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Structural Elucidation of Carotenoids in *Penaeus orientalis* Shells

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Penaeus orientalis 껍질로부터 분리 정제한 Carotenoids의 구조결정

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Abstract

6 carotenoids were separated and identified in *Penaeus orientalis* shells. β-carotene(3.1%), doradexanthin ester(16.1%), astaxanthin diester(54.8%), zeaxanthin monoester(7.9%), astaxanthin monoester(12.4%) and astaxanthin(5.7%) were structurally elucidated by UV/VIS, IR, ^{1}H-NMR, MS spectra and organic reactions such as saponification, acetylation, allylic group, epoxide test and reduction($NaBH_4$), respectively. Fatty acids esterified with carotenoid were mainly $C_{18:1}$, $C_{16:0}$ and $C_{16:1}$, and the others are minors.

Key words: *Penaeus orientalis*, carotenoids.

INTRODUCTION

The carotenoids are a class of hydrocarbons and their oxygenated derivatives. Of ca. 600 naturally occurring carotenoids[1], those of cyclic end groups, acetylenic and allylic groups, carbonyl and hydroxyl groups, epoxides and aromatic group were structurally well known and documented[2~4]. Their basic structures might reflect a mode of biosynthesis and functions. Recent interests in biological functions of carotenoides as participation in photosynthesis, provitamin A activity, photoprotection, radical quenching effects, anticarcinogenic action and immunological activity have been contiued to increase[5~7]. However new aspects on the biological functions, action and association of carotenoids still need to be completely described[7,8].

The structural studies on the carotenoids in *Penaeus orientalis* shells have been elucidated in this papers.

MATERIALS AND METHODS

Adult *Penaeus orientalis* shells were removed, the shells immediately ground with a number of anhydrous sodium sulfate, and extracted with acetone at room temperature. After the carotenoid components were concentrated with a evaporator(Eyela N-1, Japan) under reduced pressure, the concentrated carotenoids were transfered to benzene by addition of distilled water. The aqueous phase was extracted two times with benzene to effect complete pigment transfer. The extracts were combined, washed with water, dried(Na_2SO_4) and evaporated giving a crude carotenoids[9].

The crude carotenoids were developed on pre-coated silicagel $60F_{254}$ TLC glass plates with acetone/*n*-hexane(3:7, v/v) solvent mixture. Subsequently the separated individual carotenoids were quantified by scanning with densitometer(Toyo model DMU-33C). Identification of each carotenoids were accomplished by co-TLC with the reference

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carotenoids, and the structures were elucidated by UV/VIS(Perkcin Elmer 554, U.S.A), IR(IFS-120, Germany), ^{1}H-NMR(AM-200, Germany) and MS (Hewlett Packard 5890 II, U.S.A) spectra, and organic reactions such as saponification, acetylation, allylic group, epoxide test and reduction with $NaBH_4$ were also carried out, respectively[8~10].

RESULTS AND DISCUSSION

1. Carotenoids analysis

Fig. 1 is individual carotenoids separated on TLC plate in acetone/*n*-hexane(3:7, v/v) solvent system. That was quantified at 450nm by scanning with a densitometer.

The identified carotenoids band 1, 2, 3, 4, 5 and 6 were β-carotene(R_f 0.85, 3.1%), doradexanthin ester (R_f 0.79, 16.1%), astaxanthin diester(R_f 0.73, 54.8%), zeaxanthin monoester(R_f 0.49, 7.9%), astaxanthin monoester(R_f 0.43, 12.4%) and astaxanthin(R_f 0.19, 5.7%), respectively[8].

