Substituent Effects on Chemosterilant Activity of 2,4-Di-*tert*-butyl-6-(4′-substituted benzyl)phenols in the House Fly (*Musca domestica* L.)

Jan Kochansky,* Charles F. Cohen, and William R. Lusby

Insect Neurobiology and Hormone Laboratory, Agricultural Research Service, U.S. Department of Agriculture, 10300 Baltimore Avenue, Beltsville, Maryland 20705

A series of 2,4-di-*tert*-butyl-6-(4′-X-benzyl)phenols was prepared, analogous to the compound Jurd 2644 (X = OMe) in an attempt to establish a Hammett relationship between the 4′-substituent and the chemosterilant activity of the compounds after oral administration to adult *Musca domestica*. Fourteen compounds of this type were prepared, 12 of them new. Jurd 2419 (X = H) was found to be as active in our bioassay as Jurd 2644, as were four new compounds [X = $N(CH_3)_2$, CH_3, SCH_3, Cl]. All prevented successful reproduction at 30 mg/kg of diet. There was no correlation between Hammett σ and activity. Active compounds all had small substituents and molecular weights below 350. The activity seemed to be determined by the bulk of X rather than by its electronic properties.

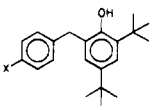
Keywords: *Reproduction; delayed toxicity; Jurd 2644*

INTRODUCTION

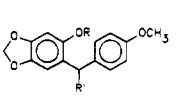
As part of a study of compounds responsible for the durability of wood from tropical trees of the genus *Dalbergia*, Jurd et al. (1971a) reported on the antimicrobial properties of obtusastyrene (4-cinnamylphenol). This study was soon extended to a series of synthetic analogs of obtusastyrene (Jurd et al., 1971b) and to benzylated phenols and benzodioxoles, summarized by Jurd and Manners (1980). More specifically, certain benzyl-substituted di-*tert*-butylphenols (particularly **Ia**, Figure 1) and benzyl-substituted 1,3-benzodioxoles (particularly **IIa,b**) were capable of sterilizing adult female house flies (*Musca domestica* L.) when fed in the diet at concentrations as low as 0.025% (Jurd et al., 1979). This sterility was not permanent, since egg viability returned when treated flies were subsequently fed untreated diets. There was also some sterilizing effect on males. The most active phenol was Jurd 2644 [2,4-di-*tert*-butyl-6-(4′-methoxybenzyl) phenol] (**Ia**, J2644) and it was used successfully for control of house flies in two screwworm rearing plants (Rawlins et al., 1982). The compound was nonmutagenic in the Ames tests (Jurd et al., 1979) and was therefore particularly attractive as a focus for further work. Chang et al. (1980) confirmed the activity of J2644 in flies treated orally, topically, or by injection but also noted the impermanence of its action. Matolczy et al. (1986) replaced the methoxy group in **Ia** with a propargyloxy in an attempt to create a cytochrome P450 oxidase inhibitor, but the compound was inactive against *Phormia regina* at 10 g/L in the diet.

The chemosterilant activity of J2644 is not limited to house flies. It also sterilized face flies (*Musca autumnalis* DeGeer) (Broce and Gonzaga 1987), screwworm flies [*Cochliomyia hominivorax* (Coquerel)] treated orally (Rawlins et al., 1979) or topically (Rawlins and Jurd, 1981), and the old-world screwworm fly (*Chrysomya bezziana* Villeneuve) (Pound and Spradbery, 1984). Activity was not universal, however, even in Diptera. J2644 and J2419 (**Ib**) were tested against the tsetse fly (*Glossina morsitans morsitans* Westwood) and showed very low activity (Langley et al., 1982). Compounds J2644 and J2419 were also poorly active against pink bollworm [*Pectinophora gossypiella* (Saunders)] (Flint et al., 1980). While J2644 and J2419 are able to interfere with mosquito larval development (albeit at concentrations higher than those required by their methyl ethers) (Dame and Jurd, 1983), treatment of mosquito eggs (Nelson et al., 1985) or pupae (Nelson and Hoosseintehrani, 1982) did not inhibit egg hatch to any useful extent.

It has been suggested (Jurd and Manners, 1980; Jurd et al., 1979) that the mode of action of these compounds involves a microsomal oxidation to unstable *o*-quinone methides, followed by reaction of these intermediates



I



II

	X	σ
a	OCH_3	-0.268
b	H	0.000
c	OH	-0.357
d	$O(CH_2)_3CH_3$	-0.320
e	$O(CH_2)_8CH_3$	
f	$N(CH_3)_2$	-0.600
g	$C(CH_3)_3$	-0.197
h	SCH_3	-0.047
i	$SOCH_3$	+0.567
j	SO_2CH_3	+0.728
k	CH_3	-0.170
l	Cl	+0.227
m	CO_2Me	+0.638 (σ⁺)
n	$N^+(CH_3)_3$	+0.859

a	$R = OCH_2CH_2CH_3$	R′ = H
b	$R = CH_2CH_3$	$R' = CH_3$

Figure 1. Structures of the 2,4-di-*tert*-butyl-6-benzylphenols **I** and benzyl-1,3-benzodioxoles **II**. [Data are taken from Jaffe (1953).]

* Author to whom correspondence should be addressed [telephone (301) 504-8668; fax (301) 504-8526].

with cell components to interfere with egg production or hatching. Among a series of papers published on chemical oxidation of this class of compounds are three on J2644 itself (Jurd and Wong, 1981a,b; Wong and Jurd, 1984). Since J2644 (X = OMe) was reported (Jurd et al., 1979) to be 4-fold more active than J2419 (X = H), we considered the possibility of a Hammett relationship (Taft, 1963) between activity and X. We realized, of course, that it would be an oversimplification to expect so simple a relationship to hold clearly in the complexity of a biological system, since it neglects partition coefficients, absorption rates, activation, and other factors. What, after all, is the solvent polarity of a house fly?

Hoping that all of the complicating factors would not totally mask the effect of ring substituents, we prepared a series of analogs of J2644 in which the substituent on the benzyl ring was varied from the strongly electron-donating $-N(CH_3)_2$ group to the strongly electron-withdrawing $-SO_2CH_3$ and $-CO_2CH_3$ groups. The compounds were fed to adult flies, and their effects on reproduction are reported here.

