

무당개구리의 복피 Carotenoid 색소에 관한 연구(제3보).  
3-Hydroxy-canthaxanthin의 분리 및 확인

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Studies on the Carotenoid Pigment in the Abdominal  
Skin of *Bombina Orientalis*(III). Occurrence of  
3-Hydroxy-canthaxanthin in the Abdominal  
Skin of *Bombina Orientalis*

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요 약 무당개구리의 복피 색소로부터 적자색 침상결정인 3-hydroxy-canthaxanthin을 분리하였다. Visible과 IR 스펙트럼의 특성, 크로마토그래피적 성질 및 물리화학적 성질을 이용하여 그의 구조를 확인하였다.

**Abstract** 3-Hydroxy-canthaxanthin, reddish purple needle shaped crystals, has been isolated from the abdominal skin of *Bombina Orientalis*. Its structure has been established by visible and IR spectral characteristics, chromatographic behaviors, and physical and chemical properties.

**Introduction**

In the preceding papers,<sup>1,2</sup> we have described the isolation of three major pigments, identified as  $\beta$ -carotene, 4'-hydroxy-echinenone and  $\alpha$ -cryptoxanthin respectively, from the abdominal skin of *Bombina Orientalis*. In the present paper, we wish to report the isolation and identification of 3-hydroxy-canthaxanthin from the abdominal skin of *Bombina Orientalis*.

3-Hydroxy-canthaxanthin(Adonirubin, 3-hydroxy-4,4'-diketo- $\beta$ -carotene) was previously isolated from green algae by Czygan and Kessler<sup>3</sup>, and also isolated from the angiosperm *annua* L. by Egger<sup>4</sup>. It has recently been separated also from two flamingo species; *Phoenicoparrus* and

*Phoenicoterus* by Fox and Hopkins<sup>5</sup>. Some other studies of this pigment had been made<sup>6-9</sup>.

In the present work, the same partition was carried out on a precoated SiO<sub>2</sub> PLC plate as before<sup>2</sup>. The pigment *F*, the most polar portion of the pigments on the SiO<sub>2</sub> PLC, was further separated and purified on the precoated SiO<sub>2</sub> plate for PLC with benzene. The major pigment of *F* was identified through its physical and chemical properties, visible and IR-spectral characteristics, and chromatographic behaviors. Its reduction product was synthesized and examined. All these properties of the pigment *F*<sub>2</sub> and its reduction product were in good agreement with 3-hydroxy-canthaxanthin as previously reported<sup>10,11</sup>.

## Experimental

**Materials and Method** The same materials and methods described in the previous reports, <sup>1,2</sup> if otherwise stated, were used.

**Preliminary Partition of Pigment** The same partition was carried out as before<sup>2</sup>. The pigment *F*, the most polar portion of pigments on SiO<sub>2</sub> PLC plate, was a broad red band. The pigment *F* zone was scraped off from the plate and the pigment was extracted from the adsorbant with acetone and little methanol. A equal volume of *n*-hexane was added to the extract of zone *F*. The resulted solution was washed twice with saturated NaCl solution, twice with water, dried over anhydrous sodium sulfate, and evaporated. The residue was dissolved in a small portion of hexane, and stood for 2 days in the refrigerator. The sterols, precipitated out as white solid, were filtered off and the filtrate was concentrated under reduced pressure. The residue was again dissolved in a small portion of benzene and the solution was stood for 2 months in a deep freezer (-15 °C) under carbon dioxide atmosphere. The solid, separated out as purple needle shaped crystals, was filtered off.

### Separation of 3-Hydroxy-canthaxanthin

The solid was dissolved in a small volume of benzene and was further purified on SiO<sub>2</sub> PLC plate with benzene. Three zones, (*F*<sub>1</sub>) a narrow orange band, (*F*<sub>2</sub>) a broad red band, and (*F*<sub>3</sub>) a narrow reddish violet band, were separated. The red colored major zone *F*<sub>2</sub> was extracted with acetone, and transferred to the petroleum ether layer. The petroleum ether layer was washed with saturated NaCl solution, finally with water, dried over anhydrous sodium sulfate, and evaporated. The purified pigment *F*<sub>2</sub> was further examined by TLC on SiO<sub>2</sub> plate with benzene-methanol(10:1), but no further separation was observed. The pure pigment *F*<sub>2</sub> was recrystallized

from hexane and little methanol, as the reddish purple needle shaped crystals, mp. 179~180 °C. The VIS-UV absorption maxima in nm: 467~468 in *n*-hexane; 485~487 in CHCl<sub>3</sub>; 498~501 in CS<sub>2</sub>; 489~491 in pyridine; 465 in petroleum ether; 480~482 in benzene as a broad round curve. IR(KBr pellet): 2.93 μ (-OH), 3.43 μ (-CH<sub>3</sub>), 6.03 μ (conjugated carbonyl), and 10.42 μ (C-H in *trans* olefin). The *R<sub>f</sub>* value, determined on SiO<sub>2</sub> TLC plate with benzene-methanol(10:1), was 0.56, and that of 4-hydroxy-echinenone was 0.71 in the same condition.

