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DIASTEREOMERIC RESOLUTION OF CAROTENOIDS

III. β,β-CAROTEN-2-OL, β,β-CAROTENE-2,2′-DIOL AND 2-HYDROXYECHINENONE

TAKASHI MAOKA and TAKAO MATSUNO*
Department of Natural Products Research, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607 Japan)
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SUMMARY

A method is described for the diastereomeric resolution of carotenoids with an 2-hydroxy-β-end group (β,β-caroten-2-ol and β,β-carotene-2,2′-diol) and an 2-hydroxy-4-oxo-β-end group (2-hydroxyechinenone). The separation of each carotenoid into individual optical isomers was achieved by using a chiral resolution column, Sumipax OA-2000, after conversion into 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 an 3-hydroxy-β-end group (zeaxanthin)[1], an 3-hydroxy-4-oxo-β-end group (astaxanthin and phoenicoxanthin)[2,3], an 3-hydroxy-ε-end group (tunaxanthin)[4] and an 3-oxo-ε-end group (ε,ε-carotene-3,3′-dione)[4] by high-performance liquid chromatography (HPLC) using a chiral resolution column, Sumipax OA-2000.

In this paper we report the separation of optical isomers of carotenoids with an 2-hydroxy-β-end group (β,β-caroten-2-ol and β,β-carotene-2,2′-diol) and an 2-hydroxy-4-oxo-β-end group (2-hydroxyechinenone = 2-hydroxy-β,β-caroten-4-one) from animals.

EXPERIMENTAL

Biological materials

Biological materials used were the stick insect *Neohirosea japonica* and the sea louse *Ligia exotica*. The details of the studies on carotenoids from *N. japonica* and *L. exotica* will be reported elsewhere.

Apparatus

HPLC was carried out on a Waters Model 510 instrument with a Waters Lamb-

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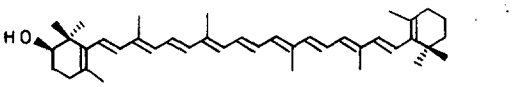
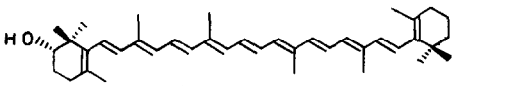
da-Max Model 418 LC spectrophotometer set at 450 nm. The column used was a 300 mm × 8 mm I.D. stainless-steel column packed with Sumipax OA-2000 (particle size 5 μm) (Sumitomo Chemical, Osaka, Japan). Visible (VIS) absorption spectra were recorded in diethyl ether on a Shimadzu UV 240 spectrophotometer. Mass spectra were recorded with an Hitachi M-80 mass spectrometer using an inonization energy of 25 eV. ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer at 300 MHz in C^2HCl_3. Circulair dichroism (CD) spectra were obtained by a Jasco J-500C spectropolarimeter in diethyl ether–isopentane–ethanol (5:5:2) (EPA) solution at 20°C.

Isolation of β,β-caroten-2-ol, β,β-carotene-2,2'-diol and 2-hydroxyechinenone from biological materials

The isolation of these carotenoids from biological materials was carried out according to our routine procedures[5]. Identification of each carotenoid was based on VIS, MS and ^{1}H NMR spectral data by comparison with those reported by Kjøsen *et al.*[6] and Foss *et al.*[7].



(2R)-β,β-Caroten-2-ol



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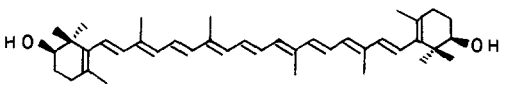
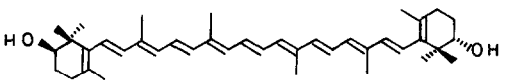
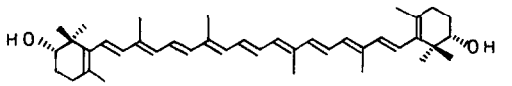


