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Note

Direct diastereomeric resolution of carotenoids

I. 3-Hydroxy-4-oxo β-end group

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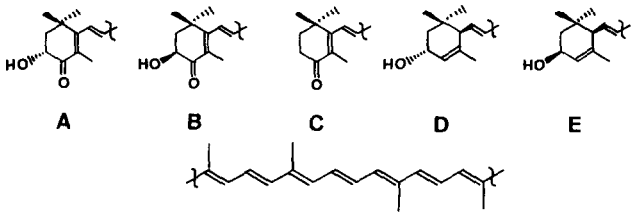
Of the 3-hydroxy-4-oxocarotenoids, astaxanthins, phoenicoxanthins, α-doradexanthin and fritschiellaxanthin are key compounds widely occurring in aquatic animals[1–4]. These carotenoids, having one to three asymmetric centres, exist as stereoisomers. Their isolation and the successful identification of their absolute configurations led to the elucidation of the stereoselective metabolism of these carotenoids in nature, but it was very difficult to resolve the intact diastereomeric carotenoids. Studies on the chromatographic resolution of the diastereoisomers of 3-hydroxy-4-oxocarotenoids via the corresponding diastereomeric camphanates have only been reported by Müller *et al.*[5].

In this paper, a simple, rapid procedure for the direct resolution of the diastereoisomers of 3-hydroxy-4-oxocarotenoids is described. The procedure is based on the use of a Sumipax OA-2000 chiral high-performance liquid chromatographic (HPLC) column.

EXPERIMENTAL

Samples

The samples used were authentic compounds from our carotenoid collections:



(3R)-Phoenicoxanthin	A—P—C
(3S)-Phoenicoxanthin	B—P—C
Fritschiellaxanthin	B—P—D
α-Doradexanthin	B—P—E
(3R,3'R)-Astaxanthin	A—P—A
(3R,3'S;meso)-Astaxanthin	A—P—B
(3S,3'S)-Astaxanthin	B—P—B

Fig. 1. Structures of the carotenoids used in this study.

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(3*R*)-phoenicoxanthin, (3*S*)-phoenicoxanthin, fritschiellaxanthin, α-doradexanthin, (3*R*,3′*R*)-astaxanthin, (3*R*,3′*S*; *meso*)-astaxanthin and (3*S*,3′*S*)-astaxanthin (Fig. 1).

Apparatus

HPLC was carried out on a Jasco Trirotar instrument with a Shimadzu SPD-M1A spectrophotometric detector.

Sumipax OA-2000 (particle size 10 μm) 250 × 4 mm I.D. and 300 × 8 mm I.D. columns (Sumitomo Chemicals, Osaka, Japan) were used for analytical and preparative chromatography, respectively.

Identification of carotenoids

The identification of each carotenoid was achieved by means of visible and circular dichroism spectral data.

RESULTS AND DISCUSSION

The seven diastereomeric mixtures of 3-hydroxy-4-oxocarotenoids were completely resolved on a Sumipax OA-2000 column, as shown in Fig. 2. Good resolution of these carotenoids was obtained with *n*-hexane–dichloromethane–ethanol (48:16:0.6) as the solvent at a flow-rate of 0.8 ml/min.

These results prompted us to use the same system for the preparative isolation of 3-hydroxy-4-oxocarotenoids from the crude carotenoids of the river crab *Potamon dehaani* (Fig. 3) and crawfish *Procambarus clarki* (Fig. 4) using slightly larger columns.

In conclusion, the described direct separation procedure using a chiral liquid chromatographic column will allow the investigation of the determinants of the stereoselective metabolism of carotenoids[6].