**Reduction of Pigment *F*<sub>2</sub>** To a solution of 0.3mg of *F*<sub>2</sub> in 15 ml of hexane-methanol(1:1), 2 mg of sodium borohydride in 5 ml absolute methanol was added dropwise at 10 °C, with stirring under CO<sub>2</sub> atmosphere. The resulting solution was kept for 15 minutes at 15 °C and the excess of sodium borohydride was decomposed by dropwise addition of water at 10 °C. The solution was washed twice with saturated NaCl solution, finally with water, dried and evaporated under reduced pressure. The residue was dissolved in a small volume of benzene and chromatographed on the column of Ca(OH)<sub>2</sub>-celite 545(1:1) with *n*-hexane-CHCl<sub>3</sub>(1:1). The three zones, (*F*<sub>2</sub>)-*a* a narrow orange band, (*F*<sub>2</sub>)-*b* a yellowish orange band, and (*F*<sub>2</sub>)-*c* a broad yellow band, were separated. The pigments in each zone were eluted out with benzene. The solvent was evaporated and the residue was examined on SiO<sub>2</sub> TLC plate with benzene-methanol(4:1). No further separation was observed. The VIS-UV absorption maxima in *n*-hexane: (*F*<sub>2</sub>)-*a* 467 nm; (*F*<sub>2</sub>)-*b* 455~456 nm; and (*F*<sub>2</sub>)-*c* 425, 448, 476nm.

## Results and Discussion

The presence and identification of 3-hydroxy-canthaxanthin has been demonstrated in the abdominal skin of Bombina Orientalis as the fourth major pigment. The visible absorption spectra of pigment *F*<sub>2</sub> as shown in Fig. 1, were almost

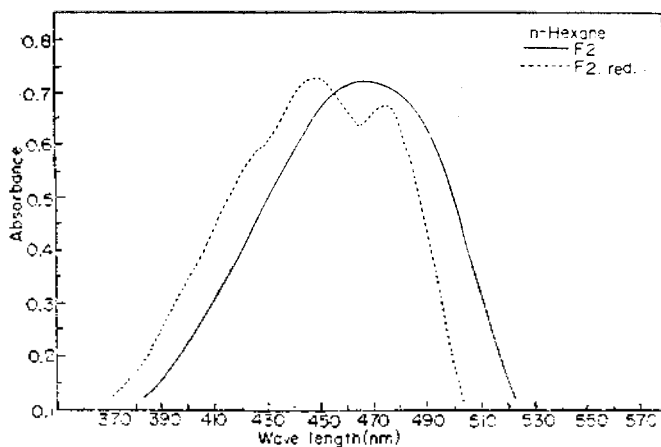


Fig. 1. Visible absorption curves of pigment  $F_2$  and  $F_2$ -reduction product; solvent, *n*-hexane.

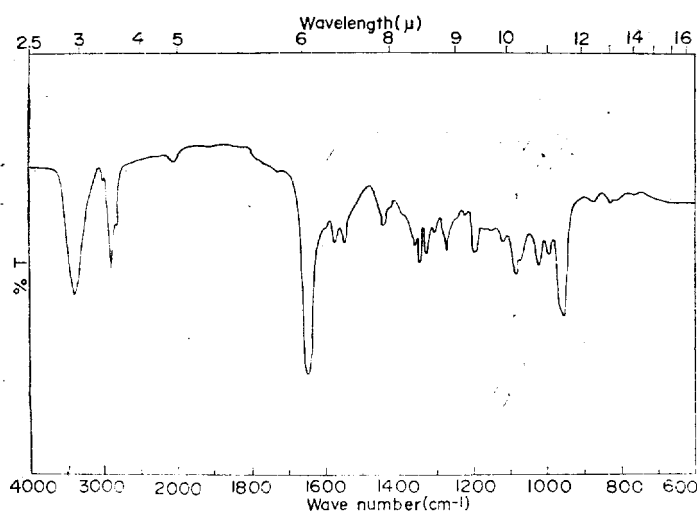


Fig. 2. IR-spectrum of pigment  $F_2$  (KBr pellet)

superimposable to that of 3-hydroxy-canthaxanthin, previously reported: 466~468 nm in *n*-hexane; 484~488 nm in  $\text{CHCl}_3$ ; 498~503 nm in  $\text{CS}_2$ ; 490~492 nm in pyridine by Czygan and Kessler<sup>10</sup>.

