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Note

Isolation of tocopherol homologues by preparative high-performance liquid chromatography

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Natural vitamin E from vegetable oils is comprised of a mixture of D-α-, β-, γ- and δ-tocopherols (Fig. 1). We were especially interested in isolating considerable quantities of D-γ-tocopherol with a purity of >90%. Preparative high-performance liquid chromatography (HPLC) seemed to be most appropriate for this objective. HPLC is a convenient method for tocopherol analyses[1–4]. Unmodified silica is usually used as the stationary phase and hexane or octane with a polar modifier as the eluent[3].

EXPERIMENTAL

For the isolation of natural D-γ-tocopherol we used a 400 mm × 100 mm column of 25–40 μm silica and the Prepbar 100 system of Merck (Darmstadt, F.R.G.). The fractions collected were evaporated in a 20-l rotary evaporator. The experimental conditions are summarized in Table I.

RESULTS

Our starting material was a vegetable oil extract from plant seeds with a total tocopherol content of 70%, 60% of which was D-γ-tocopherol (Fig. 2). A direct scale-up from the analytical to the preparative mode was achieved by only slight

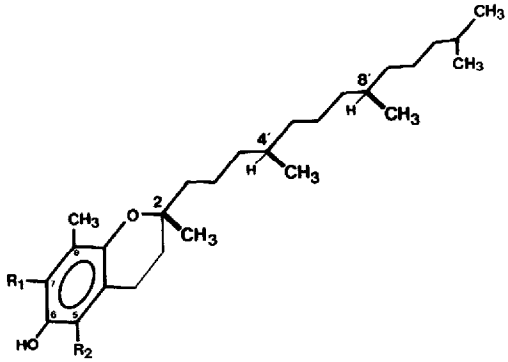

Fig. 1. Structures of the natural tocopherols: α, R_1, R_2 = CH_3; β, R_1 = H, R_2 = CH_3; γ, R_1 = CH_3, R_2 = H; δ, R_1, R_2 = H.

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TABLE I

EXPERIMENTAL CONDITIONS FOR ANALYTICAL AND PREPARATIVE HPLC

	Analytical HPLC	*Preparative HPLC*
System/pump	Kontron 420	Merck Prepbar 100
Column	250 mm × 4 mm	400 mm × 100 mm
Stationary phase	LiChrosorb Si 60, 7 μm	LiChroprep Si 60, 25–40 μm
Eluent* (*n*-hexane–*tert.*-butyl methyl ether)	96:4 (v/v)	97:3 (v/v)
Flow-rate (ml/min)	2	450
Injection volume	15 μl	50 ml
Sample amount**	1 μg	15 g
Detection	220 nm	205 nm, split 1:4
Total analysis time (min)	40	60

* HPLC solvents were used (Promochem, Wesel, F.R.G.).
** Sample dissolved in the HPLC eluent.

changes in the chromatographic conditions (Table I). Sometimes, prepurification of the crude oil extract by filtration through silica with hexane as the solvent may be helpful to prevent fouling of the preparative column. However, in our case, this was not necessary.

Whereas the efficiency of the analytical column was sufficient for separating the D-β and D-γ isomers (Fig. 2), they seemed to be overlapped in the preparative chromatogram (Fig. 3). This was due to overloading the column with 15 g of the tocopherol starting mixture in an injection volume of 50 ml. Nevertheless, suitable fractionation (Fig. 3) allowed the isolation of up to 4 g of natural D-γ-tocopherol per