EXPERIMENTAL PROCEDURE

A. Synthesis and Characterization of the Chemicals Tested. Compounds with electron-donating substituents were synthesized according to suitable modifications of Jurd's method (Jurd et al., 1979): reaction of a substituted benzyl alcohol with 2,4-di-*tert*-butylphenol in a mildly acidic medium (acetic acid or acetic/formic acid mixtures, sometimes containing a little oxalic acid) (method A). Compounds having neutral or mildly electron-withdrawing substituents (H, CH_3, Cl) were prepared according to the method of Green (1965) using the phenol, the benzyl chloride, and $ZnCl_2$ in $CHCl_3$ (method B). Attempts to prepare compounds having strongly electron-withdrawing substituents according to either of these methods failed, since more strenuous reaction conditions led to loss of *tert*-butyl groups from the starting phenol. These compounds were therefore prepared by conversion of more easily-prepared materials by oxidation (**Ii, Ij**), hydrolysis (**Im**), or alkylation (**In**) as described below.

Gas chromatography (GC) was performed on a Packard-Becker Model 421 using 2 m × 6 mm glass columns packed with 3% OV-1 on 100/120 Chromosorb W. Thin-layer chromatography was performed on silica gel plates (Analabs) using ether plus hexane (1+1) as mobile phase. Melting points were taken on a Thomas-Hoover apparatus in borosilicate glass capillaries with the use of the appropriate partial immersion thermometer. No further correction was applied (Tiers, 1990). Structures were confirmed by NMR as solutions in $CDCl_3$/TMS on a JEOL FX60Q instrument (δ scale) and by mass spectrometry on a Finnigan 4510 GC/mass spectrometer. QSAR calculations were run using HyperChem, release 4, with ChemPlus extensions, release 1 (Hypercube, Inc., Waterloo, ON).

Common solvents were of industrial grade, redistilled in glass. Unless otherwise indicated, all organic starting materials were from Aldrich. "Extractive workup" refers to partition of the product between water and an appropriate solvent (ether, if not otherwise specified) followed by washing of the organic phase with water and saturated aqueous sodium bicarbonate solution, drying with $MgSO_4$, and removal of the solvent on a rotary evaporator. Reductions with N g of $LiAlH_4$ were worked up by cautious addition of N mL of water, N mL of 15% NaOH, and $3N$ mL of water, filtration, and solvent removal.

Crude products were usually distilled, but a few could be crystallized directly. Column chromatography was carried out on 100–200 mesh Davison 923 silica gel (W. R. Grace). Even after distillation, compounds with bulky or nonpolar substituents on the benzyl group crystallized poorly, giving sticky solids of wider than normal melting ranges. These compounds were adequately pure [>98% with the exception of **Ic** (>95%)] by GC, however.

*2,4-Di-tert-butyl-6-(4′-methoxybenzyl)phenol (Jurd 2644, **Ia**)* was prepared according to method A and crystallized from methanol: mp 82–84 °C [lit. (Jurd et al., 1979) 84–85 °C].

*2,4-Di-tert-butyl-6-benzylphenol (J2419, **Ib**)* was prepared according to method B. 2,4-Di-*tert*-butylphenol (41.2 g, 200 mmol) was added to $CHCl_3$ (200 mL, dried over grade I neutral alumina), followed by $ZnCl_2$ (12 g, 88 mmol, freshly fused and powdered) and benzyl chloride (25 mL, 10% excess). After heating under reflux for 3 days, the mixture was washed once with dilute HCl (1+9) and once with water, dried, and concentrated. Distillation (155–167 °C/0.7 mmHg) yielded a fraction 83% pure by GC, which was crystallized from hexane to give 13.2 g (22%) of white crystals: mp 61–65 °C [lit. (Green, 1965) mp 54 °C]; MS 296 (32, M^+), 81 (100), 203 (28), 133 (8), 91(32), 57 (28).

2,4-Di-tert-butyl-6-(4′-methoxybenzyl)anisole (Jurd 2644 Methyl Ether). Jurd 2644 (44 g, 135 mmol) was dissolved in DMF (250 mL, dried over molecular sieves). NaH (5.3 g, 10% excess) was added in two portions and the mixture stirred until effervescence ceased. The solution was cooled in ice, and methyl iodide (14 mL, 10% excess) was added from a syringe. After about half the iodide had been added (2–3 min), the solution solidified. The cake was broken up, 50 mL of ether was added, and the mixture was warmed to give a mobile slurry. After the addition of the remaining methyl iodide and then an additional 2 mL, the slurry was stirred for 1 h (no starting material was left by GC). Dilution with 500 mL of water, followed by extractive workup, gave a crude solid which was recrystallized from EtOH/H_2O (about 450 mL) to yield 40 g (87%) of white asbestiform needles: mp 96–98 °C; NMR δ 1.24, 1.41, (18H, 2s, tBu), 3.70, 3.77 (6H, 2s, OMe), 4.00 (2H, s, CH_2), 6.7–7.3 (6H, m, aromatic.); MS 340 (63, M^+), 325 (100), 217 (45), 163 (25), 121 (85), 57 (92).

*2,4-Di-tert-butyl-6-(4′-hydroxybenzyl)phenol (**Ic**).* Jurd 2644 methyl ether (5 g, 15 mmol) was dissolved in CH_2Cl_2 (25 mL) and cooled to −80 °C in a Kjeldahl flask with a serum stopper. Boron tribromide (29.5 mL of 1 M solution in CH_2Cl_2, 2 molar equiv, Aldrich) was added slowly from a syringe. The solution was allowed to stand at −80 °C overnight and then at room temperature for 5 h. Hydrolysis of an aliquot showed one product and no starting material. The solution was poured in iced saturated $NaHCO_3$ solution, and ether was added to make the organic phase the lighter one. The organic layer was separated, washed twice with saturated aqueous sodium bicarbonate, dried, and evaporated to give a yellow oil that was dissolved in hexane and chilled to give a yellow powder: mp 108–113 °C (cloudy melt). The crude product was recrystallized from heptane (charcoal) and then from 1:1 benzene/heptane to yield 3 g (64%) of an off-white solid: mp 112–116 °C (clear melt), 98.7% pure by GC; MS 312 (47, M^+), 297 (75), 203 (100), 161 (10), 149 (18), 107 (22), 57 (16).

