Studies on the Antioxidants XVII: Photooxidation Products of Concomitantly Used Butylated Hydroxyanisole and Propyl Gallate

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ABSTRACT

The effects of antioxidants were studied with a veiw to pursuing the fate of antioxidant molecules during the antioxidation processes. In this study, with the same view, an equal mixture of butylated hydroxyanisole (BHA) and propyl gallate (PG) in ethanol was irradiated with ultraviolet rays and the chemical structure and antioxidative activities of the resulting oxides were examined. The resulting oxides were identified as 2,3,7,8-tetrahydroxy [1] benzopyrano [5,4,3-cde] [1] benzopyran-5,10-dione (ellagic acid), which was formed from PG by coupling of dehydrogenated molecules of OH group and by dealcoholization, and propyl 3,4-dihydroxy-5-(2′-hydroxy-5′-methoxy-3′-*tert*-butylphenoxy)benzoate and propyl 3,5-dihydroxy-4-(2′-hydroxy-5′-methoxy-3′-*tert*-butylphenoxy) benzoate, both of which were dimers, one formed from a BHA/PG mixture by separation of hydrogen in aromatic hydrogen and the other from PG by dehydrogenation of OH group. All of the resulting oxides showed antioxidative activity on lard, soybean oil, and methyl oleate in the stability testing by the active oxygen method (AOM).

INTRODUCTION

A series of studies have been done by the authors to pursue the fates of antioxidant molecules during the antioxidation process. So far, the chemical structures and antioxidative activities of the resulting oxides obtained by irradiating an ethanol or benzene solution of butylated hydroxyanisole (BHA) (1-3), ethyl protocatechuate (EP) (4), and an equal mixture of BHA and BHT (5), BHA and EP (6) with the ultraviolet (UV) ray have been studied. This time, using propyl gallate (PG), similar experiments were carried out, this time using PG alone and concomitantly using PG and BHA.

When the ethanol solutions of PG alone and an equal mixture of BHA and PG were irradiated with UV ray for oxidation, some oxidation products were formed. They were identified as 2,3,7,8-tetrahydroxy [1] benzopyrano [5,4,3-cde] [1] benzopyran-5,10-dione (ellagic acid) formed from PG, and propyl 3,4-dihydroxy-5-(2′-hydroxy-5′-methoxy-3′-*tert*-butylphenoxy) benzoate and propyl 3,5-dihydroxy-4-(2′-hydroxy-5′-methoxy-3′-*tert*-butylphenoxy) benzoate from BHA and PG mixture. It was also confirmed that these oxidation products were formed in the presence of oil under the same conditions. When the antioxidative activities of these products were examined, on various basic oils, it was proved that they still retained their antioxidative activities.

EXPERIMENTAL PROCEDURES

Materials

The BHA used was 2-*tert*-butyl-4-methoxyphenol (mp 64 C) which was obtained by repeatedly recrystallizing commercial product Sustane (Nikki Universal Co., Tokyo) from petroleum ether.

The PG used was obtained by repeatedly recrystallizing commercial product *n*-propyl gallate (Tokyo Kasei Co., Tokyo) from water and eliminating crystallizing water through heating at 105 C for 3 hr.

The ellagic acid was obtained by repeatedly recrystallizing synthetic product, which was prepared by potassium persulfate oxidation of gallic acid (7), from pyridine.

Commercial methyl oleate free from any antioxidants (Tokyo Kasei Co., Tokyo) was distilled under reduced pressure (5 mm Hg). Peroxide value (POV): O meq/kg; acid value (AV): 0.1; and iodine value (IV): 90. Soybean oil free from any antioxidants (Showa Sangyo Co., Tokyo) was used. POV: O meq/kg; AV: 0.1; IV: 134. The lard used was prepared by the same method as reported in our previous paper (6). POV: 2 meq/kg. The coloration test was carried out with 2,6-dichloroquinone-4-chloroimide ethanol solution.

Assay Methods

Thin layer chromatography (TLC) was carried out using a silica gel plate with 0.25 mm-thick layer. The plates with the applied samples were developed in a solvent system of chloroform/methanol/acetic acid (90:4:4, v/v).

Paper chromatography was carried out using Whatman No. 1 paper. The papers with the applied samples were developed in a solvent system of 1-butanol/acetic acid/water (4:1:5, v/v).

