⁂Studies on the Antioxidants XVIII: Oxidation Product of Tertiary Butyl Hydroquinone (TBHQ) (I)

T. KURECHI, M. AIZAWA and A. KUNUGI, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo, 192-03, Japan

ABSTRACT

To explain the mechanism of antioxidants, it is important to know their chemical changes during the process of their prevention of autoxidation. Therefore, we have carried out a series of studies on these changes. In this study, the chemical structures and antioxidative activities of the oxides isolated by irradiation with ultraviolet rays were examined by using tertiary butyl hydroquinone (TBHQ). Two of the isolated products were identified as follows: one was a golden needle crystal, 2-*tert*-butyl-*p*-benzoquinone, and the other was a white prism-like crystal, 2,2-dimethyl-5-hydroxy-2,3-dihydro-benzo(b)furan, which was formed by dehydrogenation of the OH group and the methyl group in the rearranged *tert*-butyl group at the *ortho* position, so that furan rings were formed in the molecules. When the stability of these two products was tested on three different basic oils by the active oxygen method (AOM), quinone did not show any antioxidative activity, but the benzofuran derivative showed a strong antioxidative activity on lard and methyl oleate.

INTRODUCTION

The chemical change of many organic compounds like oils through natural oxidation in the air is known as autoxidation, and this reaction can be explained as a chain reaction of free radicals. Although antioxidants used to prevent autoxidation of fat and oil have been thought to have the function of stopping the chain reaction by free radicals, the details are left to be clarified. Particularly, the antioxidant molecules during the antioxidation process are thought to involve many factors which are important in clarifying the reaction process and antioxidative effect of antioxidants; however, few studies on these subjects have been reported. Kurechi et al. have already isolated some oxidation products from several antioxidants and clarified their structures and chemical activities (1-5).

It was clarified in those studies that the formation of different kinds of oxidation products from antioxidants depends on the position of dehydrogenation. An antioxidant, tertiary butyl hydroquinone (TBHQ), is quite similar to butylated hydroxyanisole (BHA) in structure. Therefore, it is very interesting to know what compounds will be formed when TBHQ is oxidized during its antioxidative process. The authors have studied TBHQ from this point of view and examined its oxidation products.

Of the oxidation products which were formed when the benzene solution of TBHQ was irradiated with ultarviolet (UV) rays under the same conditions in the previous experiments (2,3), two products were isolated and their structures were identified. One was identified as 2-*tert*-butyl-*p*-benzoquinone (TQ), which was formed immediately after irradiation with UV rays; the other was 2,2-dimethyl-5-hydroxy-2,3-dihydro-benzo(b)furan (ARE).

When the stability of the two products on three base oils was examined by the active oxygen method (AOM), it was confirmed that only ARE still retained antioxidative activity. Moreover, it was proved that both products were formed in the presence of oil under the same conditions.

EXPERIMENTAL PROCEDURES

Materials

Commercially available reagent grade tertiary butyl hydroquinone (mp 127 C) (Wako Junyaku Co., Tokyo) was repeatedly recrystallized from hexane.

Commercially methyl oleate free from any antioxidants (Tokyo Kasei Co., Tokyo) was distilled under reduced pressure (5 mm Hg). POV: 0 meq/kg; AV: 1.8; IV: 90.

Specially prepared soybean oil was used (provided by Showa Sangyo Co., Tokyo) with POV: 0.05 meq/kg; AV: 0.08; IV: 130.

Lard used was prepared by the same method as reported in our previous paper (6).

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

Assay Methods

Thin layer chromatography (TLC). 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 (19:1, v/v).

Column chromatography. The column was prepared by packing 100-mesh silica gel (Kanto Kagaku Co., Tokyo) (20 g) into a column with 3 cm id. The column was eluted with chloroform.

Ultraviolet irradiation box (device A). In a wooden box 55 cm long, 82 cm wide and 35 cm deep inside, three fluorescent lamps (with maximum wavelength of 3050 Å made by National Electric Co.,) were set.

Ultraviolet generator for photochemistry (low-pressure type) (device B). A low-pressure 10 W mercury lamp with the wavelength of 2537-3100 Å was used.

