

First Synthesis and Deodorant Activities of Compounds Isolated from Thyme (*Thymus Vulgaris* L.) and Their Analogues

YOZO MIURA,* Fumihiko YAMAGUCHI,
and Yoshiaki Ogo

Department of Applied Chemistry, Faculty of Engineering, Osaka City University,
Sumiyoshi-ku, Osaka 558

(Received December 10, 1991)

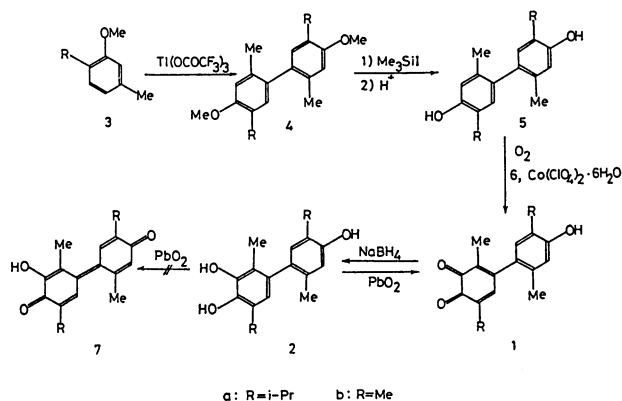
Synopsis. 4-(4-Hydroxy-5-isopropyl-2-methylphenyl)-6-isopropyl-3-methyl-3,5-cyclohexadiene-1,2-dione and 5,5'-diisopropyl-2,2'-dimethyl-3,4,4'-biphenyltriol, isolated from thyme, and their analogues, 4-(2,5-dimethyl-4-hydroxyphenyl)-3,6-dimethyl-3,5-cyclohexadiene-1,2-dione and 2,2',5,5'-tetramethylbiphenyl-3,4,4'-triol, have been synthesized and their deodorant activities against methanethiol measured.

Thyme (*Thymus vulgaris* L.) is a herb with deodorant properties and has been widely used as a culinary spice. Although the compositions of the essential oil^{1,2)} and flavonoides^{3–5)} have already been analyzed, it was only quite recently that new compounds with strong deodorant activities were isolated from the nonvolatile fraction of thyme. Nakatani et al. reported that **1a** and **2a**, isolated from thyme, are highly deodorant active against methanethiol.^{6,7)} However, the amounts of **1a** and **2a** isolated were quite small.⁸⁾ In this paper we report on the first syntheses of **1a** and **2a** as well as their analogues, **1b** and **2b**. We also discuss the deodorant activities of these synthetic compounds.

Results and Discussion

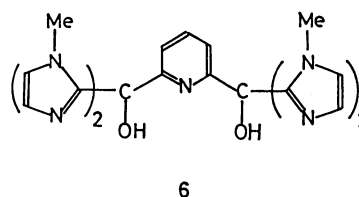
Synthesis. A synthetic outline of **1** and **2** is described in Scheme 1. An oxidative coupling reaction of **3** using thallium trifluoroacetate was performed according to the reported method,⁹⁾ and **4a** and **4b** were obtained in 42 and 31% yields, respectively.

Demethylation of the methoxyl groups of **4** was effected when **4** was treated with Me₃SiI in refluxing CH₂Cl₂ or with BBr₃ in CH₂Cl₂. The former method gave **5a** and **5b** in 63 and 55% yields, while the latter method afforded **5b** and **5b** in 48 and 61% yields, respectively. However, other reagents such as KI-Me₃SiCl, AlBr₃, and 48% HBr were found to be much less effective for demethylation.



Scheme 1.

Oxidation of **5** to **1** was successfully achieved by treating **5** with catalytic amounts of Co(ClO₄)₂·6H₂O and α,α',α',α'-tetrakis(1-methyl-2-imidazol)-2,6-pyridine-dimethanol (**6**) in acetonitrile under oxygen bubbling to afford **1a** and **1b** as dark red microprisms in 58 and 64% yields,¹⁰⁾ respectively.



