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SPECIFICITY OF INDUCTION OF CANCER PROTECTIVE ENZYMES BY ANALOGUES OF *TERT*-BUTYL-4-HYDROXYANISOLE (BHA)

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Abstract—Protection by 2(3)-*tert*-butyl-4-hydroxyanisole (BHA) and related phenols against chemical carcinogens, mutagens and other toxins has been attributed to the elevation of tissue levels of non-oxygenative detoxification enzymes. To analyze the mechanisms and specificity of these enzyme inductions, we synthesized a series of mono- and dialkyl ethers of *tert*-butylhydroquinone (R_1O-[$(CH_3)_3C$-C_6H_3]-OR_2) and its dimer. The abilities of these compounds to elevate the cytosolic specific activities of glutathione *S*-transferases (measured with 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene) and of NAD(P)H:quinone reductase in liver, upper small intestine and forestomach of female CD-1 mice were evaluated. The animals were fed five daily doses of 50 μmoles of each monomer (or 25 μmoles of each dimer). The structures of the monomers examined were: $R_1 = H$ and $R_2 = CH_3$ (I), $R_2 = C_2H_5$ (VI), $R_2 = (CH_2)_2CH_3$ (VIII), $R_2 = CH(CH_3)_2$ (X); $R_1 = CH_3$ and $R_2 = C_2H_5$ (VII), $R_2 = (CH_2)_2CH_3$ (IX), $R_2 = CH(CH_3)_2$ (XI); $R_2 = CH_3$ and $R_1 = C_2H_5$ (III), $R_1 = (CH_2)_2CH_3$ (IV) and $R_1 = CH(CH_3)_2$ (V). In addition, the monomethyl (XIII), monoethyl (XIV) and mono-*n*-propyl (XV) ethers of BHA dimer (XII; 2,2′-dihydroxy-3,3′-di-*tert*-butyl-5,5′-dimethoxybiphenyl) were also prepared. Under the conditions tested, all compounds were ineffective as enzyme inducers in the forestomach but produced coordinate induction of enzymes (generally 2- to 6-fold) in the cytosols of liver and mucosa of proximal small intestine. Increases in bulk of R_1 and R_2 beyond methyl groups tended to decrease the inductive potency of both monomers and dimers. The lack of strict structural specificity suggests that the induction depends on metabolic conversion of the analogues to common types of metabolites.

The powerful protection by 2(3)-*tert*-butyl-4-hydroxyanisole (BHA)† and related phenols against chemical carcinogens, mutagens, and other toxic agents is an interesting phenomenon of potential practical importance [1, 2]. Although numerous studies have described the conditions under which protection is observed, much less information is available on molecular mechanisms underlying this process. There is now much evidence to suggest that at least some of these protective effects result from the induction of enzymes that detoxify reactive metabolites of carcinogens and mutagens [3–16]. In this paper, we examine the capacity of a series of structural analogues of BHA to induce detoxifying enzymes with the ultimate aim of clarifying the mechanisms of induction.

The current state of our understanding of the biochemical mechanisms of protection by BHA may be summarized as follows. First, administration of BHA to rodents profoundly reduces the levels of mutagenic metabolites of benzo[*a*]pyrene in the urine and peritoneal cavity of mice [3, 4, 14]. Second, the binding of polycyclic hydrocarbons to DNA is reduced markedly in animals treated with BHA, or when DNA is exposed *in vitro* to microsomes obtained from such animals [17, 18]. Furthermore, the patterns of metabolism of polycyclic aromatics are modified qualitatively and quantitatively in response to BHA treatment [19–21]. Third, BHA protects against phorbol ester-induced promotion of skin tumors initiated with polycyclic hydrocarbons [22, 23]. Fourth, the protection by BHA is accompanied by elevations in the liver and a number of peripheral tissues of enzymes that have broad substrate specificities and can inactivate reactive electrophilic metabolites of many xenobiotics [4–6, 8–12, 24]. Thus, BHA administration elevates cytosolic glutathione *S*-transferases, cytosolic and microsomal NAD(P)H:quinone reductase, microsomal epoxide hydratase, enzymes involved in glucuronide metabolism, and those concerned with maintaining glutathione in the reduced state (glutathione reductase and glucose-6-phosphate dehydrogenase), as well as raising the tissue levels of glutathione [see review in Ref. 24]. The elevations of glutathione *S*-transferase activities which

