Isolation of α-Tocopherol (Vitamin E) from Garlic

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Vitamin E (α-tocopherol) was isolated from garlic. The method involved homogenization of garlic in phosphate-buffered saline and extraction with heptane in the presence of lithium dodecyl sulfate. An aliquot of the extract, when analyzed by HPLC on a C18 column, yielded a peak with the same retention time as the α-tocopherol standard. The presence of α-tocopherol was further confirmed by GC/MS. A total of 9.4 μg of α-tocopherol was isolated from 1 g of garlic. Oxidation of low-density lipoprotein was retarded by garlic α-tocopherol.

Keywords: *Vitamin E; garlic; α-tocopherol; LDL oxidation*

INTRODUCTION

Garlic, known botanically as *Allium sativum*, is a widely distributed common plant. It is used in all parts of the world, not only as a spice and food but also as a popular folk remedy for a variety of ailments. These include heart disease, several types of cancer, and blood clotting (Fenwick and Hanley, 1985). Despite this usage, very little is known at the molecular level about the therapeutic role of garlic in these diseases. Intrigued by the recent reports on the role of vitamin E (α-tocopherol) in preventing coronary heart attacks (Stamppfer et al., 1993; Rimm et al., 1993), we decided to investigate whether garlic contains α-tocopherol in more significant quantities than previously reported (Yamauchi and Matsushita, 1976a,b).

MATERIALS AND METHODS

Materials. Garlic was purchased from a local vegetable market. Organic solvents, heptane, methanol, chloroform, and acetonitrile (HPLC grade) were from VWR (Philadelphia, PA). Vitamin E standards (α-tocopherol, 95%; δ-tocopherol, 90%; and α-tocopherol acetate), lithium dodecyl sulfate (LiDS), ethylenediaminetetraacetic acid (EDTA), and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Company (St. Louis, MO). HPLC chromatograph model 1330 with a model 1305A UV monitor and a Hewlett Packard model 3392A integrator was from Bio Rad (Hercules, CA). Absolute ethanol (USP) was purchased from Quantum Chemical Corp. (Tuscola, IL). The Bakerbond C18, 5 μm particle size with 120 Å pore, was from J. T. Baker (Phillipsburgh, NJ). The DB-5 (0.25 μm film) 0.32 mm i.d. × 10 m length GC column was from J&W Scientific (Folsom, CA). The GC/MS was a Finnigan model 330 (San Jose, CA).

Methods. *Extraction.* For a typical preparation, 10 g of garlic (cleaned) was placed in a 400 W microwave oven for 45 s to eliminate alliinase activity. The mass of the garlic was reduced to 6 g, probably due to water loss during microwaving. This was homogenized, with an Omni International model 1000 hand-held homogenizer set at high speed (Waterbury, CT), in 2 vol of phosphate-buffered saline (10 mM potassium phosphate, 150 mM sodium chloride, pH 7.4, PBS). The resulting homogenate was vortexed for 30 s with an equal volume of 10 or 100 mM LiDS. An equal volume of ethanol was added and the mixture vortexed for an additional 30 s. An equal volume of heptane (containing 0.05% BHT as an antioxidant) was then added and the mixture vortexed for an additional 1 min. The organic phase was separated from the aqueous phase by centrifuging for 10 min at 200*g* in a IEC HN-SII centrifuge with a #958 swinging bucket rotor (Needham Heights, MA). The organic phase was carefully removed and then evaporated under a stream of nitrogen at room temperature. The residue was dissolved in 500 μL of HPLC mobile phase (acetonitrile:methanol:chloroform, 47:47:6)/mL of homogenate and filtered through a 0.45 μm pore size nylon membrane (Schleicher and Schuell, Keene, NH) (Thurnham et al., 1988). We found that 91.5% of the α-tocopherol was extracted in the first heptane treatment of the 10 mM LiDS extract and 97% with 100 mM LiDS. The remaining 8.5 and 3% were extracted with a second heptane treatment.

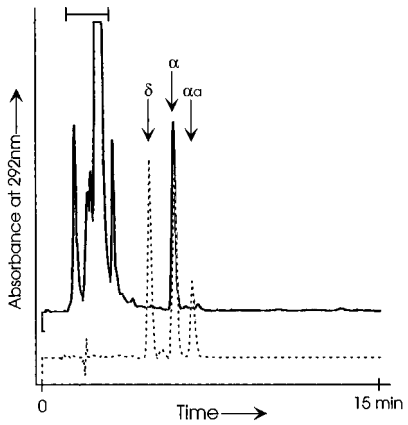

Figure 1. Liquid chromatogram of tocopherol standards (dotted line) and the heptane extract of garlic (solid line). δ indicates the position of the δ-tocopherol standard, α indicates the position of the α-tocopherol standard, and αa indicates the position of the α-tocopherol acetate standard. The bar indicates an area of substances (unidentified) contributed by the solvents used in the isolation procedure.