Dry column chromatography was carried out using a column with 3.5-cm id and packed with 100 g of 100-mesh silica gel (Kanto Kagaku Co., Tokyo). The column was eluted by benzene and benzene/acetone (95:5, and 90:10, v/v).

The nuclear magnetic resonance (NMR) spectrum was measured by a Nihondenshi Model PS-100 with DMSO-d_6 and $CDCl_3$ as solvent. The chemical shift was determined using tetramethylsilane (Me_4Si) as the internal standard, and was expressed in ppm.

The mass spectrum (MS) was measured by a Hitachi mass spectrometer Model RMU-7L. The infrared (IR) spectrum was measured using Hitachi IR spectrometer Model 285.

Procedure

UV irradiation and isolation of oxidation products. 5 g of PG alone and a mixture of each 5 g of BHA and PG were dissolved separately, each in 100-mL ethanol in a glass dish 12 cm in diameter and 3 cm deep. Each solution was placed in an UV irradiation box and irradiated continuously for 240 hr. The distance between the dish and the light source was 30 cm. Evaporized solvent was added intermittently.

The precipitate formed in the PG ethanol photoxidized solution was removed to isolate the reacted products, which were recrystallized from pyridine until yellow needle crystals (G-1) were obtained.

For the BHA-PG ethanol photoxidized solution, the same procedure was taken until the yellow crystals were obtained as in the case of PG ethanol solution. After these

crystals were removed, ethanol in solution was removed by distillation under reduced pressure. The residue was dissolved in 100 mL of benzene, the precipitate was then removed. With 10 g of Celite added, benzene in solution was removed by distillation under reduced pressure and applied to dry column chromatography, then eluted by 400 mL of benzene, 300 mL of benzene/acetone (95:5, v/v) and 400 mL of benzene/acetone (90:10, v/v) in this order. Each fraction was identified by TLC and the fractions which contained the same oxidation products were put together. The solvent was removed from each fraction to isolate oxidation products, which were purified by repeated column chromatography. Then, the refined oxidation products were recrystallized from 50% ethanol until white crystals (AG-I and AG-II) were obtained. AG-I and AG-II were acetylated as follows: 20 mg each of AG-I and AG-II, 10 mL of pyridine, 4 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 10% hydrochloric acid, 5% sodium carbonate solution and water in this sequence and recrystallized from petroleum ether.

Formation of oxidation products of BHA and PG in oil. BHA and PG of each 20 mg were dissolved in 20 mL of methyl oleate and the solution was continuously irradiated with UV for 240 hr. The products in the reaction solution were identified by TLC. An ethanol solution of BHA and PG mixture was irradiated with UV under the same condition to identify the products by TLC as a control.

Peroxide value (POV). The POV was determined according to the Wheeler method (8).

Antioxidative activity. This was determined by the active oxygen method (AOM) as reported in our previous paper (2). G-I, AG-I, AG-II, BHA and PG were added to 20 mL each of methyl oleate, lard, and soybean oil at 0.01% each and were placed in the AOM oxidation test tubes. As a control, 20 mL each of the oils were placed in AOM oxidation test 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 as the time it takes POV to reach 100 meq/kg.

RESULTS AND DISCUSSION

Formation and Isolation of Oxidation Products in Ethanol

Upon irradiating the ethanol solution of PG with UV rays, the colorless solution changed to a light yellow color, and some insoluble products were formed. The precipitate was removed to isolate the reacted products, which were recrystallized from pyridine. Then rinsed with ether, it was left to be dried at 120 C for 2 hr and light yellow needle crystals were obtained.

An ethanol solution of an equal mixture of BHA and PG was irradiated with UV. The reaction was followed by TLC to identify the products. As shown in Figure 1, in addition to the known spots, e.g., the starting compounds BHA (Rf 0.68) and PG (Rf 0.22), BHA oxidation products 2a (Rf 0.8) and 2b (Rf 0.88) and PG oxidation product G-I (Rf 0), two new spots (AG-I and AG-II) were observed with Rf value of 0.58 and 0.49, respectively. As both AG-I (Rf 0.58) and AG-II (Rf 0.49) developed color with 2,6-dichloroquinone-4-chloroimide, these were assumed to have a phenolic hydroxide group.

