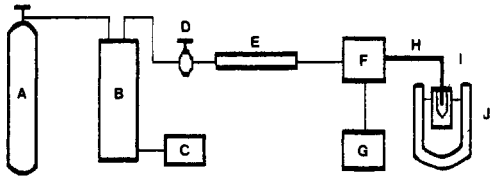

Figure 6. Schematic diagram of the NCFC purification system. (A) CO_2 tank; (B) pump; (C) microcomputer; (D) injector valve; (E) HPLC column; (F) UV detector; (G) chart recorder; (H) capillary restrictor; (I) sample microvial; (J) liquid nitrogen container–Dewar flask.

fluid" in this paper to emphasize this choice, although the use of lower temperatures appears logical in purification of labile substances.

Although purification of (*S,S*)-3,4-dehydro-*exo*-brevicomin and (*E,E*)-α-farnesene by near-critical fluid chromatography have been described here as examples, we have applied similar procedures successfully to other volatile and thermolabile compounds.

Experimental Section

The near-critical fluid chromatography system, constructed in this laboratory from various HPLC and GC hardware components, consisted of a 250-mL Varian 8500 syringe pump (Walnut Creek, CA), modified for pressure control through a microcomputer as published by Van Lenten and Rothman,[12] a UV-absorbance detector with a 3.5-μL high-pressure flow cell (Isco, Lincoln, NE), a conventional HPLC column, and an injection valve with a 10-μL loop (Rheodyne, Cotati, CA), as shown in Figure 6. Phenomenex Ultromex 5 C_{18} 25 cm × 4.6 mm, i.d., or Water Nova-Pak C_{18}, 30 cm × 3.9 mm, i.d., columns were utilized. A 50 cm × 50 μm, i.d., fused silica capillary was attached to the detector outlet as a pneumatic restrictor.

(*S,S*)-3,4-Dehydro-*exo*-brevicomin[8] and (*E,E*)-α-farnesene[9] were prepared as described.

Synthetic products and the fractions recovered by near-critical fluid chromatography were analyzed by capillary GC (a modified Varian 1400 gas chromatograph) and peak identities were ascertained by gas chromatography/mass spectrometry (GC/MS) using a Hewlett-Packard 5981 instrument with Incos data system (Finnigan MAT, San Jose, CA). The capillary column used (60 m × 0.25 mm, i.d.) was coated statically using Ucon 50-HB-2000, with benzyltriphenylphosphonium chloride added as a column deactivation agent.[13] Preparative GC of (*E,E*)-α-farnesene was carried out with a Varian 3700 gas chromatograph, using a thermal conductivity detector and a packed column (3 m × 1.2 mm, i.d.) with 6% SE-30 stationary phase on a 100–120 mesh Supelco port (Supelco, Inc., Bellefonte, PA).

The fractions from the near-critical fluid separation were collected directly into a 3-mL sample microvial immersed in liquid nitrogen (Figure 6). Typically, 5–10-μL aliquots of crude synthetic products were injected into the packed column. While pressurized carbon dioxide was used as the mobile phase, the column head pressure was held at 85 atm, and the separations were carried out at 25 °C. The fractions were collected by inserting the capillary restrictor end through a small hole in the cap of the vial. When half the volume of the collection vial became filled with frozen column effluent, the vial was removed from the liquid nitrogen. By slightly loosening the cap, carbon dioxide was allowed to escape at room temperature, while the solute remained in the vial. After collection, the vial was completely sealed with an intact cap and stored in the freezer before analysis or biological tests were performed.

Acknowledgment. This work was supported by Grant No. CHE 8605935 from the National Science Foundation.