*2,4-Di-tert-butyl-6-(4′-n-butoxybenzyl)phenol (**Id**).* Methyl *p*-hydroxybenzoate (Sigma) (76 g, 0.5 mol) was butylated in methanol (400 mL) with NaOH (1 equiv) at 0 °C and then with 1-bromobutane (1 equiv) under reflux for 4 h. The usual workup gave 82.5 g of an almost colorless oil, practically pure by GC. This crude product was reduced with $LiAlH_4$ (14 g, 370 mmol) in ether (1000 mL). Hydrolysis, filtration, and solvent removal gave 62 g of oil which was distilled at 160–161 °C/18 mmHg to yield 41.3 g (46% from methyl *p*-hydroxybenzoate) of a colorless oil which solidified to an icy solid: mp 30–33 °C [reported (Chemical Dynamics Corp. catalog) bp 154–156 °C/13 mmHg; mp 31–32 °C].

The benzylated phenol was prepared from 40 g (250 mmol) of the above 4-butoxybenzyl alcohol according to method A. The benzyl alcohol was mostly consumed after 6 h, but reflux was continued for 3 h more. Extractive workup with CH_2Cl_2 and distillation yielded 64.8 g of a pale yellow oil: bp 190–210 °C/0.8 mmHg (96% pure by GC). Fractional crystallization from methanol gave 43.3 g (47%) of a white powder: mp 54–59 °C; MS 368 (33, M^+), 353 (38), 204 (17), 203 (100), 162 (12), 161 (13), 150 (8), 107 (14), 57 (31).

*2,4-Di-tert-butyl-6-([4′-(n-decyloxy)benzyl]phenol (**Ic**)* was prepared as was the butoxy compound. Treatment of methyl

p-hydroxybenzoate with NaOH and decyl bromide gave methyl 4-decyloxybenzoate in 53% yield: mp 42–47 °C. Reduction of the ester (76 g) with $LiAlH_4/Et_2O$ as above gave 4-decyloxybenzyl alcohol (53.9 g, 78%) after recrystallization from hexane as large colorless plates: mp 58–60 °C [lit. (Anderson et al., 1986) 58–59 °C]. Reaction of this alcohol (32 g, 121 mmol) with 2,4-di-*tert*-butylphenol (30 g, 145 mmol) in HOAc (75 mL) in the presence of oxalic acid (2 g) under reflux for 10 h gave a product containing almost no decyloxybenzyl alcohol. Extractive workup with CH_2Cl_2 and removal of volatiles to a vapor temperature of 92 °C/0.6 mm gave a residue 95% pure by GC and containing only a trace of starting phenol. The product was a viscous light brown oil which crystallized very poorly. Protracted cooling (months) at 5 °C and pressing on clay gave a sticky solid of wide melting range and only slightly improved purity: MS 452 (19 M^+), 432 (11), 203 (100), 161 (21), 107 (21), 94 (27), 57 (77).

2,4-Di-tert-butyl-6-[4'-(dimethylamino)benzyl]phenol ***(If)***. 4-(Dimethylamino)benzyl alcohol was prepared by reduction of the corresponding benzaldehyde with $NaBH_4$ in ethanol at reflux for 2 h. Extractive workup and distillation gave a colorless liquid: bp 164–175 °C/24 mmHg, 132–135 °C/4 mmHg [lit. (Smith et al., 1984) 123 °C/0.8 mmHg] in 90% yield.

To 163 g (790 mmol) of 2,4-di-*tert*-butylphenol in a mixture of 200 mL each of acetic and formic acids was added 50 mL of the above (dimethylamino)benzyl alcohol. After 2 days of reflux, the balance of 100 g (660 mmol) of (dimethylamino)-benzyl alcohol was added rather too rapidly (some was lost when the solution boiled over). The solution was refluxed for 2 weeks (apparently at equilibrium as demonstrated by GC) and cooled, and the solvent was removed on a rotary evaporator. Cautious neutralization of the residue with solid Na_2CO_3, extractive workup, and distillation gave 57.4 g of an orange oil: bp 170–210 °C/2 mmHg, which was crystallized from hexane. Recrystallization of the first crop from hexane provided 20.6 g of almost white crystals (7.6% yield): mp 114–116.5 °C; NMR δ 1.37, 1.30 (18H, 2s, tBu), 2.90 (6H, s, NMe_2), 3.88 (2H, s, CH_2), 4.74 (1H, s, OH), 6.5–7.5 (aromatic, 6H); MS 339 (40, M^+), 203 (5%), 162 (15%), 121 (100, dimethylaniline).

Evaporation of the mother liquor and long standing gave a sticky solid which was recrystallized from hexane to yield 3.9 g of 2,2'-methylenebis(4,6-di-*tert*-butylphenol): mp 147–150 °C; NMR δ 1.23, 1.28 (36H, 2s, tBu), 3.93 (2H, s, CH_2), 5.85 (2H, s, OH), 7.18 (4H, weak multiplet, aromatic). An authentic sample was prepared according to the method of Gurvich et al. (1978) from 2,4-di-*tert*-butylphenol and methylal (H_2SO_4 catalyst) [reported (Gurvich et al., 1978) mp 141–142 °C]. No other products were isolated.

2,4-Di-tert-butyl-6-(4'-tert-butylbenzyl)phenol ***(Ig)***. 4-*tert*-Butylbenzoic acid was converted to the methyl ester with MeOH/2,2-dimethoxypropane/HOTs and the crude product reduced with $LiAlH_4/Et_2O$ to 4-*tert*-butylbenzyl alcohol as a pale yellow oil: bp 138–144 °C/19 mmHg (Aldrich catalog: 140 °C/20 mmHg) in 81% yield from the acid. This alcohol (40 mL, 225 mmol) was dissolved with 42 g (200 mmol) of 2,4-di-*tert*-butylphenol in 50 mL each of HOAc and formic acid and heated under reflux for 42 h. Extractive workup gave an oil which was combined with a similar preparation on one-fourth the scale for distillation (bp 160–200 °C/0.3 mmHg) as a yellow viscous oil (46 g, 65%) which was diluted with 25 mL of hexane. The product crystallized after 3 months at 5 °C. The product was collected by filtration and pressed on clay to give a sticky solid; mp 55–69 °C; MS 352 (39, M^+), 337 (85), 281 (100), 147 (40), 133 (30), 119 (29), 105 (35), 91 (30), 57 (46).