Chromatography. Filtered extract (50 μL) was injected onto a 4.6 × 250 mm C18 column which was equilibrated and developed with the mobile phase at a flow rate of 1.5 mL/min. Elution was monitored by UV absorbance at 292 nm. The α-tocopherol peak was collected, evaporated at room temperature with a nitrogen stream, and dissolved in DMSO.

GC Conditions. The column was developed at an initial temperature of 200 °C for 3 min and then programmed to rise to 300 °C at a rate of 10 °C/min. Detection was by mass spectrometry.

Preparation of LDL. LDL was prepared by a modification of the procedure of Regnström et al. (1992). Briefly, plasma was separated from human blood (provided by volunteer

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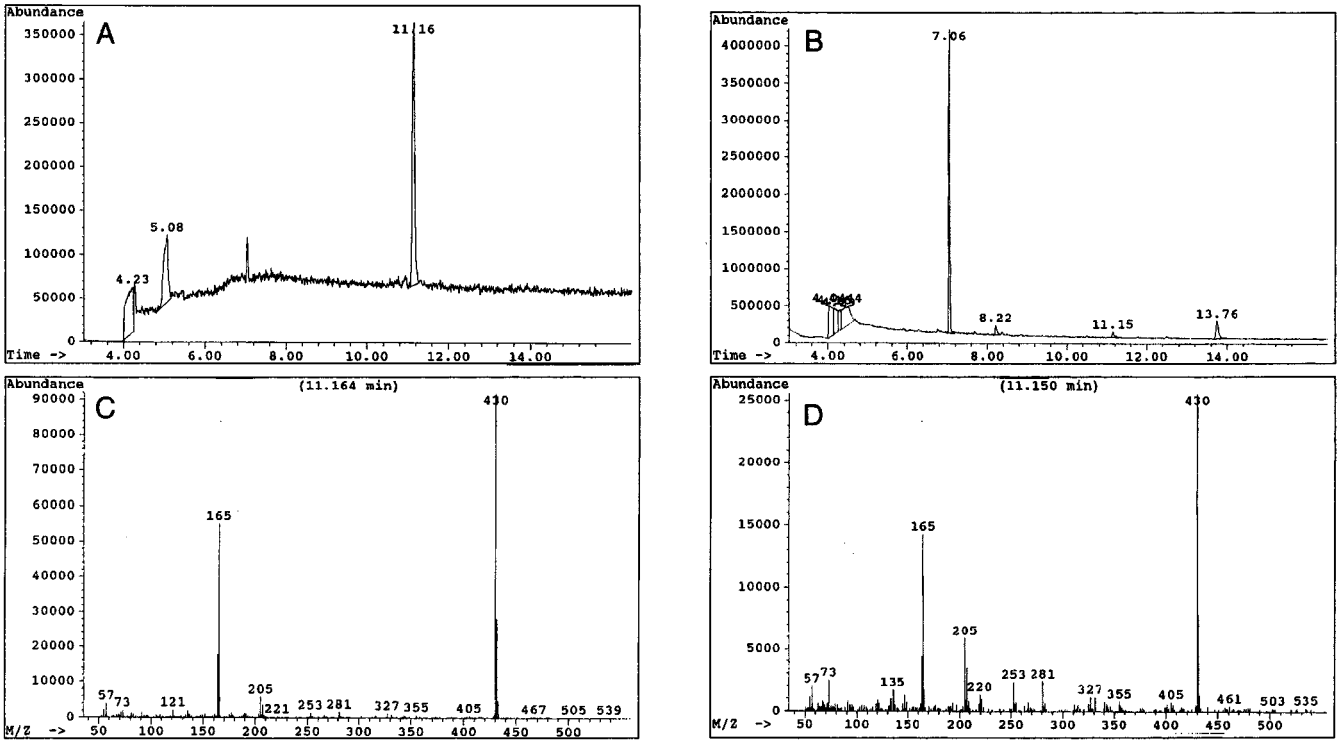

Figure 2. GC/MS of α-tocopherol standard (A, C) and garlic extract (B, D): (A, B) GC profiles of the standard and the extract, respectively, (C, D) mass spectra of the selected peaks. Retention times are indicated in parentheses.

donors), collected in the presence of EDTA, by centrifuging at 1480*g* for 20 min at 4 °C. The plasma was made to 2.637 M in NaCl. A gradient of 3 mL of 2.637 M NaCl in plasma and 3 mL each of 1.676, 0.541, and 0.189 M NaCl in 0.1 mM EDTA was formed in polyallomer tubes and centrifuged in a swinging bucket rotor at 202000*g* for 22 h at 4 °C. The band that accumulated at the interface of 0.541 and 1.676 M NaCl was collected and dialyzed overnight against 10 vol of PBS with two changes. It was protected from light.