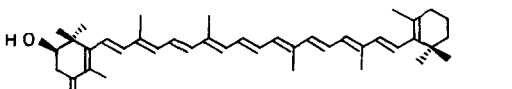
(2R,2'R)-β,β-Carotene-2,2'-diol



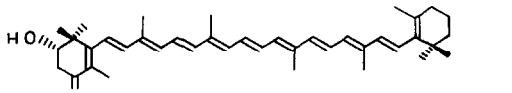
(2R,2'S;meso)-β,β-Carotene-2,2'-diol



(2S,2'S)-β,β-Carotene-2,2'-diol



(2R)-2-Hydroxy-echinenone



(2S)-2-Hydroxy-echinenone

β,β-Caroten-2-ol from *L. exotica* showed λ_{max} 425 (shoulder), 449 and 476 nm, *m/z* 552 (M^+, compatible with $C_{40}H_{56}O$), 534 $[M-18]^+$, 460 $[M-92]^+$ and 446 $[M-106]^+$ and ^1H NMR δ 1.03 s (6H, CH_3-16′,17′), 1.04 s (3H, CH_3-16), 1.08 s (3H, CH_3-17), 1.72 s (6H, CH_3-18,18′), 1.97 s (12H, CH_3-19,20,19′,20′), ≈ 2.02 m (2H, H-4′), ≈ 2.15 m (2H, H-4), 3.55 d,d (1H, H-2) and 6.1–6.7 m (14H, olefinic H) and CD at 224 [Δε (in dm^3 mol^- cm^{-1}) = −0.8], 236 (0), 245 (+1.0), 260 (0), 284 (−1.6), 325 (0) and 350 nm (+0.2).

β,β-Carotene-2,2′-diol from *N. japonica* showed λ_{max} 425 (shoulder), 449 and 476 nm, *m/z* 568 (M^+, compatible with $C_{40}H_{56}O_2$), 550 $[M-18]^+$, 532 $[M-36]^+$, 476 $[M-92]^+$ and 462 $[M-106]^+$, ^1H NMR δ 1.04 s (6H, CH_3-16,16′), 1.08 s (6H, CH_3-17,17′), 1.72 s (6H, CH_3-18,18′), 1.98 s (12H, CH_3-19,20,19′,20′), ≈ 2.15 m (2H, H-4.4′), 3.55 d,d (2H, H-2,2′) and 6.1–6.7 m (14H, olefinic H) and CD at 224 (Δε = −1.8), 236 (0), 245 (+2.0), 260 (0), 284 (−3.0), 325 (0) and 350 nm (+0.5).

2-Hydroxyechinenone from *L. exotica* showed λ_{max} 455–460 nm, *m/z* 566 (M^+, compatible with $C_{40}H_{54}O_2$), 548 $[M-18]^+$, 474 $[M-92]^+$ and 460 $[M-106]^+$, ^1H NMR δ 1.03 s (6H, CH_3-16′,17′), 1.21 s (3H, CH_3-16), 1.25 s (3H, CH_3-17′), 1.72 s (3H, CH_3-18′), 1.89 s (3H, CH_3-18), 1.98 s (9H, CH_3-20,19′,20′), 2.00 s (3H, CH_3-19), 2.62 d,d (1H, H-3_{ax}), 2.80 d,d (1H, H-3_{eq}), 3.90 d,d (1H, H-2) and 6.1–6.7 (14H, olefinic H) and CD at 225 (Δε = −2), 250 (−0.2) and 285 nm (−0.8).

Preparation of benzoates of β,β-caroten-2-ol, β,β-carotene-2,2′-diol and 2-hydroxyechinenone

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

Saponification of benzoates of β,β-caroten-2-ol and β,β-carotene-2,2′-diol

Saponification of these benzoates was carried out by a routine procedure[8].

Enzymatic hydrolysis of benzoates of 2-hydroxyechinenone

Foss *et al.*[7] have shown that 2-hydroxyechinenone is readily dehydrated to the 3,4-didehydro product by base. Therefore, hydrolysis of benzoates was carried out by enzymatic hydrolysis with lipase as described by Matsuno *et al.*[9].

Saponification and enzymatic hydrolysis caused a slight *trans/cis* isomerization of the polyene chain. Thus each hydrolysed product was further purified by HPLC on Sumipax OA-2000 with a mobile phase of *n*-hexane–dichloromethane–ethanol (48:16:0.6).

(2*R*)-β,β-Caroten-2-ol (0.08 mg available) showed λ_{max} 425 (shoulder), 449 and 476 nm and no *cis* peak and CD 224 (Δε = +1.6), 236 (0), 245 (−2.8), 260 (0), 284 (+4.2), 325 (0) and 350 nm (0.8). These data were identical to those of (2*R*)-β,β-caroten-2-ol isolated from *Trentepohlia iolithus*[6].

