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Pesticide-producing Agricultural Food Processing. The spectrophotometric properties of the pigments extracted from the heads of the melon fly, *Dacus cucurbitae* COQUILLETT (Diptera: Trypetidae)*

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I INTRODUCTION

The visual sensation of insects has been studied as a possible target physiological process for the safe, selective insecticides derived from agricultural food products[3,4]. The method of evaluating association constants of an addition reaction between all-*trans*-retinal and amino compounds was also worked out[5]. For a better performance of screening, the effect of chemicals on the visual pigments which are non-receptor pigments, accessory pigments, ought to be established, since it is expected that disturbance of the function of accessory pigments may also result in visual mulfunction via unbalance of color matching between these pigments and exposure of the receptor molecules to the excess amount of light.

For an effective screening, a rapid view of the effects of chemicals on all of the accessory pigments in the insect eye in addition to photoreceptor molecules is desirable since such a procedure could be simpler and an extent of the effect of chemicals could be examined without isolation and purification of the pigments concerned individually as usually done by previous workers [2,6]. We now report that a single column chromatography could isolate six accessory pigments satisfactorily and would be utilized for chemical screening. The spectrophotometric properties of these pigments and the effects of illumination on these pigments were also presented in this work.

II MATERIALS AND METHODS

1 Insect

Mass production of the melon fly, *Dacus cucurbitae* COQUILLETT, was carried out by the methods of Azuma and Tarama[1]. The adult flies freezed at −20°C were used.

2 Extraction and separation of accessory pigments

The 2,000 heads of the melon fly were grounded in 25 ml of M/30 (pH 7.0) phosphate buffer

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upto paste formation, extracted with the same buffer, and then centrifuged at 11,000 rpm for 20 min. The supernatant, 6 ml, was filterated by a Sephadex G-15 column (2 x 65 cm) with M/30 (pH 7.0) phosphate buffer. The flow rate was 10 ml per hr and fractions in 4 ml each were collected. The separation was based on the measurements of absorbances at 350 nm using a spectrophotometer (Hitachi, 181) and the observation of fluorescence using a fluolescence test lamp (Toshiba, Fl-3L) with the wavelength of the maximum intensity at 360 nm. All extraction and separation procedures were carried out under a dim red light at a temperature below 6°C.

3 Spectrophotometry

The UV-illumination of the fractions P-I and P-III, shown in Fig. 1 and contained in silica cells, was performed by a black light (Toshiba, FL20SBLB, 20 W). The fractions P-IV and P-VI were illuminated in a test tube for 5 min by a slide projector with a 1000 W tungsten lamp as a visible light source. The absorption spectra and differential absorption spectra of the pigments were obtained by a Hitachi spectrophotometer (Type 356). All measurements were carried out at 20°C.

III RESULTS AND DISCUSSION

The pigments extracted could be separated by a single column chromatography using Sephadex