The reduction of **1a** and **1b** with NaBH₄ gave triols **2a** and **2b** as colorless microneedles in 89 and 71% yields, respectively. The ¹H NMR and mass spectra of **1a** and **2a** completely agreed with those of natural samples,^{6,7)} and the structures of **1b** and **2b** were unequivocally confirmed by the IR, ¹H NMR, and mass spectra as well as elemental analyses. Compound **2a** was shown to contain 5.44 wt% of H₂O as a hydrate by Karl-Fischer's method. This indicates that one molecule of **2a** contains one molecule of H₂O. The elemental analysis agreed satisfactorily with the values calculated as (**2a**+H₂O).

Triol **2** in solution was found to be slowly oxidized by atmospheric oxygen to give **1**. The formation of **1** was shown by the characteristic red color of **1** [λ_{max} : 405 (**1a**) and 407 nm (**1b**) in CHCl₃]. Treatment of **2** with PbO₂ in solution accelerated the oxidation, and **1a** and **1b** were produced in 76 and 71% yields, respectively. However, no formation of **7** was found in the oxidation of **2**. Quinone **7a** was reported to be formed upon the oxidation of 2-isopropyl-5-methylphenol (thymol) with CuCl₂ under oxygen bubbling.¹¹⁾

Deodorant Assay. Deodorant activities of **1a**, **1b**, **2a**, and **2b** against methanethiol were measured by a method reported by Nakatani et al.⁶⁾ The results are illustrated in Fig. 1, in which the deodorant activities are expressed as deodorant efficiencies estimated by using Eq. 1, which is given in the Experimental Section.

As expected, the deodorant activities of synthetic **1a** and **2a** against methanethiol were almost the same as those for natural samples;^{6,7)} these were about 100-times more effective than that of sodium copper chlorophyllin, which is used as an oral deodorizer. Compounds **1b** and **2b** also showed similar strong deodorant activities against methanethiol. This suggests that the deodorant activities are not dependent on the type of alkyl groups.

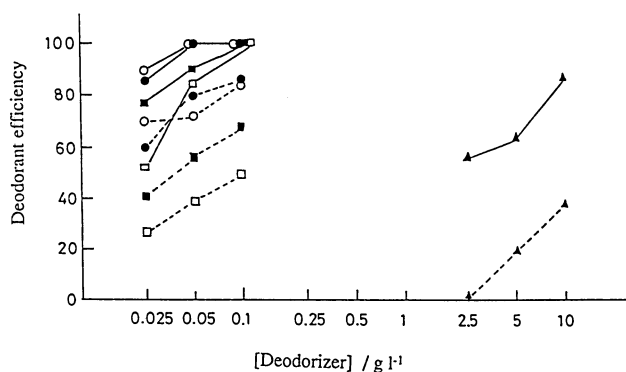


Fig. 1. Deodorant activities of **1a**, **1b**, **2a**, and **2b** against methanethiol (70 ppm). **1a** (●), **1b** (○), **2a** (■), **2b** (□), sodium copper chlorophyllin (▲); —: deodorant efficiencies measured after the samples were kept at 37 °C for 5 min; ---: deodorant efficiencies measured after the sample were kept at 37 °C for 5 min, at room temperature for 20 min, and at 37 °C for 5 min.

Experimental

The melting points were determined on a Yanagimoto micro melting-point apparatus and are uncorrected. UV-visible spectra were recorded with a Shimadzu UV-240 spectrophotometer. IR spectra were run on a JASCO A-202 spectrophotometer. ^1H NMR spectra were measured with a JEOL GX-400 spectrometer (400 MHz), with tetramethylsilane used as an internal standard. Mass spectra were obtained on a JEOL JMS-HX 100 spectrometer at 70 eV. Column chromatography was performed on silica gel (Wako gel C-200) or alumina (Merck aluminium oxide 90).

