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Biotransformation of Butylated Hydroxytoluene (BHT) to BHT-Quinone Methide in Rats

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2,6-Di-*tert*-butyl-4-methylene-2,5-cyclohexadien-1-one (BHT-quinone methide, BHT-QM) was detected in the bile of rats given butylated hydroxytoluene (BHT). The biliary excretion of BHT-QM during 24 h after administration of BHT, 3,5-di-*tert*-butyl-4-hydroxybenzyl alcohol (BHT-alcohol), or 2,6-di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (4-hydroxy-BHT) was determined by high-performance liquid chromatography. BHT-alcohol gave about 7 times as much BHT-QM as did BHT, but no BHT-QM was detected after administration of 4-hydroxy-BHT. This result suggests that the transformation of BHT to BHT-QM proceeds mainly through BHT-alcohol.

Although 1,2-bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)ethane (BHT-dimer) has been reported as a biliary metabolite of BHT in rats, most of the BHT-dimer seems to be formed artificially by dimerization of BHT-QM during the isolation process.

Keywords——butylated hydroxytoluene; metabolism in rats; BHT-alcohol; BHT-quinone methide; BHT-dimer; biliary excretion; high-performance liquid chromatography

The metabolism of butylated hydroxytoluene (BHT), an antioxidant, has been reviewed by Branen.[1] The known biliary metabolites of BHT in rats are 3,5-di-*tert*-butyl-4-hydroxybenzyl alcohol (BHT-alcohol), 3,5-di-*tert*-butyl-4-hydroxybenzaldehyde, 3,5-di-*tert*-butyl-4-hydroxybenzoic acid, 1,2-bis(3,5-di-*tert*-butyl-4-hydroxyphenyl)ethane (BHT-dimer), and S-(3,5-di-*tert*-butyl-4-hydroxybenzyl)-N-acetylcysteine.[2—4] Recently, 2,6-di-*tert*-butyl-4-methylene-2,5-cyclohexadien-1-one (BHT-quinone methide, BHT-QM) has been identified in the liver of rats given BHT.[5] Quinone methides are highly reactive compounds chemically[6] and their possible involvement in some biochemical and toxicological events has been suggested.[6—8]

Among the above compounds, BHT-dimer can be formed chemically from BHT through BHT-QM.[9] In a study with rat liver microsomes, Chen and Shaw[10] showed that 2,6-di-*tert*-butyl-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one (4-hydroxy-BHT) was transformed to BHT and BHT-alcohol, and suggested that the biotransformation proceeded through dehydration of 4-hydroxy-BHT. BHT-alcohol was also reported to be biotransformed to BHT-dimer.[11] Therefore, it seemed reasonable to postulate that BHT-QM would be formed through dehydration of 4-hydroxy-BHT and/or BHT-alcohol and then dimerized to BHT-dimer. To clarify this point, the metabolism of BHT to BHT-QM was examined *in vivo*. In the present study, we developed a method for quantitative determination of BHT-QM in the bile of rats, and determined the biliary excretion of BHT-QM after administration of BHT, BHT-alcohol, and 4-hydroxy-BHT. Artificial formation of BHT-dimer from BHT-QM in the course of the isolation process is also reported.

Materials and Methods

Materials——BHT and BHT-alcohol were purchased from Tokyo Kasei Kogyo Co., Ltd. BHT-dimer,[12] 4-hydroxy-BHT,[13] and 2,6-di-*tert*-butyl-*p*-benzoquinone[14] were synthesized by the cited methods. BHT-QM was prepared in amounts equimolar with BHT by the dissociation of 4-(2,6-di-*tert*-butyl-4-methylphen-

oxy)-2,6-di-*tert*-butyl-4-methyl-2,5-cyclohexadien-1-one dissolved at 10^{-4} M concentration in $CHCl_3$ according to the method of Becker.[15]

Animal Experiments——Male Wistar rats weighing 250—300 g were anesthetized with ether and cannulated to the common bile duct with polyethylene tubing PE-10 (INTRAMEDIC No. 7410, Becton, Dickinson and Company, U.S.A.). The test compounds, dissolved in 0.6 ml of a 1:6 emulsion of DMSO and olive oil, were injected intraperitoneally into each rat. The bile was collected at 2 h intervals for 10 h and then for up to 24 h after administration. It was kept −20°C until analyzed.

Preparation of Biliary Extracts——The bile was extracted with $CHCl_3$ (1 ml/ml of bile). The $CHCl_3$ extract was dried over anhydrous Na_2SO_4 and evaporated to dryness under a stream of N_2. The residue was redissolved in $CHCl_3$ and analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS). For the quantitative determination of BHT-QM, a portion (10 μl) of the $CHCl_3$ extract was subjected to high-performance liquid chromatography (HPLC) without any concentration.

Instrumental Analysis——GC was performed on a Shimadzu GC-5A gas chromatograph equipped with a hydrogen flame ionization detector. GC conditions are shown in the caption of Fig. 1.

