# Isolation and Identification of α-Tocopherol, a Vitamin E Factor, from Orange Flavedo

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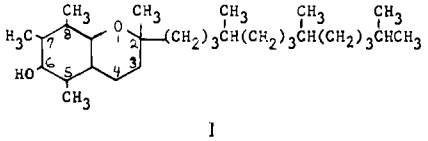
Extraction of the dried outer peel layer (flavedo) of oranges with *n*-hexane gave a viscous residue which was dewaxed by treatment with methanol. Chromatographic separation of the filtrate residue on an alumina column, a silicic acid column, and finally on silicic acid–coated thin-layer chromatographic plates afforded a pure sample of α-tocopherol.

The occurrence of a natural antioxidant and its location in the flavedo of oranges were discussed in a previous publication (*9*). It was considered likely that this was a tocopherol-like substance because it gave a positive ferric chloride–bipyridine test (*2*) and was nonpolar and soluble in petroleum ether.

The present paper describes the isolation and identification of this natural antioxidant as α-tocopherol (I), a vitamin E factor recently shown to be essential to the health and well-being of most animal species (*6*).



I

α-Tocopherol found in natural source materials is often accompanied by β- or γ-tocopherol, which are without one methyl group at the 7 or 5 position, respectively. The tocopherols in cottonseed oil, however, are α and γ in about equal amounts, whereas those in soybean oil are α, γ, and δ forms, and those in wheat germ oil are α- and β-tocopherols (*7, 1*). The δ form is without both methyl groups at positions 7 and 5.

## Experimental

**Extraction Procedure.** Fifteen standard field boxes of Valencia oranges (approximately 90 pounds per box) were passed through a Fraser-Brace peel oil extractor (*5*). In this machine, the whole fruit pass through a corridor of Carborundum rollers which rasp off the outer peel or flavedo. Water sprays directed on the fruit and the rollers washed away the oil and grated peel, which was collected on a vibrating screen to remove excess water. The wet peel, amounting to 12,360 grams, was thoroughly dried in a forced-draft oven at 75° to 80° to a dry weight of 1400 grams. The dried peel was placed in a large modified Soxhlet extractor consisting of a 4-liter aspirator bottle with a glass siphon attached. Redistilled *n*-hexane was used in the extraction, which proceeded until the extract was colorless (about 24 hours).

The extract was concentrated on a film evaporator (50°) to remove all of the *n*-hexane. The dark, viscous residue was then refluxed for 1 hour with 1 liter of anhydrous methanol. The resulting mixture was cooled and the precipitated waxes (5.35 grams) were collected on a filter. The filtrate was again concentrated to remove the methanol and finally the residue was dried under high vacuum at room temperature. This procedure gave 24.75 grams of dark, viscous oil which was dissolved in 25 ml. of *n*-hexane.

**Chromatographic Separations.** A 2-foot × 2-inch chromatographic column was filled with redistilled petroleum ether (30° to 60°) and packed (wet) with 625 grams of aluminum oxide (Fisher). The flavedo extract in hexane obtained above was placed on this column and eluted first with 2300 ml. of petroleum ether–ethyl ether 80/20 (v./v.). One hundred-milliliter fractions were collected, and each was evaporated to dryness in a stream of dry nitrogen.

Fractions 14 to 19, inclusive, contained colorless needles which were sparingly soluble in methanol. One crystallization from methanol gave a sample (m.p. 250–55°, decomposition), the infrared spectrum of which showed carbonyl absorption at 5.82 microns. This is probably the triterpenoid ketone, friedelin, first isolated by Weizmann and Mazur (*10*). Fractions 24 to 28 were eluted with 500 ml. of petroleum ether–ethyl ether 70/30 (v./v.), fractions 29 to 33 with 500 ml. of a 60/40 (v./v.) mixture, and fractions 34 to 38 with 500 ml. of a 50/50 mixture of the two solvents. Finally for fractions 39 to 60, ethyl ether alone was used. Ethyl ether containing 1% methanol was used to elute fractions 61 to 65, and 2% methanol in ethyl ether was used for the remainder of the chromatogram (total 77 fractions).

Crystals were observed in fractions 60 to 65. One crystallization from methanol of the crystals collected in fraction 62 gave colorless plates (m.p. 138–40°), whose infrared spectrum was identical with that of β-sitosterol previously reported in orange oil by Weizmann and Mazur (*10*).

The presence of reducing compounds (antioxidants) in fractions 48 to 69 was indicated by a positive ferric chloride–bipyridine test (*2*). Therefore, these fractions were combined and concentrated to dryness under high vacuum. This afforded 1.52 grams of dark, oily material which was dissolved in *n*-hexane and rechromatographed on a 1 × 8 inch silicic acid (Merck) column and eluted with 50% chloroform in hexane. Those fractions giving a positive ferric chloride–bipyridine test were combined and concentrated to dryness under high vacuum. The silicic acid column removed most of the color and the combined active fractions afforded 0.365 gram of light yellow, viscous oil. Thin-layer chromatography of this material on silicic acid coated plates showed spots with $R_f$ values similar to that of authentic α-tocopherol.