2,4-Di-tert-butyl-6-[4'-(methylthio)benzyl]phenol ***(Ih)***. 4-(Methylthio)benzyl alcohol (45 g, 291 mmol), 2,4-di-*tert*-butylphenol (65 g, 5 g excess), acetic acid (75 mL), oxalic acid (2 g), and H_2O (2 mL), along with a similar pilot reaction on a 5 g scale (carried to ca. 50% reaction) were refluxed for 45 h (GC of an aliquot at 24 h showed ca. 65% reaction). Extractive workup and two crystallizations from MeOH gave 50.5 g (51%) of pale yellowish platelets: mp 104–106.5 °C. A second crop (9.1 g, 9%) melted at 97–106 °C; MS 342 (58, M^+), 327 (65), 203 (100), 164 (34), 137 (20), 124 (40), 91 (10), 57 (40); NMR δ 1.29, 1.38 (18H, 2s, tBu), 2.45 (3H, s, SCH_3), 3.94 (2H, s, CH_2), 4.56 (1H, s, OH), 6.9–7.3 (6H, cm, aromatic).

2,4-Di-tert-butyl-6-[4'-(methylsulfinyl)benzyl]phenol ***(Ii)***. The methylthio compound **Ih** (3.4 g, 10 mmol) was dissolved in 30 mL of acetone. $NaIO_4$ (2.2 g, 10 mmol) was dissolved in 25 mL of water. The two solutions were mixed and enough acetone was added to dissolve the precipitate that formed (about 75 mL of total volume). The solution was warmed to 50 °C until reaction was essentially complete (ca. 3 h) as monitored by GC. Extractive workup and crystallization successively from hexane, benzene/heptane, and a small volume of aqueous MeOH gave 2.5 g (70%) of colorless needles: mp 130–134 °C; MS 358 (35, M^+), 343 (45), 341 (100), 328 (20), 203 (28), 187 (12), 172 (10), 164 (25), 57 (38); NMR δ 1.29, 1.41 (18H, 2s, tBu), 2.69 (3H, s, $SOCH_3$), 4.05 (2H, s, CH_2), 4.05 (2H, s, CH_2), 4.90 (1H, s, OH), 6.9–7.7 (6H, cm, aromatic).

2,4-Di-tert-butyl-6-[4'-methylsulfonyl)benzyl]phenol ***(Ij)***. The methylthio compound **Ih** (3.4 g, 10 mmol) was dissolved in acetone (30 mL), and 50% H_2O_2 (2 mL) was added; the solution was then heated to boiling. GC indicated ca. 30% reaction after 0.5 h. After 4 h, an additional 2 mL of H_2O_2 was added, and 15 minutes later, GC indicated the absence of starting material. Extractive workup gave an oil which eventually crystallized. Two recrystallizations from heptane yielded a colorless product: 1.7 g (45%); mp 128–132 °C; MS 374 (27, M^+), 359 (100), 203 (15), 140 (8), 118 (8), 107 (15), 91 (10), 57 (28); NMR δ 1.27, 1.35 (18H, 2s, tBu), 3.01 (3H, s, SO_2CH_3), 4.05 (2H, s, CH_2), 4.40 (1M, br s, OH), 7.0–8.0 (6H, cm, aromatic).

2,4-Di-tert-butyl-6-(4'-methylbenzyl)phenol ***(Ik)*** was prepared according to the same method and scale as **Ib**. Distillation (155–175 °C/0.5 mmHg) gave a yellow oil which was chromatographed on silica gel. Elution with hexane yielded a small amount of oil which was discarded. Subsequent fractions of 2.5% and 5% ether/hexane gave solids which were combined and recrystallized from methanol to give colorless crystals (5.2 g, 8%): mp 85–88 °C; MS 310 (68, M^+), 295 (100), 203 (28), 105 (10), 57(5).

2,4-Di-tert-butyl-6-(4'-chlorobenzyl)phenol ***(Il)*** was prepared similarly to **Ib** and on the same scale. Distillation (bp 155–177 °C/0.5 mmHg) gave 21.1 g of yellow oil. On chromatography, initial fractions of hexane and 2.5% ether/hexane gave small amounts of oil which were discarded. Two fractions of 5% ether/hexane gave an oil which crystallized. This solid was recrystallized from methanol to give 2.8 g (6%) of colorless crystals: mp 90–92 °C; MS 330 (33, M^+, ^{35}Cl), 332 (10, M^+, ^{37}Cl), 317 (32), 315 (100), 203 (20), 125 (10), 57 (9).

2,4-Di-tert-butylanisole. 2,4-Di-*tert*-butylphenol in ether was converted to the lithium salt with *n*-butyllithium/hexane, and the salt was methylated with dimethyl sulfate. After 16 h of reflux, unreacted Me_2SO_4 was hydrolyzed with 50 mL of 30% NH_3, followed by extractive workup and distillation (bp 130–135 °C/17 mmHg). From 412 g (2 mol) of phenol was obtained 395.8 g of product (90% pure by GC). Two crystallizations from hexane gave 183 g (42%) of pure (>99%) material. Mother liquors from this and other similar preparations were dissolved in dry DMF and treated with NaH to form the sodium salt. The salt was methylated with MeI by heating at reflux for 1 h. Results from this latter procedure were superior. Large pale greenish prisms, mp 34.5–36.5 °C, were obtained [lit. (Carpenter et al., 1951) bp 114 °C/4 mmHg, mp 36–37 °C]; MS 220 (18, M^+), 205 (100), 57 (20).

2,4-Di-tert-butyl-6-[4'-(trifluoromethyl)benzoyl)]anisole. 4-(Trifluoromethyl)benzaldehyde (120 g, 690 mmol, a gift of Hooker Chemical Co.) was suspended in water (1.5 L) and acidified with sulfuric acid (80 mL plus ice to make 300 mL), then oxidized to the acid with potassium permanganate (80 g, 8 g excess), and heated until the disappearance of the pink color. Addition of gaseous SO_2 to dissolve the MnO_2 precipitate and filtration gave the acid as a tan solid: mp 212–220 °C. Recrystallization from aqueous ethanol and then ether/toluene gave colorless crystals, 90 g (69%): mp 218–220 °C (Aldrich catalog: 219–220 °C). Oxidation in alkaline solution (NaOH) with permanganate was also successful.

The acid was converted to the chloride with excess $SOCl_2$ and a few drops of DMF. Distillation gave a colorless liquid, bp 87–90 °C/33 mmHg (Aldrich catalog: 78–79 °C/16 mmHg), in 90% yield.