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Structures of Autoxidation Products of 2-*tert*-Butyl-4-methoxyphenol in Aqueous Alkaline Solution

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2-*tert*-Butyl-4-methoxyphenol (**1**) is a major component of BHA, an extensively used antioxidant. Oxidation of **1** with various oxidants afforded 3,3′-di-*tert*-butyl-2,2′-dihydroxy-5,5′-dimethoxybiphenyl (**2**), presumably formed by coupling of the aryloxy radicals derived from **1**.[2-5] Kurechi et al. reported isolation of **2** and its isomer 2′,3-di-*tert*-butyl-2-hydroxy-4′,5-dimethoxydiphenyl ether as the products of ultraviolet-irradiated autoxidation of **1** in ethanol.[6,7] Oxidation of **1** in an aqueous alkaline solution with atmospheric oxygen was also studied and the solid that precipitated in the course of oxidation was characterized as **2**.[8] In this note we will describe the isolation and structure determination of four components other than **2** from the alkaline autoxidation mixture of **1**.

Results and Discussion

Autoxidation of 2-*tert*-butyl-4-methoxyphenol (**1**) was carried out in a 0.5 M NaOH solution. The solution of **1** was vigorously stirred to attain intimate contact with atmospheric oxygen. As oxidation proceeded, the slightly colored solution turned into a dark red mixture from which a powder precipitated gradually. After 1 week, all of **1** had been consumed and the mixture was filtered to give the crude dimeric product **2** in 46% yield. The filtrate was acidified by concentrated HCl and extracted with ether. Concentration of the ether layer induced precipitation of a white solid, which was collected by filtration. The solid was almost homogeneous, consisting of one main component (**3**, $C_{19}H_{26}O_5$; yield 1.8%). The mother liquor of **3** was evaporated to give a dark brown oil, which was chromatographed over Al_2O_3. Elution with benzene gave yellow crystals in 5.4% yield. SiO_2 TLC analysis indicated that the crystals were composed of two components, **4a** and **4b**, in the ratio of ca. 6:4. Isolation of each compound was attained by recrystallization from hexane and SiO_2 column chromatography.

Compound **4a**, mp 168–171 °C, showed a molecular ion peak $(C_{18}H_{22}O_3)^+$ in the high resolution mass spectrum. Three carbonyl stretching bands at 1720, 1680, and 1630 cm^{-1} in the IR spectrum accounted for the three oxygen atoms of the molecular formula. The ^1H NMR spectrum of **4a** taken in $CDCl_3$ exhibited five singlets, indicating the presence of two *tert*-butyl groups (δ 1.29 and 1.32), one

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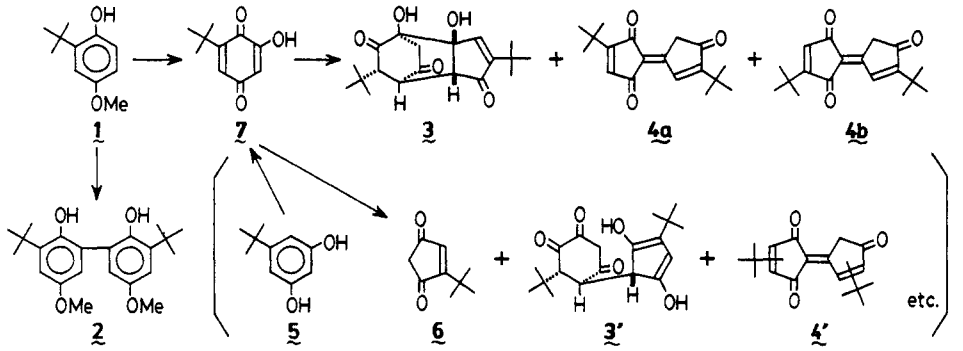
0022-3263/89/1954-4215$01.50/0

Scheme I. Autoxidation of 2-*tert*-Butyl-4-methoxyphenol (1). Autoxidation of 5-*tert*-Butylresorcinol (5) Described in Reference 9 Is Also Given in Brackets



