

무당개구리의 복피 Carotenoid 색소에 관한 연구 (제 5 보).
 γ -Carotene, Torulene, 5,6-Monoepoxy- α -carotene 유사물 및
Lutein Monoester의 분리 및 확인

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Studies on the Carotenoid Pigments in the Abdominal Skin of
Bombina Orientalis (V). Occurrence of Some Minor Carotenoids,
 γ -Carotene, Torulene, 5,6-Monoepoxy- α -caroten-like, and
Lutein Monoester

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요 약. 무당개구리의 복피색소로부터 lutein monoester, torulene, γ -carotene 및 5,6-monoepoxy- α -carotene 유사물을 분리 및 정제하여 VIS-UV 및 IR 스펙트럼 특성, 크로마토그래피적 성질 및 물리화학적 성질을 이용하여 그들의 구조를 확인하였다.

ABSTRACT. Some minor carotenoid pigments, luteins, lutein monoester, torulene, γ -carotene, and 5,6-monoepoxy- α -carotene-like have been separated and identified from the abdominal skin of *Bombina Orientalis*. Their structures have been established through the physical and chemical properties, VIS-UV and IR spectral characteristics, and chromatographic behaviors.

INTRODUCTION

In a series of papers¹⁻⁴ we have described the isolation of six major pigments, identified as β -carotene, 4-hydroxyechinenone, α -cryptoxanthin, 3-hydroxycanthaxanthin, lutein and 3,3'-dihydroxy-3,4,3',4'-bisdehydro- β -carotene respectively, from the abdominal skin of *Bombina Orientalis*. Torulene is one of the rarest carotenoids, which was first isolated

from the red yeast *Torula rubra* as a minor carotenoid by Lederer,^{5,6} and also separated by Isler,^{7,8} Goodwin,⁹ Winterstein,¹⁰ and Karrer.¹¹

Torulene appears to be fairly widely distributed in the vegetable sources, but it was not reported yet that torulene had been found in vertebrate. γ -Carotene is also one of the rarest carotenoids. Kuhn and Brockmann¹² discovered a third carotene isomer, γ -carotene, by means of chromatographic adsorption analysis.

A number of separations of γ -carotene from the vegetable kingdom had been made,¹³⁻¹⁹ but the isolation of it from the animal sources is awfully unusual. 5,6-Monoepoxy- α -carotene occurs in the blossoms of various plants,²⁰ and has been synthesized by Karrer²¹ and Kischore.²²

In our present study we wish to report the isolation and identification of γ -carotene, torulene, 5,6-monoepoxy- α -carotene-like, and lutein monoester from the abdominal skin of *Bombina Orientalis*. The same partition was carried out on a prepared SiO₂ PLC plate as before,⁴ and four minor carotenoids were further separated and purified with repeated chromatography on the prepared SiO₂ TLC plate. They were identified from their physical properties, visible and IR spectral characteristics, and chromatographic behaviors.

EXPERIMENTAL

Materials and Methods. The same materials and methods described in the previous report,⁴ unless otherwise stated, were used. SiO₂ PLC plate was prepared and activated at 110°C for two hours in an oven.

Separation of γ -Carotene. Pigment A was further separated on SiO₂ PLC plate with petroleum ether-benzene (5:1). The four zones were separated in the following order of increasing adsorption power; A1 a deep orange band, A2 a yellow band, A3 an orange band, and A4 a pink band. Pigment A1 was previously identified as β -carotene. A3 was further purified on active Al₂O₃ column by elution with 4% benzene in petroleum ether. The VIS-UV absorption spectrum of A3 was determined after purification. The visible absorption maxima are as follows (nm): 431, 461, 492 in hexane; 432, 462, 493 in pet. ether; 447, 477, 510 in benzene; 446, 475, 508 in CHCl₃, (463), 493,

533 in CS₂. On partition between hexane and 95% methanol, A3 was entirely ephiphasic. On mixed thin layer chromatography of pigment A3 and an authentic sample of γ -carotene from carrots, only one colored spot was observed.

Separation of Torulene. The pigment A4 was further eluted on an active alumina column with benzene-pet. ether (1:6) as eluent. The eluent was dried and evaporated. The residue was examined by SiO₂ TLC with benzene-pet. ether (1:5). No further separation was observed. The VIS-UV absorption maxima are as follows (nm): 459, 484, 517 in hexane; 455, 482, 515 in pet. ether; 473, 497, 532 in benzene; 469, 498, 535 in CHCl₃; 488, 521, 558 in CS₂.