Mass spectrum (MS) and infrared spectrum (IR) were measured using Hitachi Model RMU-7L and 215, respectively.

Nuclear magnetic resonance (NMR) spectrum was measured using JEOL Model PS-100 with $CDCl_3$ as solvent. The chemical shift was determined using tetramethylsilane (Me_4Si) as the internal standard and expressed in ppm.

High performance liquid chromatography (HPLC) was carried out under the following conditions with a Shimadzu Model LC-2: column: Zorbax SIL 4.6 mm x 25 cm; eluant: chloroform; flow rate: 1.0 mL/min; detector: UV 248 nm.

Procedure

UV irradiation and isolation of oxidation products. Eighteen mg of TBHQ was dissolved in 6 mL of benzene in a glass dish with 12 cm id and 1.5 cm deep. This solution was placed in device A and irradiated continuously for 18 hr. The sample solution was 8 mm in depth and the distance between the sample and the light source was set at 25 cm. The temperature within the box was kept between 10 and 20 C. The amount of benzene corresponding to the decrease by evaporation was supplemented intermittently. Another solution of 1.5 g TBHQ in 100 mL of benzene was set in device B and irradiated continuously with UV rays. The products from the irradiated solution were identified by TLC (Fig. 1 (A), (B)). Spot 2, the formation of which was first confirmed by TLC, and spot 3, which developed a bright blue tinge with BQC reagent, were selected as the products to be isolated.

To collect a large quantity of these products, they were

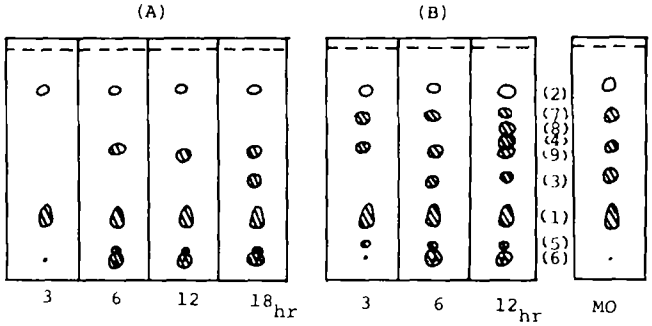


FIG. 1. TLC of oxidation products of TBHQ in benzene and methyl oleate under irradiation with UV light.

irradiated continuously for 6 hr using device B. TBHQ-benzene photooxidized solution was distilled under reduced pressure to remove benzene, and the residue was extracted with three portions of 50 mL hexane. The extracts were combined and filtered, hexane was removed under reduced pressure, and the residue was dissolved in a trace amount of chloroform. The chloroform solution was placed on the top of the column and the column was developed with chloroform. Each fraction was identified by TLC, and the fractions which contained the same products as spots 2 and 3 in Figure 1 were put together. The solvent was removed from each fraction under reduced pressure to isolate the two reaction products, which were purified by repeated column chromatography. Then the refined oxidation products were recrystallized from hexane, until golden needle crystals (TQ) and white prism-like crystals (ARE) were obtained. ARE was acetylated as follows: 8 mg of ARE, 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 with chloroform, then rinsed with 10% hydrochloric acid, 5% sodium carbonate solution and water in this order, and recrystallized from petroleum ether.

Formation of oxidation products of TBHQ in oil. The solution of 18 mg of TBHQ in 6 mL of methyl oleate was irradiated with UV rays in device A continuously for 7 days. After the solution was purified by column chromatography and eluted with chloroform to remove as much methyl oleate as possible, the products in the solution were identified by TLC.

Peroxide value (POV). The POV was measured according to the Wheeler method (7).

Antioxidative activity. The antioxidative activity was measured by the active oxygen method reported in our previous paper (6). Each of ARE, TQ, TBHQ and BHA was added to 20 mL of methyl oleate, lard and soybean oil, each at 0.01%, and was placed in an AOM oxidation test tube. As the control, 20 mL of each oil was placed in an AOM oxidation tube. All the tubes were set in the AOM apparatus and oxidized by bubbling air with heating. The POV was measured on the samples taken at certain intervals, and the antioxidative activity was determined by comparison with the standard POV curve. The stability of these sample oils was expressed by the time it took the POV to reach 100 meq/kg.

