Journal of Chromatography, 328 (1985) 387–391
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

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Note

Direct diastereomeric resolution of carotenoids

II*. All ten stereoisomers of tunaxanthin (ε,ε-carotene-3,3′-diol)

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(Received February 26th, 1985)

Tunaxanthin was first isolated from tuna, *Thunnus orientalis*, by Hirao *et al.*[1] in 1957. The planar structure of tunaxanthin was determined to be ε,ε-carotene-3,3′-diol by Crozier and Wilkie[2] in 1966 and of the ten possible stereoisomers of tunaxanthin** (Fig. 1) a total of seven have so far been isolated from natural sources: tunaxanthin A[3] (= oxyxanthin 45)[4], tunaxanthin B[5] (= oxyxanthin 51)[4], tunaxanthin C[6] (= oxyxanthin 58)[4], tunaxanthin D[7] (all from fish), tunaxanthin I (= chiriquixanthin A[8]) (from frog), tunaxanthin J (= chiriquixanthin B) (from frog[8] and fish[4]) and tunaxanthin F (= lactucaxanthin) (from plant[9] and fish[10]).

However, all these stereoisomers were poorly separated on an ordinary high-performance liquid chromatographic (HPLC) column[11]. In continuation of our previous work[12], a Sumipak OA-2000 chiral column has now been employed for the ten stereoisomers of tunaxanthin, prepared by reduction of (6*RS*,6′*RS*)-ε,ε-carotene-3,3′-dione[13,14] with $NaBH_4$. We report here a simple and rapid procedure for the resolution of all ten stereoisomers of tunaxanthin and three stereoisomers of ε,ε-carotene-3,3′-dione (Fig. 2).

EXPERIMENTAL

Samples

(6*RS*,6′*RS*)-ε,ε-Carotene-3,3′-dione was isolated from hens' egg yolk[13,14]. Tunaxanthin A, B, C, D, F (= lactucaxanthin), I (= chiriquixanthin A) and J (= chiriquixanthin B) used were authentic compounds from our carotenoid collections.

Apparatus

HPLC was carried out on a Jasco Trirotar instrument with a Shimadzu SPD-M1A spectrophotometric detector. Sumipax OA-2000 (particle size 5 μm) col-

* For Part I, see ref. 12.

** We propose that the names tunaxanthin A, B, C, D, E, F, G, H, I and J are used to describe the ten stereoisomers of ε,ε-carotene-3,3′-diol (see Fig. 1).

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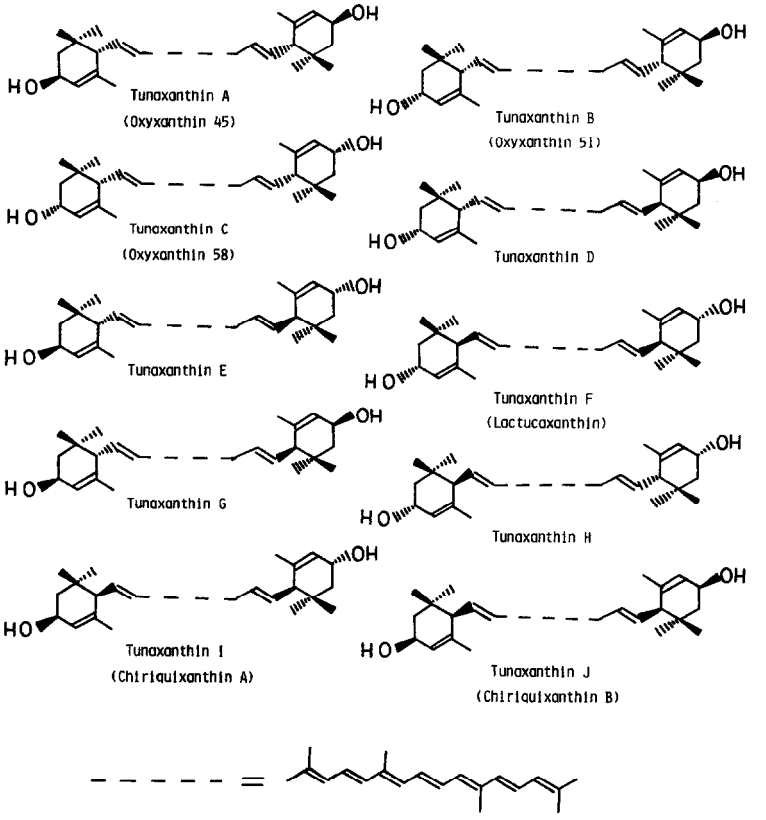

Fig. 1. Structures of the ten stereoisomers of tunaxanthin (ε,ε-carotene-3,3′-diol).

