Electronic and Structural Requirements for Metabolic Activation of Butylated Hydroxytoluene Analogs to Their Quinone Methides, Intermediates Responsible for Lung Toxicity in Mice

Kenji YAMAMOTO,*^a Sachiko KATO,^a Kazuo TAJIMA,^a and Tamio MIZUTANI^b

Department of Chemistry, Hokuriku University,^a Ho-3 Kanagawa-machi, Kanazawa 920-11, Japan and Department of Food Science and Nutrition, Kyoto Prefectural University,^b Sakyo-ku, Kyoto 606, Japan.

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Previous studies have shown that butylated hydroxytoluene (BHT) undergoes oxidation by cytochrome P450 to form BHT-quinone methide. BHT-quinone methide is probably responsible for BHT-induced lung damage in mice. In this study, we calculated the MO parameters for BHT analogs and the corresponding quinone methide intermediates. Except for the analogs with structures that form a highly sterically hindered quinone methide, correlations could be established between the lung toxicity in mice and electronic charges on the hydroxyl oxygen and 4-carbon atoms of BHT analogs. The same toxicity could also be correlated to the difference between the heat of formation of the quinone methide intermediates and the parent BHT analogs, and to the electronic charge on the carbonyl oxygen atom of the quinone methides. These results suggest that the metabolic activation of BHT analogs to their quinone methide intermediates is energetically dependent on the oxidation of the aromatic π -electron system, and that the toxic potency of BHT analogs is controlled by protonation of the oxygen atom of the quinone methides. These electronic features provide further evidence of the importance of the quinone methide intermediates in the mechanism of lung toxicity induced by BHT analogs.

Key words butylated hydroxytoluene; lung toxicity; structure-activity relationship; BHT-quinone methide; MO calculation

Butylated hydroxytoluene (BHT, 2,6-di-*tert*-butyl-4-methylphenol) is a widely used antioxidant. BHT causes lung damage in mice, as characterized by the necrosis of type I alveolar cells followed by the proliferation of type II alveolar cells and an accompanying increase in lung weight.^{1,2)} On the basis of the structure-toxicity relationships of BHT analogs³⁾ and the isotope effects on the metabolism and toxicity of BHT by the deuteration of the 4-methyl group,⁴⁾ we have proposed that BHT-quinone methide (BHT-QM), an electrophilic intermediate, is responsible for BHT-induced lung damage in mice (Fig. 1). BHT-QM is formed by the cytochrome P450-dependent oxidation of BHT in liver and lung microsomes.^{4–6)} Conjugates of BHT-QM with cellular nucleophiles, such as glutathione (GSH)^{5–7)} and cysteine,⁸⁾ have been identified in both *in vitro* and *in vivo* studies. Depletion of tissue GSH increased BHT-induced lung toxicity,⁹⁾ whereas cysteine administration protected mice from the toxicity.^{9,10)} All these data are compatible with the proposal that BHT-induced lung toxicity is mediated by the covalent binding of BHT-QM to lung macromolecules.^{11,12)} However, the toxic effect of BHT-QM cannot readily be tested by direct administration of the

compound to mice because of its high lability.^{11,12)}

The present study deals with the electronic and structural requirements for the metabolic activation of BHT analogs to their quinone methide intermediates in mice. The quantitative structure-activity relationship (QSAR) was examined between the lung toxicity of BHT analogs and the MO parameters of both the parent analogs and the corresponding quinone methides.

MATERIALS AND METHODS

Lung Toxicity The lung toxicity data for BHT and its analogs (Table 1) were taken from our previous work,³⁾ where lung damage was assessed by determining dry lung

Table 1. Structures of BHT Analogs

Compd. No.
	R ²	R ⁴	R ⁶
1	Me	Me	H
2	Me	Me	Me
3	Et	Me	H
4	Et	Me	Et
5	iso-Pr	Me	H
6	iso-Pr	Me	iso-Pr
7	<i>tert</i> -Bu	Me	H
8	<i>tert</i> -Bu	Et	H
9	<i>tert</i> -Bu	iso-Pr	H
10	<i>tert</i> -Bu	Me	Me
BHT (11)	<i>tert</i> -Bu	Me	<i>tert</i> -Bu
12	<i>tert</i> -Bu	Et	<i>tert</i> -Bu
13	<i>tert</i> -Am	Me	H
14	<i>tert</i> -Am	Me	<i>tert</i> -Am

Fig. 1. Proposed Activation and Detoxification Pathways in BHT Metabolism

Abbreviation: BHT-SG, BHT glutathione conjugate.

* To whom correspondence should be addressed.

Fig. 2. Relationship between the Lung Toxicity and Electronic Charge on the Hydroxyl Oxygen (A) or 4-Carbon (B) Atom of BHT Analogs. BHT analogs 1–14 are described in Table 1. The correlation coefficients were calculated for 11 analogs, other than 9, 12, and 14. A: $y = -23.03x - 3.86$, $r = -0.855$, $p < 0.001$. B: $y = -30.42x - 1.01$, $r = -0.931$, $p < 0.001$.

weight 4d after the administration of 2.27 mmol/kg of each compound to male ddY mice.

MO Calculations Semiempirical MO calculations were performed with Hyper Chem 4.5 software (Hypercube, Inc., Ontario, Canada). All geometric variables were fully optimized by the AM1 method.¹³⁾ The AM1 program is parameterized by fitting to experimentally determined heats of formation (H_f) for a set of molecules at 298 K. The MO calculations gave data of the molecular charge distribution and H_f for BHT analogs and the corresponding quinone methides.