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† Abbreviations: 3-BHA, the major isomer of commercial BHA, or 3-*tert*-butyl-4-hydroxyanisole; 2-BHA, the minor isomer of commercial BHA, or 2-*tert*-butyl-4-hydroxyanisole; methyl-BHA, 2-*tert*-butyl-1,4-dimethoxybenzene; BHA dimer, 2,2′-dihydroxy-3,3′-di-*tert*-butyl-5,5′-dimethoxybiphenyl; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene; and quinone reductase, NAD(P)H:quinone acceptor oxidoreductase (EC 1.6.99.2), a flavoprotein also known as DT-diaphorase or menadione reductase.

accompany BHA administration are associated with elevated enzyme protein and mRNA levels and thus presumptively with enhanced rates of enzyme synthesis [7, 25]. Fifth, there appears to be a strong correlation between the structural requirements for enzyme induction and for protection against chemical carcinogenesis [6, 11, 12, 15, 26]. Sixth, BHA produces only minor changes in the enzymes of oxygenative drug metabolism [20, 21, 27].

Although it has been suggested that BHA exerts its protective action by raising the activities of detoxifying enzymes, several questions remain unanswered. It is not known whether BHA or one of its many metabolites is primarily responsible for the protective and enzyme inductive effects. Must the ultimate protector possess antioxidant properties? Are the inductive effects mediated via a receptor, or through common metabolic processes which generate an intermediate(s) that stimulates the protein synthetic machinery?

We have recently examined aspects of the tissue specificity of induction of glutathione *S*-transferases and quinone reductases by a number of BHA analogues including the major isomer of BHA (3-BHA; 3-*tert*-butyl-4-hydroxyanisole), the minor isomer of BHA (2-BHA; 2-*tert*-butyl-4-hydroxyanisole), methyl-BHA (2-*tert*-butyl-1,4-dimethoxybenzene), 4-hydroxyanisole, and *tert*-butylhydroquinone [24, 26, 28, 29]. Although all compounds showed some capacity for inducing enzymes, the induction patterns were selective with respect to the chemical nature of inducer, the target tissue, and enzymes elevated. None of the compounds was significantly more effective than BHA itself [24, 29].

The present study is concerned with the structural requirements for enzyme induction of an extended series of alkyl ethers of *tert*-butylhydroquinone and its dimer. Dimeric derivatives of BHA are of interest as enzyme inducers not only because dimers are formed with great ease by a radical mechanism *in vitro* in the presence of base and oxygen, but also because the dimer of BHA has been isolated as a metabolite of BHA [30]. Furthermore, such dimers are formed enzymatically by the action of peroxidases [31] or of tyrosinase [32] on BHA. This facile formation of dimeric adducts raised the issue of whether BHA dimers might be responsible for the induction of detoxifying enzymes.

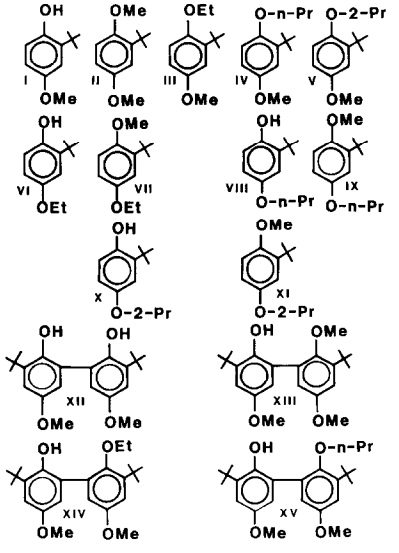


Fig. 1. Structures of monomeric and dimeric analogues of *tert*-butylhydroquinone which have been prepared for evaluation as enzyme inducers.

We describe the synthesis and characterization of a series of mono- and dialkyl derivatives of *tert*-butylhydroquinone and its dimer. Fifteen compounds (Fig. 1, I–XV) have been prepared and tested for their ability to induce glutathione *S*-transferases (EC 2.5.1.18) and NAD(P)H:quinone reductase (EC 1.6.99.2) in the cytosols of liver, mucosa of the small intestine, and the forestomach of female CD-1 mice. These organs were chosen because they were the most selectively responsive to enzyme induction by our initial series of compounds [24, 26, 29].

MATERIALS AND METHODS

Treatment of animals

Female CD-1 mice (Charles River Breeding Laboratories, Wilmington, MA) 5–6 weeks of age were housed in hanging stainless steel cages (three to four mice per cage) without bedding at 24 ± 1° with light–dark cycles of 12 hr each. The mice were fed No. 5001 Purina Laboratory Chow (Ralston-Purina, St. Louis, MO) in powder form. After allowing the mice 1 week of acclimatization, the mice were fed by gavage 50 μmoles of monomers or 25 μmoles of dimers daily for 5 days. The test compounds were dissolved in 0.1 ml of sesame oil (Fisher, Fair Lawn, NJ). Controls received 0.1 ml of vehicle only. The animals were killed by cervical dislocation 24 hr after the last dose. Four to six animals were assigned to each treatment group.