Commercially available thallium trifluoroacetate and $\text{Co}(\text{ClO}_4)_2 \cdot \text{H}_2\text{O}$ were used without purification. Compound **6** was prepared according to a reported method.¹⁰

5,5'-Diisopropyl-4,4'-dimethoxy-2,2'-dimethylbiphenyl (**4a**) and 4,4'-dimethoxy-2,2',5,5'-tetramethylbiphenyl (**4b**) were prepared by an oxidative coupling reaction of **3** using thallium trifluoroacetate, according to a reported methods.⁹

4a: Colorless prisms (from methanol); yield 42%; mp 113–114 °C; ^1H NMR (CDCl_3) δ =1.19 (6H, d, J =6.7 Hz, CHMe_2), 1.20 (6H, d, J =6.7 Hz, CHMe_2), 2.06 (6 H, s, Me), 3.30 (2H, sept, J =6.7 Hz, CHMe_2), 3.86 (6H, s, OMe), 6.74 (2H, s, Ar), and 6.96 (2H, s, Ar); MS m/z (rel intensity) 326 (M^+ , 100%), 311 (93), and 148(20). Found: C, 80.92; H, 9.45%. Calcd for $\text{C}_{22}\text{H}_{30}\text{O}_2$: C, 80.93; H, 9.26%.

4b:¹² Mp 113.5–115 °C (from methanol) (lit.⁹ 114–116 °C); yield 31%; ^1H NMR (CDCl_3) δ =2.04 (6H, s, Me), 2.19 (6 H, s, Me), 3.85 (6H, s, OMe), 6.71 (2H, s, Ar), and 6.85 (2H, s, Ar); Found: C, 79.78; H, 8.29%. Calcd for $\text{C}_{18}\text{H}_{22}\text{O}_2$: C, 79.96; H, 8.20%.

5,5'-Diisopropyl-2,2'-dimethyl-4,4'-biphenyldiol (5a). (A) By demethylation with Me_3SiI . A solution of Me_3SiI (2.98 g, 14.9 mmol) in CHCl_3 (20 ml) was added to a solution of **4a** (1.69 g, 5.18 mmol) in CH_3Cl (20 ml). After the solution was refluxed for 2 h under N_2 , water (50 ml) was added. The mixture was extracted with ether; the ether extract was washed with 10% sodium thiosulfate and brine, dried (MgSO_4), and evaporated to dryness. 15% HCl (20 ml) and THF (40 ml) were added to the residue; the mixture was stirred at room temperature for 1 d and then extracted with ether. The ether extract was dried (MgSO_4) and evaporated, and the residue was crystallized from hexane to give **5a** as colorless prisms in 63% yield (0.98 g, 3.28 mmol). Mp 162–163 °C; ^1H NMR (CDCl_3) δ =1.24 (6H, d, J =6.7 Hz, CHMe_2), 1.25 (6H, d,

J =6.7 Hz, CHMe_2), 1.98 (6H, s, Me), 3.18 (2H, sept, J =6.7 Hz, CHMe_2), 4.66 (2H, s, OH), 6.66 (2H, s, Ar), and 6.93 (2H, s, Ar); MS, m/z 298 (M^+ , 97%), 283 (100), 134 (41), 81 (21), 69 (40). Found: C, 80.06; H, 8.99%. Calcd for $\text{C}_{20}\text{H}_{26}\text{O}_2$: C, 80.49; H, 8.78%.

(B) By demethylation with BBr_3 . A solution of **4a** (0.300 g, 0.920 mmol) in CH_2Cl_2 (10 ml) was cooled to 0 °C. To this solution was added a solution of BBr_3 (2.7 g, 11 mmol) in CH_2Cl_2 (5 ml) with stirring. The resulting solution was stirred at 0 °C for 4 h and then poured into an ice-cooled 10% KOH solution. The aqueous layer was separated and acidified with 20% HCl. The deposited crystals were extracted with ether; the ether extract was washed with brine, dried (MgSO_4), evaporated, and the residue was crystallized from hexane to give **5a** as colorless micropisms in 48% yield (0.133 g, 0.446 mmol); mp 162–163 °C. The IR and ^1H NMR spectra were identical to those for the compound prepared by method (A).

2,2',5,5'-Tetramethyl-4,4'-biphenyldiol (5b). (A) By demethylation with Me_3SiI . By the same procedure as mentioned above, **5b** was obtained in 55% yield (0.490 g, 2.02 mmol) from 1.00 g (3.70 mmol) of **4b**. Colorless micropisms (from hexane); mp 137–138 °C; ^1H NMR (CDCl_3) δ =1.98 (6H, s, Me), 2.22 (6H, s, Me), 4.65 (2H, s, OH), 6.67 (2H, s, Ar), and 6.83 (2H, s, Ar); MS m/z (rel intensity) 242 (M^+ , 100%), 227 (25), 212 (23), 149 (11), 59 (14), and 43 (39). Found: C, 78.98; H, 7.27%. Calcd for $\text{C}_{16}\text{H}_{18}\text{O}_2$: C, 79.31; H, 7.49%.

