene ring. On the other hand, in the β-sclerotization, N-acetylDOPAmine does not need to become *o*-quinone, and protein is linked to the β-carbon of the side-chain of DOPAmine. If Andersen's new theory is applicable, the β-sclerotization may occur also in the wing-scales of butterflies. In this connection, whether or not arterenone and

N-acetylarterenone are released from the wing-scales depending on the condition of acid hydrolysis must be examined. In comparison with Andersen and Barrett's report (1971), it is possible that the P-4 substance which was mentioned in the section of methods may be 3,4-dihydroxyphenylglyoxal. But, as the P-4 spot was faint, the identification was not possible.

In the present paper, the above-mentioned relationship that the P-3 substance is inversely related to β-alanine in their quantities has been further confirmed by adding three new examples to the previously reported list. What does this general tendency mean? This must be a subject of further experiments. In this connection, the presence of N-(β-alanyl) DOPAmine derivative in insects would be interesting (Umebachi, 1975a,b; Umebachi and Yamashita, 1976, 1977; Rembold et al., 1978).

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References

Andersen, S. L. (1970) *J. Insect Physiol.* **16**, 1951-1959
——— (1971) *Insect Biochem.* **1**, 157-170
——— (1976) "The Insect Integument" ed. by H. R. Hepburn, Elsevier Scientific Pub. Co., p. 121-144
——— (1977) *Symp. zool. Soc. Lond.* No. **39**, 7-32
——— and F. M. Barrett (1971) *J. Insect Physiol.* **17**, 69-83
Coulson, C. B. and W. C. Evans (1958) *J. Chromatog.* **1**, 374-379
Dalgliesh, C. E. (1952) *Biochem. J.* **52**, 3-14
Rembold, H., J. Rascher, J. Eder, and Y. Umebachi (1978) *Z. Naturforsch.* **33c**, 498-503
Riley, R. F. (1950) *J. Am. Chem. Soc.* **72**, 5782-5783
Sourkes, T. L., R. L. Denton, G. F. Murphy, B. Chavez, and S. S. Cyr (1963) *Pediatrics* **31**, 660-668
Umebachi, Y. (1975a) *Insect Biochem.* **5**, 75-92
——— (1975b) *Acta Vitaminol. Enzymol.* **29**, 219-222
——— and Y. Aburano (1978) *Sci. Rep. Kanazawa Univ.* **23**, 43-55
——— and H. Yamashita (1976) *Comp. Biochem. Physiol.* **54B**, 55-62
——— and ——— (1977) *Comp. Biochem. Physiol.* **56B**, 5-8
——— and K. Yoshida (1970) *J. Insect Physiol.* **16**, 1203-1228
Voswinckel, H. (1909) *Ber. dtsch. chem. Ges.* **42**, 4651-4654