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DIASTEREOMERIC RESOLUTION OF CAROTENOIDS

IV[a]. CAROTENOIDS WITH A 4-HYDROXY-β-END GROUP

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SUMMARY

A method is described for the diastereomeric resolution of carotenoids with a 4-hydroxy-β-end group, *viz.*, β-isocryptoxanthin (β,β-caroten-4-ol), isozeaxanthin (β,β-carotene-4,4′-diol) and 4′-hydroxyechinenone (4′-hydroxy-β,β-caroten-4-one). The separation of each carotenoid into individual optical isomers was achieved by high-performance liquid chromatography using a Chiralcel OD [cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel] chiral resolution column after conversion to the corresponding benzoates.

INTRODUCTION

In the course of our stereochemical studies of naturally occurring carotenoids, we have reported on the diastereomeric resolution of carotenoids with a 3-hydroxy-β-end group (zeaxanthin)[1], 3-hydroxy-4-oxo-β-end group (astaxanthin and phoenicoxanthin)[2,3], a 3-hydroxy-ε-end group (tunaxanthin)[4], 3-oxo-ε-end group (ε,ε-carotene-3,3′-dione)[4], a 2-hydroxy-β-end group (β,β-caroten-2-ol and β,β-carotene-2,2′-diol)[5] and a 2-hydroxy-4-oxo-β-end group (2-hydroxy-echinenone)[5] by high-performance liquid chromatography (HPLC) using a Sumipax-OA 2000 chiral resolution column.

In this paper we report the separation of optical isomers of carotenoids with a 4-hydroxy-β-end group, *viz.*, β-isocryptoxanthin (β,β-caroten-4-ol), isozeaxanthin (β,β-carotene-4,4′-diol) and 4′-hydroxyechinenone (4′-hydroxy-β,β-caroten-4-one).

EXPERIMENTAL

Apparatus

HPLC was carried out on a Waters Model 510 instrument with a Waters Lambda-Max Model 418 LC spectrophotometer set at 450 nm. A 250 × 4.6 mm I.D.

[a] For Part III, see ref. 5.

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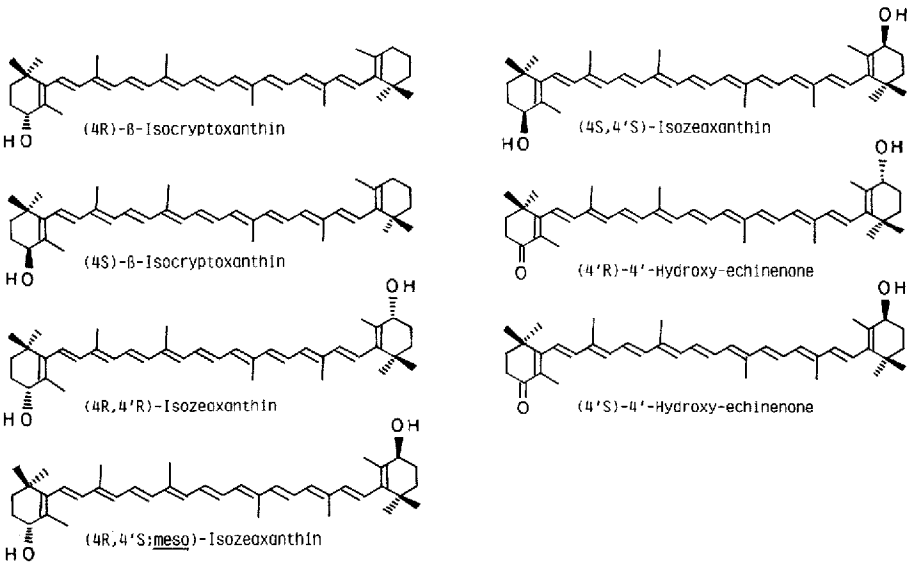

stainless-steel column packed with Chiralcel OD [cellulose tris(3,5-dimethylphenylcarbamate) coated on silica gel; Daicel Chemical Industries, Tokyo, Japan] was used. Visible light absorption spectra (VIS) were recorded in diethyl ether on a Shimadzu UV 240 spectrophotometer. Mass spectra (MS) were recorded with a Hitachi M-80 mass spectrometer using an ionization energy of 25 eV. ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer at 300 MHz in $CDCl_3$ solution. Circular dichroism (CD) spectra were obtained with a Jasco J-500 C spectropolarimeter in diethyl ether–isopentane–ethanol (5:5:2) (EPA) solution at 20°C.