Irradiated with UV rays, the ethanol solution of BHA and PG was eluted on silica gel dry column chromatography. Fractions of 2b, 2a and BHA were eluted by benzene, then

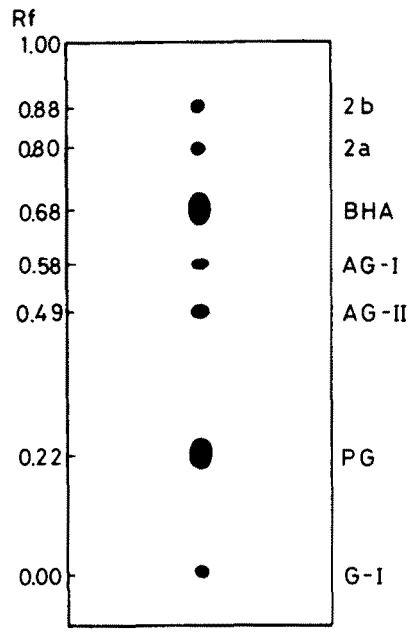


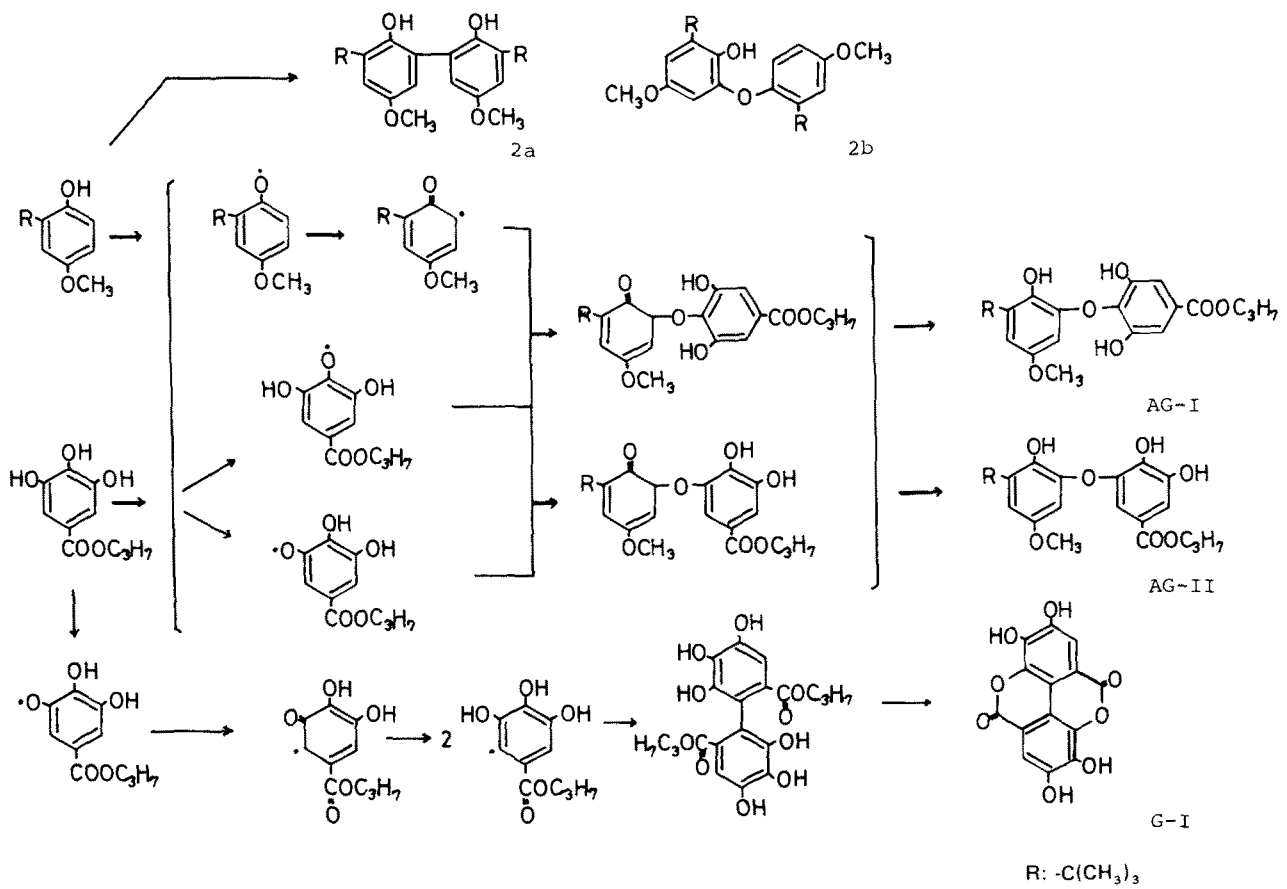
FIG. 1. TLC of photooxidation products of BHA-PG mixture in ethanol under irradiation with UV light. Solvent: $CHCl_3$-MeOH-AcOH (90:4:4).