Separation of 5,6-Monoepoxy- α -carotene-like. The pigment B was further purified on SiO₂ PLC plate with 50% pet. ether in benzene. Three zones, B1 a pink band, B2 an orange and B3 a red band. B3 was again purified on SiO₂ column with benzene-hexane (1:1). The VIS-UV absorption maxima are as follows (nm): 455, 483 in benzene; 472, 501 in CS₂; 442, 470 in pet. ether; 443, 470 in hexane.

Separation of Lutein Monoester. Pigment E was further separated on SiO₂ PLC plate with benzene-acetone (10:1). Three zones, E1 a red band, E2 a red band and E3 a purple band, were separated. Pigment E2 was purified on SiO₂ column with benzene-acetone (10:1). The VIS-UV absorption maxima are as follows (nm): 430, 444, 474 in benzene; 430, 456, 487 in CHCl₃; 447, 475, 506 in CS₂; 420, 444, 475 in hexane; 419, 445, 473 in pet. ether.

RESULTS AND DISCUSSION

The separation and identification of γ -carotene, torulene, 5,6-monoepoxy- α -carotene-like, and lutein monoester have been demonstrated in the abdominal skin of *Bombina*

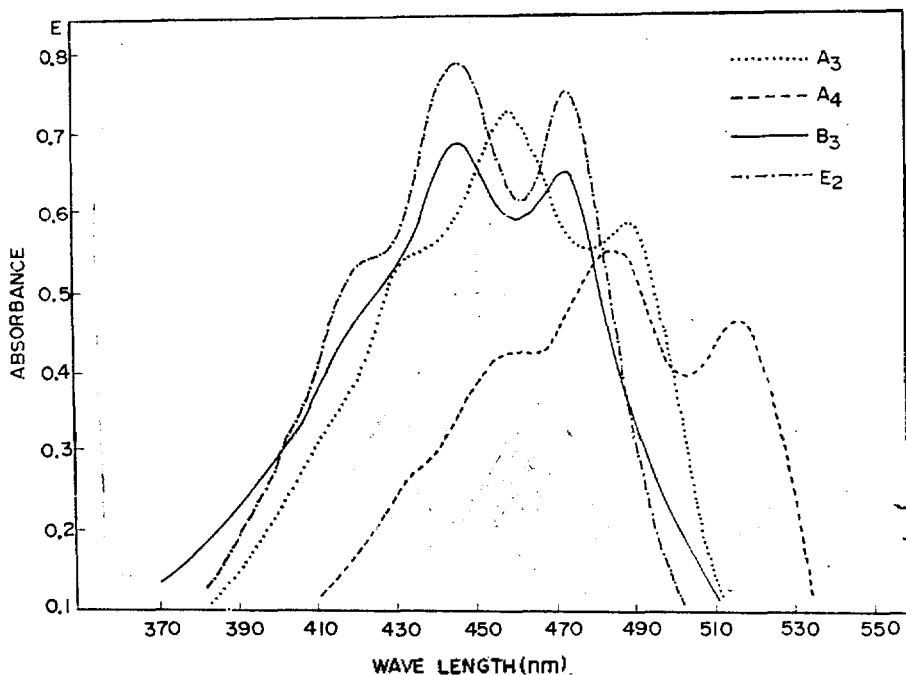
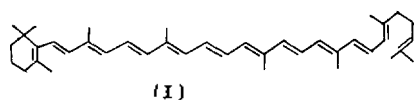


Fig. 1. Visible absorption curves of pigment A3, A4, B3 and E2. solvent in hexane.

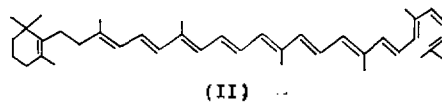
Orientalis. The shape of visible absorption curve of A3 as shown in Fig. 1 and the VIS-UV absorption maxima of A3, as shown in Table 1, were almost in consistence with that of γ -carotene(I) previously reported:⁷ 437, 462, 494 in pet. ether; 453, 496, 533 in CS₂; 447, 474, 508 in CHCl₃; 447, 477, 510 in benzene.



On partition between hexane and 95% methanol, A3 was entirely ephiphasic. Chromatographic behavior of A3 was more strongly adsorbed than β -carotene. On mixed thin layer chromatography of A3 and an authentic sample of γ -carotene from carrots, only one colored spot was observed. From all these data, pigment A3 was identified unequivocally as γ -carotene.