Preparation of racemic β-isocryptoxanthin from echinenone

Reduction of echinenone (*β,β*-caroten-4-one) (0.5 mg) isolated from sea urchins purchased at a local fish market with sodium borohydride (10 mg) in methanol (20 ml) for 30 min at 20°C provided racemic *β*-isocryptoxanthin (0.4 mg). The reduction product was purified by preparative thin-layer chromatography (TLC) on silica gel G with acetone–*n*-hexane (3:7).

Preparation of racemic 4'-hydroxy-echinenone and isozeaxanthin from canthaxanthin

Reduction of canthaxanthin (*β,β*-carotene-4,4'-dione) (1 mg) (Hoffmann-La Roche) with sodium borohydride (10 mg) in methanol (20 ml) for 15 min at 20°C provided racemic 4'-hydroxyechinenone (0.1 mg) and racemic isozeaxanthin (0.6 mg). These two reduction products were separated by preparative TLC on silica gel G with acetone-*n*-hexane (3:7).

Preparation of benzoates of β-isocryptoxanthin, isozeaxanthin and 4'-hydroxy-echinenone.

Preparation of benzoates of these carotenoids was carried out by the method described previously[1,4].

Saponification of benzoates of β-isocryptoxanthin, isozeaxanthin and 4'-hydroxyechinenone

Saponification of these benzoates was carried out by routine procedure[6].

RESULTS AND DISCUSSION

Separation of racemic β-isocryptoxanthin into (4R)- and (4S)-β-isocryptoxanthin

The separation of the two stereoisomers of β-isocryptoxanthin from a racemic mixture was achieved by HPLC on a Chiralcel OD column after conversion to the corresponding monobenzoate as shown in Fig. 1. *Cis* isomers in the polyene chain were also separated from the corresponding all-*trans* isomers. The *cis* isomers were not identified. Saponification of each separated monobenzoate gave optically pure (4*R*)- and (4*S*)-β-isocryptoxanthin. The identification of each stereoisomer was based on CD spectral data as shown in Fig. 2. Peaks 1 and 2 represent (4*R*)- and (4*S*)-β-isocryptoxanthin, respectively.

(4*R*)-β-Isocryptoxanthin (0.1 mg available) showed VIS, λ_{max} 425 (shoulder), 449 and 476 nm and no *cis* peak; MS, *m/z* 552 [M^+, compatible with $C_{40}H_{56}O$], 534 $[M-18]^+$, 460 $[M-92]^+$ and 446 $[M-106]^+$; ^{1}H NMR, δ 1.02 s (3H, CH_3-16), 1.03 s (6H, CH_3-16',17'), 1.05 s (3H, CH_3-17), 1.72 s (3H, CH_3-18'), 1.84 s (3H, CH_3-18), 1.97 s (12H, CH_3-19,20,19',20'), 2.02 m (2H, H-4'), 4.01 t (1H, H-4), 6.1–6.8 m (14H, olefinic H); and CD ($\Delta\varepsilon$), 242 (+4.0), 262 (0), 282 (−2.6), 312 (0) and 345 nm (+0.7). These data were completely identical with those of (4*R*)-β-isocryptoxanthin synthesized by Haag and Eugster[7].

