⁂Studies on the Antioxidants: X. Oxidation Products of Concomitantly Used Butylated Hydroxyanisole and Ethyl Protocatechuate

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ABSTRACT

The effects of antioxidants were studied from the viewpoints of (a) the products generated during the autoxidation process and (b) the functional mechanism of antioxidation. The chemical structure and antioxidant activity of the oxide obtained by irradiating an ethanol solution of an equal mixture of butylated hydroxyanisole (BHA) and ethyl protocatechuate (EP) with the ultraviolet (UV) ray and its secondary decomposition were studied. The oxide was identified as 2,2′-dihydroxy-3-*tert*-butyl-5-methoxy-5′-carboethoxy-diphenyl ether which was a dimer of free radicals generated by BHA and EP. The resulting oxide showed a strong antioxidant activity on lard and methyl oleate in the stability testing by active oxygen method (AOM).

INTRODUCTION

Although antioxidants which are used to prevent autoxidation of fat and oil have been thought to act as the chain reaction terminators, some of the detailed functional mechanisms are not clear. The fate of the antioxidant molecules during the antioxidation process should involve many important factors; however, few studies on these subjects have been reported. Kurechi et al. have studied this subject using butylated hydroxyanisole (BHA) (1,2), ethyl protocatechuate (EP) (3) and propyl gallate (PG) (4), as well as others. Dihydroxy diphenyl compounds such as 2,2′-dihydroxy-5,5′-dimethoxy-3,3′-di-*tert*-butyldiphenyl (2a) and 2′,3-di-*tert*-butyl-2-hydroxy-4′,5-dimethoxy-diphenyl ether (2b) were isolated from BHA, 5′-ethoxy carbonyl-3,3′,4-trihydroxydiphenyl-2,2′-carbolactone (EP_1) was isolated from EP and ellagic acid was isolated from PG as the oxidation products during their autoxidation process. Their chemical structures and antioxidant activities were clarified. Also, the quantitative changes of the compounds were examined during the process.

We have studied the fate of the antioxidants concomitantly used with other antioxidants when they were autoxidized. The autoxidation products obtained by the concomitant use of BHA and EP are described in this report.

When the ethanol solution of a mixture of BHA and EP was irradiated with ultraviolet (UV) ray for oxidation, a dimer was obtained by the dehydrogenation of aromatic hydrogen of position 6 of BHA and dehydrogenation of the OH group of position 2 of EP. The dimer was 2,2′-dihydroxy-3-*tert*-butyl-5-methoxy-5′-carboethoxy-diphenyl ether. This dimer also was formed in the presence of oil under the same conditions. The antioxidation activity of this product was examined on various base oils and it was found to retain the activity. This product was decomposed further by UV irradiation to form mother compounds, i.e., BHA and EP.

This report describes the results of using 2-*tert*-butyl-4-methoxy phenol, the major component of BHA.

EXPERIMENTAL PROCEDURES

Materials

Commercially available reagent grade ethyl protocatechuate (mp 134.5 C) was repeatedly recrystallized from benzene.

Butylated hydroxyanisole (mp 64 C) was obtained as the commercial product "Sustane" (Nikki Universal Co., Tokyo) and was repeatedly recrystallized from petroleum ether. BHA is identical to a single component of 2-*tert*-butyl-4-methoxyphenol (2-BHA). It is a white needle crystal.

Commercial methyl oleate free from any antioxidants (Tokyo Kasei Co., Tokyo) was distilled under reduced pressure (5 mm Hg). Peroxide value (POV): 0 m eq./kg; acid value (A.V.):1.8; and iodine value (I.V.):85.

The lard used was the fat portion obtained right after sacrifice (offered from Teikoku Zohki Pharmaceutical Co., Tokyo) and was boiled and prepared by dissolution method (mp 42 C). The impurities and water component in raw lard were eliminated by repeated dissolution and solidification techniques.

Specially prepared soybean oil was used (offered from Showa Sangyo Co., Tokyo) with A.V. 0.04, I.V. 136 and POV: 0.5 m eq./kg.

The coloration test was carried out with 2,6-dichloroquinone-4-chloroimide ethanol solution.