Calculation of the residual rate of TQ. TQ was added to methyl oleate at 0.01% and placed in an AOM tube. TQ evaporated from the AOM tube was dropped into 20 mL of ice-cold methanol and measured by HPLC. The residual rate of TQ was calculated from its added quantity.

RESULTS AND DISCUSSION

Formation of Oxidation Products in Benzene

On irradiating the benzene solution of TBHQ with UV rays, the color of the solution increased its yellowish tinge and gave out a characteristic odor. The progress of the reaction was followed by TLC (Fig. 1).

Eight new spots with Rf values of 0.80 (2), 0.42 (3), 0.55 (4), 0.12 (5), 0.09 (6), 0.66 (7), 0.60 (8) and 0.50 (9) were observed in addition to the known spot of the starting compound, TBHQ (Rf 0.25 [1]). All of these spots show the absorption of UV. Spot 2 appeared immediately after irradiation with UV rays and developed no color with BQC reagent. All the other spots developed blue or bluish purple with the same reagent.

Chemical Structure of Oxidation Products

TQ is a golden needle crystal with mp 53.0-54.5 C. It is soluble in ethanol and hexane, but practically insoluble in water. Its molecular weight was shown as 164 by MS spectrum. The molecular formula was agreed on by elemental analysis as $C_{10}H_{12}O_2$. UV spectrum showed the peak at 248 nm. $UV\lambda_{max}^{EtOH}$ nm (ϵ) was 248 (15681). ARE is a white prism-like crystal with mp 103.0-103.9 C. It is soluble in ethanol, hardly soluble in hexane and insoluble in water. Its molecular weight was shown as 164 by MS spectrum. The molecular formula was agreed on by elemental analysis as $C_{10}H_{12}O_2$. UV spectrum showed the peak at 304 nm. $UV\lambda_{max}^{EtOH}$ nm (ϵ) was 304 (4023). From the MS spectrum (m/e: 206; M^+) of acetylated ARE, it was confirmed that this product had an OH group.

To speculate on the basic skeletons of TQ and ARE, the IR and NMR spectra of the starting compound TBHQ were compared with those of TQ and ARE. The IR spectra of both TQ and ARE showed the absorption bands at the normal positions (3000 cm^{-1}, 1600 cm^{-1}, 1480 cm^{-1}), which is thought to result from the aromatics in the starting compound TBHQ.

The absorption at 3400 cm^{-1} observed in the IR spectrum of ARE completely disappeared in that of acetylated ARE (ARE-Ac), and an absorption at 1750 cm^{-1} resulting from the ester group was observed instead. From this fact, the presence of an OH group in ARE was confirmed. On the other hand, in the IR spectrum of TQ, the peaks at 3200 cm^{-1} and 3400 cm^{-1} resulting from the presence of an OH group in TBHQ were not observed, but a peak at 1650 cm^{-1} resulting from the -CO group was observed. This fact suggests that TQ is a quinone.

The NMR spectra (Fig. 2) show that the basic skeletons of both TQ and ARE are similar to that of TBHQ. The NMR spectra of both TBHQ and TQ gave a signal at 1.45 ppm (9H, s) which can be attributed to the *tert*-butyl group, and the NMR spectrum of ARE gave two signals at 1.45 ppm (6H, s) and at 2.98 ppm (2H, s). Therefore, the presence of two methyl groups and an Ar-CH_2 group was recognized. These data suggest that the *tert*-butyl group was changed into equivalent two methyl groups and a methylene group.