Collection and processing of tissues for enzyme studies

Livers were excised, weighed, and then perfused through the portal vein with cold 0.15 M KCl containing 2 mM EDTA, pH 7.5. The upper half of the small intestine was slit longitudinally, food was removed, and the mucosal surface was collected by scraping with a blunt-edged spatula. The stomach was dissected into forestomach and glandular portions after removal of food. All tissues were frozen in liquid nitrogen within minutes of death and stored at −80° until the enzyme activities could be assayed.

Preparation of cytosol fractions for enzyme studies

Individual tissues were homogenized in 0.25 M sucrose (3.0 ml/g tissue) at 0–4°. After centrifugation at 5000 *g* for 20 min, the supernatant fluid was collected, 0.2 vol. of 0.1 M $CaCl_2$ in 0.25 M sucrose was added to each, and the samples were stored on ice for 30 min. Centrifugation at 15,000 *g* for 20 min yielded clear cytosol fractions suitable for enzyme assays.

Determination of enzyme activities

The activity of quinone reductase was determined

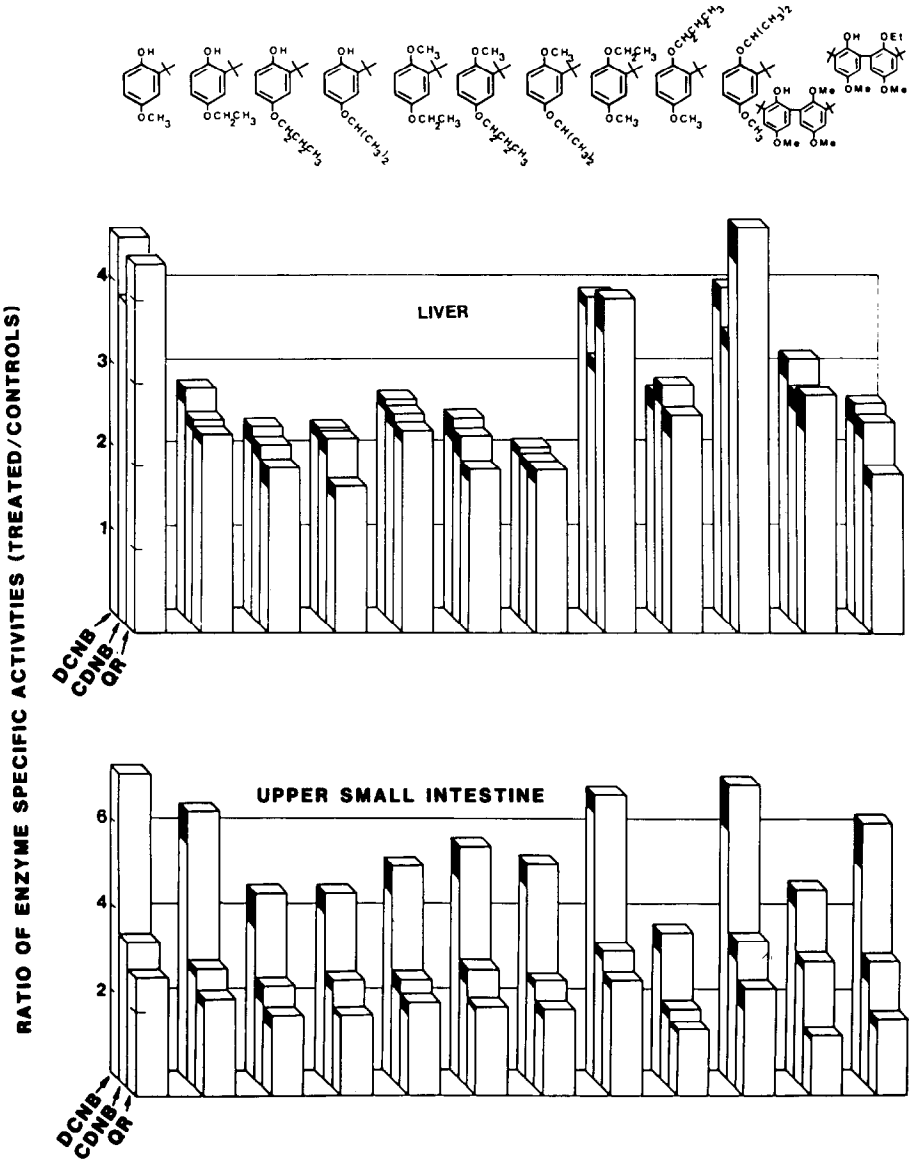


Fig. 2. Induction patterns of glutathione *S*-transferases measured with 1-chloro-2,4-dinitrobenzene (CDNB) and 1,2-dichloro-4-nitrobenzene (DCNB) and of NAD(P)H:quinone reductase (QR) in cytosols of liver (above) and upper small intestine mucosa (below) of mice treated with various analogues of *tert*-butylhydroquinone. The results are expressed as ratios of enzyme specific activities of tissues obtained from treated animals to controls (N = 4–6 per group). The standard errors (shown as black areas) have been scaled in proportion to the mean control values (Table 1). All animals received five daily doses of either 50 μmoles (monomers) or 25 μmoles (dimers) of the indicated compounds dissolved in 0.1 ml of sesame oil. The controls received 0.1 ml of sesame oil only. The control specific activities and the specific activities of tissues obtained from animals that received 50 μmoles of 3-BHA daily are shown in Table 1.

spectrophotometrically according to the method of Ernster [33] as modified and described in detail by Benson *et al.* [6]. Glutathione *S*-transferase activities were measured with 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene and glutathione as substrates at 25° according to the procedure of Habig *et al.* [34]. Protein concentrations were determined by the method of Lowry *et al.* [35].

Statistical treatment of results

The results of enzyme specific activity measurements are reported as the ratios of activities in organs of treated animals to those of control animals. The standard errors for each set of measurements (shown in Fig. 2) were calculated and divided by the means of the appropriate control values. The mean control values and their standard errors are given in Table 1.