(4*S*)-β-Isocryptoxanthin (0.1 mg available) showed the same VIS, MS and ^{1}H NMR spectral data as those of (4*R*)-β-isocryptoxanthin and showed CD ($\Delta\varepsilon$), 242 (−4.0), 262 (0), 282 (+2.6), 312 (0) and 345 nm (−0.7).

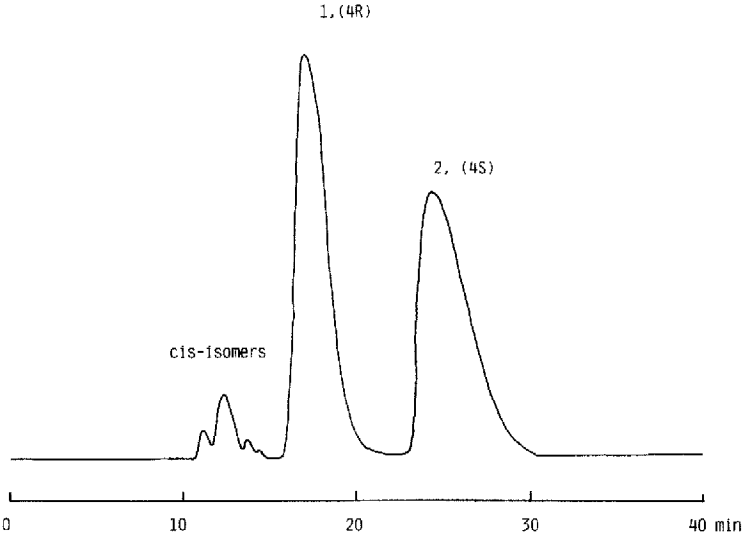

Fig. 1. Separation of (4*R*)- and (4*S*)-β-isocryptoxanthin monobenzoates (20 μg available in one separation). Column, Chiralcel OD (250 × 4.6 mm I.D.); mobile phase, *n*-hexane–ethanol–N,N-diisopropylethylamine (100:0.2:0.05); flow-rate, 0.5 ml/min; detection, 450 nm.

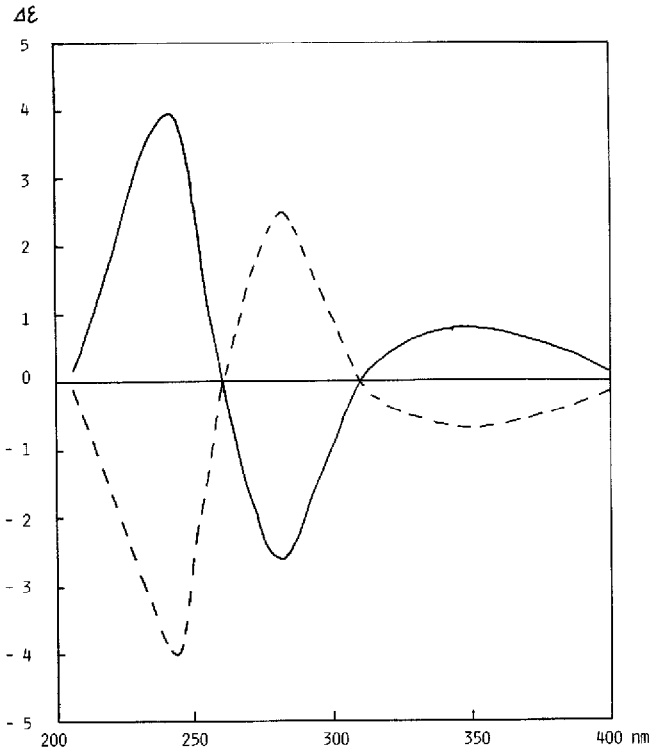

Fig. 2. CD spectra of (4*R*)-β-isocryptoxanthin (——) and (4*S*)-β-isocryptoxanthin (– – – –) in EPA at 20°C.