fraction of AG-I was eluted by benzene/acetone (95:5, v/v), followed by that of AG-II by benzene/acetone (90:10, v/v). The last two fractions were repeatedly purified by column chromatography to collect portions of having Rf values of 0.58 and 0.49 only. Each of these portions was recrystallized from 50% ethanol to obtain white needle crystals AG-I and AG-II, respectively.

Chemical Structure of Oxidation Products

Product G-I is light yellow needle crystal with mp over 360 C. It is insoluble in water and many organic solvents. It is soluble very little in ethanol and slightly soluble in heated pyridine. The mass spectrometry (MS) showed the molecular ion peak at 302 m/e. The molecular formula was agreed upon as $C_{14}H_6O_8$ as derived from the elemental analysis. IR spectrum of G-I shows a peak at 3470 cm^{-1}, which is assumed to result from an OH group and at 1720 cm^{-1}, which is assumed to result from unsaturated lactone. Its UV spectrum showed peaks at 255 and 365 nm. UVλ_{max}^{EtOH} nm (log ϵ) is 255 (4.68) and 365 (3.99). As G-I is insoluble in most of organic solvents and water, its NMR spectrum could not be measured. This compound had a single spot on the paper chromatogram with Rf value 0.33 by the solvent system of 1-butanol/acetic acid/water (4:1:5, v/v). These data agreed closely with those of natural and synthetic ellagic acid obtained by Jurd (9,10) and Burke et al (11). Thus G-I was identified as 2,3,7,8-tetrahydroxy [1] benzopyrano [5,4,3-cde] [1] benzopyran-5,10-dione. As shown in Scheme 1, the formation process can be explained thus: first by dehydrogenation of OH group of PG, coupling of particles occurred, then through dealcoholization, products were formed. It is interesting to note that G-I obtained as an oxidation product of PG is a substance that exists in the nature.

AG-I is a white crystal with mp 197 C. It is soluble in ethanol, chloroform, benzene and acetone, but insoluble in water. The mass spectrometry showed the molecular ion peak at 390 m/e. The molecular formula was agreed upon as $C_{21}H_{26}O_7$ as derived from the elemental analysis. In order to speculate the basic skeleton of this compound, IR



SCHEME 1. Formation of AG-I, AG-II and G-I.

and NMR spectra of the starting compounds, BHA and PG were compared with that of AG-I.

IR spectrum of AG-I shows absorption as observed in each one of BHA and PG, at 3420 cm^{-1}, 2970 cm^{-1} and 1695 cm^{-1} which are assumed to result from OH group, OCH_3 group and C=O group, respectively. (Analysis data of acetylated AG-I showed that it has 3 hydroxyl groups.) NMR spectrum of AG-I (Fig. 2) showed the signals at 1.39 ppm (9H, s, $-C(CH_3)_3$) and 3.52 ppm (3H, s, $-OCH_3$), which are assumed to result from *tert*-butyl group and OCH_3 group, respectively. The presence of two ethylene groups and a methyl group assumed to result from *n*-propyl group was recognized by the signals at 1.65 ppm (2H, t, $-CH_2\underline{CH}_2-CH_3$), 4.18 ppm (2H, t, $-\underline{CH}_2CH_2CH_3$) and 0.97 ppm (3H, q, $-CH_2CH_2\underline{CH}_3$), respectively. It was proved that signals at 8.05 ppm (1H, s, -OH) and 9.78 ppm (2H, s, -OH) were due to the presence of the OH group, because they disappeared when they were treated with deuterium. In the nuclear proton field, 2 doublets showing *m*-coupling were observed at 5.91 ppm (1H, J = 3 Hz) and 6.38 ppm (1H, J = 3 Hz). The doublets can be attributed to the hydrogens of positions 4′ and 6′ of BHA, respectively. A singlet showing protons of equal value was observed at 7.08 ppm (2H). This can be attributed to the hydrogens of positions 2 and 6 of PG. As is obvious from IR, MS and NMR spectra, the oxidation product AG-I is identified as propyl 3,5-dihydroxy-4-(2′-hydroxy-5′-methoxy-3′-*tert*-butylphenoxy) benzoate with the chemical structure shown in Scheme 1.