The benzoyl-substituted anisole itself was prepared according to the method of Olah et al. (1969). Sulfolane was purified according to the method of Alder and Whiting (1964) and mixed with 10% similarly purified di-*n*-propylsulfone to depress the melting point. To 400 mL of this solution under nitrogen was added silver hexafluoroantimonate, $AgSbF_6$ (Ozark-Mahoning, 71 g, 206 mmol,) and the mixture was stirred until most of the solid had dissolved. 4-(Trifluoromethyl)benzoyl chloride (43.1 g, 1 equiv) was added over ca. 20 min. The solution turned orange, and a heavy precipitate of AgCl formed. After the solution was cooled in ice, 2,4-di-*tert*-butylanisole (45.5 g, 1 equiv) in a little sulfolane/Pr_2SO_2 was added over ca. 45 min. The addition funnel was warmed periodically to prevent crystallization. After stirring for an additional 0.5 h, the reaction mixture was kept overnight at 5 °C. After addition of H_2O (100 mL) and ether (250 mL), the AgCl as removed by filtration. Extractive workup gave 78.2 g of product (orange oil), which was distilled at 100–120 °C/0.4 mmHg to give 55.5 g of a thick pale yellow oil. This crude product was crystallized from hexane to yield 25.2 g of light greenish crystals: mp 97–100 °C. Redistillation of foreruns and mother liquors gave further product. A final recrystallization from EtOH provided 29.5 g (37%) of prisms: mp 97.5–100 °C; MS 392 (23, M^+), 377 (100), 173 (55, $CF_3C_6H_4CO^+$), 159 (12), 145 (30, $CF_3C_6H_4^+$), 108 (10), 57 (45).

2,4-Di-tert-butyl-6-(4′-carbomethoxybenzyl)phenol (Im). The trifluoromethylbenzoylanisole (35.5 g, 90 mmol) was dissolved in a mixture of ethanol (50 mL), ethylene glycol (400 mL), KOH (50 g, ca. 750 mmol), H_2O (50 mL), and hydrazine hydrate (100 mL, 1.94 mol). The solution was heated under reflux for 5 h; then ca. 125 mL of distillate was removed (to a vapor temperature of 110 °C), and the solution was allowed to stand overnight. Hydrazine hydrate (50 mL) was added, and the solution was refluxed for 3 h, distilled to a vapor temperature of 190 °C, and refluxed for 3 h more. After cooling, the cake was dissolved in H_2O and extracted twice with Et_2O. The aqueous layer was poured onto 1 L of ice and acidified with HCl (pH <1). Extractive workup with Et_2O plus CH_2Cl_2 gave 23.9 g of product, which was crystallized from acetone (400 mL concentrated to 150 mL, then cooled) to provide 11.1 g of an off-white solid which was recrystallized from heptane plus a little acetone (9.7 g, mp 218–220 °C). NMR indicated that this was the carboxybenzyl anisole.

In an earlier preparation on a 15 g scale, the acetone mother liquor on crystallization from heptane gave some of the carboxybenzyl phenol (by NMR) which sintered at 168 °C and melted mostly from 180 to 189 °C. The compound resisted further purification as did its ethyl ester. The ethyl ester was saponified and acidified. All fractions from both runs were combined. The acid was divided into three fractions by crystallization from acetone and then heptane, and all fractions were methylated with diazomethane.

Methylated fraction 1 gave a solid the GC of which indicated mostly the carbomethoxyanisole. An authentic sample was prepared by methylation (CH_2N_2) of 2 g of the carboxybenzylanisole (mp 218–220 °C) prepared above. After crystallization from heptane, it melted at 110–113 °C, and the two products were identical by GC. Combination and crystallization from heptane and then methanol gave 3.5 g of crystals: mp 111.5–113.5 °C; MS 368 (50, M^+), 353 (100), 337 (5), 321 (5), 297 (5), 279 (3), 265 (10), 203 (2), 161 (30), 149 (30), 57 (43).

Methylated fraction 2 solidified at room temperature. Crystallization from heptane gave 4.4 g: mp 103–107 °C. After recrystallization from MeOH, GC indicated the material to be 98.5% pure **Im** (1% methyl ether), which was not improved by another crystallization: mp 106.5–108.5 °C; 2.1 g (5%); MS: 354 (30, M^+), 339 (100), 203 (22), 154 (25%), 149 (10), 121 (8), 91 (12), 57 (40).

Methylated fraction 3 contained multiple components and was discarded.

2,4-Di-tert-butyl-6-[4′-trimethylammonio)benzyl]phenol Methosulfate (In). To amine **If** (2.5 g, 7.4 mmol) in ether (15 mL) was added dimethyl sulfate (700 mL, 1 equiv). An oil separated and then crystallized. After standing over the weekend, the product was removed (2.7 g) and recrystallized from absolute ethanol/ether to give a white powder, 2.4 g (70%): mp 164–166 °C.

B. Bioassay. The house flies (NAIDM-1948 strain) were from our normal laboratory colony, reared according to the CSMA procedure (Chemical Specialties Manufacturers Association, 1955) on Purina 5060 Fly Larvae Media [sic]. The bioassay for chemosterilant activity was as described by Robbins et al. (1970). Briefly, unfed house flies (50 of each sex) <18 h old were placed in a cage with water and treated diet (6 g; sucrose plus dry nonfat milk plus dry whole egg, 42.5 + 42.5 + 15, coated with the experimental compound using acetone). Eggs were collected on days 6 and 12, and the flies were then provided with untreated diet for another week, after which time the final sample of eggs was collected. Aliquots of ca. 100 eggs were removed to determine hatchability. Duplicate egg samples were reared through on a sterile semidefined medium for pupal and adult survival counts. Total egg production of treated or control flies was measured by using calibrated pipets (ca. 10^4 eggs/mL). Separate controls were run for each batch of four or five compounds at each concentration. Experimental concentrations decreased in a 1, 0.3, 0.1, ... series starting at 1%, and the minimum concentration at which no viable eggs were produced was recorded. We use the term "viable" to mean capable of hatching and developing to pupation (vide infra). We did not collect the data for determination of a statistically defensible EC_{50}, since this bioassay was designed essentially as a pass/fail test.

RESULTS AND DISCUSSION

The results of the bioassays are summarized in Table 1. The data for each concentration/week are in the form eggs per female (total eggs divided by number of surviving females)/percent hatch/number of pupae produced (average of two replicates). Dashes indicate inability to test since the previous number was zero.

In contrast to Jurd's results (Jurd et al., 1979), we found that the 4′-methoxybenzyl and benzyl compounds were equally active, both compounds completely inhibiting production of viable eggs at 30 mg/kg of diet (Figure 2). In Jurd's assay, the 4′-methoxy compound J2644 (**Ia**) required 250 mg/kg of diet and the 4′-benzyl compound J2419 (**Ib**) required 10^3 mg/kg to reduce pupation to zero. Compounds **If** (X = $N(CH_3)_2$), **Ib** (X = SCH_3), **Ik** (X = CH_3), and **Il** (X = Cl) were as active as **Ia** and **Ib** in our assay, requiring only 30 mg/kg to inhibit successful reproduction.