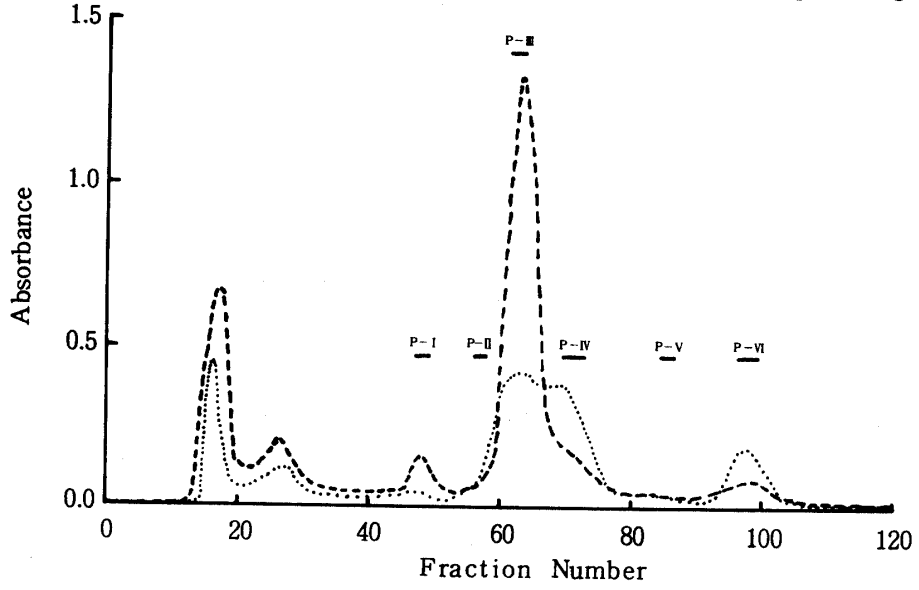


Fig 1 The Sephadex G−15 column chromatogram of the pigments extracted with M/30 (pH 7.0) phosphate buffer from the head of the melon fly

Notations, P−I through P−VI, indicate the pigments present in the fractions. ----: Absorbance at 350 nm, ...: Absorbance at 430 nm

G-15 gel as shown in Fig. 1. The separation based on absorbances at 430 nm and 350 nm showed five peaks. The fractions corresponding to 14–19 and 21–29 were the part of macromolecules and that of the pigments with featureless absorption spectra, respectively. A fluorescence test indicated the presence of two more pigments without having any noticeable peaks in the chromatogram. Only one of them was obtained as a single component. From these observations, the fractions P-I through P-VI were named as shown in Fig. 1.

The UV- and visible absorption spectrum of P-I is presented in Fig. 2. There were two maxima

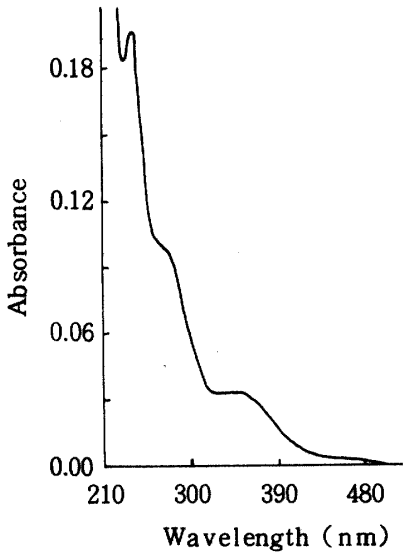


Fig 2 The absorption spctrum of P－I at pH 7.0

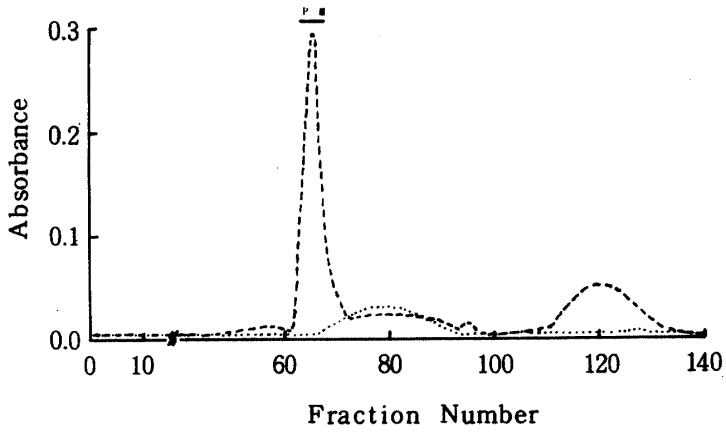
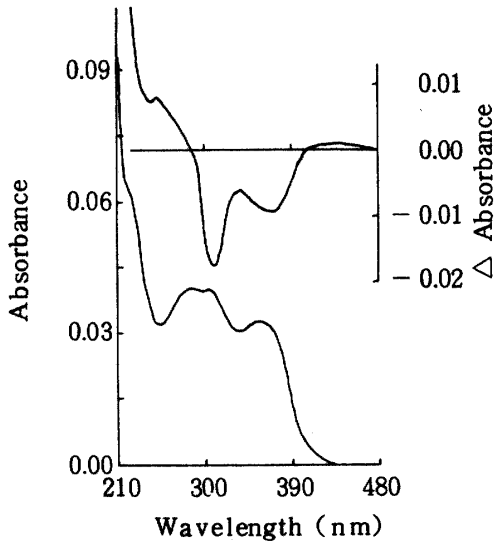