RESULTS AND DISCUSSION

BHT and thirteen of its analogs, with structures that can potentially form a *p*-quinone methide, were examined for possible QSAR between the lung toxicity and electronic features. BHT analogs, except 2-*tert*-butyl-4-isopropylphenol (9), 2,6-di-*tert*-butyl-4-ethylphenol (12), and 2,6-di-*tert*-amyl-4-methylphenol (14), had good significant QSARs between the toxic potency and the electronic charges on both the hydroxyl oxygen and the 4-carbon atoms (Fig. 2). Higher toxic analogs, 2-*tert*-butyl-4-methylphenol (7), 2-*tert*-amyl-4-methylphenol (13), 2-*tert*-butyl-4,6-dimethylphenol (10), and BHT (11), showed more minus charges on both atoms as compared to the non-toxic analogs 1–6. Similarly, the moderately toxic analog, 2-*tert*-butyl-4-ethylphenol (8), showed more minus charges. Although 9, 12, and 14 also had more minus charges, they were essentially ineffective in inducing lung damage. For reasons to be described later, the quinone methide intermediates formed from these three analogs seem to be unreactive to lung macromolecules. The electronic charge on the hydroxyl oxygen atom was about 2.5-fold that on the 4-carbon atom for each analog. Previous studies indicated that the cytochrome P450-catalyzed oxidation of the aromatic π -electron system of BHT to form the corresponding phenoxy radical is the first step in the bioactivation of this compound.^{12,14)} The resulting radical can subsequently produce BHT-QM by the loss of a second electron. Therefore, the QSARs shown in Fig. 2 agree with the previous proposal that the lung toxicity of BHT analogs is mediated by their quinone methide intermediates.³⁾

Fig. 3. Relationship between the Lung Toxicity of BHT Analogs and the Difference in the Heat of Formation between the Quinone Methide Intermediates and the Parent BHT Analogs.

BHT analogs 1–14 are described in Table 1. The correlation coefficient was calculated for 10 analogs, other than 8, 9, 12, and 14. $y = -0.077x + 5.47$, $r = -0.779$, $p < 0.01$.

The mechanics of quinone methide formation were calculated as the difference between the heat of formation of the quinone methide intermediates and the parent BHT analogs (ΔH_f). Figure 3 shows a significant QSAR between the toxic potency and the ΔH_f value for BHT analogs, except in 8, 9, 12, and 14. The higher toxic analogs, 7, 13, 10, and 11, showed smaller ΔH_f values than the non-toxic analogs 1–6. Although 8, 9, and 12 were energetically favorable to the formation of quinone methides, methyl substituent(s) on the terminal methylene carbon of the resulting quinone methides may reduce the reactivity of intermediates,³⁾ resulting in a partial or complete loss of toxicity. The observed difference in toxicity between 8 and 12 can be ascribed to the difference in the hindering effects of *ortho-tert*-butyl group(s) on the carbonyl group in their quinone methides. Irrespective of its low ΔH_f value, analog 14 was also ineffective in inducing lung toxicity. Possible reasons for this discrepancy may be that the hindering effect of two *ortho-tert*-amyl groups on the hydroxyl group extremely reduces the cytochrome P450-catalyzed oxidation of 14 to its quinone methide intermediate, and that the carbonyl group of the intermediate is highly sterically hindered. The latter effect may result in a lowering of the reactivity of quinone methide. Non-toxic analogs 1–6 themselves, in contrast, were energetically less favorable

Fig. 4. Relationship between the Lung Toxicity of BHT Analogs and the Electronic Charge on the Carbonyl Oxygen Atom of the Quinone Methide Intermediates

BHT analogs 1–14 are described in Table 1. The correlation coefficient was calculated for 10 analogs, other than 8, 9, 12, and 14. $y = -33.00x - 7.57$, $r = -0.827$, $p < 0.01$.

to the formation of quinone methides. These results suggest that the metabolic activation of BHT analogs to their quinone methide intermediates, and hence the toxic potency of BHT analogs, are primarily energetically dependent on the oxidation of the aromatic π -electron system.

Quinone methides are susceptible to Michael type additions by many biological nucleophiles.^{12,15} The addition may occur stepwise in two ways, that is, protonation of the carbonyl oxygen atom may occur prior to the addition of the nucleophile, or *vice versa*.^{16,17} This reactivity of quinone methides is dependent on the nucleophilic nature of the carbonyl oxygen, in addition to the electrophilic nature of the methylene carbon. Figure 4 shows a significant QSAR between the toxic potency of BHT analogs, except for 8, 9, 12, and 14, and the electronic charge on the carbonyl oxygen of the corresponding quinone methides. Quinone methides formed from 7, 13, 10, and 11 showed more minus charges on the oxygen atom as compared to those from 1–6. The former quinone methides seem to be more susceptible to protonation of the oxygen atom, leading to the development of higher toxic potency. Quinone methides formed from 8, 9, 12, and 14 showed much too large minus charges on the oxygen atom to account for the moderately toxic or non-toxic potency of the parent analogs. As mentioned above, the unexpectedly lower toxicity of these analogs

may be ascribed to the hindered structures of the corresponding quinone methides. There was no significant relationship between the toxic potency and the electronic charge on the terminal methylene carbon (data not shown). Therefore, the observed QSAR suggests that the toxic potency of BHT analogs is controlled mainly by protonation of the carbonyl oxygen of quinone methide intermediates. Further studies are required to elucidate whether other electronic features of BHT analogs and their quinone methide intermediates are responsible for the lung toxicity of the parent analogs.

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