The IR-spectrum of pigment  $F_2$  showed the presence of a conjugated carbonyl group (6.03  $\mu$ ), hydroxy group (2.93  $\mu$ ) and C-H in *trans*-olefin (10.42  $\mu$ ), as shown in Fig. 2, and no

absorption of non-conjugated carbonyl group (ca. 5.83  $\mu$ ).

From the broad round shape of visible spectrum curve, it is suggested that pigment  $F_2$  possesses a conjugated carbonyl group on the terminal ring group of the carotenoid.

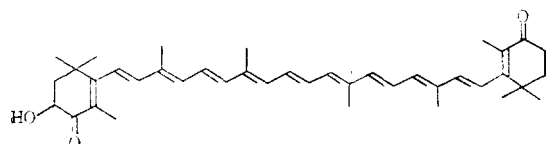
The intensities of the conjugated carbonyl absorption of pigment  $F_2$  in IR-spectrum was greater than that of 4'-hydroxy-echinenone which has one conjugated carbonyl group in its molecule<sup>1</sup>. This result suggests the presence of more than one conjugated carbonyl groups in its molecule. The melting point of pigment  $F_2$ , the reddish purple shaped crystals from hexane and little methanol, was 179~180 °C (uncorr. under  $\text{CO}_2$  gas). The  $R_f$  value of pigment  $F_2$ , determined on  $\text{SiO}_2$  TLC plate with benzenemethanol (10:1), was 0.56, less than that of 4'-hydroxy-schinenone ( $R_f$ ; 0.71) isolated from the *Bombina orientalis*.

For the further confirmation,  $F_2$  was reduced with sodium borohydride and the resulted

reduction products were examined. The reduction products, chromatographed on the column of  $\text{Ca}(\text{OH})_2$ -celite (1:1) with *n*-hexane- $\text{CHCl}_3$  (1:1), were separated into three zones. After final purification of these reduction products, the VIS-UV absorption maxima of them were determined in *n*-hexane. The least polar pigment ( $F_2$ )-a,  $\lambda_{\text{max}}$ ; 467 nm as a round broad curve, was identified as a starting material itself, the

medium polar reduction product( $F_2$ )-b,  $\lambda_{\max}$ ; 455~456 nm as a round broad curve, was identified as the partially reduced product, in which one of the conjugated carbonyl group of  $F_2$  remained intact, and the most polar portion of the reduction product( $F_2$ )-c,  $\lambda_{\max}$ ; 425, 448, 476 nm having fine structure of well defined three peaks, was exactly consistent with that of 3, 4, 4'-trihydroxy- $\beta$ -carotene, previously reported:  $\lambda_{\max}$ ; 426, 448, 476 nm in *n*-hexane, by Czygan and Kessler<sup>10</sup>(Fig. 1).

From all these data the pigment  $F_2$  was proved unequivocally as 3-hydroxy-canthaxanthin.



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#### References

1. Sae Hee Chang and Ui Chun Chong, *This Journal*, **18**, 289(1974).
2. Sae Hee Chang and Ui Chun Chong, *ibid*, **18**, 368(1974).
3. E. Kessler and F. C. Czygan, *Per., dtsh. bot Ges.*, **78**, 342(1965).
4. K. Egger, *Phytochemistry*, **4**, 609(1965).
5. D. L. Fox and T. S. Hopkins, *Biochem. Physiol.*, **17**, 841(1966).
6. E. Kessler and F. C. Czygan, *Arch. Mikrobiol.*, **55**, 320 and 346(1967).
7. F.-C. Czygan and W. Heumann, *ibid*, **57**, 123(1967).
8. H. Kleinig, *Z. Naturforsch.* **22b**, 977(1967).
9. K. Egger and H. Kleinig, *Phytochemistry*, **6**, 437, 611 and 903(1967).
10. F. C. Czygan and E. Kessler, *Z. Naturforsch.*, **22b**, 1085(1967).
11. G. E. Ungers and J. J. Cooney, *J. Bacteriology*, **96**, 234(1968).