Assay Methods

Thin layer chromatography (TLC) and column chromatography were used. The TLC plates were prepared with 250-μ-thick layers and were activated by heating at 110 C for 2 hr. The plates with the applied samples were developed in a solvent system of chloroform/methanol/acetic acid (90:2:2 v/v).

The column was prepared by packing silica gel (50 g) into a column with id 3.5 cm so that the total height was adjusted to 12 cm. The eluting solution systems used were benzene and a mixture of benzene and acetone (45:3, v/v).

The nuclear magnetic resonance (NMR) spectrum was determined using Model JAOLPS 100 with $CDCl_3$ as solvent. The chemical shift was determined using tetramethylsilane (Me_4Si) as the internal standard and expressed in ppm. Mass spectrum (MS) and infrared (IR) spectrum were measured using Hitachi Models RMU-7L and 215, respectively.

Procedure

UV irradiation and isolation of oxidation products. One each of BHA and EP was dissolved in 20 ml ethanol in a glass dish 12 cm in diameter and 2 cm deep. The solution was placed in a UV irradiation box and irradiated continuously for 100 hr. The distance between the dish and the light source was 30 cm. Evaporated ethanol was added intermittently. Two separate sample systems of BHA alone and EP alone were irradiated under the same conditions as the controls; the reaction products after the completion of UV irradiation were identified by TLC. Ethanol in solution was removed by distillation under the reduced pressure and the residue was dissolved in a trace amount of benzene; the precipitate was then removed. The benzene solution was

placed on the top of the column and the column was developed until the fractions of 2a, 2b and BHA were eluted and was then developed by benzene/acetone (45:3, v/v). The solvent was removed from each fraction under reduced pressure to isolate the reaction products, which were recrystallized from petroleum ether until white needle crystals (M_2) were obtained. M_2 was acetylated as follows: 10 mg of M_2, 5 ml of pyridine and 2 ml of acetic anhydride were mixed and allowed to stand overnight. The mixture was poured into ice-cold water and extracted by chloroform, then rinsed with hydrochloric acid solution followed by water and recrystallized from petroleum ether.

Formation of oxidation products of BHA and EP in oil. Methyl oleate solutions (0.02%) of BHA and EP were irradiated with UV for 7 days. The products in the reaction solutions were identified by TLC. An ethanol solution of a BHA and EP mixture was irradiated with UV under the same conditions to identify the products by TLC as a control.

Peroxide value (POV). The POV was determined according to the Wheeler method (5).

Antioxidative activity. This was determined by active oxygen method (AOM) as reported in our previous paper (2). BHA, M_2 and EP were added to 20 ml each of methyl oleate, lard and soybean oil at 0.005%, 0.01% and 0.02%, respectively, and were placed in the AOM oxidation test tubes. As the control, 20 ml each of the oils were placed in AOM tubes. All the tubes were set in the AOM apparatus and oxidized by bubbling air with heating. POV was measured on the samples taken at certain intervals and the antioxidative activity was determined by comparison with the standard POV curve. Stability of these sample oils was expressed by the time it takes POV to reach 100 m equiv/kg.

Secondary decomposition of the oxidation product M_2. M_2 (10 mg) was dissolved in 20 ml ethanol and was irradiated with UV for 8 days. Evaporated ethanol was supplemented to keep the solution volume constant. The progress of the reaction was followed by TLC. The same procedures were taken with BHA and EP as controls.

RESULTS AND DISCUSSION

Formation and Isolation of Oxidation Products in Ethanol

Upon irradiating the ethanol solution of BHA and EP with UV rays, the solution color was first changed to light yellow, and then turned yellow and dark brown. The reaction was followed by TLC (Fig. 1 shows the chromatogram).

Three new spots with Rf values 0.43, 0.26 and 0.20 were observed in addition to the known spots, e.g., the starting compounds BHA (Rf: 0.63), EP (Rf: 0.15), BHA oxidation products 2a (Rf: 0.78) and 2b (Rf: 0.87) and EP oxidation product EP_1 (Rf: 0.05). Among the 3 new spots, 2 with Rf values 0.43 (M_1) and 0.26 (M_2) developed color with 2,6-dichloroquinone-4-chloroimide, these were assumed to have an aromatic hydroxide group. M_3 (Rf: 0.20) did not develop color with this reagent.