AG-II is a white crystal with mp 89 C. It is soluble in ethanol, chloroform, benzene and acetone, but insoluble in water. The mass spectrometry showed the molecular ion peak at 390 m/e. The molecular formula was agreed upon as $C_{21}H_{26}O_7$ as derived from the elemental analysis. NMR spectrum of AG-II (Fig. 2) showed signals at 1.36 ppm (9H, s, $-C(CH_3)_3$) and 3.56 ppm (3H, s, $-OCH_3$), which can be attributed to *tert*-butyl group and methoxyl group, respectively. The presence of two methylene groups and a methyl group of propyl-origin was recognized by the signals at 1.66 ppm (2H, t, $-CH_2\underline{CH}_2CH_3$), 4.10 ppm (2H, t, $-\underline{CH}_2CH_2CH_3$) and 0.90 ppm (3H, q, $-CH_2CH_2\underline{CH}_3$), respectively. In the nuclear protons field, 2 doublets showing *m*-coupling were observed at 6.19 ppm (1H, J = 3 Hz) and 6.46 ppm (1H, J = 3 Hz). The doublets can be attributed to the hydrogens at positions of 4′ and 6′ of BHA, respectively. Two doublets showing *m*-coupling were also observed at 6.91 ppm (1H, J = 3 Hz) and 7.21 ppm (1H, J = 3 Hz), which can be attributed to the hydrogens at positions of 2 and 6 of PG, respectively. As is obvious from these spectra data, the oxidation product AG-II was identified as propyl 3,4-dihydroxy-5-(2′-hydroxy-5′-methoxy-3′-*tert*-butylphenoxy) benzoate with the chemical structure shown in Scheme 1.

Reviewing the structures of oxidation products AG-I and AG-II obtained from the mixture of BHA and PG, both these products were considered to be, as shown in Scheme 1, the dimers formed by etherification after dehydrogenation of the aromatic hydrogen adjacent to the OH group of BHA and dehydrogenation of the OH group of position 3 or 4 of PG. It is notable that, as with the result reported in our previous paper, the dimerization between the free radicals of different kinds of antioxidants resulted in oxidation