We use the term "successful reproduction" to mean the production of viable eggs. "Viable eggs", in turn, are those that produce larvae which can develop to pupation (and usually adult emergence). All of the compounds active at 30 mg/kg in our assay allowed, at this or higher concentrations, production of eggs which hatched. However, the larvae from these all died, usually in the first instar. For the purposes of this assay, these eggs were regarded as nonviable. In one case (compound **Ib** at 30 mg/kg) pupae were produced but no adults emerged. Jurd may have observed this, too, since his definition of hatch (Jurd et al., 1979) is "proportion of progeny reaching the pupal stage from 100 eggs". This is reported to be common for chemosterilants in flies, with egg hatch alone not considered to be a good criterion for sterilization (LaBrecque, 1968; Fye et al., 1966; Morgan and LaBrecque, 1964). They also recommend the pupae per 100 eggs standard.

With the exception of the 4′-hydroxy analog **Ic**, all other compounds were inactive; i.e., viable eggs were produced at diet levels of 10^4 mg/kg. **Ic** prevented successful reproduction at 10^4 mg/kg but allowed it at

Table 1. Chemosterilant Activity of Compounds I against *M. domestica* Adults When Fed in the Diet at Various Concentrations

	substituent	concn (mg/kg)	week 1	week 2	week 3
Ia	OCH_3	10^4	0/–/–[a]	0/–/–	0/–/–[b]
		500	0/–/–	27/53/0	43/60/12
		300	67/40/0	70/64/0	61/94/23
		30	28/0/0	63/0/0	61/80/35
		10	14/69/49	79/94/32	77/85/49
Ib	H	10^4	0/–/–	0/–/–	0/–/–
		10^3	3/0/–	43/0/–	32/58/0[b]
		300	6/68/0	51/23/0	37/83/8
		30	3/nd/0	26/0/–	55/55/18[c]
		10	80/95/68	96/83/41	59/82/52
Ic	OH	10^4	8/50/0	83/88/0	0/–/–[b]
		10^3	68/76/33	60/81/23	57/87/44
Id	*n*-butoxy	10^4	27/75/56	76/81/12	65/84/36
Ie	*n*-decyloxy	10^4	6/94/60	88/89/44	103/86/58
If	$N(CH_3)_2$	10^4	45/26/0	51/38/0	0/–/–[b]
		10^3	61/0/–	12/21/0	30/46/12
		300	76/40/0	60/67/0	55/98/24
		30	56/0/–	44/0/–	36/83/22
		10	6/100/0	73/87/27	82/84/56
Ig	*tert*-butyl	10^4	21/84/46	65/89/48	66/85/35
Ih	SCH_3	10^4	36/0/0	65/31/0	27/56/0
		10^3	14/6/0	53/55/0	22/63/0
		300	13/76/0	62/1/0	52/71/48
		30	20/0/–	57/0/–	77/82/49
		10	67/86/75	89/84/43	63/86/41
Ii	$SOCH_3$	10^4	63/53/27	49/80/41	66/85/60
Ij	SO_2CH_3	10^4	0/–/–	44/82/31	30/80/39
Ik	CH_3	10^4	0/–/–	26/44/0	4/75/0
		10^3	47/10/0	0/–/–	31/65/31
		300	37/65/0	70/12/0	77/73/3
		30	70/0/–	81/0/–	89/0/–
		10	4/0/–	60/97/59	64/94/47
Il	Cl	10^4	0/–/–	0/–/–	0/–/–
		10^3	0/–/–	40/1/0	45/68/0
		300	28/56/0	55/16/0	68/82/25
		30	5/nd/–	23/0/–	47/0/–
		10	75/97/75	104/93/53	79/92/50
Im	CO_2CH_3	10^4	16/28/8	33/44/2	6/0/–[b]
In	$N^+(CH_3)_3$	10^4	20/20/0	43/72/20	55/89/50
control[d]			61/92/50	78/88/50	66/87/50

[a] Eggs per female/% hatch/pupated successfully. nd, not determined. Mean of two determinations. [b] All males died. [c] No adults emerged from 35 pupae in the two replicates. [d] Means of all controls, standard deviations in text.

10^3 mg/kg. **Ic** was not tested at 3×10^3 mg/kg, but this would only make the difference between "not very active" and "hardly active at all." Activity is therefore shown as a range in Figure 2.

Under these bioassay conditions, control flies produced 61.0 ± 17.4 eggs/female the first week, 78.5 ± 14.8 eggs/female the second, and 66.0 ± 15.9 eggs/female the third ($N = 13$). Egg hatch for controls was 92.2 ± 3.5% ($N = 12$), 88.5 ± 4.4%, and 87.9 ± 5.3%, respectively. Production of pupae and adults in the rear-through controls was 50 ± 13 and 34 ± 13 per replicate, respectively. Of 177 rear-through assays on these and related compounds, in only 3 did one replicate produce pupae and the other did not. For **Ik** at 300 ppm, the two replicates produced 5 and 0 pupae. The other two cases (7, 0 and 3, 0) were for analogs of these compounds not reported here. Variation on pupal production in the two replicates was generally within ca. 20% but obviously increased with smaller numbers.

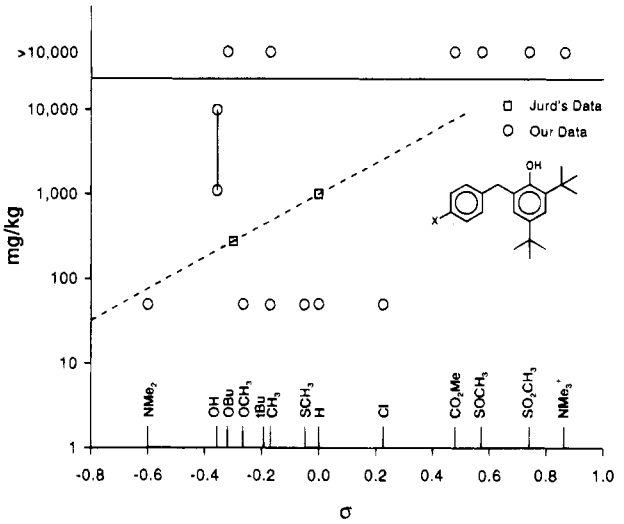


Figure 2. Plot of minimum sterilizing concentration (the lowest concentration at which eggs produced no "viable" larvae, see text) of compounds **I** vs σ constants of substituents. The *n*-decyloxy compound **Ic** is not plotted, since we could find no s constant for it. It should be very close to that of the *n*-butoxy compound **Id** and was also inactive at 1%.