An ethanol solution of a BHA and EP mixture was irradiated with UV and eluted on silica gel column chromatography as already described. Fractions of 2b, 2a and BHA were eluted by benzene. (These fractions previously showed Rf values 0.87, 0.78 and 0.63, respectively, on the TLC plates.) The fractions with TLC-Rf values 0.43, 0.26 and 0.20 were eluted by benzene/acetone (45:3, v/v). These 3 fractions were repeatedly purified by column chromatography to collect a portion having only Rf value 0.26. This portion was recrystallized from petroleum ether to obtain

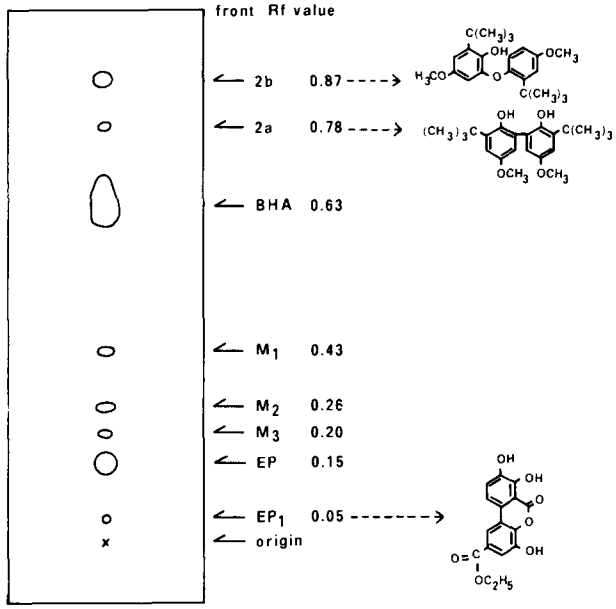


FIG. 1. TLC of BHA, EP and their photodegradation products in ethanol.

white needle crystals which showed the single spot with Rf value 0.26. Because its Rf value agreed with that of a previous M_2 test in TLC, it was confirmed to be one of the oxidation products and it is called M_2 hereafter.

Chemical Structure of Oxidation Product

M_2 is a white needle crystal with mp 140 C. It is soluble in ethanol, chloroform and benzene, but insoluble in water. Its molecular weight (MW) is shown as 360 by MS (theoretical value: 360.43). The molecular formula was agreed upon as $C_{20}H_{24}O_6$. Acetylated M_2 is a white crystal and is soluble in many solvents, e.g., benzene. Melting point is 130 C and MW is 444. MS of acetylated M_2 showed that it has 2 hydroxyl groups. In order to speculate the basic skeleton of this compound, IR and NMR spectra of the starting compounds, EP and BHA, were compared with those of M_2.

IR spectrum (Fig. 2) of M_2 shows a peak at 3450 cm^{-1} (which is assumed to result from an OH group) and other peaks at 1700 cm^{-1} and 1300 cm^{-1} which were observed in EP but not in BHA and are assumed to result from the ester portion. NMR spectrum of M_2 (Fig. 3) showed the triplet at 1.30 ppm (J = 7 Hz) and quadlet at 4.31 ppm (2 H, J = 7 Hz). The presence of an EP-origin $COOC_2H_5$ group is therefore recognized and the presence of BHA-origin $C(CH_3)_3$ and OCH_3 groups is recognized by the peaks at 1.45 ppm (9 H) and 3.64 ppm (3 H), respectively. In the nuclear protons, 2 doublets showing m-coupling were observed at 6.23 ppm (1 H, J = 3 Hz) and 6.65 ppm (1 H, J = 3 Hz). The doublets are derived from the hydrogens at positions 3 and 5 of BHA. Two doublets were observed at 7.75 ppm (1 H, J = 3 Hz) and 7.56 ppm (1 H, J = 3Hz) suggesting m-coupling. These are derived from the hydrogens of positions 3 and 5 of EP, respectively. One doublet was observed at 7.05 ppm (1 H, J = 8 Hz) which showed o-coupling. This is derived from the hydrogen of position 2 of EP. Figure 4 shows the NMR spectrum of acetylated M_2.

