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Note

Separation of three dimers of α-tocopherol by high-performance liquid chromatography

J. CILLARD, J. GOBAILLE and P. CILLARD*

Laboratoire de Botanique et Biologie Cellulaire, 2 avenue Léon-Bernard, U.E.R. "Médicament", 35043 Rennes Cedex (France)

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The oxidation of α-tocopherol (vitamin E, α-T) leads to the formation of different compounds, particularly dimeric products, which result from divalent oxidation of α-T.

Dimers can be formed either by alkaline ferricyanide oxidation of α-T[1–5] or during the autoxidation of unsaturated fatty acids with α-T[6–9]. A dimer has also been considered as the major metabolite of Vitamin E[2,10].

Nevertheless, there were conflicting proposals on the final structure of the dimer. It was suggested that the dimer was a mixture[11]. In earlier studies, the purification of the dimer of α-T was achieved by column chromatography on magnesium silicate[1] or neutral alumina[2,4]. In our laboratory, we separated a dimer from α-T and α-tocopherylquinone by reversed-phase high-performance liquid chromatography (HPLC)[12].

The purpose of this paper is to describe a rapid method for purification and separation of a crude alkaline ferricyanide oxidation product of α-T into three dimers, using both analytical and preparative normal-phase high-performance liquid chromatography.

EXPERIMENTAL

Reagents and chemicals

α-T was a gift from Hoffmann-laRoche (France). Potassium ferricyanide, petroleum ether (40–60°C), isopropanol and di-isopropyl ether were supplied by Merck (Darmstadt, F.R.G.), sodium hydroxide by Prolabo and *n*-heptane (chromasol) by S.D.S.

Instrumentation

An LDC high-performance liquid chromatograph was purchased from Sopares-France and equipped with a Valco 7000 p.s.i. injector and a constametric III pump which can be modified for preparative chromatography. The spectromonitor III detector was set at 300 nm.

The UV absorption spectra were recorded in *n*-heptane on a Pye-Unicam SP 8-400 spectrophotometer. Mass spectra were obtained on a Varian MAT 311 instrument.

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Preparation of a crude oxidation product of α-T

The crude alkaline ferricyanide oxidation product of α-T was prepared according to the procedure of Skinner and Alaupovic[4]. A sample of α-T (2.2 g) was dissolved in 100 ml of light petroleum (b.p. 40–60°C) and shaken vigourously in a separating funnel with a solution of 6.4 g of potassium ferricyanide in 64 ml of 0.2 *M* sodium hydroxide for 3 min. The petroleum ether layer was separated, washed with water and dried over anhydrous sodium sulphate. The solvent was removed *in vacuo* leaving *ca.* 2 g of a yellow oily oxidation product of α-T.

Chromatography of the crude oxidation product of α-T

Chromatographies were performed either on a 25 × 0.49 cm I.D. column for analytical chromatography or on a 25 × 2.5 cm I.D. column for preparative chromatography. Both columns were packed with Lichrosorb Si 60 (particle size 5 μm).

Two solvent systems were used: *n*-heptane–isopropanol (99.85:0.15) at a flow-rate of 2 ml/min for analytical chromatography; *n*-heptane–diisopropyl ether (97.5:2.5) at a flow-rate of 22.5 ml/min for preparative chromatography.

The crude oxidation product of α-T was dissolved in *n*-heptane. Samples of 20 μl (*ca.* 10 μg of crude oxidation product) and 2 ml (*ca.* 10 mg) were injected into the chromatograph for analytical and preparative chromatography, respectively.