GC–MS was performed on a JEOL JMS-D100 mass spectrometer coupled with a JGC-20K gas chromatograph. The ionizing energy was 22 eV. Isothermal GC was conducted on a 1 m × 2 mm i.d. glass column packed with 2% XE-60 on Gas Chrom Z at 110°C and 200°C. The carrier gas was helium at 0.5 kg/cm².

HPLC was performed on a Hitachi 638-30 liquid chromatograph equipped with a UV spectrophotometer. The stainless steel column (25 cm × 4 mm i.d.) was packed with LiChrosorb Si 60 (E. Merck, mean particle size 10 μm). The other conditions are given in the caption of Fig. 2.

Results and Discussion

Two peaks (I and II) were found near that of BHT in the gas chromatogram of the biliary extract 2 h after administration of BHT (Fig. 1a). The mass spectrum of peak I showed a molecular ion at *m/e* 218, which is 2 mass units lower than that of BHT. The fragment ions at *m/e* 203 ($M^+ - CH_3$), 175 ($M^+ - CH_3 - C_2H_4$), and 161 ($M^+ - C_4H_9$) correspond to the fragment ions of BHT at *m/e* 205, 177, and 163, respectively. These data suggested the presence of BHT-QM. An authentic sample of BHT-QM[15] was synthesized and proved to be consistent with peak I by GC and GC–MS comparisons. The mass spectrum of peak II showed a molecular ion at *m/e* 220 and a base peak at *m/e* 205 ($M^+ - CH_3$), suggesting that the compound was 2,6-di-*tert*-butyl-*p*-benzoquinone (BHT-quinone) previously identified in our laboratory.[16] Its identify was confirmed by GC–MS comparison with an authentic sample of BHT-quinone.[14]

BHT-dimer in the biliary extract had a retention time of 9.0 min on the XE-60 column at 180°C (Fig. 1b). It was identified by GC–MS comparison with an authentic sample[12] and showed a molecular ion at *m/e* 438 and a base peak at *m/e* 219 (BHT-benzyl cation).

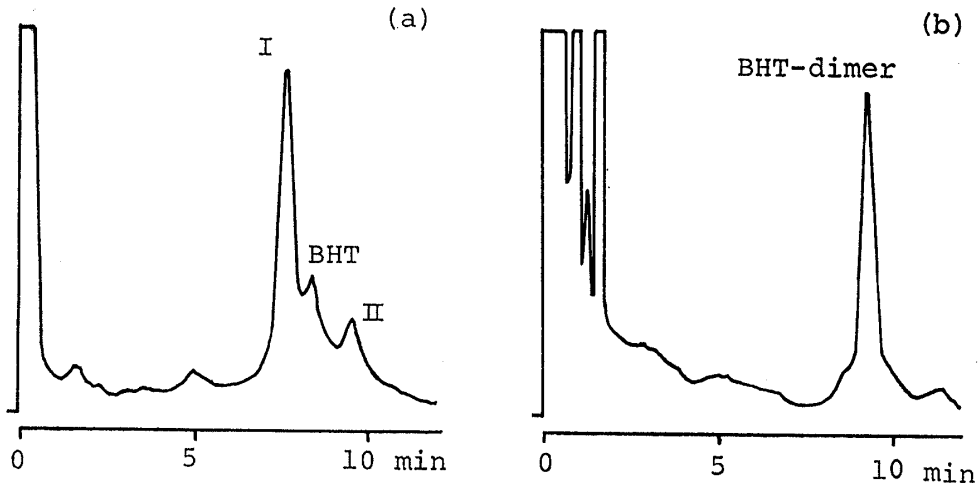

Fig. 1. Gas Chromatograms of the Biliary Extract 2 h after Intraperitoneal Administration of BHT: I, BHT-QM; II, BHT-quinone

Conditions: column, 2% XE-60 on Gas Chrom Z 1 m × 3 mm i.d.; column temp., 80°C (a) and 180°C (b); carrier gas, N_2 50 ml/min.

When BHT-QM added to normal bile was extracted with $CHCl_3$ and subjected to GC after concentration, the recovery was low and variable (20—40%) probably because of accompanying formation of BHT-dimer. On the other hand, the amount of BHT-QM in the bile of rats given BHT was too low to be analyzed by GC without concentration.