Both products gave two broad singlets at 6.58 ppm (1H) and 6.68 ppm (2H) which can be attributed to nuclear protons. This fact shows that TQ and ARE also have the same partial structure as that of the starting compound TBHQ. It was found from the following results that ARE had one OH group in its molecule. The IR spectrum on ARE-Ac did not show an absorption peak at 3400 cm^{-1} due to OH group, but instead it showed one absorption peak at 1750 cm^{-1} due to the -CO group. One OH group was acetylated as was proved by mass spectrum (m/e: 206;

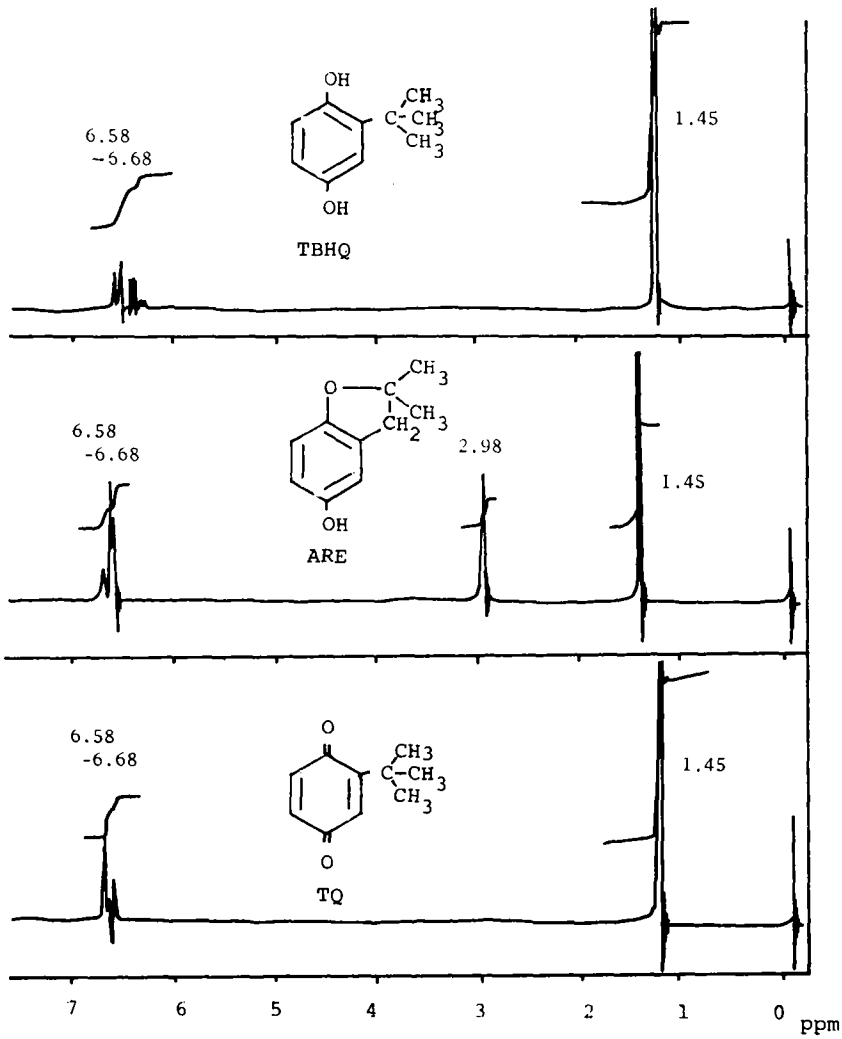
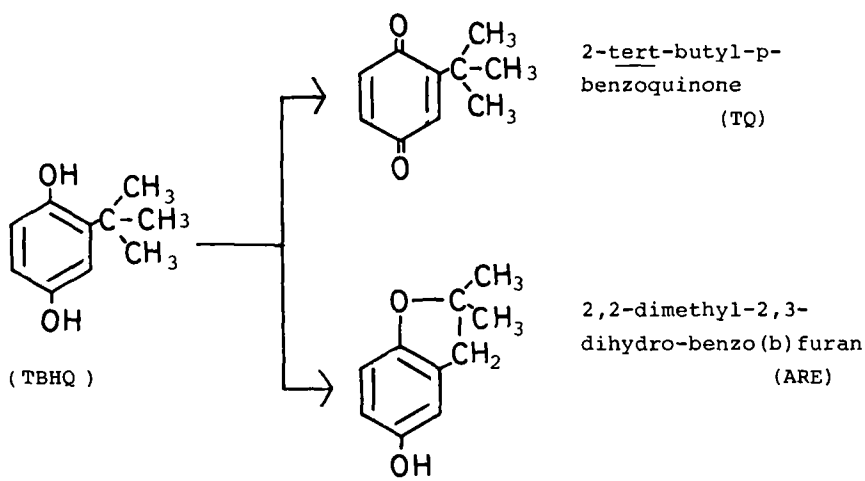


FIG. 2. NMR spectra of TBHQ, ARE and TQ.