The above 0.365 gram was dissolved in *n*-hexane and streaked on ten 8 × 8 inch glass thin-layer chromatographic plates coated 0.5 mm. thick with silica gel G (Brinkmann), and these were developed in redistilled chloroform. After drying, the guide edge of each plate was sprayed with 0.2% ferric chloride in acetone, followed immediately by 0.5% 2,2′-bipyridine in acetone. A red-colored area indicated the location of the band containing the desired material. The area of the band corresponding to the location of the positive bipyridine test was scraped from each plate and the scrapings were combined and eluted with methanol. Concentration of the filtrate from these silicic acid plates yielded 0.096 gram of pale yellow viscous oil.

## Results and Discussion

The infrared absorption spectrum of this isolated material was determined on a Beckman IR 4 infrared spectrophotometer and found to be identical with that of an authentic sample of *d*-α-tocopherol (Figure 1). Both samples were examined in carbon tetrachloride solution using a cell with a path of 0.10255 mm. The entire extraction procedure and isolation of *d*-α-tocopherol were repeated using a different sample of Valencia oranges. Similar results were obtained and a pure sample of *d*-α-tocopherol was again isolated. There was no indication

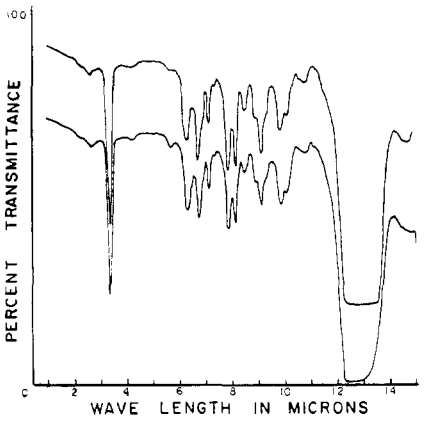



Figure 1. Infrared absorption in chloroform

*Upper.* Authentic sample of *d*-α-tocopherol
*Lower.* Antioxidant compound from orange flavedo

that any tocopherol other than the alpha form was present in the sample. This was verified by thin-layer chromatography of samples of the β-, γ-, and δ-tocopherols.

The presence of α-tocopherol in orange oil was suspected by Proctor and Kenyon (*7*), since it appeared to be especially effective as an antioxidant for this oil. Rakieten (*8*), using the bipyridine test of Emmerie and Engel (*3*), reported the α-tocopherol content of orange juice to be not more than 99 mg. per 100 ml. Our results would indicate that the α-tocopherol content of orange flavedo is less than 1 mg. per 100-gram fresh weight. However, the α-tocopherol content of the crude dewaxed oil (24.75 grams) obtained from the *n*-hexane extract of orange peel was 3.9 mg. per gram. This is a high concentration compared to that of many natural oils. While no isolation and identification of α-tocopherol from orange juice were attempted in this study, the authors were unable to detect the presence of any tocopherol-like substance in the juice using the procedure of Emmerie and Engel (*3*). The juice of orange contained little or no antioxidant activity (*9*), and it is doubtful that the α-tocopherol content of the juice would be greater than that of the flavedo.

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**PEEL JUICE FLAVOR**

# Proximate Analyses of Florida Orange Peel Juice Extract for the 1962–63 and 1963–64 Seasons

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**Juice was obtained from whole orange peel with a hydraulic press at intervals during the 1962–63 and 1963–64 seasons. In general, maturity and variety did not appear to have much influence on the peel juice bitterness except at the last of the seasons when bitterness was greater. The quantity of benzene extract was considerably higher during the latter season. There appeared to be some agreement between peel juice bitterness and the content of extract neutral fraction. From threshold flavor values, the neutral fraction was estimated to be responsible for 35 to 75% of the total bitterness.**

Swift and Veldhuis (*4*) measured soluble solids, reducing sugars, sucrose, total sugars, ascorbic acid, soluble pectic substances, acidity, flavonoids, diacetyl, pH, specific gravity, viscosity, color, and fluorescence in commercial orange juice, peel juice, and segment juice throughout the 1954–55 operating season. Marked differences were noted. Composite peel juice samples for any quarter of the season could be detected by a taste panel when added to good quality segment juice at the 3% level. These experiments did not reveal whether or not bitter substances from peel were present in commercial orange juice. No objective test existed at the time by which such an admixture could be measured.

Subsequently, Swift (*3*) found that the chief volatile constituents present in orange peel juice were linaloöl and α-terpineol and developed a method for their estimation in orange juice. However, as these compounds could not account for the major bitterness of orange peel juice, it was necessary to investigate the nonvolatile substances present. Preliminary work indicated that most of the bitterness was extractable with benzene and that much of the bitterness of the extract resided in its neutral fraction.

The present investigation was undertaken to learn more about the effects of variety and maturity on bitterness and to investigate the relative importance of the neutral fraction in this respect.

### Experimental

Orange peel, relatively free of pulp and cell membranes, was obtained from local processing plants over the 1962–63 and 1963–64 seasons. It was frozen at −20° C. and then ground in a Fitzpatrick Model D comminuting mill using a screen with 0.25-inch openings. The ground, frozen peel was weighed, mixed

Freezing weather occurred early in the 1962–63 season, so it was decided to continue the work for an additional year to obtain more nearly normal results. It is intended to use the information gained as a guide in future work on the identity and abundance of individual compounds related to bitterness.