Oxidation of LDL. The dialyzed LDL was adjusted to 0.25 mg/mL with PBS; 100 μL (25 μg) of LDL and 10 μL of DMSO or 0.25 μg of α-tocopherol standard or α-tocopherol from garlic in DMSO were brought to 990 μL with PBS and incubated at 37 °C for 3 h. The oxidation reaction was initiated by the addition of 10 μL of 0.166 mM $CuSO_4$ (final concentration of 1.66 μm) and monitored at 234 nm for up to 4 h. The molar ratio of α-tocopherol to LDL was 5.8:1 (Regnström et al., 1992; Esterbauer et al., 1991).

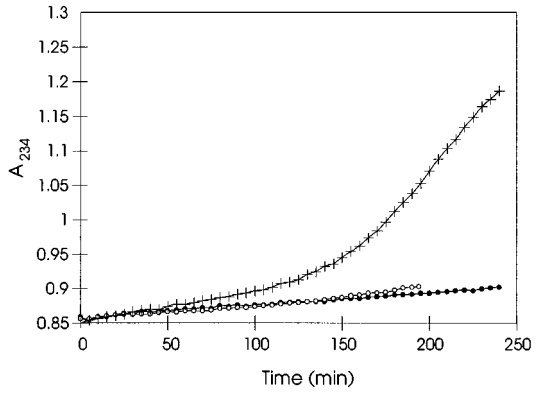

Figure 3. Oxidation of LDL. The reactions were performed as described under Methods: (+) LDL with DMSO, (○) LDL with standard α-tocopherol, (●) LDL with α-tocopherol from garlic.

RESULTS AND DISCUSSION

When the HPLC column was developed isocratically (see Methods), a peak was found with the same retention time as the α-tocopherol standard (Figure 1). These data suggest the presence of α-tocopherol in garlic. This was further confirmed by injecting the same garlic extract into a GC with MS for detection. Again, a peak with the same retention time and mass spectrum as the α-tocopherol standard was found, thus confirming the presence of α-tocopherol in garlic (Figure 2). A total of 9.4 μg of α-tocopherol is isolated from 1 g of garlic. This represents 47.7% of heptane extractable compounds. This value compares favorably with those reported in the literature as the highest abundance (0.1−0.3% of the oils) natural sources of vitamin E (Schudel et al., 1972). It has previously been reported that garlic contains 0.1 μg of α-tocopherol/g of garlic (Yamauchi and Matsushita, 1976a,b; Souci et al., 1986). The isolation method used by these investigators (Yamauchi and Matsushita, 1976a,b) is lengthy, and the yield is one one-hundredth of what we obtained.

Garlic has been shown to be effective in lowering blood cholesterol level and as an antiatherosclerotic agent (Sainani et al., 1976; Harenberg et al., 1988), but what causes this reduction in atherosclerosis by the consumption of garlic has been largely undetermined. Recent evidence suggests that oxidation of LDL contributes to atherosclerosis. The addition of α-tocopherol to LDL increases its resistance to oxidation (Regnström et al., 1992; Esterbauer et al., 1991). We confirmed this effect with α-tocopherol isolated from garlic (Figure 3). Thus, the presence of α-tocopherol in garlic could partially explain its antiatherosclerotic property.

When used as therapy, large quantities of garlic, or its equivalent, are consumed. Garlic oil capsules contain up to 16 850 mg of oil (Super Garlic, Schiff Products, Inc., Salt Lake City, UT). Since garlic contains an average of 1.2 mg of fat/g (Souci et al., 1986), each capsule could contain approximately 132 mg of vitamin E. The recommended daily dosage of vitamin E is 10 mg/day. Therefore, the consumption of one

capsule of garlic oil could give a substantially higher dose than recommended.

ABBREVIATIONS USED

BHT, butylated hydroxytoluene; C18, octadecyl; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; GC, gas chromatography; HPLC, high-performance liquid chromatography; LDL, low-density lipoprotein; MS, mass spectrometer; PBS, phosphate-buffered saline.

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