(2*S*)-β,β-Caroten-2-ol (0.12 mg available) showed λ_{max} 425 (shoulder), 449 and 476 nm and no *cis* peak and CD at 224 (Δε = −1.6), 236 (0), 245 (+2.8), 260 (0), 284 (−4.2), 325 (0) and 350 nm (−0.8).

(2*R*, 2′*R*)-β,β-Carotene-2,2′-diol (0.04 mg available) showed λ_{max} 425 (shoulder), 449 and 476 nm and no *cis* peak, and CD at 224 (Δε = +4.6), 236 (0), 245 (−4.2), 260 (0), 284 (+7.2), 325 (0) and 350 nm (+1.2). These data were identical to those of (2*R*,2′*R*)-β,β-carotene-2,2′-diol isolated from *T. iolithus*[6].

(2*R*,2′*S*;*meso*)-β,β-Carotene-2,2′-diol (0.1 mg available) showed λ_{max} 425 (shoulder), 449 and 476 nm and no *cis* peak and no CD activity.

(2*S*,2′*S*)-β,β-Carotene-2,2′-diol (0.06 mg available) showed λ_{max} 425 (shoulder, 449 and 476 nm and CD at 224 ($\Delta\varepsilon$ = −4.6), 236 (0), 245 (+4.2), 260 (0), 284 (−7.2), 325 (0) and 325 nm (−1.2).

(2*R*)-2-Hydroxyechinenone (0.04 mg available) showed λ_{max} 455–460 nm and no *cis* peak and CD at 225 ($\Delta\varepsilon$ = +4.2), 250 (+0.5) and 285 nm (+2.8). These data were identical to those of (2*R*)-2-hydroxyechinenone isolated from *Daphnia magna*[7].

(2*S*)-2-Hydroxyechinenone (0.06 mg available) showed λ_{max} 455–460 nm and no *cis* peak and CD at 225 ($\Delta\varepsilon$ = −4.2), 250 (−0.5) and 285 nm (−2.8).

RESULTS

Separation of β,β-caroten-2-ol into (2R)- and (2S)-β,β-caroten-2-ol

β,β-Caroten-2-ol obtained from *L. exotica* showed an opposite and weaker Cotton effect to that of (2*R*)-β,β-caroten-2-ol isolated from the green alga *Trentepohlia iolithus*[6]. This suggested that β,β-caroten-2-ol obtained from *L. exotica* is a mixture of two enantiomers.

The diastereomeric separation of these two compounds was achieved by HPLC on a chiral resolution column, Sumipax OA-2000, after conversion into the corresponding monobenzoate. Good resolution was accomplished by recycling ten times (Fig. 1). *cis*-Isomers of the polyene chain were also separated from the corresponding all-*trans*-isomers and they were removed before recycling. The *cis*-isomers have not been identified.

Saponification of each monobenzoate separated gave optically pure (2*R*)- and (2*S*)-β,β-caroten-2-ol- The identification of each enantiomer was based on CD spectral data as shown in Fig. 2. Peaks 1 and 2 represent (2*R*)- and (2*S*)-β,β-caroten-2-ol, respectively.

Separation of β,β-carotene-2,2′-diol into (2R,2′R)-, (2R,2′S;meso)- and (2S,2′S)-β,β-carotene-2,2′-diol

β,β-Carotene-2,2′-diol obtained from *N. japonica* showed an opposite and weaker Cotton effect to that of (2*R*,2′*R*)-β,β-carotene-2,2′-diol isolated from *T.*

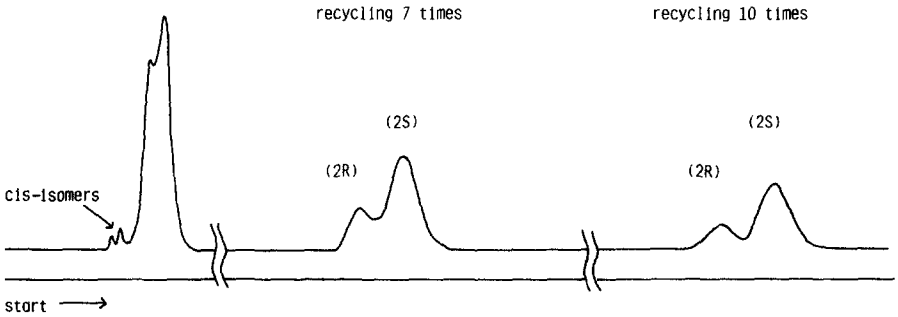


Fig. 1. Separation of (2*R*)- and (2*S*)-β,β-caroten-2-ol monobenzoates (0.2 mg available in one operation). Column: Sumipax OA-2000, 5 μm (300 mm × 8 mm I.D.). Mobile phase: *n*-hexane–dichloromethane–ethanol (48:8:0.01). Flow-rate: 2.0 ml/min. Detection 450 nm.