Table 1. Glutathione *S*-transferase and quinone reductase specific activities of cytosols of liver and upper small intestinal mucosa in female CD-1 mice treated with 3-BHA*

Expt.	Treatment group	Glutathione *S*-transferase specific activities (nmoles/min/mg)				Quinone reductase specific activities (nmoles/min/mg)	
		CDNB		DCNB			
		Liver	Intestine	Liver	Intestine	Liver	Intestine
1	Control	2176 ± 272	714 ± 80	29.7 ± 3.8	5.67 ± 0.54	101 ± 11	512 ± 61
	3-BHA	8454 ± 696	2127 ± 221	132 ± 11	35.7 ± 4.7	434 ± 27	1284 ± 211
		(3.89)†	(2.98)	(4.44)	(6.30)	(4.30)	(2.51)
2	Control	2026 ± 279	624 ± 54	31.2 ± 3.1	4.93 ± 0.50	92.6 ± 5.8	442 ± 21
	3-BHA	7278 ± 167	2454 ± 176	139 ± 7	40.1 ± 4.5	426 ± 25	1314 ± 173
		(3.59)	(3.93)	(4.46)	(8.13)	(4.60)	(2.97)

* 3-BHA was given by gavage at a dose of 50 μmoles daily in 0.1 ml of sesame oil for 5 days. Controls received sesame oil only. Cytosols were prepared and enzyme assays were performed as described in the text. Values are means ± S.E.M. based on analysis of livers of five animals and samples of intestinal mucosa of four animals.

† Values in parentheses are the ratios of mean specific activities in treated to control groups.

SYNTHESIS AND CHARACTERIZATION OF COMPOUNDS

The preparation, purification and characterization of Compounds I, II and *tert*-butylhydroquinone (Fig. 1) have been described [29]. The BHA dimer was synthesized according to the method of Hewgill and Hewitt [36]. Four general synthetic protocols described below were employed to synthesize the remaining compounds. All reactions were carried out under positive nitrogen pressure. Aliquots from reactions were analyzed periodically by thin-layer chromatography, and reflux was terminated when starting phenols were no longer detectable. NMR spectra were obtained on a Varian 60 MHz spectrometer. All samples were dissolved in $CDCl_3$ with tetramethylsilane as internal standard. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN).

Compounds III–V

These were synthesized by refluxing a mixture of 3-BHA, K_2CO_3, acetone and the appropriate alkyl bromide (mole proportions were 1:2:7:10). Reflux was maintained for 6–14 days, after which reaction solvents were removed under reduced pressure and water was added. The product was extracted with hexane, and this fraction was washed repeatedly with 0.1 N NaOH and with water, dried over $MgSO_4$, and decolorized with Florisil. Hexane was removed under reduced pressure and vacuum distillation of the residues produced colorless oils.