No changes were observed in the signals of $COOC_2H_5$, $C(CH_3)_3$ and OCH_3 groups. In the nuclear protons, however, a signal at 7.05 ppm (1 H, J = 8 Hz) is shifted by 0.1 ppm. From this it is considered that the OH group of posi-

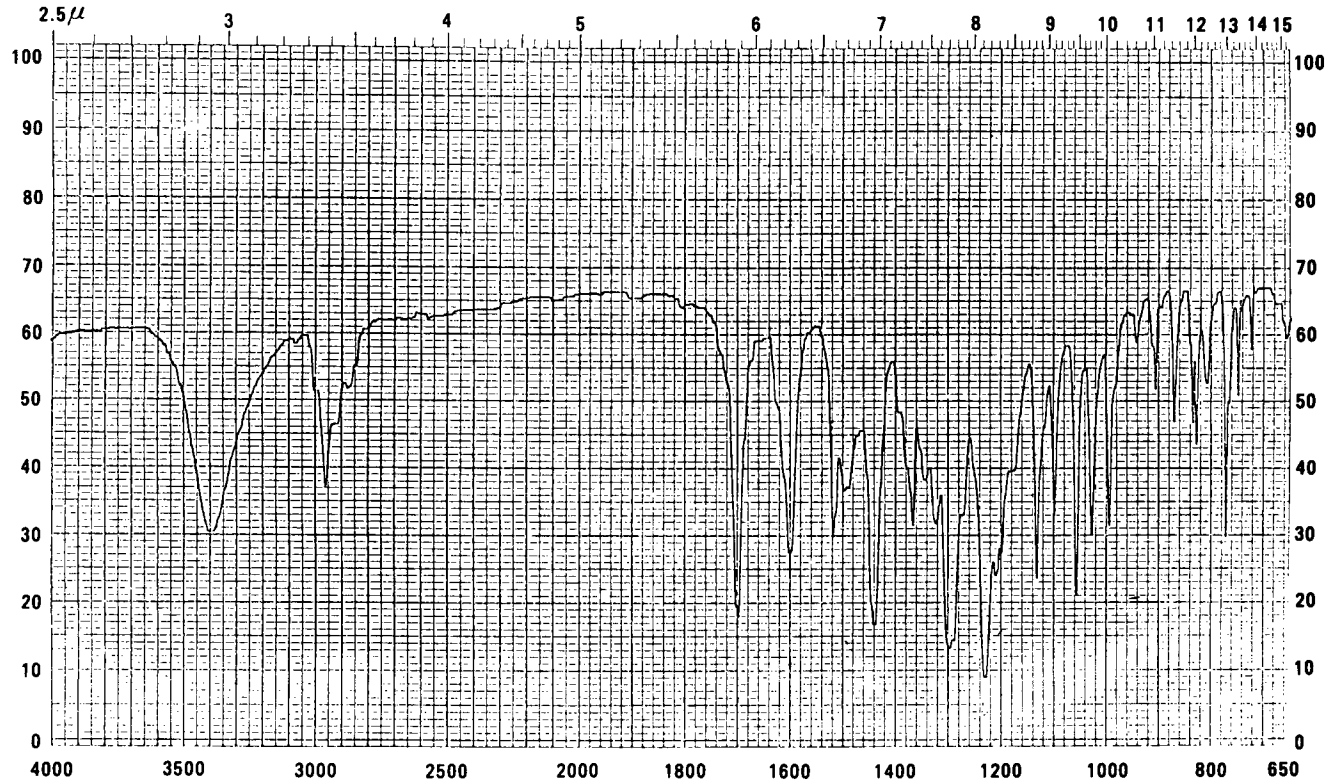


FIG. 2. IR spectrum of M_2.

tion 1 was acetylated, because when the OH group of position 2′ was acetylated among 2 OH groups of position 2 of EP-origin, 2 signals at 7.75 and 7.56 ppm showing the m-coupling should be shifted; actually, o-coupling shown by 7.05 ppm (1 H, J = 8 Hz) was shifted.

As is obvious from IR, MS and NMR spectra, the oxidation product M_2 is identified as 2,2′-dihydroxy-3-*tert*-butyl-5-methoxy-5′-carboethoxy-diphenyl ether with the chemical structure shown in Scheme I.

Reviewing the structure, M_2 was considered to be the dimer formed by etherification after dehydrogenation of the aromatic hydrogen of position 6 of BHA and dehydrogenation of the OH group of position 2 of EP – the same as the oxidation product of BHA (1) which was reported previously.