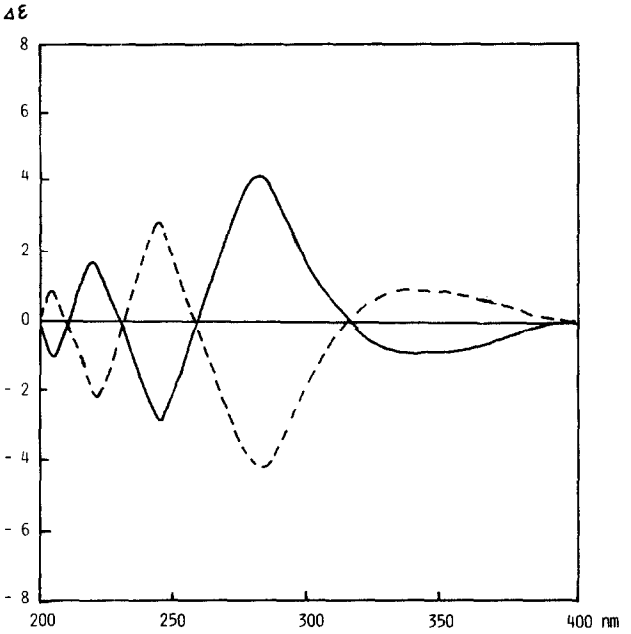


Fig. 2. CD spectra of (2*R*)-β,β-caroten-2-ol (———) and (2*S*)-β,β-caroten-2-ol (– – –) in EPA at 20°C.

iolithus[6]. This fact suggested that the β,β-carotene-2,2'-diol fraction from *N. japonica* was partly racemized.

The diastereomeric separation of three stereoisomers was achieved by the method described above (Fig. 3). Saponification of each dibenzoate separated gave optically pure (2*R*,2'*R*)-, (2*R*,2'*S*;*meso*)- and (2*S*,2'*S*)-β,β-carotene-2,2'-diol. The identification of each stereoisomer was based on CD spectra data as shown in Fig. 4.

cis-Isomers were also separated from the corresponding all-*trans*-isomers and they were removed before recycling. The *cis*-isomers have not been identified.

Separation of 2-hydroxyechinenone into (2R)- and (2S)-2-hydroxyechinenone

2-Hydroxyechinenone obtained from *L. exotica* showed an opposite and weaker Cotton effect to that of (2*R*)-2-hydroxyechinenone from *Daphnia magna*[7]. This

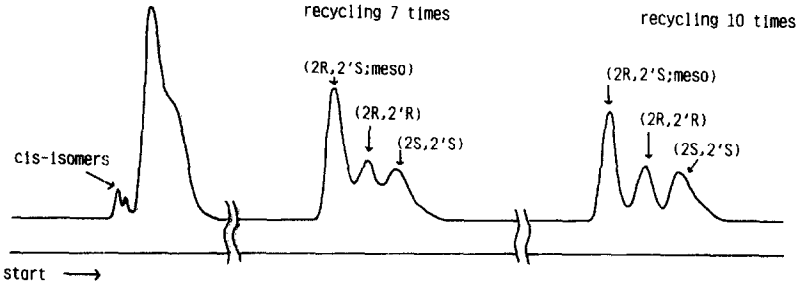


Fig. 3. Separation of (2*R*,2'*R*)-, (2*R*,2'*S*;*meso*)- and (2*S*,2'*S*)-β,β-carotene-2,2'-diol dibenzoates (0.2 mg available in one operation). Conditions as in Fig. 1.