Compound III. Yield 58%; b.p.$_{0.1}$ 65–67°. ^{1}H NMR δ 1.37 (s,9,Ar-C(CH_3)$_3$), 1.43 (t,3,Ar-OCH$_2$$\underline{CH_3}$), 3.73 (s,3,Ar-OCH$_3$), 3.98 (q,2,Ar-OC$\underline{H_2}CH_3$), 6.57–6.87 (m,3,Ar-H). Anal. Calc. for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 74.71; H, 9.73.

Compound IV. Yield 53%; b.p.$_{0.12}$ 83–84°. ^{1}H NMR δ 1.05 (t,3,Ar-O(CH$_2$)$_2$C$\underline{H_3}$), 1.33 (s,9,Ar-C(CH_3)$_3$), 1.83 (m,2,Ar-OCH$_2$C$\underline{H_2}$CH$_3$), 3.69 (s,3,Ar-OCH$_3$), 3.86 (t,2,Ar-OC$\underline{H_2}$C$_2$H$_5$), 6.65–6.80 (m,3,Ar-H). Anal. Calc. for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 75.62; H, 10.15.

Compound V. Yield 28%; b.p.$_{0.1}$ 66–68°. ^{1}H NMR δ 1.32 (d,6,Ar-OCH(C$\underline{H_3}$)$_2$), 1.33 (s,9,Ar-C(CH_3)$_3$), 3.74 (s,3,Ar-OCH$_3$), 4.57 (m,1,Ar-OC$\underline{H}$(CH$_3$)$_2$), 6.57–6.93 (m,3,Ar-H). Anal. Calc. for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 76.29; H, 9.94.

Compounds VI, VIII, and X

These were synthesized by combining *tert*-butylhydroquinone, K_2CO_3 and the appropriate alkyl bromide (mole ratio, 1:4:10) and then refluxing for 1–3 days. The inorganic salts were removed by filtration, and the liquid phase was dissolved in hexane and washed repeatedly with 0.5 M K_2CO_3 and then with water. The organic phase was dried over $MgSO_4$, and the hexane was removed under reduced pressure. The resulting oil crystallized on cooling, and the product was isolated by washing in cold hexane and filtering. These off-white crystals were recrystallized from hexane to give white needles.

Compound VI. Yield 38%; m.p. 97.0–97.3°. ^{1}H NMR δ 1.36 (t,3,Ar-OCH$_2$C$\underline{H_3}$), 1.37 (s,9,Ar-C(CH_3)$_3$), 3.93 (q,2,Ar-OC$\underline{H_2}$CH$_3$), 4.73 (s,1,Ar-OH), 6.37–6.80 (m,3,Ar-H). Anal. Calc. for $C_{12}H_{18}O_2$: C, 74.19; H, 9.34. Found: C, 74.63; H, 9.30.

Compound VIII. Yield 24%; m.p. 62.0–62.4°. ^{1}H NMR δ 1.02 (t,3,Ar-O(CH$_2$)$_2$C$\underline{H_3}$), 1.62 (s,9,Ar-C(CH_3)$_3$), 1.78 (m,2,Ar-OCH$_2$C$\underline{H_2}$CH$_3$), 3.82 (t,2,Ar-OC$\underline{H_2}$C$_2$H$_5$), 4.27 (s,1,Ar-OH), 6.38–6.78 (m,3,Ar-H). Anal. Calc. for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 75.68; H, 9.91.

Compound X. Yield 17%; m.p. 80.9–81.3°. ^{1}H NMR δ 1.30 (d,6,Ar-OCH(C$\underline{H_3}$)$_2$), 1.38 (s,9,Ar-C(CH_3)$_3$), 4.30 (m,1,Ar-OC$\underline{H}$(CH$_3$)$_2$), 4.35 (s (broad), 1,Ar-OH), 6.60–6.90 (m,3,Ar-H). Anal. Calc. $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 75.50; H, 9.78.

Compounds VII, IX, and XI

These were prepared by dissolving VI, VIII, or X, respectively, in acetone with an excess of K_2CO_3 and a 10–100% molar excess of dimethyl sulfate. The mixture was refluxed for 1–3 days. Inorganic salts were removed by filtration and acetone was evaporated under reduced pressure. The golden oily residue was dissolved in hexane, and the organic phase was then extracted with 1 N NaOH, dried with $MgSO_4$, and decolorized with alumina. The product was concentrated under reduced pressure, and vacuum distillation resulted in a colorless oil.