methylene (δ 3.49), and two olefinic hydrogens (δ 6.90 and 8.64). The remaining four carbon atoms in the molecular formula of **4a** were shown to be olefinic carbon atoms bearing no hydrogen atoms by the ^{13}C NMR spectrum measured in $CDCl_3$. All spectral data of the other product **4b**, mp 112 °C, closely resembled those of **4a**, e.g., the ^1H NMR spectrum of **4b** was almost superimposable on that of **4a**, showing five singlets at δ^{CDCl_3} 1.30, 1.34, 3.49, 6.89, and 8.66. One of the autoxidation products of 5-*tert*-butylresorcinol (**5**) exhibits spectral data very similar to those of **4a** and **4b** as reported by Musso and Borman,[9] who assigned structure **4'** to their compound since it was also obtained by dimerization of 4-*tert*-butyl-4-cyclopentene-1,3-dione (**6**), but the position of the *tert*-butyl groups in **4'** was not determined (see Scheme I). Taking into account the steric hindrance due to the bulky *tert*-butyl group, one of the *tert*-butyl groups of **4'** can be located at the 3' position. Thus, the structure of **4'** is considered to be either the *Z* or *E* isomer of 4-*tert*-butyl-2-(3-*tert*-butyl-4-oxo-2-cyclopenten-1-ylidene)-4-cyclopentene-1,3-dione. Since the melting point reported for **4'** was 184–185 °C, we assumed that our autoxidation product **4a** corresponds to **4'** and that the other product **4b** whose melting point is much lower than **4a** is the geometrical isomer of **4'**. For determination of the geometry of the pivotal double bond, the effect of the addition of lanthanoid shift reagent tris(dipivaloylmethanato)europium (Eu-DPM) upon the proton chemical shifts of **4a** and **4b** was studied. A prominent downfield shift of the methine resonance (H-2', δ 8.64) was observed for **4a**, while in the case of **4b** the lanthanoid induced shift of the methylene signal was the largest as shown in Figure 1. Assuming that the reagent coordinates preferentially to the carbonyl oxygen at the C-1 position, which is the least hindered of the three carbonyl groups, the C-2' methine in **4a** and the C-5' methylene in **4b** were considered to be cis to the C-1 carbonyl group. Thus, the *E* and *Z* configurations were assigned to **4a** and **4b**, respectively. The structure of **4a** was finally confirmed by X-ray crystallographic analysis, the details of which will be published elsewhere.

For the purpose of obtaining experimental support for the *E*–*Z* isomeric relationship of **4a** and **4b**, the photoinduced isomerization reaction[10] was attempted. As expected, UV irradiation of a benzene solution of **4a** afforded a mixture which contained **4a** and **4b** in a ratio of 5:7, indicating the configurational isomerism of these compounds. It was also found that dissolution of **4a** or **4b** in pyridine-d_5 gave a 1:1 mixture of **4a** and **4b** as analyzed by ^1H and ^{13}C NMR spectroscopy.

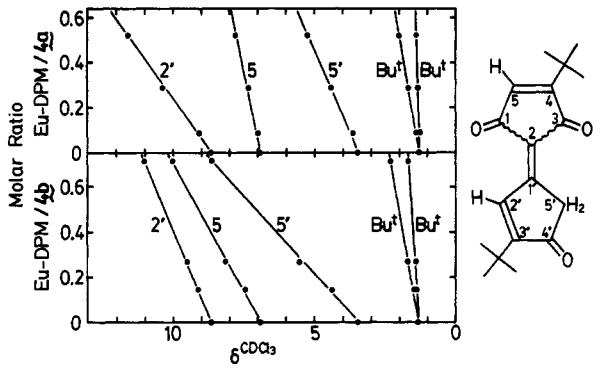


Figure 1. Dependence of proton chemical shifts of **4a** and **4b** upon the addition of Eu-DPM.

Since **4a** was formed from **1** in the present study and also from **5** as described in ref 9, both reactions were considered to proceed through common intermediates. According to Musso and Borman,[9] oxidation of **5** affords 2-*tert*-butyl-6-hydroxy-*p*-benzoquinone (**7**), which undergoes a benzilic acid rearrangement followed by oxidative decarboxylation yielding cyclopentenedione **6**. Hence it was considered that **7** and **6** were formed from **1** as intermediates to **4a** and **4b**. Although the presence of **6** could not be ascertained, the benzoquinone **7** was actually isolated from the reaction mixture. The yield of **7** was shown to reach a maximum (7.7%) after 2 days and then it decreased gradually to become negligible after 10 days. When **7** was subjected to the alkaline autoxidation, **3**, **4a**, and **4b** were formed, indicating that oxidation of **1** to **3** also proceeds via the intermediate **7**.