RESULTS AND DISCUSSION

The crude oxidation product of α-T was separated into four fractions by both analytical and preparative chromatography (Fig. 1). It should be noted that the elut-

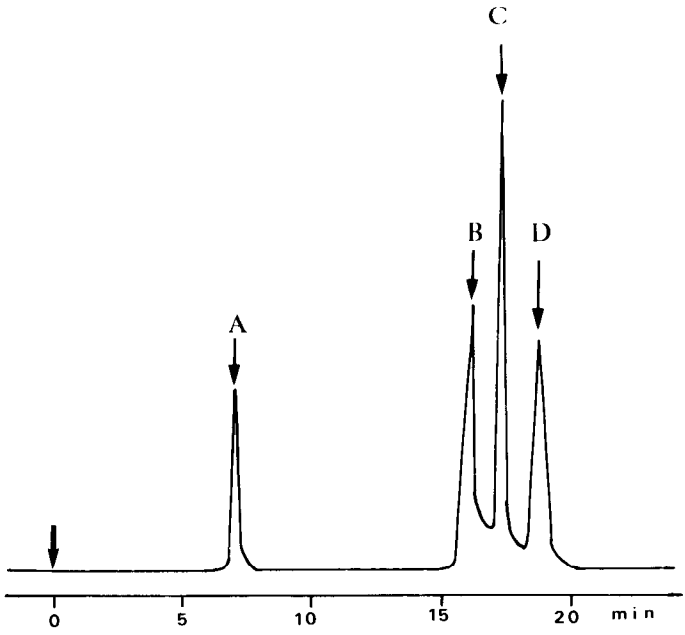

Fig. 1. Separation of the alkaline ferricyanide oxidation product of α-T by preparative HPLC using *n*-heptane–diisopropyl ether (97.5:2.5) as solvent. Peak A: unidentified compound; peaks B, C and D: dimers of α-T.

ing solvent used for analytical chromatography does not allow an efficient separation of fractions B, C and D by preparative chromatography. So, in this latter case, we shall have to use a solvent that is slightly less polar.

Fraction A is a colourless product, which showed a maximum absorption spectra at 294 nm (Table I).

TABLE I

RETENTION TIMES AND UV ABSORPTION OF THE FRACTIONS ISOLATED FROM THE CRUDE ALKALINE FERRICYANIDE OXIDATION PRODUCT OF α-T

Fraction	*Retention time (min)*	*UV absorption maximum (nm)*
A	7	294
B	16	300–337
C	17.5	300–337
D	19	300–337

Fractions B, C and D, which are eluted between 16 and 19 min, have a bright yellow colour and exhibited the same absorption spectra with two maxima at 300 and 337 nm (Table I). An identical UV, absorption was mentioned for the dimer of α-T by various authors[1,2,4].

Mass spectra of fractions B, C and D showed, for each fraction, a peak for a molecular ion at *m/e* 858 which corresponded to the dimerization of α-T and the removed of two hydrogen atoms.

In the literature, there is much controversy about the structure of the dimer of α-T. Nelan and Robeson[1] postulated that the dimer is a spirenone ether with a molecular weight of 856. Csallany[13] carried out a reappraisal of the structure of the dimer and concluded that dimerization entails the formation of a nine-membered chelate ring through strong intramolecular hydrogen-bonding. The resulting compound has a molecular weight of 858.

To compare our results to those previously found by other workers, we have also chromatographed the crude oxidation product of α-T according to the procedure of Skinner and Alaupovic[4] using a neutral alumina column (Brockmann activity I) and a solvent composed of petroleum ether–diethyl ether (9:1) at low pressure.

This procedure leads to the elution of a single yellow fraction which corresponded to a dimer of α-T according to the authors. This fraction, rechromatographed by HPLC according to the method described in the present paper, gives three fractions B, C and D. Fraction A was not detected in this case.

Considering the physical properties (UV spectra and mass spectra) of fractions B, C and D, we can conclude that these fractions correspond to three distinct dimers of α-T which can be separated by normal-phase HPLC. Further investigations will be undertaken to elucidate the difference of structure between these three dimers. We have noted that fraction C is quite stable, while fractions B and D both lead rapidly to a mixture of B + D.

Fraction A, which is eluted before the three dimers, could presumably be the trimer of α-T described by Skinner and Alaupovic[4].

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