Compound VII. Yield 76%, b.p.$_{0.4}$ 80–84°. ^{1}H NMR δ 1.31 (t,3,Ar-OCH$_2$C$\underline{H}_3$), 1.32 (s,9,Ar-C(C$\underline{H}_3$)$_3$), 3.65 (s,3,Ar-OC$\underline{H}_3$), 3.85 (q,2,Ar-OC$\underline{H}_2$CH$_3$), 6.42–6.75 (m,3,Ar-H). Anal. Calc. for $C_{13}H_{20}O_2$: C, 74.96; H, 9.68. Found: C, 75.29; H, 9.75.

Compound IX. Yield 72%; b.p.$_{0.2}$ 90–91°. ^{1}H NMR δ 1.03 (t,3,Ar-O(CH$_2$)$_2$C$\underline{H}_3$), 1.35 (s,9,Ar-C(C$\underline{H}_3$)$_3$), 1.77 (m,2,Ar-OCH$_2$C$\underline{H}_2$CH$_3$), 3.72 (s,3,Ar-OC$\underline{H}_3$), 3.82 (t,2,Ar-OC$\underline{H}_2$C$_2$H$_5$), 6.57–6.90 (m,3,Ar-H). Anal. Calc. for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 76.16; H, 10.06.

Compound XI. Yield 73%; b.p.$_{0.1}$ 68°. ^{1}H NMR δ 1.33 (d,6,Ar-OCH(C$\underline{H}_3$)$_2$), 1.34 (s,9,Ar-C(C$\underline{H}_3$)$_3$), 3.81 (s,3,Ar-OC$\underline{H}_3$), 4.40 (m,1,Ar-OC$\underline{H}$(CH$_3$)$_2$), 6.66–6.96 (m,3,Ar-H). Anal. Calc. for $C_{14}H_{22}O_2$: C, 75.63; H, 9.97. Found: C, 75.53; H, 9.88.

Compounds XIII, XIV, and XV

These were prepared by refluxing a mixture of BHA dimer, K_2CO_3, and excess alkylating agent (dimethyl sulfate, ethylbromide or *n*-propylbromide) in acetone for 2–4 hr. The acetone was evaporated under reduced pressure, and the residue was dissolved in hexane. Inorganic salts were removed by filtration, and the hexane fraction was washed with water, dried over $MgSO_4$, and concentrated under reduced pressure to a golden oil. Addition of boiling ethanol resulted in the formation of crystals. Recrystallization of the crude product from ethanol produced white needles.

Compound XIII. Yield 69%; m.p. 102.5–104.0°. ^{1}H NMR δ 1.42 (s,18,Ar-C(C$\underline{H}_3$)$_3$), 3.42 (s,3,Ar-OC$\underline{H}_3$), 3.80 (s,6,Ar-OC$\underline{H}_3$), 6.65 (s,1,Ar-OH), 6.7–7.0 (m,4,Ar-H). Anal. Calc. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.58; H, 8.87.

Compound XIV. Yield 91%; m.p. 100.0–101.0°. ^{1}H NMR δ 1.16 (t,3,Ar-OCH$_2$C$\underline{H}_3$), 1.43 (s,18,Ar-C(C$\underline{H}_3$)$_3$), 3.63 (m,2,Ar-OC$\underline{H}_2$CH$_3$), 3.80 (s,6,Ar-OC$\underline{H}_3$), 6.6–7.0 (m,4,Ar-H), 7.0 (s,1,Ar-OH). Anal. Calc. for $C_{24}H_{34}O_4$: C, 74.58; H, 8.87. Found: C, 74.80; H, 8.91.

Compound XV. Yield 88%; m.p. 119.8–120.3°. ^{1}H NMR δ 0.82 (t,3,Ar-(CH$_2$)$_2$C$\underline{H}_3$), 1.43 (s,18,Ar-C(C$\underline{H}_3$)$_3$), 1.4–1.6 (m,2,Ar-OCH$_2$C$\underline{H}_2$CH$_3$), 3.53 (t,2,Ar-OC$\underline{H}_2$C$_2$H$_5$), 3.80 (s,6,Ar-OC$\underline{H}_3$), 6.6–7.0 (m,4,Ar-H), 6.75 (s,1,Ar-OH). Anal. Calc. for $C_{25}H_{36}O_4$: C, 74.96; H, 9.06. Found: C, 75.37; H, 8.99.