SCHEME 1. Structure of oxidation products (ARE and TQ).

M^+). As mentioned before, ARE was considered to have an OH group.

In view of the above data, the oxidation products TQ and ARE were identified as 2-*tert*-butyl-*p*-benzoquinone and 2,2-dimethyl-5-hydroxy-2,3-dihydro-benzo(b)furan, respectively. Their structures are shown in Scheme 1.

In reviewing the structures, it is considered that ARE was formed in the following manner. TQ, formed by dehydrogenation of the OH groups in TBHQ, was n-π^* excited, and then through the intramolecular hydrogen abstraction at the γ-position and the cleavage of the produced intermediates, ARE was formed. Moreover, the structure shown in Scheme 1 can be supported by the general consideration that the NMR signal of Ar-CH_2 appears at 2.5-3.1 ppm and that of Ar-O-CH_2 appears at a lower magnetic field than 3.5 ppm (Fig. 2) (see Scheme 1).

It has been clarified by many reports that antioxidants are dehydrogenated during their antioxidative process to generate free radicals, which eventually dimerize with the same or different species of the free radicals to form the oxidation products. However, it has never been reported that, the *tert*-butyl group like ARE is involved in the reaction that forms oxidation products. From the fact that an oxidation product with a specific structure like ARE was obtained from antioxidant TBHQ, it seems very interesting to study and clarify the cases for BHA and BHT, both of which have similar structures to that of TBHQ.

Formation of Oxidation Products in Methyl Oleate

When the methyl oleate solution of TBHQ was irradiated with UV rays, five spots (spot 1, 2, 3, 4 and 7) were observed by TLC (Fig. 1). As the Rf values of these spots were the same as those of the oxidation products formed in the benzene solution, similar oxidation products were confirmed to be produced in oils. Among them, the spots with the Rf values of 0.42 and 0.80 were confirmed to be identical to the isolated ARE and TQ, respectively, by TLC.

Antioxidative Activity of Oxidation Products

The stability of the two oxidation products, TQ and ARE, against AOM was evaluated from the change of POV (Fig. 3) and the antioxidative ratio (AR) using three kinds of oils (Table I). The antioxidative ratio was calculated

TABLE I

Antioxidative Effect of Oxidation Products of TBHQ on Lard, Soybean Oil and Methyl Oleate

Compound[a]	Lard		Soybean oil		Methyl oleate	
	IP[b]	AR[c]	IP	AR	IP	AR
	hr		hr		hr	
None	5.1		10.3		7.0	
TQ	10.8	0.10	10.8	0.02	8.0	0.05
ARE	35.1	0.51	9.6	-0.03	33.5	1.73
TBHQ	64.0	1.00	35.1	1.00	22.3	1.00
BHA	47.8	0.72	12.5	0.09	38.8	2.08

[a]Concentration: 0.01%.
[b]IP: induction period.
[c]AR: antioxidation ratio = $\frac{IP_{sample}-IP_{control}}{IP_{TBHQ}-IP_{control}}$.

Antioxidative activity
Lard: TBHQ > BHA > ARE > TQ
Soybean oil: TBHQ > BHA > TQ > ARE
Methyl oleate: BHA > ARE > TBHQ > TQ