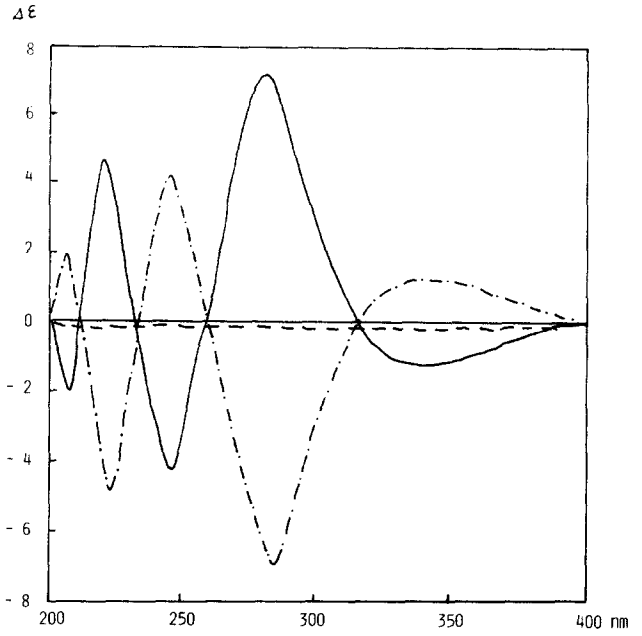


Fig. 4. CD spectra of (2*R*,2′*R*)-β,β-carotene-2,2′-diol (———), (2*R*,2′*S*;*meso*)-β,β-carotene-2,2′-diol (– – –) and (2*S*,2′*S*)-β,β-carotene-2,2′-diol (– · – · –) in EPA at 20°C.

suggested that 2-hydroxyechinenone from *L. exotica* was a mixture of two enantiomers.

The separation into each enantiomer was accomplished by recycling HPLC on Sumipax OA-2000 after conversion into the corresponding monobenzoate (Fig. 5). Enzymatic hydrolysis of each separated monobenzoate with lipase gave optically pure (2*R*)- and (2*S*)-2-hydroxyechinenone. The identification of each enantiomer was based on CD spectral data (Fig. 6). *cis*-Isomers were also separated from the corresponding all-*trans*-isomers and they were removed before recycling. The *cis*-isomers have not been identified.

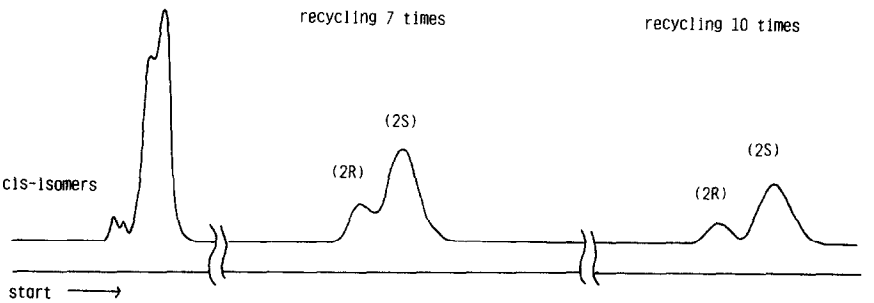


Fig. 5. Separation of (2*R*)- and (2*S*)-2-hydroxyechinenone monobenzoates (0.1 mg available in one operation). Conditions as in Fig. 1.

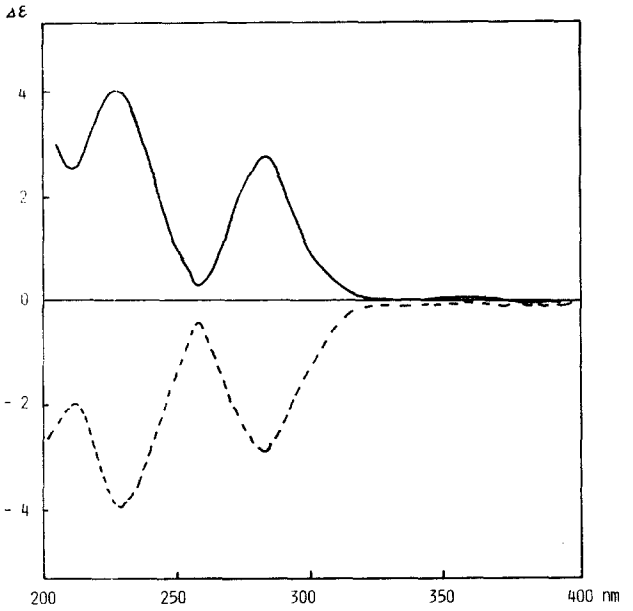


Fig. 6. CD spectra of (2*R*)-2-hydroxyechinenone (———) and (2*S*)-2-hydroxyechinenone (– – – –) in EPA at 20°C.

