

## The thin-layer chromatographic characterization of some oxidation products of vitamin E

The chemistry of vitamin E, *dl*- $\alpha$ -tocopherol, is of increasing interest in view of its important role in metabolism and the intensified research on its metabolic products. Methods for the separation and identification of the tocopherols, their metabolites, and oxidation products in the past have largely been confined to column and coated-paper chromatography. An interesting review covering these earlier methods and some more recent data on the use of thin-layer chromatography with the isomeric tocopherols was presented by KOFLER *et al.*<sup>1</sup>. The petroleum jelly, silicone or liquid paraffin impregnated paper chromatographic systems that have been used for the tocopherols and their oxidation products are time-consuming to use, and are difficult to prepare so that uniform results are obtainable.

In recent years, the various isomeric tocopherols or their acetates have been characterized by SEHER<sup>2</sup>, DÁVIDEK AND BLATTNÁ<sup>3</sup> and BOLLIGER<sup>4</sup>, using silica gel or alumina thin-layer chromatography. The latter workers also used secondary magnesium phosphate thin-layer plates.

In connection with our studies on the oxidation of the tocopherols, we have found the use of silica gel thin-layer chromatography as indispensable for separation and establishment of purity of the various oxidation products produced. Table I summarizes the results of the use of silica gel G thin-layer chromatography for the separation of *dl*- $\alpha$ -tocopherol and some of its oxidation products and derivatives. Optimum separation of these compounds was obtained using cyclohexane-chloroform (2:1) as

TABLE I

SILICA GEL THIN-LAYER CHROMATOGRAPHY OF *d,l*- $\alpha$ -TOCOPHEROL AND ITS OXIDATION PRODUCTS

<i>dl</i> -Tocopherol derivative	Relative mobility ( $R_f \times 100$ )			Detection sprays			
	Benzene	Chloroform	Cyclohexane Chloroform (2:1)	A	B	C	D
Tocopheryl quinone <sup>a</sup>	01-07	17-25	01-06	blue	tan	—	blue
Toco Red <sup>b</sup>	01-02	14-16	02-04	blue	tan	orange	blue
Tocopherol <sup>c</sup>	37-43	80-87	23-29	blue	yellow	pink	blue
Keto ether dimer <sup>d</sup>	59-63	96-98	40-45	blue	grey	pink	blue
Compound B <sup>d</sup>	80-90	96-99	59-65	blue	brown	red	blue
Compound A <sup>d</sup>	92-96	96-99	78-85	blue	brown	red	blue
Tocopheryl acetate <sup>e</sup>	41-54	91-96	40-51	blue <sup>f</sup>	yellow	yellow	blue
Toco Purple <sup>e</sup>	01-06	15-27	01-06	blue	tan	—	blue
Dihydroxy dimer <sup>d</sup>	05-08	70-75	03-07	blue	grey	tan	blue

A: 5% aqueous  $K_3FeCN_6$  followed by 5% aqueous  $FeCl_3$ .

B: 60%  $H_2SO_4$  with heating to 150°.

C: 20%  $SbCl_5$  in  $CHCl_3$ .

D: 10% ammonium molybdate in 10%  $H_2SO_4$  with heating to 150°.

<sup>a</sup> Prepared by  $FeCl_3$  oxidation of *dl*- $\alpha$ -tocopherol<sup>5</sup>.

<sup>b</sup> Prepared by  $HNO_3$  oxidation of *dl*- $\alpha$ -tocopherol<sup>6</sup>.

<sup>c</sup> Merck and Co., Inc.

<sup>d</sup> See *Science*, 140 (1963) 803.

<sup>e</sup> Prepared by  $FeCl_3$  oxidation of *dl*- $\alpha$ -tocopherol<sup>7</sup>.

<sup>f</sup> After 10 min.

solvent. Results are presented on the use of four spray reagents (5% aqueous potassium ferricyanide followed by 5% aqueous ferric chloride, 60% sulfuric acid with heating to 150°, 20% antimony pentasulfide in chloroform, and 10% ammonium molybdate in 10% sulfuric acid with heating to 150°). The most useful spray reagent for these compounds was the Turnbull Blue reagent ( $K_3FeCN_6-FeCl_3$ ).

### Experimental

The thin-layer plates (8 in. × 8 in.) were prepared from "Silica-Gel G. acc. to Stahl" (Brinkmann Instruments Co., Great Neck, Long Island, N.Y.) by mixing 30 g of the dry powder with 60 ml of distilled water and applying to the glass plates with a 250-micron spreader. After drying in the air the plates were baked in an oven for one hour at 110°.

All chromatograms were prepared by the ascending method at 20° in a solvent-saturated atmosphere. The compounds (2  $\gamma$  from ethanol solution) were placed 2 cm from the bottom of the thin layer which was submerged in solvent to a depth of 3-5 mm and the solvent allowed to run a distance of 15 cm.

Purity of the oxidation products utilized in this study was established by comparison of their infrared spectra and thin-layer chromatographic behavior with that of authentic samples.

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