The Effect of Antioxidants on the Decomposition of Methyl 13-Hydroperoxyoctadecadienoate

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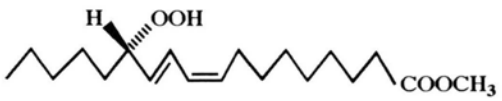
Phenolic antioxidants such as butylated hydroxytoluene, α-tocopherol, eugenol, isoeugenol, sesamol and quercetin effectively prevented thermal decomposition of methyl 13-hydroperoxyoctadecadienoate in methanolic solutions. Ascorbic acid, however, was found to promote the decomposition and the acidic nature of the vitamin was suggested to be involved in the mechanism.

Introduction

Prevention of autoxidation of unsaturated lipids by antioxidants is of central importance in medical and food sciences. Complete prevention of autoxidation, however, is impossible and lipid hydroperoxides are formed inevitably being dependent on long time exposure to oxygen, contamination of metal ions, heating and other factors. Unstable hydroperoxides thus formed decompose further to give a number of products including aldehydes and other yet unidentified polymerization as well as degradation products. Some of them are known to be highly toxic against mammals (Kaneko *et al.*, 1987, Tovar and Kaneda, 1977) and to exert deteriorative effects on fresh foods. From this view, prevention of the decomposition of lipid hydroperoxides is another important aspect . Since the mechanism of the thermal decomposition of lipid hydroperoxide is known to involve alkoxy and hydroperoxy radicals, the chemical process should be prevented by some antioxidants or radical scavengers. In fact, it was demonstrated that tocopherol, a typical phenolic antioxidant, could significantly reduce the decomposition products of unsaturated fatty acid hydroperoxides and the product spectrum was strongly dependent on the chemical structure of the antioxidant as well as the reaction conditions (Frankel and Gardner, 1989).

The present study focused on the chemical stability of unsaturated fatty acid hydroperoxide in the presence of various antioxidants including butylated hydroxytoluene (BHT), α-tocopherol, eugenol, isoeugenol, sesamol, quercetin, ascorbic acid (AA), sodium ascorbate and ß-carotene in which the first seven antioxidants are known to function as radical scavenger and can donate hydrogen radical to any other carbon or oxygen radicals like allylic, alkoxy as well as peroxy ones formed during autoxidation process of unsaturated lipids. Methyl 13-hydroperoxyoctadecadienoate (**1**) (ML-OOH) was employed as an example of lipid hydroperoxide.



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Materials and Methods

Methyl 13-hydroperoxyoctadecadienoate (**1**) was prepared in a pure state and in a large amount by soybean lipoxygenase-catalyzed hydroperoxidation of linoleic acid and esterification with diazomethane followed by silica gel column purification (Baba *et al.*, 1989). All the antioxidants were purchased from Aldrich Chemicals and Nacalai Tesque Inc. Kyoto Japan. ^{1}H NMR spectra ($CDCl_3$) were recorded on a Varian VXR 500 NMR spectrometer (500MHz). Column chromatography was done on silica gel 60 (Nacalai Tesque, 230–400 mesh) and TLC was on Kieselgel 60 F_{254} (Merck, Art. 5554, 0.2 mm). The spot intensity was determined by a densitometer, Model Shimadzu dual-wavelength flying spot scanner, model CS-9000. The decomposition experiment was conducted at 60 °C in methanol (1.0 ml) containing ML-OOH (20 mg, 0.06 mmol) and antioxi-

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dant (4.5 mM) in the dark and an aliquot (0.5 μl) of the solution was analyzed in one-hour intervals. The remaining hydroperoxide (%) undecomposed was determined by analyzing the reaction mixture with silica gel TLC (hexane/ ethyl acetate, 9:1). The spot intensity was determined by the densitometer at 234 nm. The amount of the remaining hydroperoxides (%) in each experiment is shown in Figs 1 and 2.