DISCUSSION

β,β-Caroten-2-ol and β,β-carotene-2,2′-diol were first isolated from the green alga *T. iolithus* by Kjøsen *et al.*[6], and the absolute configurations were determined to be (2*R*) and (2*R*,2′*R*), respectively by Buchecker *et al.*[10,11]. These carotenoids were also obtained from the moth *Cerula vinula*[12] and stick insect *Carausius morosus*[13], but the CD spectra of both compounds from insects showed opposite and weaker Cotton effects to those of β,β-caroten-2-ol and β,β-carotene-2,2′-diol from *T. iolithus*. These facts suggested that β,β-caroten-2-ol and β,β-carotene-2,2′-diol from insects were mixtures of stereoisomers.

However, diastereomeric derivatization with (−)-camphanyl chloride[14] and with (*S*)-(+)-α-(1-naphthyl)ethyl isocyanate[15] did not result in chromatographic separation by HPLC. On the other hand, Aareskjold and Liaaen-Jensen[16] and Kayser *et al.*[17] succeeded in the configurational analysis by using ^{1}H NMR spectroscopy in the presence of a shift reagent, Eu(fod)$_3$ (fod is 1,1,1,2,2,3,3-heptafluoro-7,7′-dimethyl-4,6-octanedionate), after conversion into the corresponding methoxy α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) esters. However, separation of the diastereomeric MTPA esters by HPLC was not achieved.

On the other hand, we have succeeded in the separation of β,β-caroten-2-ol, β,β-carotene-2,2′-diol and 2-hydroxyechinenone into their individual stereoisomers by using a chiral resolution column, Sumipax OA-2000, after conversion into the corresponding benzoates as described above. Saponification or enzymatic hydrolysis with lipase of the benzoates gave optically pure stereoisomers of β,β-caroten-2-ol, β,β-carotene-2,2′-diol and 2-hydroxyechinenone.

In conclusion, this is the first report on the HPLC-separation of enantiomeric or diastereomeric mixtures of (2*R*)- and (2*S*)-β,β-caroten-2-ol, (2*R*,2′*R*)-, (2*R*,2′*S*;*meso*)- and (2*S*,2′*S*)-β,β-carotene-2,2′-diol and (2*R*)- and (2*S*)-2-hydroxyechinenone.

REFERENCES

1 T. Maoka, A. Arai, M. Shimizu and T. Matsuno, *Comp. Biochem. Physiol.* B, 83 (1986) 121.
2 T. Matsuno, T. Makao, M. Katsuyama, M. Ookubo, K. Katagiri and H. Jimura, *Nippon Suisan Gakkaishi*, 50 (1984) 1598.
3 T. Maoka, K. Komori and T. Matsuno, *J. Chromatogr.*, 318 (1985) 122.
4 Y. Ikuno, T. Maoka, M. Shimizu, T. Komori and T. Matsuno, *J. Chromatogr.*, 328 (1985) 387.
5 T. Matsuno and T. Maoka, *Nippon Suisan Gakkaishi*, 47 (1981) 377.
6 H. Kjøsen, N. Arpin and S. Liaaen-Jensen, *Acta Chem. Scand.*, 26 (1972) 3053.
7 P. Foss, V. Partali, Y. Olsen, G. Borch and S. Liaanen-Jensen, *Acta Chem. Scand. Ser. B*, 40 (1986) 157.
8 T. Matsuno, T. Akita and M. Hara, *Nippon Suisan Gakkaishi*, 39 (1973) 51.
9 T. Matsuno, M. Ookubo, T. Nishizawa and I. Shimizu, *Chem. Pharm. Bull.*, 32 (1984) 4309.
10 R. Buchecker, C. H. Eugster and S. Liaaen-Jensen, *Helv. Chim. Acta*, 56 (1973) 299.
11 R. Buchecker, C. H. Eugster, H. Kjøsen and S. Liaaen-Jensen, *Acta Chem. Scand., Ser. B*, 28 (1974) 499.
12 H. Kayser, *Z. Naturforsch., Teil C*, 31 (1976) 122.
13 H. Kayser, *Z. Naturforsch., Teil C*, 31 (1976) 646.
14 R. K. Müller, K. Bernhard, H. Mayer, A. Ruttiman and M. Vecchi, *Helv. Chim. Acta*, 63 (1980) 1654.
15 A. Rüttiman, K. Schiedt and M. Vecchi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 6 (1983) 612.
16 K. Aareskjold and S. Liaaen-Jensen, *Acta Chem. Scand., Ser. B*, 36 (1982) 499.
17 H. Kayser, K. Aareskjold, G. Borsch and S. Liaaen-Jensen, *Insect Biochem.*, 14 (1984) 51.