The VIS-UV absorption maxima of A4, as shown in Table 1, were nearly superimposable

to that of 3',4'-dehydro- γ -carotene (torulene), previously reported;^{7,9} 460, 484, 518 in hexane; 488, 522, 563 in CS₂; 469, 501, 539 in CHCl₃. The pigment A4, with pink color, is one of the less polar pigments, but characterized by much bathochromic shift (increment of 23 nm in hexane) in visible region from γ -carotene. From all these data the pigment A4 was presumed to be torulene (II).



The VIS-UV absorption maxima of B3, as shown in Table 1, were consistent to that of 5,6-monoepoxy- α -carotene (III) reported as 442, 471 in hexane; 455, 484 in benzene; 454, 483 in CHCl₃; 471, 503 in CS₂ by Goodwin.⁹ The shape of visible curve is similar to that of α -carotene. From these data, the pigment B3 is supposed to be 5,6-monoepoxy- α -carotene-like.

Table 1. Visible absorption maxima of A3, A4, B3, and E2 in various solvents.

Pigment	Solvent				
	Hexane	Pet. ether	Benzene	CHCl ₃	CS ₂
A3	431, 461, 492	432, 462, 493	447, 477, 510	446, 475, 508	463, 496, 533
A4	459, 484, 517	455, 482, 515	473, 497, 532	469, 498, 535	488, 521, 558
B3	443, 470	442, 470	455, 483	454, 484	472, 501
E2	420, 444, 475	419, 445, 473	430, 457, 487	430, 456, 487	447, 475, 505

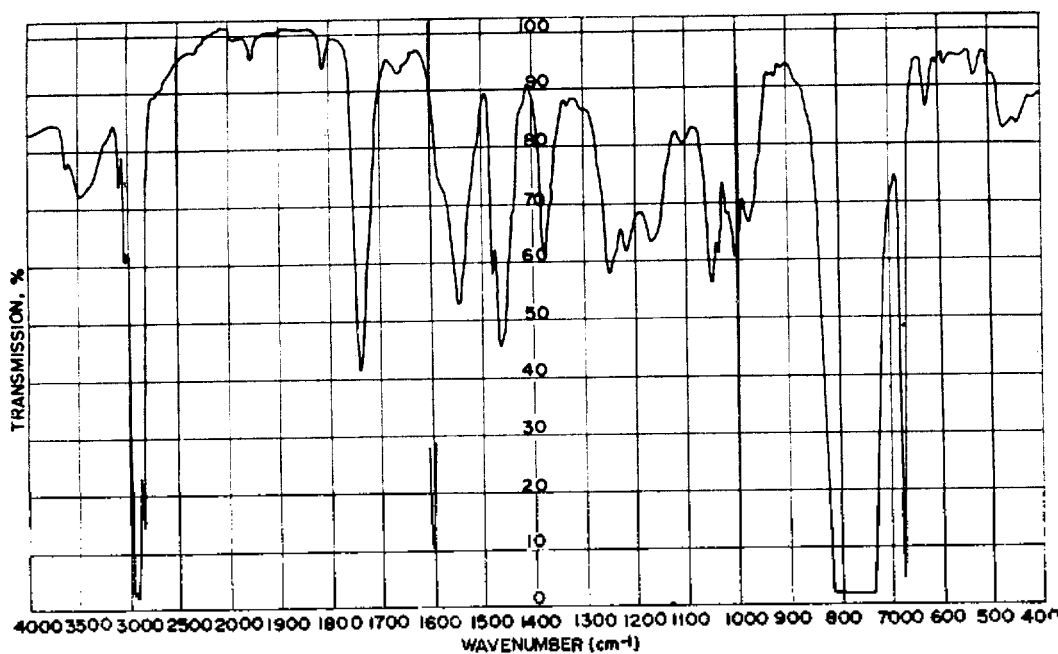
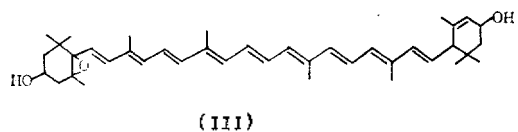
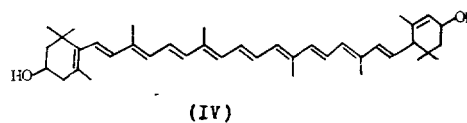


Fig. 2. IR spectrum of pigment E2 in CCl₄.



The shape of visible absorption curve of E2 was nearly identical with that of α -carotene and the VIS-UV absorption maxima of E2 was identical with that of lutein (IV). The chromatographic behavior is more strongly adsorptive than 4-hydroxyechinenone and less adsorptive than 3-hydroxycanthaxanthin and lutein. The IR spectrum of E2 shows an ester carbonyl group and hydroxyl group as shown in Fig. 2. From all these data the pigment E2 was

identified as lutein monester itself.



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