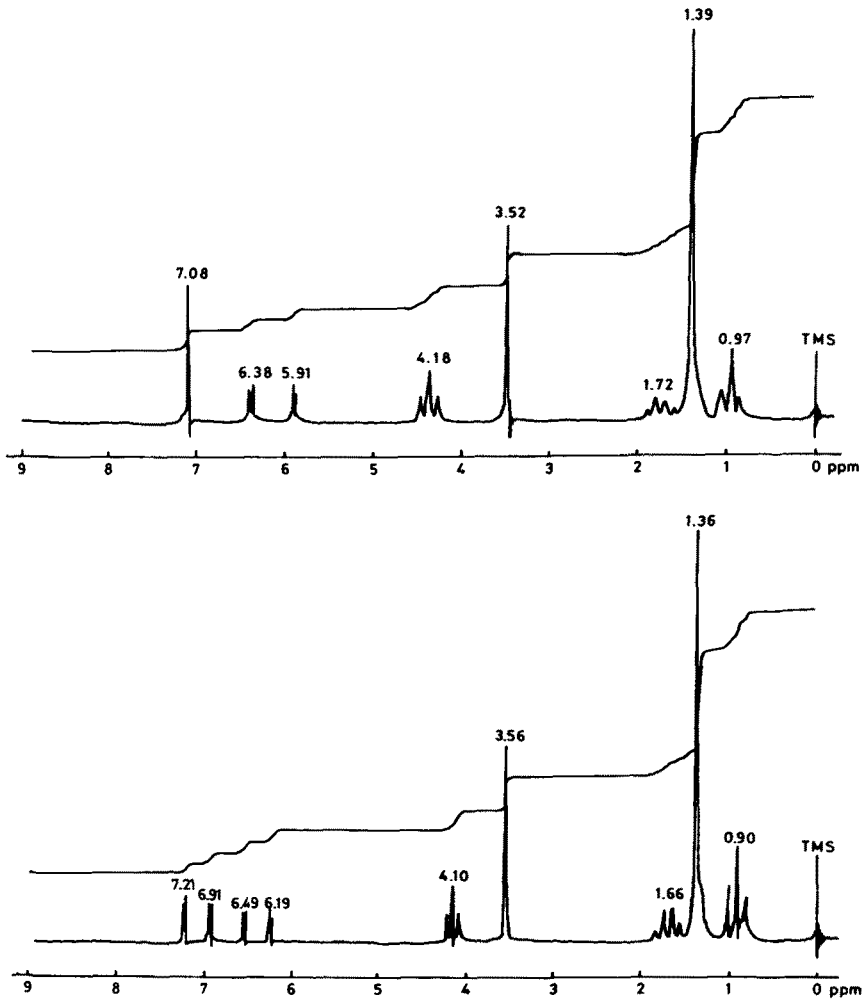


FIG. 2. NMR spectra of AG-I and AG-II in DMSO-d_6.

products in addition to the formation of oxidation products between the radicals derived from the same antioxidants. The formation process of G-I, AG-I and AG-II gives an indication on the fate of active radicals generated from the antioxidant molecules during the autoxidation processes.

Formation of Oxidation Products of BHA and PG in Oil

Two spots were observed on TLC plates which have Rf values of 0.49 and 0.58 in addition to oxidation products of BHA and PG, when methyl oleate solution of a BHA and PG mixture was irradiated with UV. Through the identification of TLC plate, these were confirmed to be AG-I and AG-II, identical to the oxidation products formed in ethanol solution. It was confirmed, therefore, that oxidation products were produced from a BHA and PG mixture in oil like in ethanol solution.

Antioxidative Activity of Oxidation Products

The antioxidative activity of an oxidation product G-I was measured by active oxygen method (AOM) in various oils.

Figure 3 shows the change in POV after adding G-I and PG at 0.01% each to methyl oleate. As is obvious from the POV curves, G-I was confirmed to have its antioxidative activity on methyl oleate, though it is weaker than that of its base compound, PG.

Antioxidative activities of AG-I and AG-II measured by

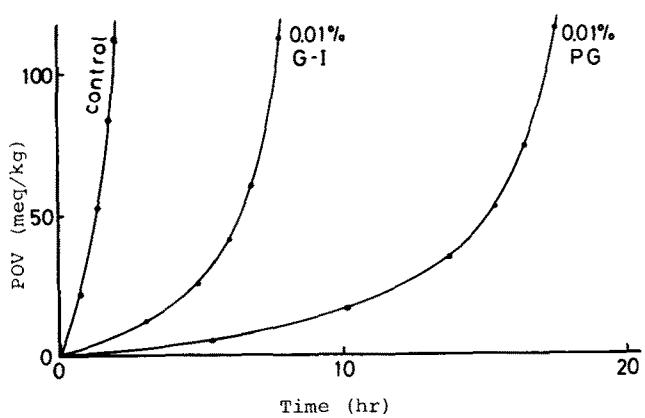
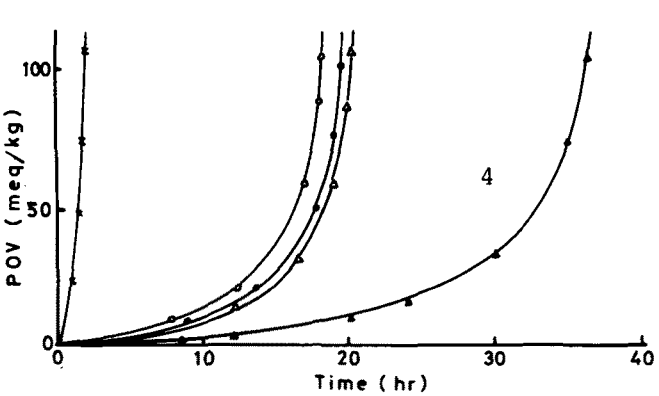