2. β-Carotene(Band 1)

R_f 0.85; UV/VIS: λ_{max}(hexane)(425), 450 and 477nm, (p.e) (425), 448 and 475nm, (EtOH) (427), 449 and 475nm, (acetone) (429) 452 and 478nm, ($CHCl_3$) 435, 461 and 485nm, (benzene) (435), 462 and 487nm, (CS_2) 450, 485 and 520nm;

IR: ν_{max}($CHCl_3$) 3050~2800cm^{-1}(C-H), 1625, 1560cm^{-1}(C=C), 1455, 1390, 1365cm^{-1}(gem. dimethyls), 1010, 970cm^{-1}(trans, CH=CH) and 830cm^{-1} (C=CH-);

^{1}H-NMR: δ ppm($CDCl_3$) 6.90~6.0(14H, olefinic) 1.97s(12H, in-chain CH_3), 1.72s(6H, end of chain), 1.53(CH_2) and 1.03s(12H, gem. dimethyls);

MS: 536(M), M-92, M-79, M-106, M-137 and M-158, respectively[2~5].

3. Doradexanthin ester(Band 2)

R_f 0.79; UV/VIS: λ_{max}(hexane) 452 and (473) nm, (benzene) 465 and (486)nm, and (CS_2) 483 and (503)nm, respectively.

After saponification, IR and ^{1}H-NMR datas were nearly similiar with those of astaxanthin. A small sample reduced with $NaBH_4$ in EtOH had changed from red to yellow, UV/VIS: λ_{max}(EtOH) (451)nm with a subsidiary maximum at 476nm and an inflexion at ca. 426nm.

MS: 568(M), M-18, M-167 and M-233, respectively[9,11,12].

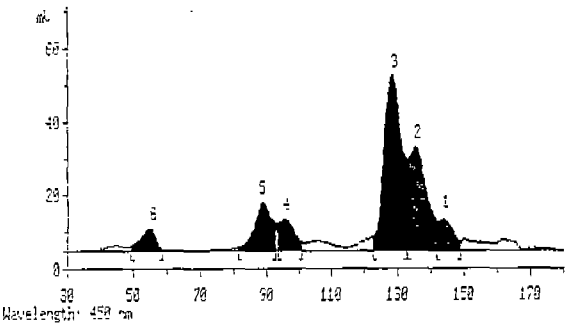

Fig. 1. *P. orientalis* carotenoids separated on the silicagel 60F_{254} TLC plate using acetone/n-haxane(3:7, v/v) system. Individual carotenoids were quantified by scanning with densitometer at 450nm. Band 1, β -carotene ; Band 2, doradexanthin ester; Band 3, astaxanthin diester; Band 4, zeaxanthin monoester, Band 5, astazanthin monoester and Band 6, astaxanthin; respectively.

4. Astaxanthin diester(Band 3)

R_f 0.73; UV/VIS: λ_{max}(hexane) 468nm, (p.e) 468nm, (EtOH) 478nm, (acetone) 480nm, ($CHCl_3$) 485nm, (benzene) 478nm and (CS_2) 503nm;

IR: ν_{max}($CHCl_3$) 2930cm^{-1}(C-H), 1660cm^{-1} (C=O, conj.), 1605, 1575cm^{-1}(C=C, conj.), 1440cm^{-1} (CH_2), 1385, 1370cm^{-1}(gem. dimethyls), 1280, 990 and 970cm^{-1}(trans, CH=CH);

MS: 1096~988(M), M-92 and M-106 mass units, respectively[8].

After saponification with 5% KOH/90% MeOH for 1hr at room temperature, carotenoids were purified.

IR: ν_{max}($CHCl_3$) 3506m(OH), 1664s(C=O, conj.), 1564m(C=C, conj.), 1374m, 1365m(gem. dimethyls), 1079s(sec. OH) and 970(trans, CH=CH);

^{1}H-NMR: δ ppm($CDCl_3$) 1.21(6H), 1.32(6H) (methyls at C-1 and C-1´), 1.94(6H), 2.0 (12H) (methyls on C=C) and 3.66(2H)(O-H, removed by deuteriation);

MS: 592(M), M-16, M-92 and M-106, respectively[2,3,8~14].