(B) By demethylation with BBr_3 . A solution of BBr_3 (2.7 g, 11 mmol) in CH_2Cl_2 (5 ml) was added to a stirred solution of **4b** (0.300 g, 1.11 mmol) at 0 °C. After completing of the addition, the resulting mixture (heterogeneous) was warmed to room temperature and stirred for 2 h to give a homogeneous solution. The same workup as mentioned above gave **5b** in 61% yield (0.163 g, 0.672 mmol). Mp 137–138 °C. The IR and ^1H NMR spectra were identical with those for the compound prepared by method (A).

4-(4-Hydroxy-5-isopropyl-2-methylphenyl)-6-isopropyl-3-methyl-3,5-cyclohexadiene-1,2-dione (1a). Into a stirred solution of $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ (10.8 mg, 0.030 mmol) and **6** (10.8 mg, 0.023 mmol) in acetonitrile (50 ml) was bubbled oxygen at room temperature for 1 h. Biphenyldiol **5a** (0.595 g, 0.200 mmol) was added; the bubbling of oxygen was continued for 30 min with stirring. After the bubbling was stopped, the mixture was stirred at room temperature for 1 d under an oxygen atmosphere. The reaction mixture was evaporated, and the residue was chromatographed on silica gel using 1:2 ethyl acetate–hexane as the eluant. Collection of the red zone, evaporation of the solvent, and crystallization of the residue (hexane–ethyl acetate) gave **1a** as dark red micropisms in 58% yield (0.360 g, 0.115 mmol). Mp 174–175 °C (lit.⁶ 170 °C); IR (KBr) 3400, 2950, 1650, 1635, 1605, 1570, 1500, 1460, 1385, 1320, 1220, 1170, 1110, 1050, 1020, 960, 900, 850, and 610 cm^{-1} UV-vis_{max} (CHCl_3) 241 (ϵ 9030), 277 (3440), and 405 nm (3090); ^1H NMR (CDCl_3) δ =1.11 (6H, d, J =6.7 Hz, CHMe_2), 1.25 (3H, d, J =6.7 Hz, CHMe_2), 1.26 (3H, d, J =6.7 Hz, CHMe_2), 1.76 (3H, s, Me), 2.17 (3H, s, Me), 2.98 (1 H, sept, J =6.7 Hz, CHMe_2), 3.21 (1H, sept, J =6.7 Hz, CHMe_2), 5.05 (1H, s, OH), 6.62 (1H, s, Ar), 6.72 (1H, s, Ar), and 6.91 (1H, s, Ar); MS m/z (rel intensity) 314 (M^+ +2, 100%), 299 (76), 284 (67), 269 (32), 241 (21), 227 (17), 177 (15), and 142 (23). Found: C, 76.75; H, 7.74%. Calcd for $\text{C}_{20}\text{H}_{24}\text{O}_3$: C, 76.89; H, 7.74%.

4-(2,5-Dimethyl-4-hydroxyphenyl)-3,6-dimethyl-3,5-cyclohexadiene-1,2-dione (1b). By the same procedure as mentioned above, **1b** was obtained in 64% yield (0.336 g, 1.31 mmol) from 0.494 g (2.04 mmol) of **5b** by using 18.6 mg (0.0508 mmol) of $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$ and 52.0 mg (0.112 mmol) of **6**. Dark-red micropisms (from hexane–ethyl acetate); mp 160–161 °C; IR (KBr) 3400, 2950, 1640, 1610, 1570, 1500,

