

무당개구리의 복피 Carotenoid 색소에 관한 연구(제 2 보).  
 $\alpha$ -Cryptoxanthin(3-hydroxy- $\alpha$ -carotene)의 분리 및 확인

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**Studies on the Carotenoid Pigment in the Abdominal  
Skin of Bombina Orientalis(II). Occurrence of  
 $\alpha$ -Cryptoxanthin(3-hydroxy- $\alpha$ -carotene) in the  
Abdominal Skin of Bombina Orientalis**

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**요 약.** Bombina Orientalis(무당개구리)의 복피로부터 추출한 암적색의 색소를 TLC, PLC(preparative thin layer chromatography) 및 column chromatography로 분리 및 정제하여 물리, 화학적 성질 visible 및 IR-spectral 특성을 이용하여 세면체주색소인  $\alpha$ -cryptoxanthin(3-hydroxy- $\alpha$ -carotene)을 확인하였다.

**Abstract.** Dark red extract of abdominal skin of Bombina Orientalis was separated and purified with TLC, PLC(preparative thin layer chromatography) and column chromatography. Through the physical and chemical properties, visible and infrared spectral characteristics the third major pigment was identified as  $\alpha$ -cryptoxanthin(3-hydroxy- $\alpha$ -carotene).

**Introduction**

In the previous paper<sup>1</sup>, it was reported that two major pigments separated from the abdominal skin of Bombina Orientalis were  $\beta$ -carotene and 4-hydroxy-echinenone respectively. In the present work, the third major pigment was separated and identified as  $\alpha$ -cryptoxanthin (3-

hydroxy- $\alpha$ -carotene). Zeinoxanthin was first isolated from the maize<sup>2</sup>, and has recently been shown<sup>3,4</sup> to be identical with  $\alpha$ -cryptoxanthin (3-hydroxy- $\alpha$ -carotene) isolated from Capsicum annum<sup>5</sup>. Livingston and Knowles<sup>6</sup> had been reported as a major carotenoid in the alfalfa, Medicago sativa, and some investigations of  $\alpha$ -cryptoxanthin had also been made.<sup>7-10</sup>

In the previous work, the pigment extract was partitioned by the PLC on a precoated alumina plate. The most polar portion in the extract tended to adhere so strongly on alumina to make the recovery impossible of this portion of pigment by solvent extraction after development of chromatogram. In the present work, the initial partition was carried out on a precoated SiO<sub>2</sub> PLC plate. Somewhat different chromatogram was obtained. Although the widths of each colored band were varied with the chromatogram on alumina plate, the sequence and the color shade on the bands were identical with the later.

$\alpha$ -Cryptoxanthin was reddish orange crystals from the moderately polar portion on the SiO<sub>2</sub> chromatogram by further separation and purification with repeated chromatography on the activated SiO<sub>2</sub>.

It was identified from its physical, spectral, and chromatographic behaviors. Its acetate and dehydration product were synthesized and examined. All of the properties of itself and its derivatives unequivocally matched with the data reported for an authentic sample of  $\alpha$ -cryptoxanthin.

### Experimental

**Materials and Methods.** The same materials and methods described in the previous report,<sup>1</sup> if otherwise stated, were used. The acid chloroform reagent<sup>11</sup> was obtained by saturating Wako chloroform with dry HCl gas at the room temperature for 10 min. The separation and purification of each pigment was followed the same procedure described before<sup>1</sup> even if not stated below.

**Preliminary Partition of Pigment.** The dark red hexane solution obtained by concentration of the original extract, without saponification, was separated on the precoated SiO<sub>2</sub> plate for PLC by Merck with petroleum ether—

benzene—methanol(5:1:0.1) solution as developing solvent. Six main zones were separated in the following order of increasing adsorptive power: (A) a broad orange band, (B) a narrow yellow band, (C) a narrow deep red band, (D) a broad red band, (E) a broad reddish orange band, and (F) a broad red band. Each zone was scraped off from the plate and the pigments were extracted from the adsorbant with benzene for (A) and (B) and acetone for other zones. Each extract was concentrated and examined by TLC.

(A), This was further separated on SiO<sub>2</sub> PLC-plate with petroleum ether—benzene(5:1). The three zones were separated in the following order of increasing adsorptive power: (A<sub>1</sub>) a deep orange band, (A<sub>2</sub>) a orange band, and (A<sub>3</sub>) a pink band. (A<sub>1</sub>) was previously identified as  $\beta$ -carotene.