Several fatty acids esterified with the astaxanthin diester were analyzed by GC(GC-9AG, Shimadzu, Japan), mainly $C_{10:0}$(3.7%), $C_{14:0}$(1.9%), $C_{16:0}$(21.2%), $C_{16:1}$(15.4%), $C_{17:1}$(1.3%), $C_{18:0}$(7.4%), $C_{18:1}$(23.3%), $C_{18:2}$(2.2%), $C_{20:0}$(1.7%), $C_{20:1}$(3.9%), $C_{20:4}$(1.2%), $C_{20:5}$(7.3%) and $C_{22:1}$(5.2%) were involved[15].

5. Zeaxanthin monoester(Band 4)

R_f 0.49; UV/VIS: λ_{max}(hexane) (426), 450 and 480nm, (p.e) 424, 449 and 476nm, (EtOH) 428, 450 and 488nm, (acetone) 452 and 479nm, ($CHCl_3$) (434), 459 and 488nm, (benzene) (440), 463 and 491nm, (CS_2) 450, 482 and 517nm;

IR: ν_{max}($CHCl_3$) 3500cm^{-1}(OH), 1665cm^{-1}(C=O, conj.), 1567(C=C, conj.), 1372, 1366cm^{-1}(gem. dimethyl) and 972cm^{-1}(CH=CH, trans);

^{1}H-NMR: δ ppm($CDCl_3$) 1.07(12H, gem. dimethyl), 1.74(6H, C-18, 18´), 1.98(12H, in-chain methyl), 3.53(-OR), 5.8~6.4(14H, olefinic) and 7.3~7.5(aromatic), respectively[12~14].

After saponification, MS: 568(M), M-2, M-18, M-92, M-106 and M-153, respectively[2,3].

6. Astaxanthin monoester(Band 5)

R_f 0.43; UV/VIS, IR, ^{1}H-NMR and MS datas were a good agreement with those of astaxanthin diester(3)[9~14].

After saponification the fatty acid composition of the astaxanthin monoester involved namely $C_{10:0}$ (6.0%), $C_{12:0}$(0.2%), $C_{14:0}$(4.1%), $C_{16:0}$(17.0%), $C_{16:1}$ (10.0%), $C_{17:1}$(2.6%), $C_{18:0}$(8.9%), $C_{18:1}$(22.8%), $C_{18:2}$(3.4%), $C_{20:0}$(2.4%) and $C_{20:5}$(11.7%), $C_{22:1}$ (6.3%), respectively.[15]

7. Astaxanthin(Band 6)

R_f 0.19; UV/VIS: λ_{max}(hexane) 468nm, (p.e) 468nm, (EtOH) 478nm, (acetone) 480nm, ($CHCl_3$) 485nm, (benzene) 478nm and (CS_2) 503nm;

IR: ν_{max}($CDCl_3$) 3506m(OH), 1664s(C=O, conj.), 1564m(C=C, conj.), 1374m, 1365m(gem. dimethyl), 1079s(sec. OH), 970S(CH=CH, trans);

^{1}H-NMR: δ ppm($CDCl_3$) 1.21(6H), 1.32(6H) (methyl at C-1 and C-1´), 1.94(6H) and 2.0(12H) (methyl on C=C);

MS: 596(M), M-2, M-16, M-18, M-16-16, M-79, M-92, M-2-92, M-106, M-2-106, M-154, M-219 and M-233, respectively[2,8~14]. This mass data was also same as that of authentic astaxanthin.

요 약

Penaeus orientalis 껍질에서 6종류의 carotenoids가 분리정제되었다. β-carotene(3.1%), doradexanhin ester(16.1%), astaxanthin diester(54.8%), zeaxanthin monoester(7.9%), astaxanthin monoester(12.4%) 그리고 astaxanthin(5.7%)의 함량과 구조들은 UV/VIS, IR, ^{1}H-NMR, MS 스펙트라와 검화반응, 아세탈화반응, 알릴릭그룹, 에폭시 반응 그리고 환원반응을 이용하여 결정되었다. Carotenoids와 에스테르 결합된 지방산들은 주로 $C_{18:1}$, $C_{16:0}$, $C_{16:1}$이고 나머지 지방산들도 소량 함유되었다.

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