Separation of racemic isozeaxanthin into (4R,4′R)-, (4R,4′S;meso)- and (4S,4′S)-isozeaxanthin

The separation of the three stereoisomers of isozeaxanthin from a racemic mixture was achieved by HPLC on a Chiralcel OD column after conversion to the corresponding dibenzoate as shown in Fig. 3. *Cis* isomers were also separated from the corresponding all-*trans* isomers. The *cis* isomers were not identified. Saponification of each separated dibenzoate gave optically pure (4*R*,4′*R*)-, (4*R*,4′*S*;*meso*)- and (4*S*,4′*S*)-isozeaxanthin. The identification of each stereoisomer was based on CD spectral data as shown in Fig. 4. Peaks 1, 2 and 3 represent (4*R*,4′*R*)-, (4*R*,4′*S*;*meso*)- and (4*S*,4′*S*)-isozeaxanthin, respectively.

(4*R*,4′*R*)-Isozeaxanthin (0.1 mg available) showed VIS, λ_{max} 425 (shoulder), 449 and 476 nm and no *cis* peak; MS, *m*/*z* 568 [M^+, compatible with $C_{40}H_{56}O_2$], 550 [$M-18]^+$, 532 [$M-36]^+$, 476 [$M-92]^+$ and 462 [$M-106]^+$; ^{1}H NMR, δ 1.02 s (6H, CH_3-16,16′), 1.05 s (6H, CH_3-17,17′), 1.84 s (6H, CH_3-18,18′), 1.97 s (12H, CH_3-19,20,19′,20′), 4.01 t (2H, H-4,4′) and 6.1–6.8 m (14H, olefinic H); and CD ($\Delta\varepsilon$), 243 (+5.0), 262 (0), 282 (−5.9), 312 (0) and 346 nm (+1.0). These data were completely identical with those of (4*R*,4′*R*)-isozeaxanthin synthesized by Haag and Eugster[8].

(4*R*,4′*S*;*meso*)-Isozeaxanthin (0.2 mg available) showed the same VIS, MS and ^{1}H NMR data as those of (4*R*,4′*R*)-isozeaxanthin and showed no optical activity in CD.

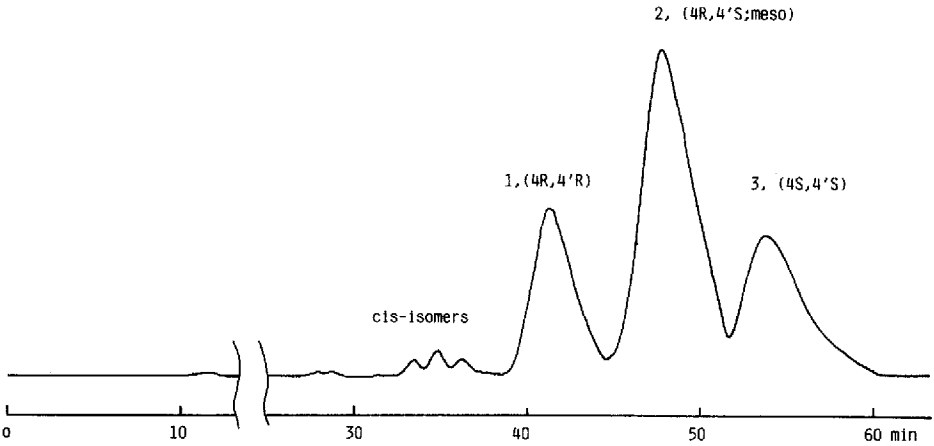

Fig. 3. Separation of (4*R*,4′*R*)-, (4*R*,4′*S*;*meso*)- and (4*S*,4′*S*)-isozeaxanthin dibenzoates (10 μg available in one operation). Column, Chiralcel OD (250 × 4.6 mm I.D.); mobile phase, *n*-hexane–ethanol–N,N-diisopropylethylamine (100:0.5:0.05); flow-rate, 0.5 ml/min; detection, 450 nm.