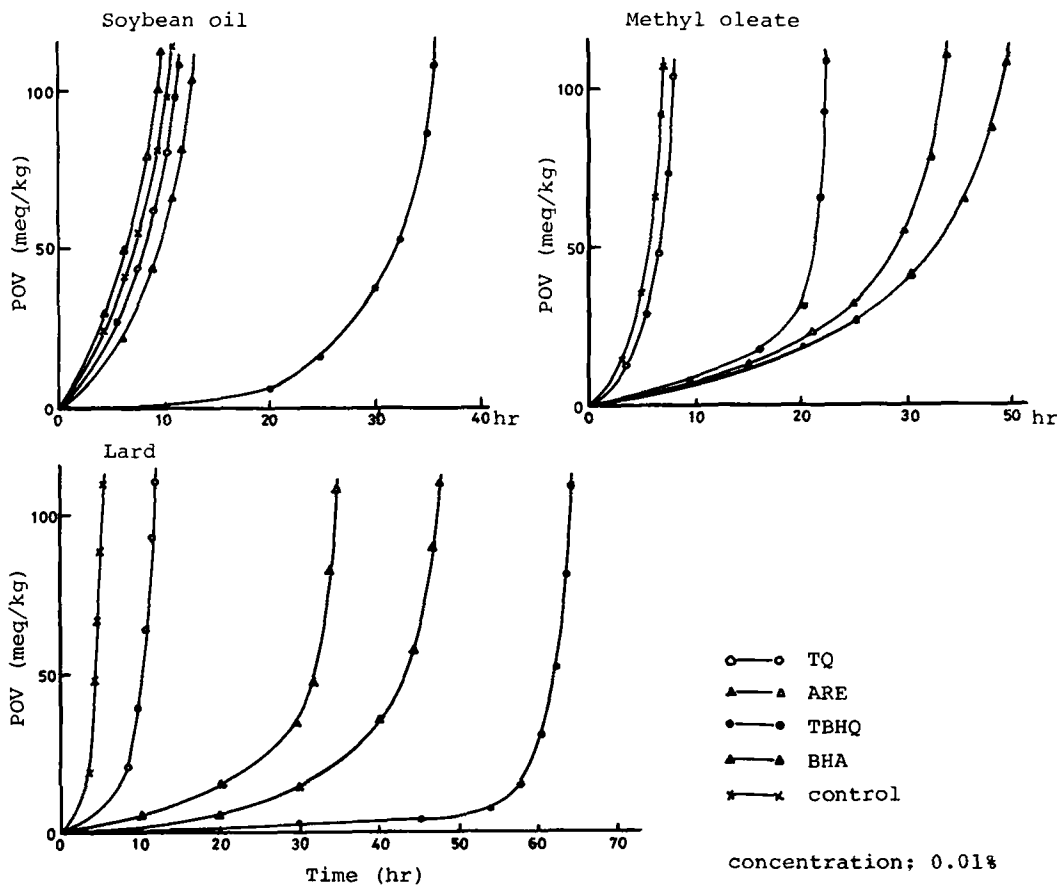


FIG. 3. Antioxidative effect of oxidation products of TBHQ on lard, soybean oil and methyl oleate.

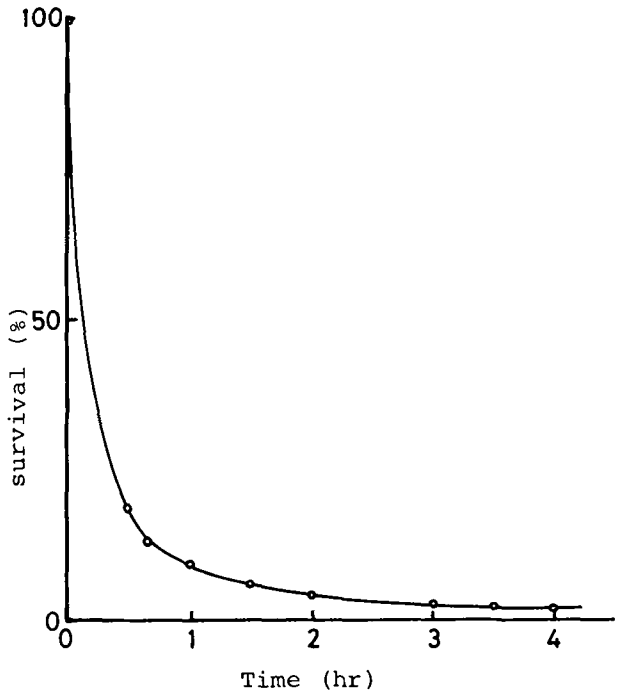


FIG. 4. Change of residual 2-*tert*-butyl-*p*-benzoquinone with time under AOM in methyl oleate.

from the relative ratio of the induction period on oil of TBHQ to that of its oxidation products.