High concentrations (10^3–10^4 mg/kg) of **Ia, Ib, Ic, If, Ih, Ik,** and **Il** gave permanent inhibition of reproduction (no viable eggs produced the third week), although this was sometimes the result of complete male mortality. In all cases at lower concentrations, males survived, and viable eggs were usually produced in the third week of the test, during which the flies were fed on untreated diet. No viable eggs were produced with compounds **Ik** and **Il** at 30 mg/kg the third week, but both compounds had allowed formation of at least a few pupae at 300 mg/kg.

Several of these compounds, particularly **Ik** and **Il** but to a lesser extent **Ia, Ib,** and **If,** showed a "bimodal" activity curve. That is, they were more active at high (>1000 ppm) and low (30 ppm) concentrations than at 300 ppm. Since the assays were not all run at the same time, it is unlikely to be a systematic error in the assay. A possible rationalization of these data is a slow spontaneous oxidation in the insect to an active species (quinone methide?) at high concentrations, and enzymatic activation at lower concentrations. We have no data to support this speculation, but it at least seems plausible.

Figure 2 plots the results of minimal effective concentration vs Hammett σ constant (Jaffe, 1953) for the various substituents. Data from Jurd et al. (1979) are also plotted. None of the compounds with $\sigma > 0.4$ were active. Compounds having more electron-donating substituents seem to have activity based more on steric grounds, since compounds having large (*tert*-butyl and the two large alkoxy groups) substituents were inactive, whereas **Il** (X = Cl, $\sigma = 0.227$) is as active as **If** [X = $N(CH_3)_2$, $\sigma = -0.600$]. Compounds **Ia, Id,** and **Ic** provide a steric probe. The 4-butoxy compound **Id** ($\sigma = 0.320$) is inactive, as is **Id** with unknown but probably similar σ for *n*-decyloxy. It seems unlikely that there would be a very large difference in polarity in such a large molecule with the addition of three CH_2 groups, but the size does increase. The 4′-hydroxy compound **Ic** ($\sigma = -0.357$) is anomalous, its poor activity possibly due to hydrogen-bonding effects leading to poor absorp-

tion or activation or to metabolic conversion to an inactive conjugate.

The best correlation of activity was with molecular weight. The receptor site seems to be very sensitive to the size of the active species. Of six compounds active at 30 mg/kg, all have molecular weights between 296 and 342. Six compounds inactive at 10^4 mg/kg all had molecular weights from 352 to 452. **In** has a molecular weight of 354 for the cation and is inactive, but probably should not be included in the correlation because of its charge. **Ic** again does not fit.

Casual inspection of the relationship between structure and calculated values for various QSAR parameters generally supported the size/activity postulate. The dividing line used was activity at 30 mg/kg in the diet. Presentation is in the form parameter (active compound values, inactive compound values, value for *p*-OH compound **Ic**): solvent accessible surface area, Å^2 (530–626, 603–845, 540), van der Waals surface area, Å^2 (343–403, 387–552, 352), solvent accessible volume, Å^3 (307–352, 344–482, 314), log *P* (octanol/water) (6.8–7.6, 8.05–10.43, 6.81), and refractivity, Å^3 (95–109, 108–143, 97). There was no obvious correlation with polarizability. These data, in spite of some overlap between active/inactive value ranges, support the smaller, not-too-lipophilic nature of active materials. **Ic** is anomalous in essentially all of these tests.

CONCLUSIONS

It is clear from Figure 2 that no good correlation of activity with Hammett σ value exists for this series of compounds in this bioassay. All active compounds had small substituents and correspondingly small molecular weights (under 350). Among the new compounds prepared, four [**If**, X = $N(CH_3)_2$, **Ih**, X = SCH_3, **Ik**, X = CH_3, and **Il**, X = Cl) were as active as J2644 and J2419. Because certain compounds in this work allowed female flies to produce eggs that hatched, but failed to produce surviving larvae, care should be taken in interpreting previous data on these or similar compounds.

LITERATURE CITED

Alder, R. W.; Whiting, M. C. An exceptionally powerful oxidant: the ion $H_3O_2^+$. *J. Chem. Soc.* **1964**, 4707–4712.

Anderson, R. C.; Reitter, B. E.; Winslow, C. M. Platelet-activating factor analogs. II. Synthesis of the 1-*O*-(4-decyloxymethylphenyl)- and 1-*O*-(4-decyloxyphenylmethyl) analogs. *Chem. Phys. Lipids* **1986**, *39*, 73–82.

Broce, A. B.; Gonzaga, V. G. Effects of substituted benzylphenols and triflumuron on the reproduction of the face fly (Diptera: Muscidae). *J. Econ. Entomol.* **1987**, *80*, 37–43.

Carpenter, M. S.; Easter, W. M.; Wood, T. F. Nitro musks. I. Isomers, homologs, and analogs of musk ambrette. *J. Org. Chem.* **1951**, *16*, 586–617.

Chang, S. C.; Borkovec, A. B.; DeMilo, A. B. Effects of substituted benzylphenols on reproduction of house flies. *J. Econ. Entomol.* **1980**, *73*, 745–747.

Chemical Specialties Manufacturers Association. Peet-Grady method. *Soap Chem. Spec. Blue Book* **1955**, 249–250, 267.

Dame, D. A.; Jurd, L. Assessment of benzyl and cinnamyl substituted phenol and 1,3-benzodioxole derivatives as IGR's against mosquitoes. *Mosq. News* **1983**, *43*, 50–54.

Flint, H. M.; Jurd, L.; Merkle, J. R. Pink bollworm sterilizing effects of benzylphenols and benzyl-1,3-benzodioxoles. *J. Econ. Entomol.* **1980**, *73*, 710–714.

Fye, R. L.; LaBrecque, G. C.; Gouck, H. K. Screening tests of chemicals for sterilization of adult house flies. *J. Econ. Entomol.* **1966**, *59*, 485–487.

Green, H. A. U.S. Pat. 3 193 526, 1965.

Gurvich, Y. A.; Grinberg, A. F.; Liakumovich, A. G.; Michurov, J. I.; Starikova, D. F.; Yanshevsky, V. A.; Kumok, S. T.; Styskin, E. L.; Rutman, G. I. U.S. Pat. 4 087 469, 1978.