1440, 1380, 1370, 1320, 1255, 1230, 1210, 1160, 1100, 1000, 890, and 560 cm^{-1} ; UV-vis $_{\text{max}}$ (CHCl_3) 241 (ϵ 8200), 277 (3430), and 407 nm (2780); $^1\text{H NMR}$ (CDCl_3) δ =1.74 (3H, s, Me), 1.96 (3H, s, Me), 2.16 (3H, s, Me), 2.24 (3H, s, Me), 4.96 (1H, s, OH), 6.66 (1H, s, Ar), 6.72 (1H, s, Ar), and 6.84 (1H, s, Ar); MS m/z (rel intensity) 258 (M^+ + 2, 100%), 225 (29), 167 (17), 149 (67), 71 (16), 57 (30), and 43 (17). Found: C, 74.75; H, 6.28%. Calcd for $\text{C}_{16}\text{H}_{16}\text{O}_3$: C, 74.98; H, 6.29%.

5,5'-Diisopropyl-2,2'-dimethyl-3,4,4'-biphenyltriol (2a). To a solution of **1a** (0.50 g, 1.60 mmol) in methanol (30 ml) was added NaBH_4 (0.40 g, 10.6 mmol) in one portion. After the resulting mixture was stirred at room temperature for 1 h, 5% HCl (50 ml) was added, and the mixture was stirred for 5 min. Methanol was evaporated, and the resulting mixture was extracted with ether. The ether extract was washed with brine, dried (MgSO_4), evaporated, and the residue was crystallized from hexane to give **2a** as colorless microneedles in 89% yield (0.449 g, 1.43 mmol). Mp 85—87 $^{\circ}\text{C}$; ^{13}C (lit.⁷ 75 $^{\circ}\text{C}$); IR (KBr) 3550, 3350, 2950, 1680, 1610, 1580, 1500, 1430, 1380, 1360, 1340, 1260, 1180, 1100, 1050, 970, 950, 900, 870, 800, and 710 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ =1.240, 1.244, 1.250, 1.257 (12H, all d, J =6.7 Hz, CHMe_2), 1.94 (3H, s, Me), 1.98 (3H, s, Me), 3.178 (1H, sept, J =6.7 Hz, CHMe_2), 3.183 (1H, sept, J =6.7 Hz, CHMe_2), 4.68 (1H, s, OH), 5.03 (1H, s, OH), 5.18 (1H, s, OH), 6.58 (1H, s, Ar), 6.65 (1H, s, Ar), and 6.92 (1H, s, Ar); MS m/z (rel intensity) 314 (M^+ , 100%), 299 (85), and 142 (18). Found: C, 72.48; H, 8.09%. Calcd for $\text{C}_{20}\text{H}_{28}\text{O}_4$ (**2a**+ H_2O): C, 72.26; H, 8.49%.

2,2',5,5'-Tetramethyl-3,4,4'-biphenyltriol (2b). By the same procedure as above, **2b** was obtained in 71% yield (0.294 g, 1.14 mmol) from 0.41 g (60 mmol) of **1b**. Colorless microneedles (from hexane-benzene); mp 87—88.5 $^{\circ}\text{C}$; IR (KBr) 3300, 2930, 1620, 1580, 1490, 1460, 1400, 1380, 1320, 1255, 1170, 1070, 1035, 1000, 950, 910, 870, 855, 820, 770, 700, 670, and 600 cm^{-1} ; $^1\text{H NMR}$ (CDCl_3) δ =1.93 (3H, s, Me), 1.97 (3H, s, Me), 2.22 (3H, s, Me), 2.24 (3H, s, Me), 4.58 (1H, s, OH), 4.97 (1H, s, OH), 5.07 (1H, s, OH), 6.48 (1H, s, Ar), 6.66 (1H, s, Ar), and 6.82 (1H, s, Ar); MS m/z (rel intensity) 258 (M^+ , 100%), 242 (15), 225 (28), and 78 (23). Found: C, 74.55; H, 6.91%. Calcd for $\text{C}_{16}\text{H}_{18}\text{O}_3$: C, 74.94; H, 7.02%.