**Separation of  $\alpha$ -cryptoxanthin.** (C) was further separated on SiO<sub>2</sub> PLC plate with benzene—petroleum ether(1:3). Three zones, (C<sub>1</sub>) a narrow red band, (C<sub>2</sub>) a broad deep red band, and (C<sub>3</sub>) a narrow red band, were separated. The pigments in each zone were extracted with acetone, transferred to petroleum ether layer by adding water. The petroleum ether layer was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. From the color intensities of each band, the C<sub>2</sub> band was assumed as the major constitution of this fraction. The fraction C<sub>2</sub> was dissolved in benzene and chromatographed further on SiO<sub>2</sub> column with benzene containing 5% of acetone. The major red zone was eluted out. The eluted solution was washed with water, dried, and evaporated. The residue was examined by TLC on SiO<sub>2</sub> with benzene—acetone(20:1). No further separation was observed. Since the residue was obtained only as semisolid mass and failed to give crystals, the hexane solution of C<sub>2</sub> was saponified with 10%

KOH-methanol solution, by allowing to stand at room temperature for 60 hours. The reaction mixture was transferred to a separatory funnel and added a small portion of benzene. It was washed with several portions of water and saturated NaCl aqueous solution. The benzene layer was separated and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. The residue was dissolved in a little amount of hexane and the resulting solution was allowed to stand overnight in a refrigerator.

The precipitate was filtered and recrystallized from benzene-hexane solvent. Reddish orange plate with metallic luster  $C'_2$ , mp. 159~160°C. The VIS-UV absorption maxima in nm: 421, 443.5, 471 in hexane; 433, 457.5, 488 in benzene; 421, 444, 471 in petroleum ether; 425.5, 450, 474.5 in  $CHCl_3$ ; 449, 472, 502 in  $CS_2$ . IR (KBr): 2.86  $\mu$  (-OH), 3.47  $\mu$  and 10.4  $\mu$ .

**Acetylation of  $C'_2$ .** To the solution of  $C_2$  in pyridine, acetic anhydride was added dropwise at room temperature. The acetylation product was purified on alumina column with benzene containing 5% of acetone as eluent. IR ( $CCl_4$ ): 3.47  $\mu$ , 5.83  $\mu$ .

**Dehydration of  $C'_2$ .** One mg of the carotenol pigment  $C'_2$  in 5 ml of chloroform was treated with 5 drops of acid-chloroform reagent under nitrogen atmosphere. No appreciable change took place and unchanged  $C'_2$  was recovered. The dehydration was achieved in  $POCl_3$ -pyridine at 40°C. The dehydration product was purified on  $SiO_2$  column with benzene. The VIS-UV absorption maxima in nm: 455 in hexane, 462 in benzene as a round-shaped curve.

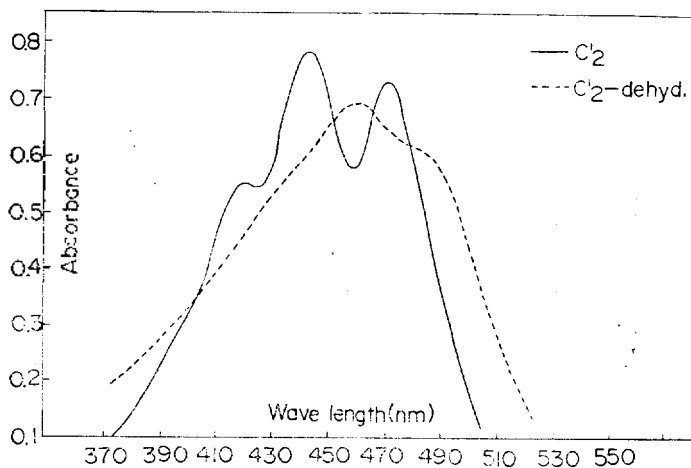


Fig. 1. Visible absorption curves of pigment  $C'_2$  and  $C'_2$ -dehydration product; solvent, in hexane

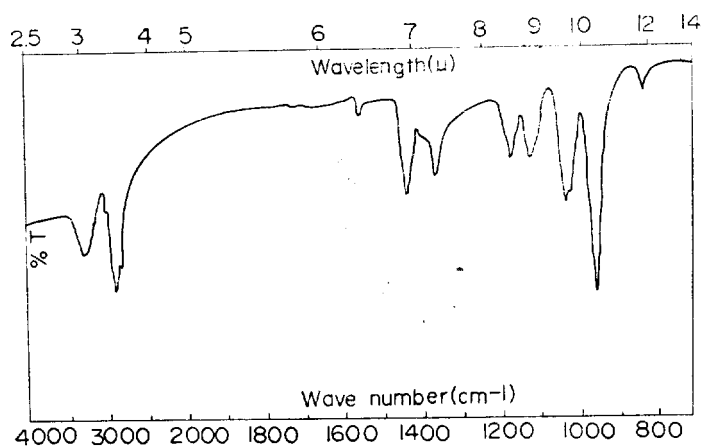


Fig. 2. IR-spectrum of pigment  $C'_2$  (KBR pellet)