Compound **3**, mp 293–293.5 °C, was shown to possess the molecular formula $C_{19}H_{26}O_5$ by elemental analysis and a molecular ion peak at *m/z* 334 in the mass spectrum. The IR spectrum exhibited carbonyl absorptions (1740 and 1710 cm^{-1}) and hydroxyl bands, and the UV spectrum showed absorption due to a conjugated enone system (225 nm, ε 3800). The ^1H NMR spectrum of **3** in DMSO-d_6 indicated the presence of two *tert*-butyl groups (δ 1.00, s and 1.15, s), one methylene (δ 2.10, d, *J* = 19.8 and 2.29, d, *J* = 19.8 Hz), one deshielded olefinic proton (δ 7.12, s), two tertiary hydroxyls (δ 5.60, s and 5.75, s), and three adjacent methines (δ 2.67, d, *J* = 1.3; 3.17, dd, *J* = 1.3 and 3.7; and 2.84, d, *J* = 3.7 Hz). Since a product, mp 294 °C, reported from the alkaline autoxidation of **5** possessed spectral data very similar to those of **3** but was assigned structure **3'** as shown in Scheme I,[9] it seemd highly probable that it was identical with our **3**. The ^{13}C NMR spectral data of **3**, however, demonstrated the presence of two olefinic carbons ($\delta^{pyridine\text{-}d_5}$ 154.1 and 157.0), in addition to three carbonyl groups ($\delta^{pyridine\text{-}d_5}$ 204.2, 207.2, and 210.8),

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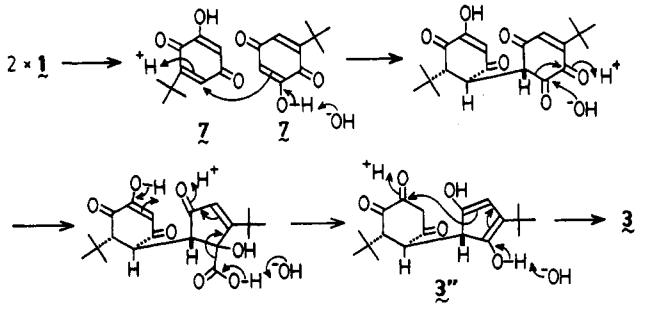
Scheme II



instead of four olefinic carbons as suggested by the structure 3′. Taking into account the molecular formula $C_{19}H_{26}O_5$, the presence of a carbon–carbon double bond and three carbon–oxygen double bonds indicated that **3** is tricyclic, presumably a tricyclo[5.2.2.0^{2,6}]undecane system formed by a cycloaddition of six-membered and five-membered rings. Detailed analysis of the nuclear Overhauser effect in the ^{1}H NMR spectra revealed the structure and stereochemistry of **3** as shown in Scheme I. An X-ray crystallographic analysis of the dibenzoyl derivative, which will be reported independently elsewhere, has confirmed the spectroscopically proposed structure of **3** as (1*R**,2*R**,6*S**,7*S**,9*R**)-4,9-di-*tert*-butyl-6,7-dihydroxytricyclo[5.2.2.0^{2,6}]undec-4-ene-3,8,10-trione.

The tricyclo[5.2.2.0^{2,6}]undecane system of **3** suggests that **3** is formed by cycloaddition of five-membered and six-membered ring intermediates. Although benzoquinone **7** and cyclopentenedione **6** seem to be good candidates for such intermediates, the addition of **6** to **7** would give a product with formula $C_{19}H_{24}O_5$ having fewer hydrogen atoms than **3** ($C_{19}H_{26}O_5$). One of the possible mechanisms for the formation of **3** is illustrated in Scheme II. A base-catalyzed dimerization product of **7** undergoes a benzilic acid rearrangement followed by decarboxylation, giving intermediate **3″**, which corresponds to a stereoisomer of **3′** proposed by Musso and Borman.[9] Transannular reaction of **3″** furnishes the tricyclic system **3**.

