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(36) See footnote 33 in ref 31 for a summary of our previous findings relating to the amino acid analysis of *O*-methyltyrosine containing analogues of arginine-vasopressin. Hydrolysis of the products reported here which contained *O*-methyltyrosine with hydrochloric acid in the normal way yielded tyrosine. This demethylation was reportedly avoided by hydrolysis with sulfuric acid.[10] The quantitation of *O*-methyltyrosine reported here was by reference to the TFA cleavage product of Boc-*O*-methyltyrosine,[31] which emerged from the short column before lysine and contained no tyrosine. In samples containing *S*-benzylcysteine, there was overlap, hence, the indication of data as approximate. Hydrolysis of Boc-*O*-methyltyrosine with sulfuric acid[10] under the conditions used for hydrolysis of peptides was shown to allow an 85–90% recovery, 80% as material identical to the TFA cleavage product, and assumed, therefore, to be *O*-methyltyrosine and 5–10% as tyrosine. There was in the case of protected peptides indication that hydrolysis by sulfuric acid of the peptide chain was incomplete, small amounts of material eluting in the void volume of the long column.
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Notes

Synthesis and Chemical Carcinogen Inhibitory Activity of 2-*tert*-Butyl-4-hydroxyanisole

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The title compound **1** was selectively synthesized in its pure isomeric form by means of the hydroxyl-protecting reagent dimethyl-*tert*-butylchlorosilane. Exclusive silylation occurred at the less hindered hydroxyl group of **3**. Dimethyl sulfate methylation of **4** gave **5** in excellent yield. Compound **1** was then obtained by acid hydrolysis of **5**. The two BHA isomers, **1** and **2**, were tested on their inhibitory effects toward benzo[*a*]pyrene-induced neoplasia in the forestomach of the ICR/Ha mouse. Both isomers, when added to the diet, reduced the number of mice with tumors and the number of tumors per mouse. Isomer **1**, which has the less hindered free hydroxyl group, showed higher inhibitory effect in the present experimental model.

Butylated hydroxyanisole (BHA) has been widely used as an antioxidant to stabilize fatty foods since 1947.[1] Recently, BHA has been found to protect laboratory animals from chemically induced neoplasia under various experimental conditions.[2] While the detailed mechanism of protection is still under investigation, in vitro observations using benzo[*a*]pyrene (BP) as a model carcinogen indicate the BHA decreases the formation of active BP metabolites as well as the level of BP–DNA binding.[3] Commercially available BHA, which contains two isomers in an approximately 15:85 ratio of **1** and **2**, has been used for tumor protection and mechanistic studies. When

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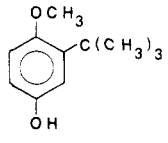
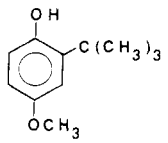


1



2

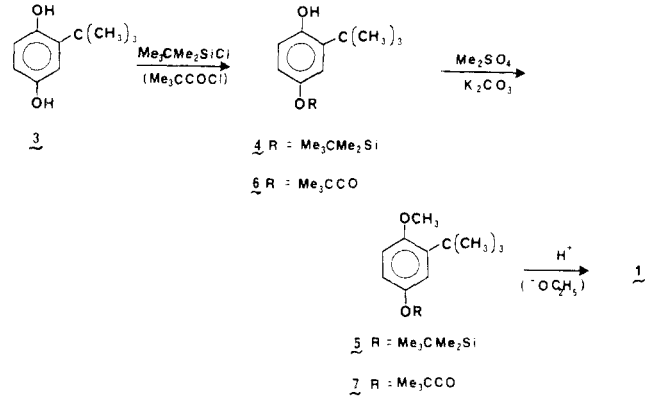
subjected to various oxidizing agents, 1 and 2 produced different products of oxidation due to the different *tert*-butyl group orientation relative to the phenolic function.[4] While the oxidation potentials of the two isomers are similar, their relative antioxidant efficiencies are different.[5] Consequently, the isomers may exhibit similar or different inhibitory effects toward chemical carcinogens.

The synthesis of BHA, either by tert-butylation of hydroxyanisole[6] or by methylation of *tert*-butylhydroquinone,[7] resulted in a mixture of 1 and 2 in approximately the same proportion as that of the commercial product. Isomer 2 can be obtained routinely in >99.5% purity from fractonal crystallization of the commercial mixture from acetone. Attempts to isolate 1 from the commercial mixture in large quantity, however, have been complicated by its rapid autoxidation in solution. In this work, isomer 1 was selectively synthesized and was tested along with the purified isomer 2 as inhibitors of BP-induced neoplasia in the ICR/Ha mouse.