RESULTS AND DISCUSSION

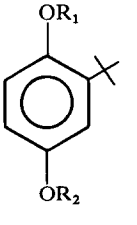
The enzyme inductive effects of all compounds were examined according to a protocol in which 50 μmoles of monomers or 25 μmoles of dimers were fed by gavage daily for 5 days, and the tissues were removed for enzyme assays (glutathione *S*-transferases measured with CDNB and DCNB, and quinone reductase) 24 hr after administration of the last dose. These lower doses were selected since in our earlier studies [24, 28, 29] 100-μmole daily doses of 3-BHA or methyl-BHA produced near maximal inductive effects, and we wished to identify potentially more potent compounds. The cytosols of liver, mucosa of the proximal small intestine, and the forestomach were examined. The results are most economically displayed as three-dimensional bar graphs (Fig. 2) which show the ratios of the induced to control enzyme specific activities in the tissue cytosols. The specific activities of tissues of control animals and those that received 3-BHA are given in Table 1. The principal findings are as follows.

(1) In the liver all compounds at the doses tested increased the specific activities of glutathione *S*-transferases and quinone reductases coordinately (2- to 6-fold over control values). The hepatic elevations of glutathione *S*-transferase activities measured with CDNB and DCNB were similar, as previously observed with commercial BHA and some related phenols [4, 29].

(2) In cytosols prepared from the mucosa of the proximal small intestine, the quinone reductase specific activities were elevated 2-fold or more by all compounds except IV and XIII, which were only weak inducers. The patterns of induction of glutathione *S*-transferases in this tissue were quite different from those observed in liver. Although all compounds produced significant inductions of these transferases, the activities toward DCNB rose much more than those for CDNB. This phenomenon probably involves the differential induction in this tissue of the transferase with pI of 8.7. It has been shown [37] that BHA administration causes marked changes in the ratio of glutathione *S*-transferases with pI 8.7 and 9.3 in the small intestine. The former, but not the latter, has substantial reactivity with CDNB.

(3) At the doses tested none of the compounds induced enzymes in the forestomach. These data are not shown, but the conclusions are based on the assay of at least four forestomachs in each experimental group. This finding complements our earlier observations [24, 28, 29] that 3-BHA and methyl-BHA are not enzyme inducers in the forestomach, and contrasts with the observations that 2-BHA, *tert*-butylhydroquinone and 4-hydroxyanisole act as inducers in this tissue [11, 29].

(4) More detailed examination of the effect of alkyl substitutions in compounds of the general structure:



revealed the following. When $R_2 = CH_3$ and $R_1 = CH_3$ [29], C_2H_5, or $CH(CH_3)_2$, induction was comparable to that obtained with 3-BHA ($R_1 = H$). However, when $R_1 = (CH_2)_2CH_3$, the inductive potency was considerably lower. When $R_1 = CH_3$ or H and $R_2 = C_2H_5$, $(CH_2)_2CH_3$, or $CH(CH_3)_2$, the inductive potency was also reduced.

(5) Dimeric species were also inducers but were less effective than their component monomers at equivalent (half) molar concentrations. Unfortunately, the dimer of BHA itself could not be tested because of its extreme insolubility.

The present study clearly demonstrates that the inductive capacity of BHA analogues is not highly dependent on the structure of the compounds administered, making it unlikely that enzyme induction is determined by the structures of the parent compounds. This conclusion is not unexpected since many structurally unrelated compounds such as lactones, coumarins, azo dyes, certain sulfur compounds (e.g. disulfiram and dithiolthiones) and other antioxidants such as 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) and ethoxyquin are inducers of these enzymes [11, 12, 15, 16, 24, 26, 38].

BHA and BHT undergo extensive metabolism in rodents and in man. The metabolic patterns usually involve oxidations of the *tert*-butyl and other alkyl groups [39, 40]. One product of BHA metabolism is *tert*-butylhydroquinone [40] which is a good enzyme inducer in mice [24, 29] and exerts the broadest tissue specificity for induction, including not only liver and upper small intestine, but also forestomach and glandular stomach, where other compounds are ineffective. It is reasonable to speculate therefore that the monomers tested in this study are converted in the tissues to a series of metabolites resembling *tert*-butylhydroquinone or hydroquinone.