## Results and Discussion

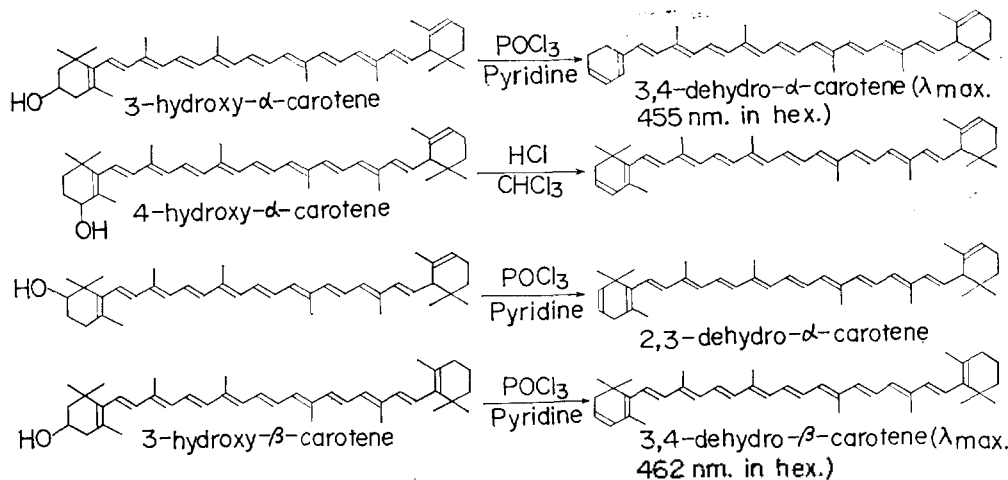
This investigation has demonstrated the occurrence of  $\alpha$ -cryptoxanthin(3-hydroxy- $\alpha$ -carotene) in the abdominal skin of *Bombina Orientalis* as the third major pigment. The absorption spectrum of pigment  $C'_2$  in visible region, as shown in Fig. 1, was exactly superimposable to

that of  $\alpha$ -cryptoxanthin, previously reported: 471.5, 443.5, and 420.5 nm in hexane; 433, 457, 488 nm in benzene by Cholnoky<sup>5</sup>.

The melting point of pigment  $C'_2$ , the reddish orange plate, was 159~160°C. (uncorr. evacuated capillary under CO<sub>2</sub> gas), and in good consistence with that of  $\alpha$ -cryptoxanthin measured by Cholnoky<sup>5</sup>. The IR-spectrum of pigment  $C'_2$  shows a distinct absorption band at 2.86  $\mu$  (—OH), 3.4  $\mu$  and 10.4  $\mu$  (Fig. 2). The  $R_f$ -value of pigment  $C'_2$  on Al<sub>2</sub>O<sub>3</sub>(F254, Merck 0.02 mm) with benzene—methanol(1 : 1) was 0.70, similar with that of cryptoxanthin (3-hydroxy- $\beta$ -carotene,  $R_f$ : 0.68) isolated from the Calyx of physalis.

To confirm the position of OH-group, the dehydration with HCl—CHCl<sub>3</sub><sup>12</sup> and POCl<sub>3</sub>—pyridine<sup>13</sup> were studied.

With HCl—CHCl<sub>3</sub> treatment, no appreciable change occurred. However, POCl<sub>3</sub>—pyridine gives epiphasic product with conjugated polyene. Absorption maximum of dehydration product was consistent with that of 3,4-dehydro- $\alpha$ -carotene, previously reported: 455 nm in hexane by Zeichmeister<sup>14</sup> (Fig. 1). An acetate of  $C'_2$  was prepared with acetic anhydride and pyridine, and checked by IR-absorption spectra. The absorption of OH-group at 2.86  $\mu$  disappeared and a strong absorption at 5.83  $\mu$  (=CO) was observed. These data strongly supported that pigment  $C'_2$  has no tertiary or allylic hydroxy group, and the hydroxy group should be attached at 3 position of carbon on the  $\beta$ -ring. From all these data pigment  $C'_2$  was proved unequivocally as 3-hydroxy- $\alpha$ -carotene( $\alpha$ -cryptoxanthin).



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#### References

1. Sae Hee Chang and Ui Chun Chong, *This journal*, 18, 289 (1974).
2. E. N. Petzold and F. W. Quackenbush, *Arch. Biochem. Biophys.*, 86, 163(1960).
3. I. Sjabolcs and A. Ronai, *Acta. Chim. Acad. Sci. Hung.*, 61, 309(1969).
4. L. Bartlett, *et al*, *J. Chem. Soc. (C)*, 2527 (1969).

5. L. Cholnoky, *et al.*, *Annalen*, **616**, 207(1958).
6. A.L. Livingston and R.E. Knowles, *Phytochem.*, **8**, 1311(1969).
7. O. Isler *et al.*, *Helv. Chim. Acta*, **44**, 994 (1961).
8. E. Lederer, *C.R. Acad. Sci.*, **197**, 1694(1933).
9. E. Lederer, *Bull. Soc. Chim. Biol.*, **20**, 611 (1938).
10. A. Winterstein, *Angew. Chem.*, **72**, 902(1960).
11. F.J. Petracek and L. Zechmeister, *J. Amer. Chem. Soc.*, **78**, 1427(1956).
12. E.C. Grob and R.O. Pflugstapt, *Helv. Chim. Acta*, **45**, 1592(1962).
13. D. Sarmatis and A. Ofner, *J. Org. Chem.*, **28**, 2735(1863).
14. L. Zechmeister and G. Karmakar, *J. Amer. Chem. Soc.*, **77**, 55(1954).