Chemistry. Two different protecting groups have been used in the selective synthesis of 1 (Scheme I). These are the dimethyl-*tert*-butylsilyl group and the pivaloyl group. Pivaloyl chloride has been used successfully in the synthesis of peptides as a carboxyl-protecting group.[8] Dimethyl-*tert*-butylchlorosilane was developed as an hydroxyl protecting group in the prostaglandin synthesis[9] and has been used effectively in the syntheses of deoxynucleotides[10] and steroids.[11] Each reagent has a sufficiently bulky group to optimize steric interaction with the *tert*-butyl group on 3. The result was exclusive esterification or silyl ether formation at the less hindered hydroxyl group of 3. The pivaloyl chloride method for the synthesis of isomer 1 has been discussed in detail elsewhere.[12] Dimethyl-*tert*-butylchlorosilane in the presence of imidazole reacts with 3 almost instantaneously. The reaction was usually complete as soon as dropwise addition of the reagent was over in 1–2 h. Compound 4 can be isolated in very good yield. Dimethyl sulfate methylation of 4 or 6 in acetone in the presence of potassium carbonate gave 5 or 7 in excellent yield. Isomer 1 was obtained by saponification of 7 or acid hydrolysis of 5. The silyl ether method of synthesis is preferred when a relatively small quantity of 1 is required because of the ease in the incorporation and removal of the protecting group. The pivaloyl chloride method is recommended, however, when a large quantity is needed.

Biological Activity. The effect of the BHA isomer on BP-induced neoplasia of ICR/Ha mouse forestomach is given in Table I. In the experimental format employed, the autoxidants were mixed in the diet and the carcinogen was given by oral intubation in eight 1-mg doses over a period of 4 weeks. The experimental diet was given 1 week before the first carcinogen administration and continued until 2 additional days after the last dose of carcinogen. With this animal model, there was a 30 and 10% decrease in the number of mice with forestomach tumor when isomer 1 and 2 were added to the diet respectively. The number of forestomach tumors per mouse was decreased by approximately 70% for isomer 1 and by approximately 50% for isomer 2. Comparable protection in the forestomach was observed in previous experiments where the BHA mixture of 15:85 ratio of 1 and 2 was added to the diet.[2a]

Scheme I



Several studies of the effects of BHA on the microsomal metabolism of BP have been carried out in order to determine the mechanism by which this antioxidant inhibits BP-induced neoplasia. Detailed LC analysis of BP metabolites extracted from mouse liver microsomal incubations has shown a significant decrease of active BP metabolites as a result of BHA feeding.[3a] Incubation of BP with microsomes from mice fed BHA results in less binding of BP metabolites to DNA than with microsomes from control mice. In addition, the cytochrome P-450 enzyme characteristics from mice fed BHA are altered.[3b] Chung and co-workers reported the inhibition of the monooxygenase system by in vitro addition of BHA in rat liver microsomal systems. BHA was found to bind to cytochrome P-450 and induce "type I" binding spectra.[13] Because of the data showing an altered BP metabolism by BHA, LC analysis of the BP metabolites, extracted from mouse liver microsomal incubations prepared from mice fed 1 or 2, was initiated. In preliminary work, a similar pattern of active and nonactive metabolites has been obtained with both isomers. Using a polarographic method, Penketh correlated the oxidation potential and antioxidant activity of various phenolic antioxidants. He assigned values of relative antioxidant efficiencies based on relative induction periods of the antioxidants. The relative efficiencies of 1 and 2 are 1.1 and 0.40, respectively.[5] Since a low relative efficiency is indicative of a high antioxidant activity and vice versa, 2 is expected to be a better antioxidant than 1. The results indicate that high antioxidant activity does not necessarily give a high inhibitory effect in the forestomach. This was also observed when BHA and BHT were found equally effective in the inhibition of the forestomach tumor, while the former has much higher antioxidant activity.[2a] The mechanism of protection by BHA isomers is most likely the result of an alteration of enzyme systems that are responsible for the biotransformation of BP.

Experimental Section

Melting points were taken with a Fisher-Johns melting-point apparatus and are uncorrected. IR spectra were recorded on a Beckman Acculab 5 spectrometer. UV spectra were recorded on a Beckman Model 25 spectrophotometer. NMR were obtained on a Varian 80-MHz HFT spectrometer with tetramethylsilane as internal standard. TLC was done on silica gel GF plates (Analtech) and was sprayed with a 1:1 mixture of 0.1 M $FeCl_3$ and 0.1 M $K_3Fe(CN)_6$ solutions. Elemental analyses were done by M-H-W Laboratories, Phoenix, Ariz.