It is notable that the dimerization between the free radicals of different kinds of antioxidants resulted in oxidation products in addition to the formation of oxidation products between the radicals derived from the same antioxidants.

Formation of Oxidation Products in Methyl Oleate

Two spots were observed on TLC plates which have Rf values 0.43 and 0.26 in addition to BHA, EP and their oxidation products when methyl oleate solution of a BHA and EP mixture was irradiated with UV. Because the Rf values of these spots were the same as those of the oxidation products formed in the ethanol solution, similar oxidation

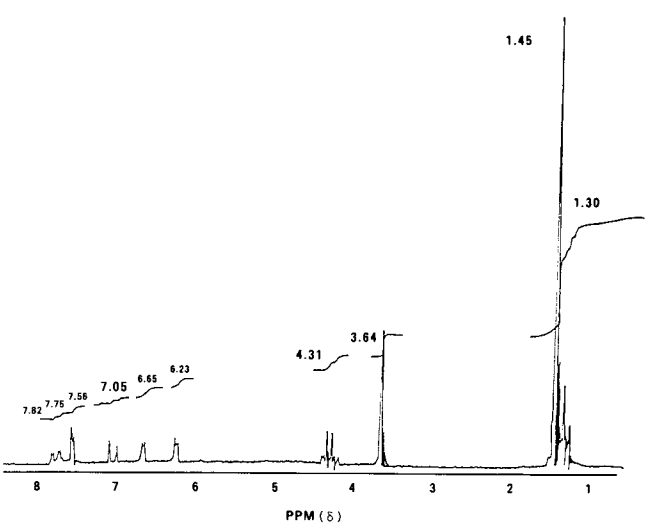


FIG. 3. NMR spectrum (60 MHz) of M_2.

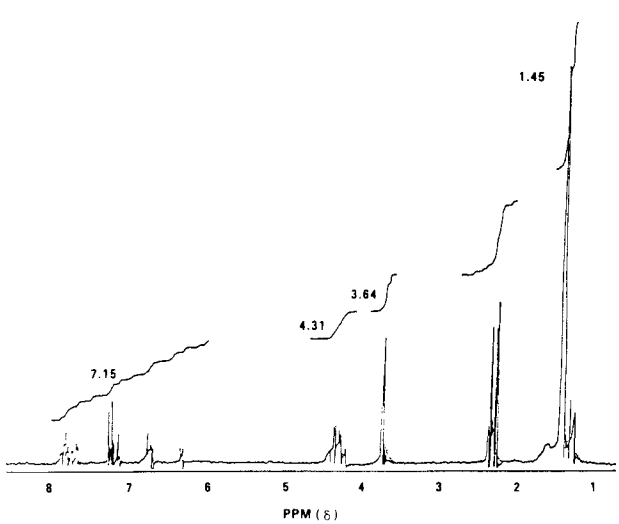
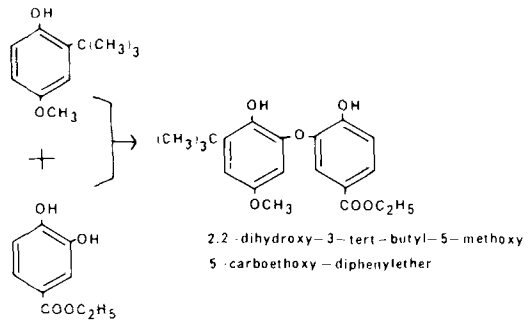


FIG. 4. NMR spectrum (60 MHz) of M_2-Ac.



SCHEME I. Structure of oxidation product (M_2).

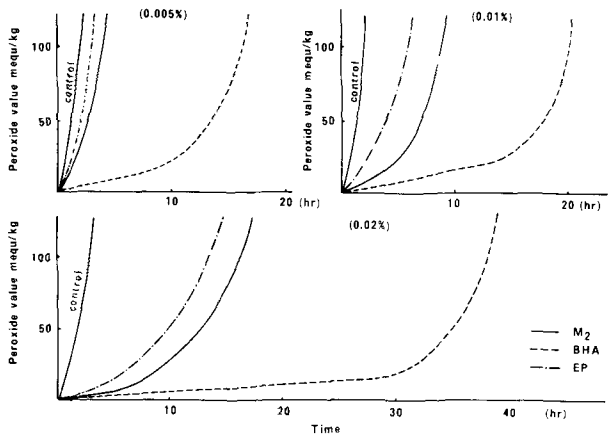


FIG. 5. Antioxidative effect of oxidation product (M_2), BHA and EP on methyl oleate.

products were confirmed to be produced in oils. It also was confirmed that M_2 with Rf value 0.26 formed in methyl oleate was identical to the purified material by TLC.