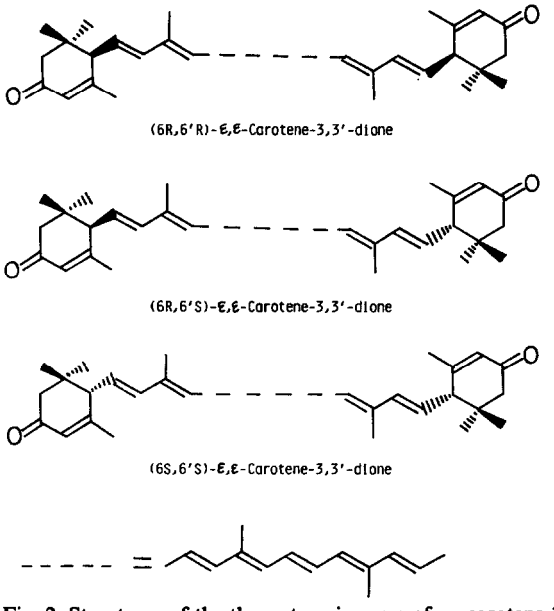

Fig. 2. Structures of the three stereoisomers of ε,ε-carotene-3,3′-dione.

umns of dimensions 250 × 4 mm I.D. and 300 × 8 mm I.D. (Sumitomo Chemical, Osaka, Japan) were used for analytical and preparative chromatography, respectively.

Operating conditions

Details are given in Figs. 3–6.

Identification of carotenoids

The identification of each carotenoid was achieved by means of visible and circular dichroism spectral data and HPLC of a mixture with an authentic sample.

Preparation of tunaxanthin diacetates or dibenzoates

In a small vial were placed 0.5 mg of tunaxanthin sample, 1 ml of dry pyridine and 1 ml of acetic anhydride or 0.2 ml of benzyl chloride. The mixture was left to stand at room temperature for 30 min, then the reaction product was extracted with *n*-hexane by addition of water and chromatographed on a silica gel G plate with acetone–*n*-hexane (3:7). Elution of the product ($R_F \approx 0.7$) with diethyl ether and evaporation of the solvent gave a yellowish residue, which was submitted directly to HPLC analysis.

RESULTS

Separation of three stereoisomers of (6RS,6'RS)-ε,ε-carotene-3,3'-dione

The three stereoisomers of (6*RS*,6'*RS*)-ε,ε-carotene-3,3'-dione were completely resolved on the Sumipax OA-2000 column, as shown in Fig. 3.

Separation of stereoisomers of tunaxanthin (method A)

Fig. 4 shows a chromatogram of the ten stereoisomeric mixtures of tunaxanthin prepared from (6*RS*,6'*RS*)-ε,ε-carotene-3,3'-dione by reduction with $NaBH_4$. Nine peaks were obtained. Tunaxanthin A and tunaxanthin F (= lactucaxanthin)

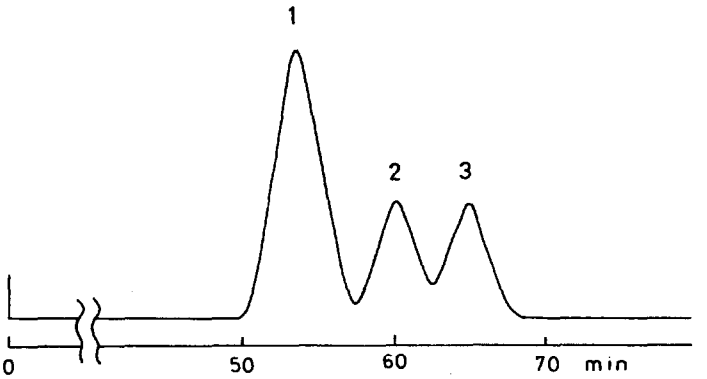

Fig. 3. Separation of three stereoisomers of ε,ε-carotene-3,3'-dione. Column: Sumipax OA-2000, 5 μm (250 × 4 mm I.D.). Mobile phase: *n*-hexane–dichloromethane–ethanol (54:10:0.3). Flow-rate: 0.6 ml/min. Detection: 440 nm. Peaks: 1 = (6*R*,6'*S*)-ε,ε-carotene-3,3'-dione; 2 = (6*S*,6'*S*)-ε,ε-carotene-3,3'-dione; 3 = (6*R*.6'*R*)-ε,ε-carotene-3,3'-dione.

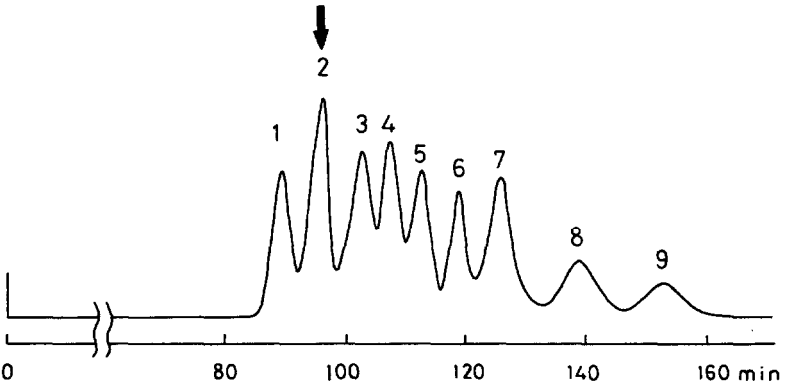

Fig. 4. Separation of the ten stereoisomeric of tunaxanthin. Conditions as in Fig. 3. Peaks: 1 = tunaxanthin E; 2 = tunaxanthin F + tunaxanthin A; 3 = tunaxanthin G; 4 = tunaxanthin H; 5 = tunaxanthin I; 6 = tunaxanthin B; 7 = tunaxanthin D; 8 = tunaxanthin J; 9 = tunaxanthin C. Assignment of peak 3 and 4 may be reversible.