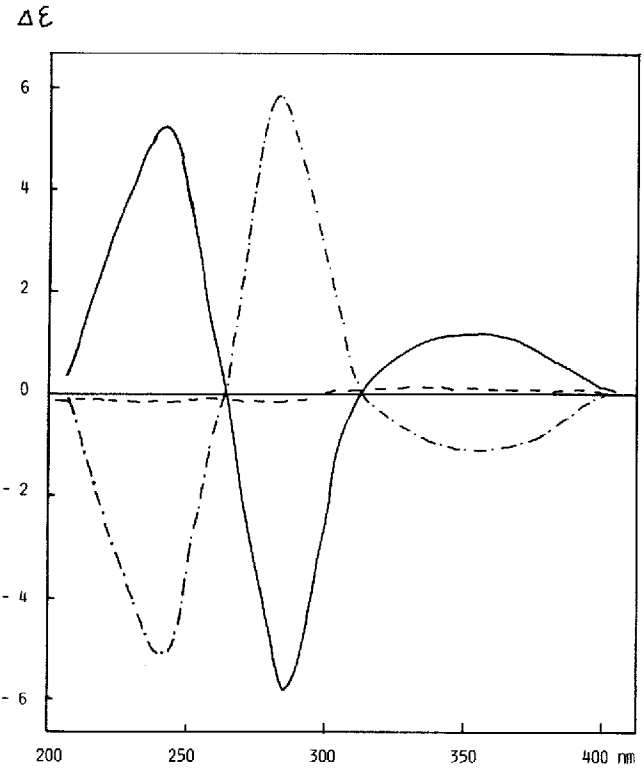

Fig. 4. CD spectra of (4*R*,4′*R*)-isozeaxanthin (——) (4*R*,4′*S*;*meso*)-isozeaxanthin (– – – –) and (4*S*,4′*S*)-isozeaxanthin (– · – · –) in EPA at 20°C.

(4*S*,4′*S*)-Isozeaxanthin (0.1 mg available) showed the same VIS, MS and ^{1}H NMR data as those of (4*R*,4′*R*)-isozeaxanthin and showed CD ($\Delta\varepsilon$), 243 (−5.0), 262 (0), 282 (+5.9), 312 (0) and 346 nm (−1.0).

Separation of racemic 4′-hydroxyechinenone into (4′R)- and (4′S)-4′-hydroxyechinenone

The separation of the two stereoisomers of 4′-hydroxyechinenone from a racemic mixture was ahieved by HPLC on a Chiralcel OD column after conversion to the corresponding monobenzoate as shown in Fig. 5. *Cis* isomers were also separated from the corresponding all-*trans* isomers. The *cis* isomers were not identified. Saponification of each separated monobenzoate gave optically pure (4′*R*)- and (4′*S*)-4′-hydroxyechinenone. The identification of each stereoisomer was based on CD spectral data as shown in Fig. 6. Peaks 1 and 2 represent (4′*R*)- and (4′*S*)-4′-hydroxyechinenone, respectively.

(4′*R*)-4′-Hydroxyechinenone (0.05 mg available) showed VIS, λ_{max} 455–460 nm and no *cis* peak; MS, *m/z* 566 [M$^+$, compatible with $C_{40}H_{54}O_2$], 548 [M−18]$^+$, 474 [M−92]$^+$ and 460 [M−106]$^+$; ^{1}H NMR, δ 1.02 s (3H, CH_3-16′), 1.05 s (3H, CH_3-17′), 1.20 s (6H, CH_3-16,17), 1.84 s (3H, CH_3-18′), 1.88 s (3H, CH_3-18), 1.98 s (9H, CH_3-20,19′,20′), 2.00 s (3H, CH_3-19), 2.51 t (2H, H-3) 4.01 t (1H, H-4′) and 6.1–6.8 m (14H, olefinic H); and CD ($\Delta\varepsilon$), 240 (0), 252 (+1.1), 270 (0), 298 (−1.8), 330 (0) and 350 nm (+0.5). These data were completely identical with those of (4′*R*)-4′-hydroxyechinenone synthesized by Haag and Eugster[7].