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REFERENCES

1. L. W. Wattenberg, *Adv. Cancer Res.* **26**, 197 (1978).
2. L. W. Wattenberg and L. K. T. Lam, in *Inhibitors of Tumor Induction* (Eds. M. S. Zedeck and M. Lipkin), p. 1. Plenum Press, New York (1981).
3. R. P. Batzinger, S-Y. L. Ou and E. Bueding, *Cancer Res.* **38**, 4478 (1978).
4. A. M. Benson, R. P. Batzinger, S-Y. L. Ou, E. Bueding, Y-N. Cha and P. Talalay, *Cancer Res.* **38**, 4486 (1978).
5. A. M. Benson, Y-N. Cha, E. Bueding, H. S. Heine and P. Talalay, *Cancer Res.* **39**, 2971 (1979).
6. A. M. Benson, M. J. Hunkeler and P. Talalay, *Proc. natn. Acad. Sci. U.S.A.* **77**, 5216 (1980).
7. A. M. Benson, M. J. Hunkeler and J. F. Morrow, *Cancer Res.* **44**, 5256 (1984).
8. Y-N. Cha and E. Bueding, *Biochem. Pharmac.* **28**, 1917 (1979).
9. Y-N. Cha and H. S. Heine, *Cancer Res.* **42**, 2609 (1982).
10. Y-N. Cha, F. Martz and E. Bueding, *Cancer Res.* **38**, 4496 (1978).
11. V. L. Sparnins and L. W. Wattenberg, *J. natn. Cancer Inst.* **66**, 769 (1981).
12. V. L. Sparnins, J. Chuan and L. W. Wattenberg, *Cancer Res.* **42**, 1205 (1982).
13. P. Talalay and A. M. Benson, *Adv. Enzyme Regulat.* **20**, 287 (1982).
14. P. Talalay, R. P. Batzinger, A. M. Benson, E. Bueding and Y-N. Cha, *Adv. Enzyme Regulat.* **17**, 23 (1979).
15. L. W. Wattenberg, J. B. Coccia and L. K. T. Lam, *Cancer Res.* **40**, 2820 (1980).
16. S. S. Ansher, P. Dolan and E. Bueding, *Hepatology* **3**, 932 (1983).
17. M. W. Anderson, M. Boroujerdi and A. G. E. Wilson, *Cancer Res.* **41**, 4309 (1981).
18. Y. M. Ioannou, A. G. E. Wilson and M. W. Anderson, *Cancer Res.* **42**, 1199 (1982).
19. J. L. Speier and L. W. Wattenberg, *J. natn. Cancer Inst.* **55**, 469 (1975).
20. J. L. Speier, L. K. T. Lam and L. W. Wattenberg, *J. natn. Cancer Inst.* **60**, 605 (1978).
21. L. W. Wattenberg, J. Speier and A. Kotake, *Adv. Enzyme Regulat.* **14**, 313 (1976).
22. T. J. Slaga and W. M. Bracken, *Cancer Res.* **37**, 1631 (1977).
23. W. J. Kozumbo, J. L. Seed and T. W. Kensler, *Cancer Res.* **43**, 2555 (1983).
24. M. J. De Long, H. J. Prochaska and P. Talalay, in *Protective Agents in Cancer* (Eds. D. C. H. McBrien and T. F. Slater), p. 175. Academic Press, London (1983).
25. W. R. Pearson, J. J. Windle, J. F. Morrow, A. M. Benson and P. Talalay, *J. biol. Chem.* **258**, 2052 (1983).
26. M. J. De Long, H. J. Prochaska and P. Talalay, in *Extrahepatic Drug Metabolism and Chemical Carcinogenesis* (Eds. J. Rydström, J. Montelius and M. Bengtsson), p. 181. Elsevier, Amsterdam (1983).
27. A. D. Rahimtula, B. Jernström, L. Dock and P. Montelius, *Br. J. Cancer* **45**, 935 (1982).
28. H. J. Prochaska, M. J. De Long, H. Bregman and P. Talalay, *Fedn Proc.* **43**, 562 (1984).
29. M. J. De Long, H. J. Prochaska and P. Talalay, *Cancer Res.* **45**, 546 (1985).
30. A. Guarna, L. D. Corte, M. G. Giovanni, F. De Sarlo and G. Sgaragli, *Drug Metab. Dispos.* **11**, 581 (1983).
31. G. Sgaragli, L. D. Corte, R. Pulitt, F. De Sarlo, R. Francalanci and A. Guarna, *Biochem. Pharmac.* **29**, 763 (1980).
32. M. J. Nilges and H. M. Swartz, *J. biol. Chem.* **259**, 2446 (1984).
33. L. Ernster, *Meth. Enzym.* **10**, 309 (1967).
34. W. H. Habig, M. J. Pabst and W. J. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
35. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
36. F. R. Hewgill and D. G. Hewitt, *J. chem. Soc.* (C) 726 (1967).
37. R. E. Parchment and A. M. Benson, *Biochem. biophys. Res. Commun.* **119**, 1015 (1984).
38. A. M. Benson and P. B. Barretto, *Fedn Proc.* **43**, 590 (1984).
39. M. Matsuo, K. Mihara, M. Okuno, H. Ohkawa and J. Miyamoto, *Fd chem. Toxic.* **22**, 345 (1984).
40. R. El-Rashidy and S. Niazi, *Biopharm. Drug Dispos.* **4**, 389 (1983).