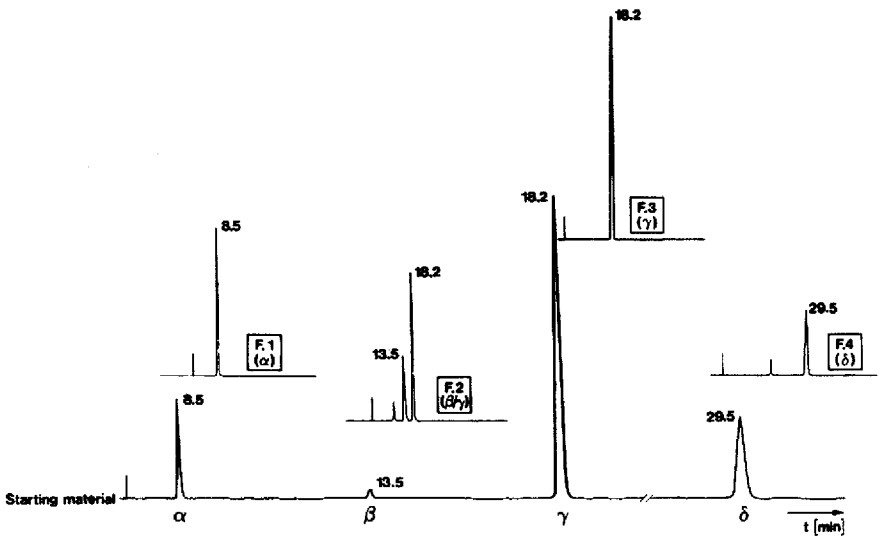

Fig. 2. Analytical HPLC of the starting material and of the D-α (F.1) to D-δ (F.4) fractions, isolated by preparative HPLC.

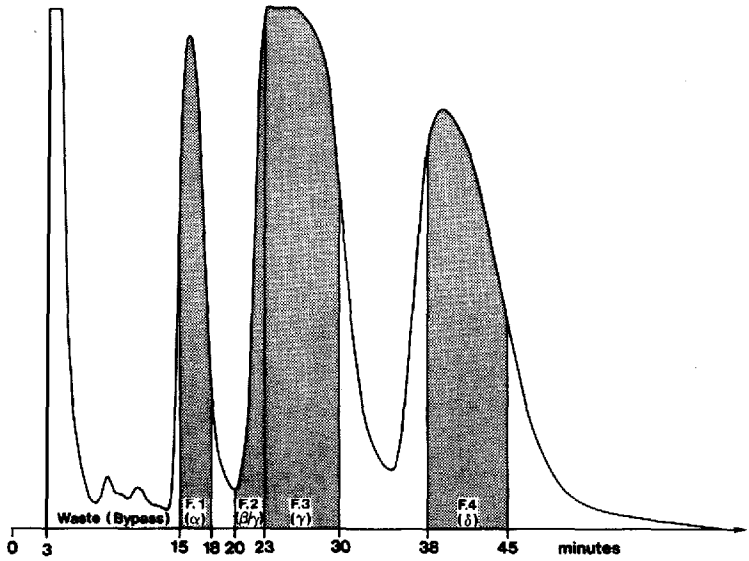

Fig. 3. Preparative chromatogram with time marks for the cuts of fractions F.1–F.4.

TABLE II

ANALYTICAL DATA FOR ISOLATED NATURAL D-TOCOPHEROL FRACTIONS FROM 350 g STARTING MATERIAL CONTAINING 70% TOTAL TOCOPHEROL, 60% OF WHICH WAS D-γ-TOCOPHEROL

Fraction	*Content* (%, w/w)*				*Yield (g)*
	D-α	D-β	D-γ	D-δ	
(1) D-α	88.2	–	–	–	20
(2) D-β	2.5	10.4	39.0	–	8
(3) D-γ	–	–	95.4	–	95**
(4) D-δ	–	–	3.6	90.1	49

* HPLC data, calibrated with tocopherol standards from Merck.
** 65% D-γ-Tocopherol yield relative to the D-γ-tocopherol content of the starting material.

experiment in 95.4% purity, without contamination by other tocopherols. The analytical data for the fractions isolated are summarized in Table II.

Thus, preparative HPLC has proved to be a rapid and economic method for isolating natural tocopherol homologues.

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