(4′*S*)-4′-Hydroxyechinenone (0.05 mg available) showed the same VIS, MS and ^{1}H NMR data as those of (4′*R*)-4′-hydroxyechinenone and showed CD ($\Delta\varepsilon$), 240 (0), 252 (−1.1), 270 (0), 298 (+1.8), 330 (0) and 350 nm (−0.5).

Concerning the stereoisomers of carotenoids with a 4-hydroxy-β-end group, only (4*R*)-stereoisomers, *i.e.* (4*R*)-β-isocryptoxanthin, (4*R*,6′*R*)-α-isocryptoxanthin, (4′*R*)-4′-hydroxyechinone, (4*R*)-isorubixanthin and (4*R*,4′*R*)-isozeaxanthin, have so far been synthesized by Haag and Eugster[7,8]; (4*S*)-stereoisomers have not yet been reported.

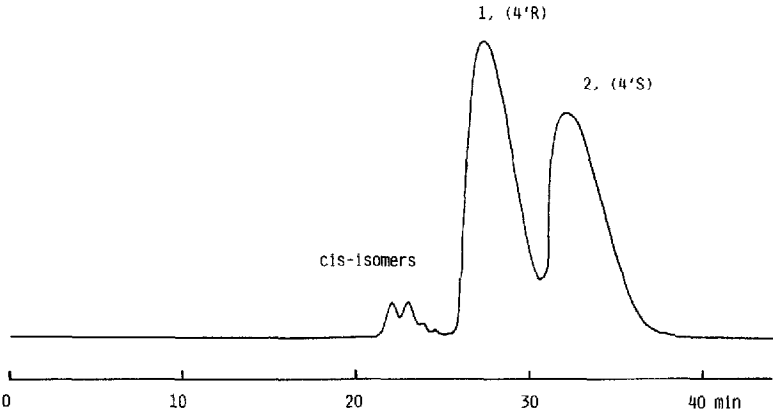

Fig. 5. Separation of (4′*R*)- and (4*S*′)-4′-hydroxyechinenone monobenzoates (10 μg available in one separation). Column, Chiralcel OD (240 × 4.6 mm I.D.); mobile phase, *n*-hexane–ethanol–N,N-diisopropylethylamine (100:0.2:0.05); flow-rate, 0.5 ml/min; detection at 450 nm.

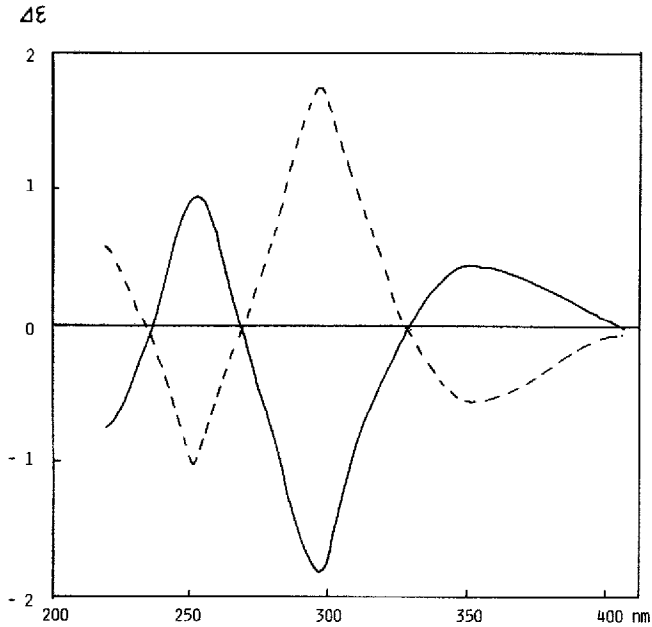

Fig. 6. CD spectra of (4′*R*)-4′-hydroxyechinenone (——) and (4′*S*)-4′-hydroxyechinenone (– – – –) in EPA at 20°C.

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