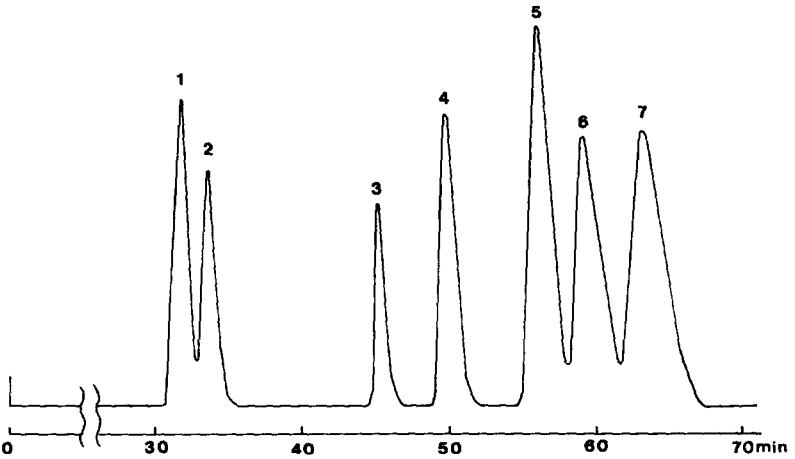

Fig. 2. Separation of diastereometric mixtures of 3-hydroxy-4-oxocarotenoids. Column: Sumipax OA-2000, 10 μm (250 × 4 mm I.D.). Mobile phase: *n*-hexane–dichloromethane–ethanol (48:16:0.6). Flow-rate: 0.8 ml/min. Detection: 470 nm. Peaks: 1 = (3*R*)-phoenicoxanthin; 2 = (3*S*)-phoenicoxanthin; 3 = fritschiellaxanthin; 4 = α-doradexanthin; 5 = (3*R*,3′*R*)-astaxanthin; 6 = (3*R*,3′*S*; *meso*)-astaxanthin; 7 = (3*S*,3′*S*)-astaxanthin.

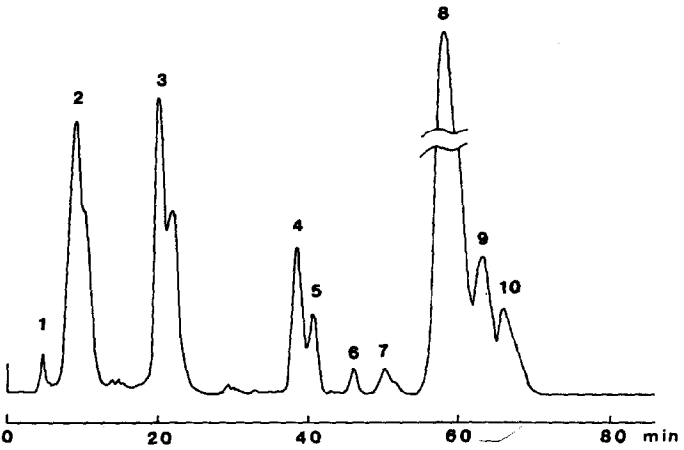

Fig. 3. Separation of the crude carotenoids from the river crab *Potamon dehaani*. Conditions as in Fig. 2, except column, 300 × 8 mm I.D., and flow-rate, 3.0 ml/min. Peaks 1 = β-carotene; 2 and 3 = esterified carotenoids; 4 = lutein; 5 = zeaxanthin; 6 = fritschiellaxanthin; 7 = α-doradexanthin; 8 = (3*R*,3′*R*)-astaxanthin; 9 = (3*R*,3′*S*; *meso*)-astaxanthin; 10 = (3*S*,3′*S*)-astaxanthin.

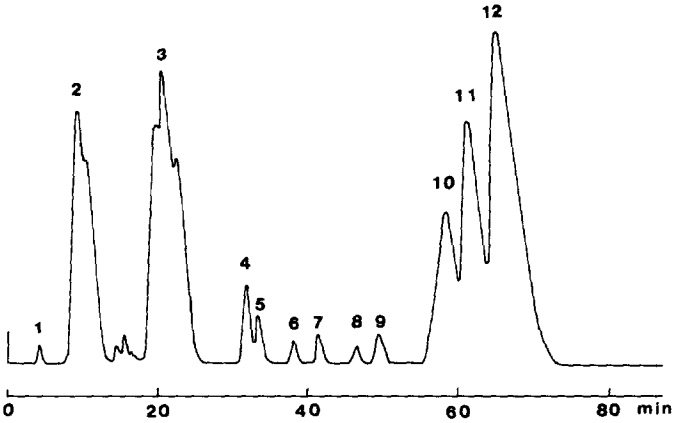

Fig. 4. Separation of the crude carotenoids from the crawfish *Procambarus clarki*. Conditions as in Fig. 2. Peaks 1 = β-carotene; 2 and 3 = esterified carotenoids; 4 = (3*R*)-phoenicoxanthin; 5 = (3*S*)-phoenicoxanthin; 6 = lutein; 7 = zeaxanthin; 8 = fritschiellaxanthin; 9 = 4-ketozeaxanthin; 10 = (3*R*,3′*R*)-astaxanthin; 11 = (3*R*,3′*S*; *meso*)-astaxanthin; 12 = (3*S*,3′*S*)-astaxanthin.

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