BHT-dimer was not detected by GC in the biliary extract samples after administration of BHT when these samples were manipulated without concentration. However, when the biliary extract samples were once concentrated and then diluted to their original volume with $CHCl_3$, BHT-dimer could be detected. Although BHT-dimer has been reported as a biliary metabolite of BHT in rats,[2,4] these results suggest that most of the BHT-dimer may be an artifact formed in the course of the isolation process, since BHT-QM is known to give dimeric products at high concentration.[9,17]

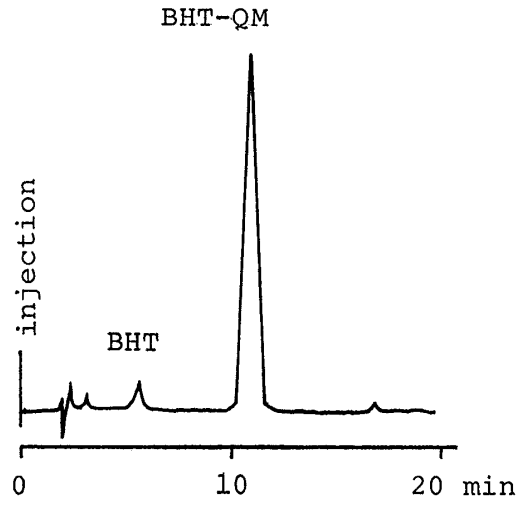

Fig. 2. High-Performance Liquid Chromatogram of the Biliary Extract 2 h after Intraperitoneal Administration of BHT

Conditions: column, LiChrosorb Si 60 (10 μm) 25 cm × 4.0 mm i.d.; mobile phase, *n*-hexane; flow rate, 1.5 ml/min at ambient temp.; detection; UV 285 nm; 0.04 AUFS.

Since BHT-QM has a strong absorbance (ε 27000) at 285 nm,[15,17] BHT-QM was determined by HPLC with a UV detector. BHT-QM in the biliary extract of rats given BHT was analyzed by the HPLC method without concentration. Fig. 2 shows the chromatogram of the biliary extract. BHT-QM was identified on the basis of the retention time and co-chromatography with an authentic sample of BHT-QM.[15] The calibration curve of peak area against amount of BHT-QM was linear in the range of 10—400 ng and the detection limit was 10 ng. The recovery of BHT-QM was 85—90%.

To identify the precursor of BHT-QM, the biliary excretion of this compound was determined by HPLC after intraperitoneal administration of BHT, BHT-alcohol, and 4-hydroxy-BHT (Table I). The excretion of BHT-QM in 24 h after dosing with BHT-alcohol was about 7-fold greater than that after dosing with BHT. However, when 4-hydroxy-BHT was dosed, no BHT-QM was detected. The 2-hourly excretion of BHT-QM was also determined for 10 h after dosing with BHT and BHT-alcohol. The excretion of BHT-QM after dosing with BHT-alcohol was greater and more rapid than that after dosing with BHT. These results

Table I. Biliary Excretion of BHT-QM after Intraperitoneal Administration of BHT, BHT-alcohol, and 4-Hydroxy-BHT

Period (h)	BHT	Substrate[a] BHT-alcohol	4-Hydroxy-BHT
	Excreted % of dose[b]		
0—24	0.086±0.009	0.628±0.076	ND
	Amount excreted, μg/head[b]		
0— 2	7.3±1.0	120.2±15.5	—
2— 4	13.1±2.5	93.5±14.5	—
4— 6	7.9±1.6	79.0±13.2	—
6— 8	6.5±1.9	43.5±5.7	—
8—10	6.4±1.6	32.9±4.9	—

a) BHT, BHT-alcohol, and 4-hydroxy-BHT were each administered as a single dose of 280, 280, and 210 mg/kg, respectively.
b) Each value is the mean±S.E. of four rats.

are in accord with those of Holder *et al.*,[4] who found that BHT-alcohol was absorbed, metabolized, and excreted in the bile more rapidly than BHT after intraperitoneal administration to rats.

The present results suggest that the biotransformation of BHT to BHT-QM proceeds mainly through BHT-alcohol. A possible explanation of this pathway is that BHT-alcohol is dehydrated directly or after being converted to some conjugate (s). It is generally accepted that metabolic reduction of alcohols proceeds through dehydration followed by hydrogenation.[18] On the other hand, 4-hydroxy-BHT does not seem to be dehydrated *in vivo* to BHT-QM, although it was reported to be transformed *in vitro* to BHT and BHT-alcohol.[10]

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Fundamental Pharmacokinetic Behavior of Sulfadimethoxine, Sulfamethoxazole and Their Biotransformed Products in Dogs[1]

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Plasma concentration profiles of sulfadimethoxine, sulfamethoxazole and their biotransformed products (N^4-acetate and N^1-glucuronide) in dogs were determined and their pharmacokinetic parameters were calculated by using a two-compartment open model. The apparent partition coefficients between chloroform and phosphate buffer were also determined. Decline in plasma levels of sulfadimethoxine and sulfamethoxazole was considerably accelerated by N^4-acetylation and N^1-glucuronidation. The elimination of sulfadimethoxine-N^1-glucuronide from plasma was more rapid than that of sulfadimethoxine-N^4-acetate, and a similar tendency was observed for sulfamethoxazole and its biotransformed products. N^4-Acetylation or N^1-glucuronidation of sulfadimethoxine and