Fig 3 The Sephadex G－15 column chromatogram of Fr. Nos. 61－63 shown in Fig. 1

- - - - - : Absorbance at 350 nm, ····· : Absorbance at 430 nm

Fig 4 The absorption spectrum and photosensitivity of P－Ⅲ

The lower part indicates the absorption spectrum (pH 7.0) and the upper part indicates the difference spectrum of the P－Ⅲ illuminated for 120 min with near UV－light, taken against an unilluminated sample.



at 350 nm and 240 nm and a sholder at 277 nm. The fraction P-I emitted blue fluorescence by the illumination using a fluorescence test lamp and showed no change in absorption spectrum by BLB illumination. An absorbance at 350 nm decreased to about 40% by allowing to stand at 20°C under a dark condition for 2 days.

The absorption spectrum of P-II was not obtained, since P-II was not separated from P-III. Nevertheless, emittion of yellow fluorescence indicated the presence of the pigment being distinct from P-III.

The fraction P-III was separated by rechromatography using Sephadex G-15 (Fig. 3) due to a larger content in the extract. The UV- and visible absorption spectrum of P-III is shown in Fig. 4. There were three maxima at 355 nm, 305 nm, and 278 nm. The P-III emitted blue fluorescence. An illumination with BLB decreased absorbances at 369 nm and 312 nm while it increased that at 255 nm. The absorbance at 355 nm decreased 30% on standing at a temperature of 20°C in darkness for 2 days.

The fraction P-IV was dark yellow and had no fluorescence. There were the absorbance maxima at 435 nm and 228 nm as shown in Fig. 5. Absorbances at 450 nm and 235 nm decreased

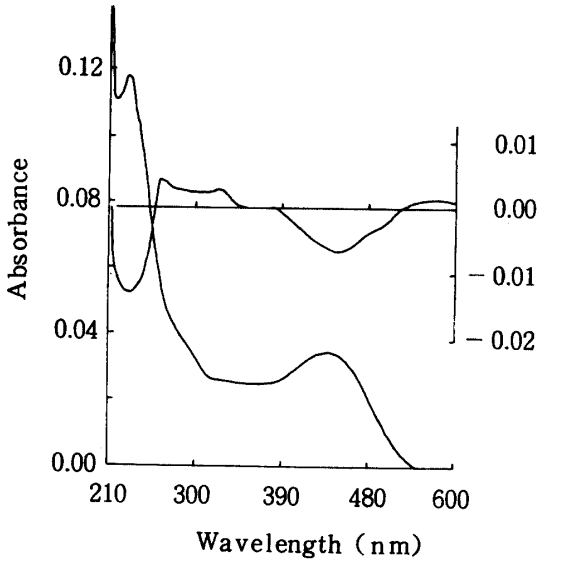


Fig 5 The absorption spectrum and photosensitivity of P−Ⅳ

The lower part indicates the absorption spectrum (pH 7.0) and the upper part indicates the difference spectrum of the P−Ⅳ illuminated for 5 min with visible light, taken against an unilluminated sample.

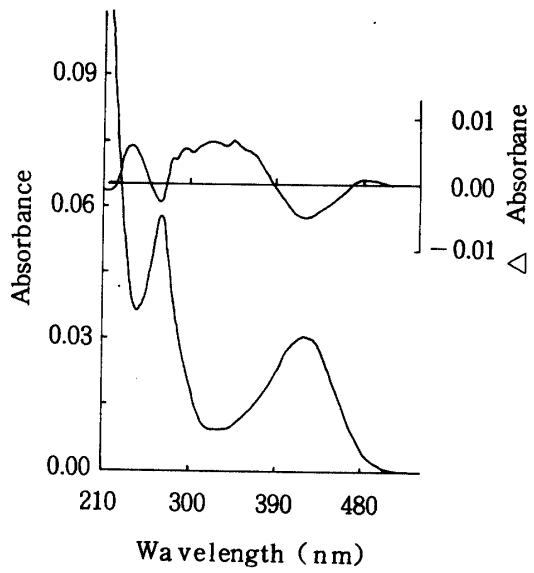


Fig 6 The absorption spectrum and photosensitivity of P−Ⅵ

The lower part indicates the absorption spectrum (pH 7.0) and the upper part indicates the difference spectrum of the P−Ⅵ illuminated for 5 min with visible light, taken against an unilluminated sample.