2-*tert*-Butyl-4-[(dimethyl-*tert*-butylsilyl)oxy]phenol (4). To a solution of 3 (8.3 g, 0.05 mol) in 100 mL of dry DMF at 10

Table I. Effect of BHA on BP-Induced Neoplasia of the Forestomach of ICR/Ha Mice

expt no.	carcinogen[a] (8 × 1 mg/mouse)	exptl diet: BHA isomer (5 mg/g of diet)	no. of mice	diet intake (g/mouse/day)	body wt (g) gained	tumors of forestomach: no. of mice with tumors	tumors of forestomach: % of mice with tumors	tumors of forestomach: no. of tumors/mouse[b]
1	BP	none	16	4.3	10.3	15	94	4.2 ± 0.6
	BP	2	19	3.7	7.4	15	79	2.3 ± 0.5
	BP	1	20	3.6	10.8	12	60	1.1 ± 0.4
2	BP	none	20	4.3	11.0	18	90	4.7 ± 0.6
	BP	2	19	4.2	13.5	15	79	2.5 ± 0.6
	BP	1	20	4.2	15.0	13	65	1.7 ± 0.6

[a] BP (1 mg/0.2 mL of corn oil) was administered by oral intubation. [b] $p < 0.02$; mean ± SE.

°C was added dropwise with stirring a solution of dimethyl-*tert*-butylchlorosilane (9 g, 0.06 mol) and imidazole (8 g, 0.0117 mol) in 100 mL of DMF. After the addition was completed, the reaction mixture was diluted with 500 mL of ether and was washed five times with 200 mL of water. The ether layers was then washed with 10% sodium bicarbonate solution and water and dried over anhydrous magnesium sulfate. The ether layer was removed in vacuo and **4** was obtained in 90% yield. Recrystallization from benzene gave pure **4**: mp 59 °C; UV λ_{max} (95% EtOH) 204 nm (ϵ 17.0), 227 (5.80), 284 (2.67); IR (CCl_4) 3610 (free OH) and 3390 (bonded OH), 1255 ($SiCH_3$), 865, 840 cm^{-1} ($SiCH_3$); NMR ($CDCl_3$) δ 0.15 (s, 6), 0.96 (s, 9), 1.36 (s, 9), 4.58 (s, 1), 6.49–6.74 (m, 3); mass spectrum (70 eV) *m/e* (relative intensity) 280 (64), 223 (53), 167 (100), 57 (57). Anal. ($C_{16}H_{28}O_2Si$) C, H.

2-*tert*-Butyl-4-[(dimethyl-*tert*-butylsilyl)oxy]anisole (5). A solution containing **4** (28 g 0.1 mol), dimethyl sulfate (25.2 g, 0.2 mol), and powdered anhydrous potassium carbonate (50 g, 0.36 mol) in 350 mL of dry acetone was heated under reflux for 5 h. The reaction was cooled and the solid removed by filtration. The acetone solution was concentrated by reduced-pressure distillation. The residue was dissolved in 350 mL of ether, and the ether solution was washed with 3 × 50 mL of H_2O, 2 × 50 mL of 10% sodium bicarbonate, and again with 50 mL of H_2O. The ether layer was dried over anhydrous magnesium sulfate and the solvent removed in vacuo. The crude product was distilled under reduced pressure: yield 80%; mp 20.5–21.0 °C; IR (CCl_4) 1255 ($SiCH_3$), 865, and 840 cm^{-1} ($SiCH_3$); UV λ_{max} (95% EtOH) 204 nm (ϵ 17.1), 227 (7.57), 284 (2.80); NMR ($CDCl_3$) δ 0.17 (s, 6), 0.97 (s, 9), 1.34 (s, 9), 3.76 (s, 3), 6.66–6.76 (m, 3); mass spectrum (70 eV) *m/e* (relative intensity) 294 (87), 279 (9), 237 (26), 223 (10), 181 (100), 57 (23). Anal. ($C_{17}H_{30}O_2Si$) C, H.

2-*tert*-Butyl-4-hydroxyanisole (1). To a solution of **5** (10 g, 0.034 mol) in 50 mL of THF was added 50 mL of 6 N HCl. The reaction mixture was vigorously stirred for 2 h. Ether was then added. The ether solution was washed with 150 mL of H_2O, 2 × 50 mL of 10% sodium bicarbonate solution, and again with 50 mL of H_2O. The ether layer was dried over anhydrous magnesium sulfate. The solvent was removed in vacuo, the crude product was purified by a silica gel column eluted with 4:1 petroleum ether–ether, and **1** was recrystallized from cyclohexane: yield 75%; mp 62.0–62.5 °C, lit. 62.5 °C;[6] NMR[14] ($CDCl_3$) δ 1.33 (s, 9), 3.77 (s, 3), 5.11 (s, 1), 6.68–6.82 (m, 3).

Forestomach Tumor Study. At 11 or 12 weeks, female ICR/Ha mice were randomized by weight and placed on experimental diets of powdered Purina Rat Chow to which BHA isomers had been added. One week after the start of the experimental diet, BP (1 mg/0.2 mL of cottonseed oil) was administered by oral intubation twice a week for a total of eight administrations. Two days after the carcinogen administration, the mice were taken off the experimental diet and were returned to a diet of Purina Rat Chow pellets. The mice were killed 20 weeks after the first dose of carcinogen, and the stomachs were fixed in an expanded state produced by intragastric injection of formalin; subsequently, they were split longitudinally. The tumors were counted under a dissecting microscope.

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