Development of 3-Aminopropyl-DL-α-tocopherylphosphate as a Potent Antioxidant: Focus on Linoleic Acid Autoxidation

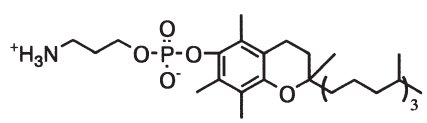
Sir:

Lipid oxidation is a degradative, free radical-mediated process responsible for the development of unpleasant odors and tastes in oils, fats, and foods containing them (1,2). Moreover, oxidation of the polyunsaturated fatty acids of the biomembranes causes functional abnormalities and pathological changes. Tocopherol is well recognized for its effective inhibition of lipid oxidation in foods, cosmetics, and biological systems (1,2). However, because of its unfavorable stability and solubility, tocopherol has limited use, especially in aqueous products (1,2). Therefore, its derivatives such as tocopheryl acetate have been extensively studied to overcome these problems of tocopherol (3).

Recently, in a continuation of our study on the development of stable tocopherol derivatives, we have had much interest in 3-aminopropane phosphoric acid because it is reported to stimulate collagen production in cultured human fibroblasts and is utilized as an active ingredient in cosmetic products (4). To provide tocopherol with improved physical properties and bioactivities, we prepared 3-aminopropyl-DL-α-tocopherylphosphate (APTP) (Scheme 1) in good yield (84%) by the reaction of DL-α-tocopherylphosphodichloridate and 3-aminopropan-1-ol in the presence of triethylamine at 15°C in tetrahydrofuran (THF) followed by hydrolysis in a mixture of THF and H_2O (1:1). APTP: White solid; mp 248–249°C (acetone); ^{1}H nuclear magnetic resonance (300 MHz, $CDCl_3$) δ 0.83–0.88 (*m*, 12H), 1.08–1.57 (*m*, 24 H), 1.69–1.76 (*m*, 4 H), 2.04 (*s,* 3 H), 2.15 (*s*, 3 H), 2.19 (*s*, 3 H), 2.53 (*t, J* = 6 Hz, 2 H), 2.80 (*br*, 2 H), 4.05 (*br*, 2 H), 8.40 (*br*, 3 H); fast atom bombardment mass spectroscopy (*m/z*) 568 (M^+ + 1: 15.39), 510 (2.60), 430 (47.42), 245 (77.87), 165 (43.59), 58 (100), 43 (46.14).

APTP is very stable and has improved physical properties compared with tocopherol. In addition, to our surprise, it showed a much higher antioxidative property than DL-α-tocopherol (T) and DL-α-tocopheryl acetate (TA) against autoxidation of linoleic acid.

The comparison of various compounds for antioxidant activity was carried out in two ways. First, 10 μmol of each antioxidant was admixed to 2.0 g of linoleic acid in a petri dish. The mixture was stored at 60°C in a dark room. Weight gain was recorded every day to estimate the degree of oxidation of linoleic acid. When there was no more weight gain, the ^{1}H nuclear magnetic resonance (NMR) spectrum of each mixture was obtained to investigate the degree of autoxidation of linoleic acid, which was determined by the ratio of integral of peaks corresponding to vinyl protons (4H, C9, C10, C12, and C13) of unoxidized (A of Fig. 1) to oxidized (B,C) linoleic acid. The experiment was repeated twice, and the percentage error was ± 1.0%. Second, the increase of peroxides in the autoxidation of linoleic acid was also determined by the peroxide value at 37°C. Each mixture of T, TA, or APTP with linoleic acid was kept at 37°C in a dark room. Aliquots (3 g) of the mixtures were taken for the determination of peroxide value (PV) every 7 d. The peroxide formed was estimated iodometrically, where the mixture was reacted with a saturated aqueous solution of potassium iodide. The iodine liberated by the peroxide was titrated with a standard solution of sodium thiosulfate. The experiment was repeated twice, and the percentage error was ±0.5%. Determination of PV was carried out at 37°C because peroxides formed are known to be more unstable at 60°C.



SCHEME 1

According to our results, in the presence of TA and T, the weight of the mixtures increased by about 7% within 7 d, which was due to oxidation of linoleic acid. The ^{1}H NMR spectra in the case of T (C of Fig. 1) showed that about 86% of linoleic acid had been oxidized within 7 d, in which the peaks corresponding to vinyl (C9, C10, C12, and C13) and allyl (C11) protons of linoleic acid disappeared almost completely. The overall patterns of the ^{1}H NMR spectra were different from that of unoxidized linoleic acid. On the other hand, in the case of APTP, the weight of the mixture increased by only 0.6% even after 30 d. The ^{1}H NMR spectrum also showed that only 3% of linoleic acid had been oxidized after 30 d. Figure 1 showed that the ^{1}H NMR spectrum (B) of the mixture was almost the same as that (A) of linoleic acid. The same experiments were performed at 37°C. Despite the lengthened induction period of autoxidation (20 and 11 d in the case of T and TA, respectively), the order of efficiency was unchanged.