85% by visible light illumination.

The presence of P-V was detected by purple fluorescence. The absorption spectrum of P-V had three sholders at 460 nm, 345 nm, and 280 nm.

The fraction P-VI was green yellow and had yellow fluorescence. Its absorbance maxima were at 420 nm and 268 nm as shown in Fig. 6. The fraction showed decreasing absorbances at 420 nm and 268 nm and increasing absorbances at 285–360 nm and 240 nm by visible light illumination as presented in Fig. 6. The absorbance at 420 nm decreased about 10% by standing at a temperature of 20°C in darkness for 2 days.

The spectrophotometric characteristics of the six pigments described above are summarized in Table 1. The fractions P-IV, P-V, and P-VI were capable of absorbing visible light and the rest of

Table 1. The spectrophotometric properties of the pigments extracted from the heads of the melon fly

Pigment	Fraction Number	λ max (nm)	Fluorescence	Photosensitivity**	Remark
P-I	47–49	350, 277*, 240	Blue	–	
P-II	56–58		Yellow		
P-III	61–62	355, 305, 278	Blue	+	
P-IV	69–73	435, 228	–	+	Dark yellow
P-V	84–86	460*, 345*, 280*	Purple		
P-VI	96–99	420, 268	Yellow	+	Green yellow

* Sholders
** +: Partially bleached, –: Unbleached

the fractions absorbed only near-UV. Except P-IV, all pigments emitted distinct fluorescence.

The photosensitivity was observed in P-III at 355 nm, in P-IV at 435 nm, and in P-V at 440 nm. Since these pigments may function as the light deterctors of the pigment granules in the accessory cells[2,6], the effects of visual inhibitors should be studied in relation to the modification of the photosensitivity of these pigments as well as formation of the photostable pigments from these pigments.

It is now evident from the results of this work that a single chromatographic procedure isolates major accessory pigments in the heads of the melon flies. This separation procedure would be utilized for a rapid screening of the interaction of possible visual inhibitors with the accessory pigments.

IV SUMMARY

For the purpose of application to rapid biochemical screening of the insect-vision inhibitors derived from agricultural food products, single chromatographic process for separation of the

major water-soluble pigments extracted from the heads of the melon fly, *Dacus cucurbitae* COQUILLETT, was established. The existence of six small-molecular weight pigments (P-I – P-VI) were confirmed on Sephadex G-15 column chromatography of an extract with M/30 (pH 7.0) phosphate buffer from the insect heads. The fractions P-I through P-III had absorbance peaks at the near UV region while P-IV through P-VI were capalbe of absorbing visible light. Except P-IV, all pigments emitted distinct fluorescence. The fractions P-III, P-IV, and P-VI were photosensitive. Only P-VI formed the new absorbance maximum at the near UV region by bleaching with white light. From these results, it was suggested that the present separation procedure would be used for screening purpose.

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農薬生産用農産食料製造－ウリミバエ頭部より抽出された水溶性色素の分光学的性質

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要　　約

食料起源の昆虫視覚阻害剤の迅速な生化学的スクリーニングに応用する目的で，ウリミバエ頭部から抽出された主な水溶性色素を一回のクロマトグラフィにより分離する方法を確立した。ウリミバエ頭部（2,000）からM／30（pH 7.0）リン酸緩衝液で得た抽出物のセファデックスG－15カラムクロマトグラフィにより小分子色素が6種（P－I～P－VI）存在することが認められた。P－IからP－IIIは，近紫外部に吸収帯を持ち，P－IVからP－VIは，可視光線を吸収した。P－IVを除いて，全色素が特異的な蛍光を発した。得られた6色素中，P－III，P－IV，及びP－VIは光感受性を示した。P－VIのみは，白色光で退色すると共に，近紫外部に新しい吸収帯を形成した。これらの結果により，本分離法が，スクリーニングに利用できることが示された。

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