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-carotene-like, and Lutein Monoester

Sae Hee Chang and Ui Chun Chong *
Department of Chemistry, Seoul National University, 151, Korea
*Department of Pre-Medicine, College of Medicine, Ewha Womans University, Seoul 120, Korea
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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-hydroxyxanthophyll, lutein and 3,3′-dihydroxy-3,4,4′-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 \( \gamma \)-carotene from the vegetable kingdom had been made,\textsuperscript{12-15} but the isolation of it from the animal sources is awfully unusual. 5,6-Monoepoxy-\( \alpha \)-carotene occurs in the blossoms of various plants,\textsuperscript{20} and has been synthesized by Karrer\textsuperscript{21} and Kishore.\textsuperscript{22}

In our present study we wish to report the isolation and identification of \( \gamma \)-carotene, torulene, 5,6-monoepoxy-\( \alpha \)-carotene-like, and lutein monoester from the abdominal skin of Bombina Orientalis. The same partition was carried out on a prepared \( \text{SiO}_2 \) PLC plate as before,\textsuperscript{4} and four minor carotenoids were further separated and purified with repeated chromatography on the prepared \( \text{SiO}_2 \) 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,\textsuperscript{4} unless otherwise stated, were used. \( \text{SiO}_2 \) PLC plate was prepared and activated at 110\textdegree C for two hours in an oven.

Separation of \( \gamma \)-Carotene. Pigment A was further separated on \( \text{SiO}_2 \) 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 \( \beta \)-carotene. A3 was further purified on active \( \text{Al}_2\text{O}_3 \) 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, 506 in \( \text{CHCl}_3 \). 463, 493, 533 in \( \text{CS}_2 \). On partition between hexane and 95\% methanol, A3 was entirely ephipsic. On mixed thin layer chromatography of pigment A3 and an authentic sample of \( \gamma \)-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 \( \text{SiO}_2 \) 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 \( \text{CHCl}_3 \); 488, 521, 553 in \( \text{CS}_2 \).

Separation of 5, 6-Monoepoxy-\( \alpha \)-Carotene-like. The pigment B was further purified on \( \text{SiO}_2 \) 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 \( \text{SiO}_2 \) column with benzene-hexane (1:1). The VIS-UV absorption maxima are as follows (nm): 455, 483 in benzene; 472, 501 in \( \text{CS}_2 \); 442. 470 in pet. ether; 443, 470 in hexane.

Separation of Latein Monoester. Pigment E was further separated on \( \text{SiO}_2 \) 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 \( \text{SiO}_2 \) column with benzene-acetone (10:1). The VIS-UV absorption maxima are as follows (nm): 430. 444, 474 in benzene; 430. 455, 487 in \( \text{CHCl}_3 \); 447, 475, 506 in \( \text{CS}_2 \); 429, 444, 475 in hexane; 419, 445, 473 in pet. ether.

**RESULTS AND DISCUSSION**

The separation and identification of \( \gamma \)-carotene, torulene, 5,6-monoepoxy-\( \alpha \)-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 \(\gamma\)-carotene(I) previously reported: \(7; 437, 462, 494\) in pet. ether; \(453, 496, 533\) in \(CS_2\); \(447, 474, 508\) in \(CHCl_3\); \(447, 477, 510\) in benzene.

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

The VIS-UV absorption maxima of A4, as shown in Table 1, were nearly superimposable to that of \(3',4'dehydro-\gamma\)-carotene (torulene), previously reported: \(7; 460, 484, 518\) in hexane; \(488, 522, 563\) in \(CS_2\); \(469, 501, 539\) in \(CHCl_3\). 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 \(\gamma\)-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-\(\alpha\)-carotene (III) reported as \(442, 471\) in hexane; \(455, 484\) in benzene; \(454, 483\) in \(CHCl_3\); \(471, 503\) in \(CS_2\) by Goodwin. The shape of visible curve is similar to that of \(\alpha\)-carotene. From these data, the pigment B3 is supposed to be 5,6-monoepoxy-\(\alpha\)-carotene-like.
Table 1. Visible absorption maxima of A3, A4, B3, and E2 in various solvents.

<table>
<thead>
<tr>
<th>Pigment</th>
<th>Hexane</th>
<th>Pet. ether</th>
<th>Benzene</th>
<th>CHCl3</th>
<th>CS2</th>
</tr>
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<tbody>
<tr>
<td>A3</td>
<td>431, 461, 492</td>
<td>432, 462, 493</td>
<td>447, 477, 510</td>
<td>446, 475, 508</td>
<td>463, 496, 533</td>
</tr>
<tr>
<td>A4</td>
<td>459, 484, 517</td>
<td>455, 482, 515</td>
<td>473, 497, 532</td>
<td>469, 498, 535</td>
<td>488, 521, 558</td>
</tr>
<tr>
<td>B3</td>
<td>443, 470</td>
<td>442, 470</td>
<td>455, 483</td>
<td>454, 484</td>
<td>472, 501</td>
</tr>
</tbody>
</table>

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 carboxyl group and hydroxyl group as shown in Fig. 2. From all these data the pigment E2 was identified as lutein monester itself.

REFERENCES
7. O. Isler and P. Schudel, "In Carotine und Carotinoide, (Sociokopf, Darmstadt)", Vol. 9, P. 54 1963.