There was a sharp increase in PV of linoleic acid over time in the presence of T and TA (Fig. 2). However, in the case of APTP, the PV did not increase significantly, which indicates that APTP is a much more potent antioxidant against autoxidation of linoleic acid.

It was reported (5) that autoxidation of lipids includes not only formation of hydroperoxides but also several sequences of radical-mediated reactions including isomerization and formation of secondary products from hydroperoxides. Antioxidants not only inhibit free radical-induced lipid autoxidation but they also inhibit the oxidations induced by the electronically excited singlet oxygen (1). We presume APTP would

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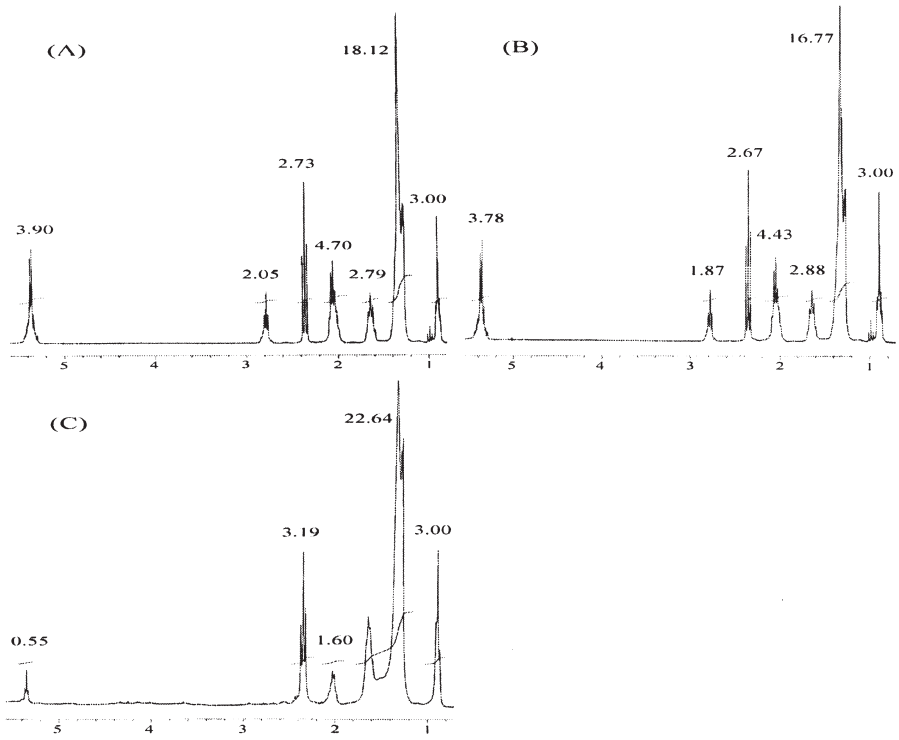

FIG. 1. Portions of ^{1}H nuclear magnetic resonance spectra including vinyl protons of unoxidized linoleic acid (A), oxidized linoleic acid in the presence of 3-aminopropyl-DL-α-tocopherylphosphate (B), or DL-α-tocopherol (C).

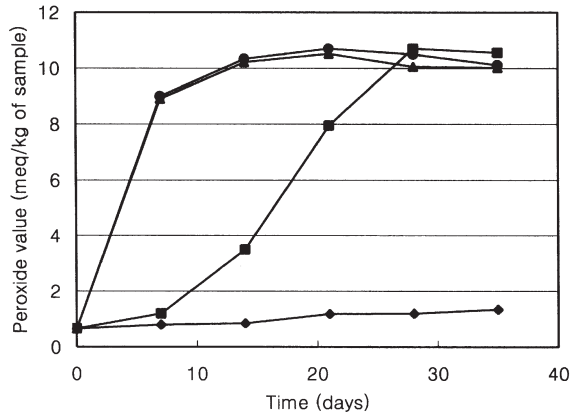

FIG. 2. Peroxide value of linoleic acid in the absence of antioxidant (●) and in the presence of DL-α-tocopherol (■), DL-α-tocopheryl acetate (▲), or 3-aminopropyl-DL-α-tocopherylphosphate (◆).

mainly inhibit lipid autoxidation by reaction with singlet oxygen by physical and chemical quenching. However, we did not focus on the detailed mechanism of the autoxidation of linoleic acid in the presence of APTP.

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