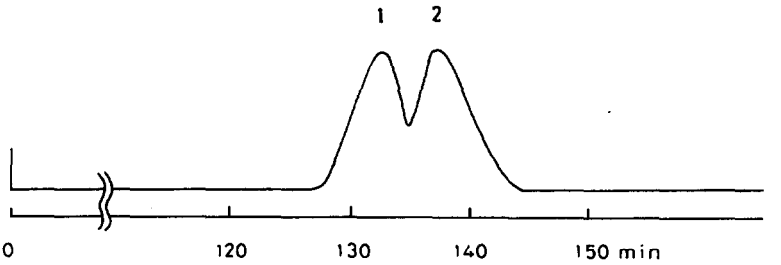

Fig. 5. Separation of a mixture of the diacetates of tunaxanthin A and tunaxanthin F. Column: Sumipax OA 2000, 5 μm (300 × 8 mm I.D.). Mobile phase: *n*-hexane–dichloromethane (90:10). Flow-rate: 2.0 ml/min. Detection: 440 nm. Peaks: 1 = tunaxanthin F diacetate; 2 = tunaxanthin A diacetate.

overlapped, which is shown by an arrow in Fig. 4, and were resolved by conversion to the corresponding diacetates (Fig. 5).

Separation of stereoisomers of tunaxanthin by conversion to the corresponding dibenzoates (method B)

Fig. 6 shows a chromatogram of a mixture of the ten stereoisomeric tunaxanthin dibenzoates. All ten stereoisomers were resolved.

DISCUSSION

For the separation of tunaxanthins, the most advanced method previous was that reported by Vecchi *et al.* in 1982[11], using HPLC [Spherisorb CN; *n*-hexane–dichloromethane–methanol–N-ethyldiisopropylamine (60:40:0.1:0.1)]. With their method, tunaxanthin A, B, C and D were effectively separated, but tunaxanthin F (= lactucaxanthin), I (= chiriquixanthin A) and J (= chiriquixanthin B) could not be separated from the enantiomers tunaxanthin A, B and C, respectively. It was also very difficult to separate directly a set of enantiomers by traditional HPLC tech-

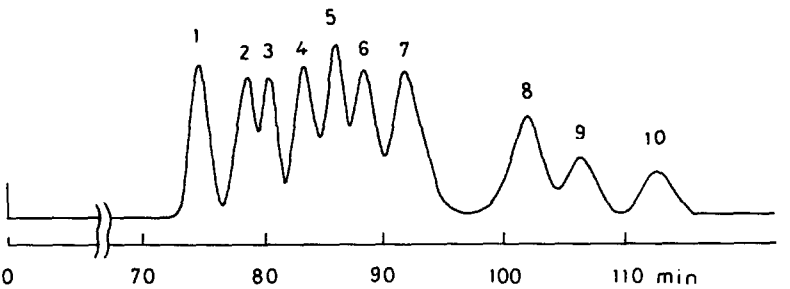

Fig. 6. Separation of the ten stereoisomers of the dibenzoates of tunaxanthins. Column: Sumipax OA 2000, 5 μm (300 × 8 mm I.D.). Mobile phase: *n*-hexane–dichloromethane–ethanol (54:10:0.1). Flow-rate: 1.2 ml/min. Detection: 440 nm. Peaks: 1 = tunaxanthin E dibenzoate; 2 = tunaxanthin F dibenzoate; 3 = tunaxanthin A dibenzoate; 4 = tunaxanthin G dibenzoate; 5 = tunaxanthin H dibenzoate; 6 = tunaxanthin I dibenzoate; 7 = tunaxanthin B dibenzoate; 8 = tunaxanthin D dibenzoate; 9 = tunaxanthin J dibenzoate; 10 = tunaxanthin C dibenzoate. Assignment of peak 4 and 5 may be reversible.

niques. This led us to employ a chiral column of Sumipax OA-2000. By using this column, all ten possible stereoisomers of tunaxanthin could be separated directly at the same time, except for tunaxanthin A and F (= lactucaxanthin). These two overlapping carotenoids were resolved by conversion to their corresponding diacetates or dibenzoates.

In conclusion, all ten stereoisomers of tunaxanthin were completely resolved by applying either method A or B.

A major advantage of this system is that it can be used for the study of natural products chemistry, comparative biochemistry and the metabolism of carotenoids in plants and animals. Details on these applications will be given elsewhere.

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