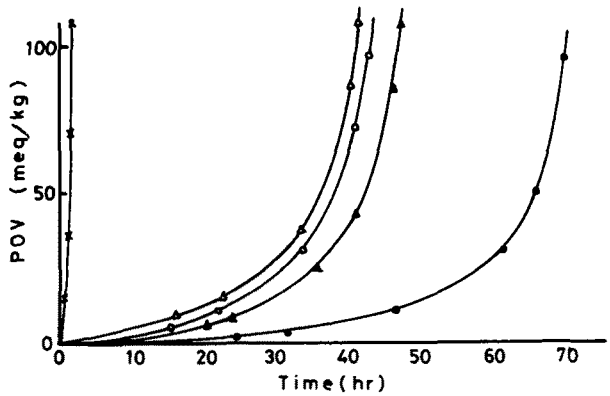
FIG. 3. Antioxidative effect of PG and its oxidation product on methyl oleate.

AOM in methyl oleate, lard and soybean oil were as follows:

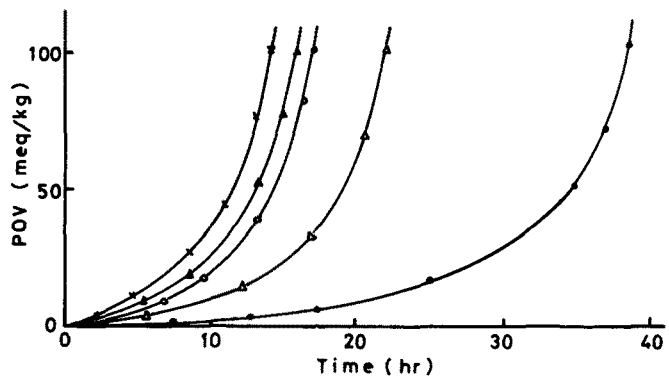
Antioxidative activity on methyl oleate. From the POV changes (Fig. 4a) after AG-I, AG-II, BHA and PG being added at 0.01% each to methyl oleate, either of AG-I and



(a)



(b)



(c)

Antioxidation ratio

Compound	Methyl oleate	Lard	Soybean oil
AG-I	0.53	0.88	4.00
AG-II	0.47	0.91	1.50
BHA	1.00	1.00	1.00
PG	0.52	1.50	12.25

(d)

FIG. 4. Antioxidative effect of BHA, PG and their oxidation products on different fats and oils. Concentration: 0.01%.

(a) Methyl oleate; (b) Lard; (c) Soybean oil; (d) Antioxidation ratio. —△—: AG-I; —○—: AG-II; —●—: PG; —▲—: BHA; —X—: control.

AG-II was confirmed to have antioxidative activity on methyl oleate. The order of antioxidative activity on methyl oleate, observed from POV change, was this: BHA > AG-I > PG > AG-II.

Antioxidative activity on lard. Figure 4b shows the change in POV after adding AG-I, AG-II, BHA and PG at 0.01% each to lard. From the POV curves, AG-I and AG-II were confirmed to have antioxidative activity on lard, too. The order of antioxidative activity is this: PG > BHA > AG-II > AG-I.

Antioxidative activity on soybean oil. By the change in POV after adding AG-I, AG-II, BHA and PG at 0.01% each to soybean oil, AG-I showed its antioxidative activity on soybean oil, but AG-II, like BHA, showed little antioxidative activity on soybean oil. The order of the antioxidative activity is this: PG > AG-I > AG-II ≥ BHA.

From the results mentioned above, it was confirmed that G-I increases the stability of methyl oleate over AOM, and AG-II and AG-II increase that of three base oils, and that all of these three have antioxidative activity. Figure 4 shows the antioxidative activity rates of them. Both AG-I and AG-II developed remarkable antioxidative activity on methyl oleate, and the rate of the activity was almost as same as that of PG. It is interesting that both AG-I and AG-II showed higher antioxidative effect on soybean oil than BHA.

It was confirmed that two new kinds of oxidation products were formed from different radicals of BHA and PG, in addition to 2a and 2b from BHA, and G-I (ellagic acid) from PG, when BHA and PG were concomitantly used.

All of these five oxidation products, except G-I, were proved to contribute to prevention of oxidation in three base oils. These results are interesting enough to note, because they seem to have some relation to the evaluation of synergism of antioxidatns when they are concomitantly used.

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