Results and Discussion

As column A in Fig. 1 shows, more than 80% of the hydroperoxide decomposed at 60°C after 72 h in the control experiment. Against this decomposition, as shown in column B, no preventive effect was found using β-carotene which is known to be a singlet oxygen scavenger. On the other hand, BHT (column C), α-tocopherol (D), eugenol (E), isoeugenol (F), sesamol (G) and quercetin (H) showed remarkably high preventive activity against the decomposition. The structural integrity of the remaining hydroperoxides was confirmed by ^{1}H NMR after isolating it from the reaction mixture. As column I in Fig. 1 shows, however, all the ML-OOH disappeared after 72 h at 60°C in the presence of AA. Also, as shown in column B and A in Fig. 2, the amount of the remaining ML-OOH was 15% and 80% in the presence and absence of AA respectively. The cause of this unexpected effect

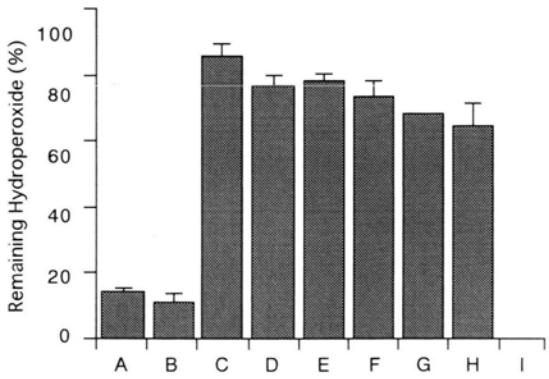


Fig. 1. Preventive action of antioxidants against decomposition of methyl 13-hydroperoxyoctadecadienoate in methanol after 72 h.
A: Control (60 mM); B: β-Carotene (4.5 mM); C: Butylated hydroxytoluene (BHT) (4.5 mM); D: α-Tocopherol (4.5 mM); E: Eugenol (4.5 mM); F: Isoeugenol (4.5 mM); G: Sesamol (4.5 mM); H: Quercetin (4.5 mM); I: Ascorbic acid (4.5 mM).

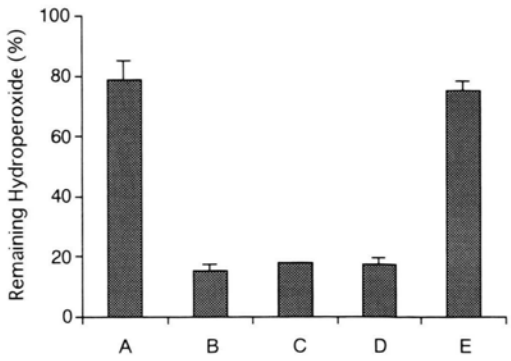
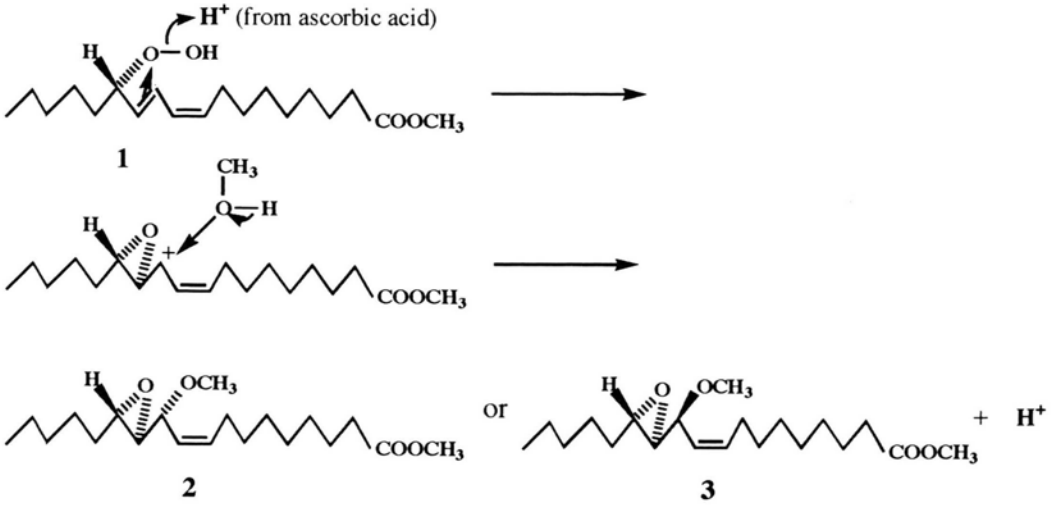


Fig. 2. Effect of ascorbic acid on decomposition of methyl 13-hydroperoxyoctadecadienoate in the presence and absence of phytic acid and of sodium ascorbate after 10 h.
A: ML-OOH control (60 mM); B: Ascorbic Acid (4.5 mM); C: Sesamol (4.5 mM) + Ascorbic Acid (4.5 mM); D: Ascorbic Acid (4.5 mM) + Phytic Acid (3.5 μM); E: Sodium Ascorbate (4.5 mM).

of AA could not be explained since it is known to be a good radical scavenger, too. On the other hand, AA has two additional characteristics, i.e., its reducing activity and acidic nature.