Antioxidative Activity of Oxidation Product

The antioxidative activity of an oxidation product, M_2, is shown by active oxygen method (AOM) in various base oils.

Antioxidative activity on methyl oleate. Figure 5 shows the change in POV after adding BHA, EP and M_2 at 0.005%, 0.01% and 0.02%, respectively, to methyl oleate.

As is obvious from the POV curves, M_2 showed its antioxidative activity on methyl oleate. The activity was increased in proportion to its concentration and was greater than that of EP. Independent of the concentration, the order of the activity was: BHA, M_2, EP.

Antioxidative activity on lard. Figure 6 shows the change in POV after adding BHA, EP and M_2 at 0.005%, 0.01% and 0.02%, to lard.

From the POV curves, it is obvious that all these compounds significantly stabilized lard when it was tested by AOM. The order of the activity was the same as in tests with methyl oleate.

Antioxidative activity on soybean oil. All BHA, EP and M_2 added at 0.005% to soybean oil showed almost the same behavior as the control. At this concentration, these compounds did not develop their antioxidative activities on soybean oil. The induction period was not significantly

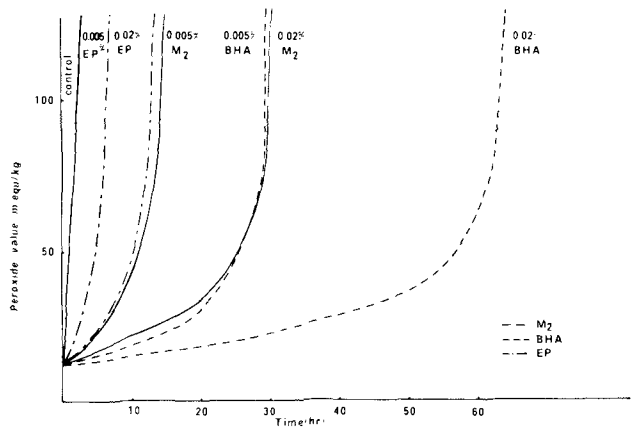


FIG. 6. Antioxidative effect of oxidation product (M_2), BHA and EP on lard.

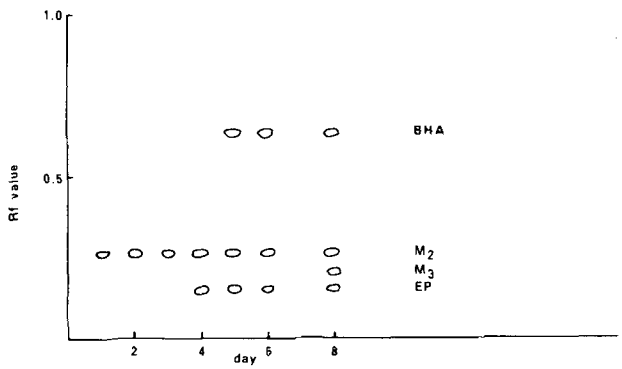


FIG. 7. TLC of oxidation product of M_2 in ethanol under UV light (3050 Å).

extended by these 3 compounds at the 0.02% level.

The soybean oil test showed that the oxidation products formed by ineffective antioxidants on the base oil have any of the same antioxidative activities as their base antioxidants.

Secondary Decomposition of Oxidation Product M_2

Photosensitivity was studied for the oxidation products formed from various kinds of antioxidants. Figure 7 shows the chromatogram of M_2 ethanol solution irradiated with UV.

An EP spot on the TLC plate was observed on the fourth day; a BHA spot and a yellow spot also were observed on the 5th day and 8th days, respectively. This suggests that the oxidation product M_2 was secondarily decomposed to form BHA and EP by the photo-irradiation. The antioxidant molecules behaved in a complex manner.

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