ARE has antioxidative activity on methyl oleate and lard. It was observed particularly that its antioxidative activity on methyl oleate was remarkably stronger than that of the base compound TBHQ. In contrast to this, on soybean oil, it was shown to have some effect to promote oxidation.

Measurement of Residual TQ Quantity

TQ showed little antioxidative activity on any of the basic oils used, so further experiment was carried out to determine the reason. The evaporated quantity of TQ during the AOM test on methyl oleate was measured by HPLC. As shown in Figure 4, a large quantity of TQ was evaporated during the AOM test: its residual rate was ca. 10% after 1 hr and 5% after 2 hr. It seems that this result is closely related to the lack of the antioxidative activity of TQ on basic oils.

REFERENCES

1. Kurechi, T., Eisei Kagaku 13:191 (1967).
2. Kurechi, T., Ibid. 15:301 (1969).
3. Kurechi, T., and T. Taki, Yakugaku Zasshi 97:1174 (1977).
4. Kurechi, T., and A. Kunugi, JAOCS 60:109 (1983).
5. Kurechi, T., K. Kikugawa and S. Aoshima, Chem. Pharm Bull. 29:2351 (1981).
6. Kurechi, T., and T. Yamaguchi, JAOCS 57:216 (1980).
7. Wheeler, D.H., Oil Soap (Chicago) 9:89 (1932).
8. Orlando, C.M., Jr, H. Mark, A.K. Bose and M.S. Manhas, J. Am. Chem. Soc. 89:6526 (1967).

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⁂ Studies on the Antioxidants XIX: Photooxidation Products of Tertiary Butyl Hydroquinone (TBHQ) (II)

T. KURECHI and **A. KUNUGI**, Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo, 192-03, Japan

ABSTRACT

A series of studies have been made with a view to pursuing the fate of antioxidant molecules during the antioxidation processes. In this study following that reported in the preceding paper, tertiary butyl hydroquinone (TBHQ) in benzene solution was irradiated with ultraviolet rays and the chemical structure and antioxidative activities of the resulting photooxidation products were examined. They were identified as the following three compounds: 2-(2-[3'-*tert*-butyl-4'-hydroxyphenoxy]-2-methyl-1-propyl)hydroquinone, which was a dimer of the intermediate generated by photorearrangement of the *tert*-butyl group and the substrate TBHQ, 2-(2-hydroxy-2-methyl-1-propyl)hydroquinone, which was formed by interaction of the rearranged *tert*-butyl group with water, and 2-*tert*-butyl-4-ethoxy-phenol, which was formed from a secondary decomposition of the dimer. All of the resulting oxidation products showed antioxidative activity on methyl oleate, soybean oil and lard in the stability test by the active oxygen method (AOM).

INTRODUCTION

In the course of the investigation with the view to pursuing the fate of antioxidant molecules during the antioxidation processes, Kurechi et al. have studied the chemical structures of various oxidation products obtained from butylated hydroxyanisole (BHA) (1-3), ethyl protocatechuate (EP) (4), propyl gallate (PG) (5) and their mixtures (6,7). The fact that their oxidation products were formed corresponding to the positions of dehydrogenation of antioxidants has been clarified through structural analysis of the resulting products.

In the present study, the chemical structures and the antioxidative activity of photooxidation products of tertiary butyl hydroquinone (TBHQ) were examined.

When benzene solution of TBHQ was irradiated with ultraviolet (UV) rays for oxidation, some oxidation products were formed. Besides 2-*tert*-butyl-*p*-benzoquinone and 2,2-dimethyl-2,3-dihydro-5-hydroxybenzo (b) furan, 2-(2-[3'-*tert*-butyl-4'-hydroxyphenoxy]-2-methyl-1-propyl) hydroquinone, 2-(2-hydroxy-2-methyl-1-propyl)hydroquinone and 2-*tert*-butyl-4-ethoxy-phenol were newly identified. Moreover, when the antioxidative activity of these products was examined in various base oils, it was proved that they still retained their antioxidative activity.

EXPERIMENTAL PROCEDURES

Materials

Commercially available reagent grade tertiary butyl hydroquinone (mp 127 C) (Wako Junyaku Co., Tokyo) was repeatedly recrystallized from hexane.