In summary, autoxidation of **1** afforded **2**, **3**, **4a**, **4b**, and **7**. The products **3**, **4a**, and **4b** were formed through the intermediate **7**. It was already known that **7** was also formed by the oxidation of **5** and other reported products from **5** seem to include **3** and **4a**, but the structure of **3** was erroneously assigned and a distinction among the possible isomers of **4a** was not made.[9] Our study has unambiguously established the structures and stereochemistry of **3**, **4a**, and **4b**.

Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus and are not corrected. IR and UV spectra were recorded on Jasco IRA-1 and Hitachi 124 spectrometers, respectively. NMR spectra were taken on Hitachi R-24A, Varian XL-200, or JEOL GX-400 spectrometers. Mass spectra (MS) were obtained on a Hitachi M-52 or a Hitachi M-2000 mass spectrometer at 70 eV.

Autoxidation of 2-*tert*-Butyl-4-methoxyphenol (1). Recrystallization of commercially available BHA (Nakarai Chemicals) from water afforded **1**, mp 61–62 °C, as colorless fine needles. A solution of **1** (18.02 g, 0.1 mol) in 0.5 M NaOH (1000 mL) was vigorously stirred under atmospheric oxygen for 7 days at room temperature. The reaction mixture was filtered to afford crude **2** (8.24 g, 46%) as a light brown solid. The filtrate was washed with ether, acidified with concentrated aqueous HCl, and extracted with ether. The ether extract was concentrated by evaporation and colorless precipitates of **3** (306 mg, 1.8%) were collected by filtration. The filtrate was evaporated to afford a dark oil, which was chromatographed over active Al_2O_3 (Sumitomo KCG-30), using benzene as eluent, to give a mixture of **4a** and **4b** (ca. 6:4) as yellow crystals (778 mg, 5.4%). Recrystallization from hexane yielded **4a** (321 mg) and SiO_2 (Merck Silica gel 60) column chromatography of the mother liquor afforded **4b** (112 mg).

Autoxidation of **1** (4.50 g, 25 mmol) in 0.5 M NaOH (250 mL) was undertaken for 2 days under the same conditions as described above. The biphenol **2** (1.35 g, 30%) was isolated and the filtrate was also treated similarly and was then subjected to SiO_2 column chromatography. Elution with chloroform yielded trace amounts of **4a** and **4b**, the intermediate **7** (348 mg, 7.7%), and **3** (20 mg, 0.5%).

3,3′-Di-*tert*-butyl-2,2′-dihydroxy-5,5′-dimethoxybiphenyl (2): colorless crystals from benzene, mp 237–238 °C (lit.[4] mp 228–229 °C, lit.[8] mp 237–238 °C); ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 18 H, 2 × Bu^t), 3.78 (s, 6 H, 2 × OMe), 5.05 (s, 2 H, 2 × OH), 6.64 (d, *J* = 3 Hz, 2 H, 2 × Ar H), 6.98 (d, *J* = 3 Hz, 2 H, 2 × Ar H).

(1*R,2*R**,6*S**,7*S**,9*R**)-4,9-Di-*tert*-butyl-6,7-dihydroxytricyclo[5.2.2.0^{2,6}]undec-4-ene-3,8,10-trione (3):** colorless crystals from EtOH, mp 293–293.5 °C; IR (KBr) 3540, 3400, 1740, 1710 cm^{-1}; ^{1}H NMR (DMSO-d_6; NOE data are given in brackets as peak enhancement in percent upon the irradiation of the proton designated in parentheses) δ 1.00 (s, 9 H, Bu^t on C-9), 1.15 (s, 9 H, Bu^t on C-4), 2.10 (d, *J* = 20 Hz, 1 H, H-11), [1 (H-5)], 2.29 (d, *J* = 20 Hz, 1 H, H-11′) [3 (Bu^t on C-9)], 2.67 (d, *J* = 1.3 Hz, 1 H, H-9) [19 (Bu^t on C-9), 6 (H-2), 5 (H-1)], 2.84 (d, *J* = 3.7 Hz, 1 H, H-2) [10 (H-1), 7 (H-9)], 3.17 (dd, *J* = 3.7 and 1.3 Hz, 1 H, H-1) [23 (Bu^t on C-9), 6 (H-2), 6 (H-9)], 7.12 (s, 1 H, H-5) [23 (Bu^t on C-4), 2 (H-11)]; ^{13}C NMR (pyridine-d_5) δ 28.2 and 29.0 (Me of Bu^t), 32.2 and 34.7 (C^{quat} of Bu^t), 43.1 (C-11), 52.0 and 57.2 (C-1, C-2, C-9, overlapped), 78.7 and 81.1 (C-6, C-7), 154.1 and 157.0 (C-4, C-5), 204.2, 207.2, and 210.8 (C-3, C-8, C-10); UV (MeOH) λ_{max} 225 nm (ε 3800); MS *m*/*z* 334 (M$^+$). Anal. Calcd for $C_{19}H_{26}O_5$: C, 68.24; H, 7.83. Found: C, 68.01; H, 7.79.