Jaffe, H. H. A reexamination of the Hammett equation. *Chem. Rev.* **1953**, *53*, 191–261.

Jurd, L.; Manners, G. D. Wood extractives as models for the development of new types of pest control agents. *J. Agric. Food Chem.* **1980**, *28*, 183–188.

Jurd, L.; Wong, R. Y. Quinones and quinone methides. VIII. Oxidation products of the screwworm chemosterilant, 2,4-bis(1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)phenol. *Aust. J. Chem.* **1981a**, *34*, 1633–1644.

Jurd, L.; Wong, R. Y. Quinones and quinone methides. IX. Oxidation of 2,4-bis(1,1-dimethylethyl)-6-(4-methoxyphenylmethyl)phenol in alcoholic solvents: Monomeric products and the crystal structure of an unusual dimer, 2-[3,5-bis-(1,1-dimethylethyl)-2-{methoxy(4-methoxyphenyl)methoxy}-phenoxy]-1,5-bis(1,1-dimethylethyl)-3-[methoxy(4-methoxyphenyl)methyl]benzene. *Aust. J. Chem.* **1981b**, *34*, 1645–1654.

Jurd, L.; King, A. D., Jr.; Mihara, K.; Stanley, W. L. Antimicrobial properties of natural phenols and related compounds. I. Obtusastyrene. *Appl. Microbiol.* **1971a**, *21*, 507–510.

Jurd, L.; Stevens, K. L.; King, Jr.; A. D.; Mihara, K. Antimicrobial properties of natural phenols and related compounds II: cinnamylated phenols and their hydrogenation products. *J. Pharm. Sci.* **1971b**, *60*, 1753–1755.

Jurd, L.; Fye, R. L.; Morgan, J., Jr. New types of insect chemosterilants. Benzylphenols and benzyl-1,3-benzodioxole derivatives as additives to housefly diet. *J. Agric. Food Chem.* **1979**, *27*, 1007–1016.

LaBrecque, G. C. Laboratory procedures. In *Principles of Insect Chemosterilization*; LaBrecque, G. C., Smith, C. N., Eds.; Appleton-Century Crofts: New York, 1968; Chapter 3, pp 41–98.

Langley, P. A.; Trewern, M. A.; Jurd, L. Sterilising effects of benzyl-1,3-benzodioxoles on the tsetse fly *Glossina morsitans morsitans* Westwood (Diptera: Glossinidae). *Bull. Entomol. Res.* **1982**, *72*, 473–481.

Matolczy, G.; Feyereisen, R.; Van Mellaert, H.; Pál, Á.; Varjas, L.; Bélai, I.; Kulcsár, P. Molecular modifications of benzylphenol and benzyl-1,3-benzodioxole types of insect chemosterilants. *Pestic. Sci.* **1986**, *17*, 13–24.

Morgan, P. B.; LaBrecque, G. C. Studies on the effect of meat exposed to gamma radiation or chemosterilants on the reproductive capacity of a blow fly *Phaenicia cuprina* (Wiedemann). *Fla. Entomol.* **1964**, *47*, 31–33.

Nelson, F. R. S.; Hoosseintehrani, B. Effects of benzylphenol and benzyl-1,3-benzodioxole derivatives on fertility and longevity of the yellow fever mosquito (Diptera: Culicidae). *J. Econ. Entomol.* **1982**, *75*, 877–878.

Nelson, F. R. S.; Mohamed, A. K.; Vattikutti, P. Efficacy of three insect growth regulators on the development of *Aedes aegypti*. *J. Am. Mosq. Control Assoc.* **1985**, *1*, 240–242.

Olah, G. A.; Lukas, J.; Lukas, E. Aromatic substitution. XXVI. Propionylation and benzoylation of benzene and alkylbenzenes with ethyloxocarbonium (propionylium) and phenyloxocarbonium (benzoylium) hexafluoroantimonate in nitromethane and tetramethylene sulfone solution. *J. Am. Chem. Soc.* **1969**, *91*, 5319–5323.

Pound, A. A.; Spradbery, J. P. Chemosterilisation of the screwworm fly, *Chrysomya bezziana* Villeneuve with benzylphenols. *J. Aust. Entomol. Soc.* **1984**, *23*, 99–103.

Rawlins, S. C.; Jurd, L. Influence of the mode of administration of benzylphenols and benzyl-1,3-benzodioxoles on screwworm fertility. *J. Econ. Entomol.* **1981**, *74*, 215–217.

Rawlins, S. C.; Jurd, L.; Snow, J. W. Antifertility effects of benzylphenols and benzyl-1,3-benzodioxoles on screwworm flies. *J. Econ. Entomol.* **1979**, *72*, 674–677.

Rawlins, S. C.; Woodard, D. B.; Coppedge, J. R.; Jurd, L. Management of an insecticide-resistant house fly (Diptera: Muscidae) population by the strategic use of a benzylphenol chemosterilant. *J. Econ. Entomol.* **1982**, *75*, 728–732.

Robbins, W. E.; Kaplanis, J. N.; Thompson, M. J.; Shortino, T. J.; Joyner, S. C. Ecdysones and synthetic analogs:

molting hormone activity and inhibitive effects on insect growth, metamorphosis and reproduction. *Steroids* **1970**, *16*, 105–125.

Smith, J. R. L.; Linford, J. M.; McKeer, L. C.; Morris, P. M. Amine oxidation. Part 14. Acid-catalysed deoxygenation of some *NN*-dimethylaniline *N*-oxides and the reactions of the resultant iminium-benzenium dications. *J. Chem. Soc., Perkin Trans. 2* **1984**, 1099–1105.

Taft, R. W., Jr. Separation of polar, steric, and resonance effects in reactivity. In *Steric Effects in Organic Chemistry*, 2nd printing; Newman, M. S., Ed.; Wiley: New York, 1963; Chapter 13, pp 556–675.

Tiers, G. V. D. "Melting points are uncorrected"—true or false? *J. Chem. Educ.* **1990**, *67*, 258–259.

Wong, R. Y.; Jurd, L. Quinones and quinone methides. X. The crystal structure of a dimeric oxidation product from 2,4-bis(1,1-dimethylethyl)-6-[(4-methoxyphenyl)methyl]phenol. *Aust. J. Chem.* **1984**, *37*, 2593–2597.

Received for review December 14, 1994. Revised manuscript received June 2, 1995. Accepted August 23, 1995.[⊗] Mention of a commercial product does not constitute an endorsement by the U.S. Department of Agriculture.

JF940708V

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1995.