If AA acts as an acid, the following reaction may occur as reported by Gardner et al (Gardner et. al. , 1984) in their study using dilute aqueous HCl. In this case, the expected products are the epoxides (**2**) and (**3**) since the bond energy of the -O-O- bond is very week (30–40 kcal/mol). In our experiment, the reaction mixture with AA and without AA (thermal decomposition) both showed a number of spots on silica gel TLC (hexane/EtOAc, 95:5, 6 times development) detected by anisaldehyde-H_2SO_4/heating. Two intense spots, however, appeared on the former TLC (**X**: Rf = 0.23, 14% and **Y**: Rf = 0.28, 6.4%) which were not observed without AA. The structural identification of these compounds should provide us clue to the role of AA in ML-OOH decomposition. The fractions were isolated by silica gel column chromatography. Compound **X** showed a ^{1}H NMR ($CDCl_3$) spectrum as follows δ(ppm): 1.60 (2H, m, CH_2-C-$COOCH_3$), 2.08(2H, m, C=C-CH_2), 2.30(2H, m, $OCOCH_2$), 2.75(1H, dd, J = 5, 2 *Hz*, proton at 12-C), 2.93 (1H, dt, J = 5, 2 Hz, proton at 13-C), 3.30 (3H, s, -OCH_3), 3.66 (3H, s, $COOCH_3$), 4.03 (1H, dd, J = 5, 10 Hz, proton at 11-C), 5.26 (1H, m, proton at 10-C), 5.72 (1H, m, proton at 9-C). These signals explain well the



structure (**2**) and was found to coincide completely to that reported by Gardner et al in their study of HCl-promoted degradation of ML-OOH. Compound **Y** showed very similar ^{1}H NMR ($CDCl_3$) spectrum to those of (**2**) as follows δ(ppm): 1.60 (2H, m, CH_2-C-$COOCH_3$), 2.05(2H, m, C=C-CH_2), 2.30(2H, m, $OCOCH_2$), 2.75(1H, m, J = 5, 2 Hz, proton at 13-C), 2.78 (1H, dt, J = 5, 2 Hz, proton at 12-C), 3.34 (3H, s, -OCH_3), 3.66 (3H, s, $COOCH_3$), 3.75 (1H, dd, J = 5, 10 Hz, proton at 11-C), 5.33 (1H, m, proton at 10-C), 5.69 (1H, m, proton at 9-C). This compound was found to have the structure (**3**) which is a stereoisomer of (**2**) according to the data by Gardner et al (Gardner *et al.*, 1984). Thus, the molecular structure of the two major fractions, **X** and **Y** were identified as (**2**) and (**3**) respectively. This outcome unambiguously suggested us that the acidic nature of ascorbic acid is largely responsible for the accelerated decomposition of ML-OOH in the reaction conditions concerned. This conclusion was supported by additional facts, i.e., as shown in columns C and D in Fig. 2, an addition of sesamol as a radical scavenger or an addition of phytic acid as an inactivator of metal ions like Fe^{2+} and Cu^+ could not prevent the AA-catalyzed decomposition of ML-OOH. It is known that, in the presence of metal ions such as Fe^{2+}, AA operates as a prooxidant and promotes oxidation of unsaturated lipids via a radical pathway and it is thought that the function of ascorbic acid is to reduce Fe^{3+} to Fe^{2+} in the metal-catalyzed autoxidation (Hochstein and Ernster, 1963, Haase and Dunkley, 1969). Also, sodium ascorbate (column E) which is no more acidic was found to show no effect on the decomposition of ML-OOH.

Relating to the activity of the antioxidants examined here, very similar results were obtained in the aqueous solution, too, i.e., phenolic antioxidants exhibited preventive effect but AA accelerated the decomposition. Additional experiments in aqueous system showed that, at pH 5, AA promoted greatly the decomposition of ML-OOH which disappeared completely after 48 h at 60 °C, while at pH 9, 26% remained undecomposed after the same period. This unambiguously support the conclusion that acidic nature of AA is largely responsible for the accelerated decomposition also in the aqueous system. The present study showed a new aspect of the behavior of antioxidants on the decomposition of lipid hydroperoxides.

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