Reoxidation of 2a to 1a with PbO_2 . Biphenyltriol **2a** (0.500 g, 1.59 mmol) was dissolved in THF (30 ml) with stirring. After K_2CO_3 (5.0 g) was added in one portion, PbO_2 (5.0 g) was added in three or four portions over 1—2 min. After the mixture was stirred for 10 min, K_2CO_3 and PbO_2 were removed by filtration and the filtrate was evaporated. Crystallization of the residue (hexane-ethyl acetate) gave **1a** in 76% yield (0.357 g, 1.14 mmol). Mp 174—175 $^{\circ}\text{C}$. The IR and $^1\text{H NMR}$ spectra agreed completely with those of the compound described above.

Reoxidation of 2b to 1b with PbO_2 . By the same procedure as mentioned above, 0.500 g (1.94 mmol) of **2b** was reoxidized to give **1b** in 71% yield (0.351 g, 1.37 mmol), mp 160—161.5 $^{\circ}\text{C}$ (from hexane-ethyl acetate). The IR and $^1\text{H NMR}$ spectra completely agreed with those of the compound described above.

Measurements of Deodorant Activities. Deodorant activi-

ties were measured according to the method of Nakatani et al.⁶ Phosphate buffer (pH 7.5) (0.1 mol dm^{-3} , 15 ml) was placed in a Nessler tube and mixed with 5 ml of known concentration of the test sample in acetone and 0.1 ml of a 0.15% aqueous methanethiol solution. After the mixture was shaken for 5 s and kept at 37 $^{\circ}\text{C}$ for 5 min, the first measurement of the concentration of the remaining methanethiol was made with a gas analyzer (which was purchased from Gastec). After the sample solution was kept at room temperature for 20 min and at 37 $^{\circ}\text{C}$ for 5 min, a second measurement of the concentration of the remaining methanethiol was made by the same method. The sample used for a blank test was prepared using adding a pure (deodorant-free) acetone solvent (5 ml) instead of a deodorant-containing acetone solution. The deodorant efficiencies were estimated using

$$\text{Deodorant efficiency} = \frac{[\text{MeSH}]_{\text{blank}} - [\text{MeSH}]_{\text{sample}}}{[\text{MeSH}]_{\text{blank}}} \times 100 \quad (1)$$

The authors wish to thank Professor N. Nakatani of Osaka City University for valuable suggestions. They also wish to thank Mr. H. Tsuda of Nippon Terpene Chemical Company for the measurements of deodorant activities.

References

- 1) V. W. Messerschmidt, *Plant Med.*, **13**, 56 (1965).
- 2) R. Granger, J. Passet, and J. P. Girard, *Phytochemistry*, **11**, 2301 (1972).
- 3) W. Awe, J. F. Schaller, and H. J. Kummel, *Naturwissenschaften*, **46**, 558 (1959).
- 4) T. Adzet and F. Martinez-Verges, *Planta Med.*, (Suppl.), **1980**, 52.
- 5) C. O. Van Den Broucke, R. A. Dommissie, E. L. Esmans, and J. A. Lemli, *Phytochemistry*, **21**, 2581 (1982).
- 6) N. Nakatani, K. Miura, and T. Inagaki, *Agric. Biol. Chem.*, **53**, 1375 (1989).
- 7) K. Miura, T. Inagaki, and N. Nakatani, *Chem. Pharm. Bull.*, **37**, 1816 (1989).
- 8) Nakatani et al. reported that 18.7 mg of **1a** and 2.1 mg of **2a** were isolated from 2 kg of dried ground leaves of thyme.^{6,7}
- 9) A. McKillop, A. G. Turrell, D. W. Young, and E. C. Taylor, *J. Am. Chem. Soc.*, **102**, 6504 (1980).
- 10) S. Tamagaki, Y. Kanamaru, M. Ueno, and W. Tagaki, *Bull. Chem. Soc. Jpn.*, **64**, 165 (1991).
- 11) Y. Takizawa, T. Munakata, Y. Iwasa, T. Suzuki, and T. Mitsuhashi, *J. Org. Chem.*, **50**, 4383 (1985).
- 12) Since only the melting point was given in the literature,⁹ we have fully analyzed the compound by the $^1\text{H NMR}$ spectrum and elemental analysis.
- 13) Benzoquinone **2a** melted at 85—87 $^{\circ}\text{C}$ and solidified with increasing temperature. The wax-like solid remelted at 136—138 $^{\circ}\text{C}$.