(*Z*)-4-*tert*-Butyl-2-(3-*tert*-butyl-4-oxo-2-cyclopenten-1-ylidene)-4-cyclopentene-1,3-dione (4a): yellow prisms from hexane, mp 168–171 °C; IR (KBr) 1720, 1680, 1630, 1590, 1570 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 9 H, Bu^t), 1.32 (s, 9 H, Bu^t), 3.49 (s, 2 H, H-5′), 6.90 (s, 1 H, H-5), 8.64 (s, 1 H, H-2′); ^{13}C NMR ($CDCl_3$) δ 28 (Me of Bu^t), 34 (C^{quat} of Bu^t), 39.5 (C-5′), 141 and 145 (C-5, C-2′), 119.5, 149, 166.5, and 171 (C-2, C-4, C-1′, C-3′), 192, 193, and 202 (C-1, C-3, C-4′); UV (EtOH) λ_{max} 230 (ε 13 000), 302 nm (34 000); MS *m*/*z* 286 (M$^+$). Anal. Calcd for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74. Found: C, 75.53; H, 7.87.

(*E*)-4-*tert*-Butyl-2-(3-*tert*-butyl-4-oxo-2-cyclopenten-1-ylidene)-4-cyclopentene-1,3-dione (4b): yellow fine crystals from hexane, mp 112 °C; IR (KBr) 1720, 1680, 1630, 1590, 1570 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.30 (s, 9 H, Bu^t), 1.34 (s, 9 H, Bu^t), 3.49 (s, 2 H, H-5′), 6.89 (s, 1 H, H-5), 8.66 (s, 1 H, H-2′); ^{13}C NMR ($CDCl_3$) δ 28 (Me of Bu^t), 33.5 (C^{quat} of Bu^t), 40 (C-5′), 141 and 145 (C-5, C-2′), 119.5, 149.5, 167 and 171 (C-2, C-4, C-1′, C-3′), 192.5, 194, and 202 (C-1, C-3, C-4′); UV (EtOH) λ_{max} 232 (ε 17 800), 303 nm (36 000); MS calcd for $C_{18}H_{22}O_3$ *m*/*z* 286.1568, found 286.1590.

2-*tert*-Butyl-6-hydroxy-*p*-benzoquinone (7): yellow plates from hexane, mp 124–127 °C (lit.[9] mp 138–139.5 °C, lit.[11] mp 124–128 °C); ^{1}H NMR ($CDCl_3$) δ 1.25 (s, 9 H, Bu^t), 5.96 (d, *J* = 2.2 Hz, 1 H, Ar H), 6.43 (d, *J* = 2.2 Hz, 1 H, Ar H), 7.4 (s, 1 H, OH).

Autoxidation of 7. A solution of **7** (90 mg, 0.5 mmol) in 0.5 M NaOH (5 mL) was stirred for 7 days at room temperature. The reaction mixture was acidified with concentrated aqueous HCl and extracted with ether. From the ether extract **3** (3.8 mg, 4.5%) was isolated and the presence of **4a** and **4b** was ascertained by SiO_2 TLC (Merck Silica gel 60 F_{254}; R_f 0.4 and 0.25; benzene).

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