Structure and Activity of New Deodorant Biphenyl Compounds from Thyme (*Thymus vulgaris* L.)

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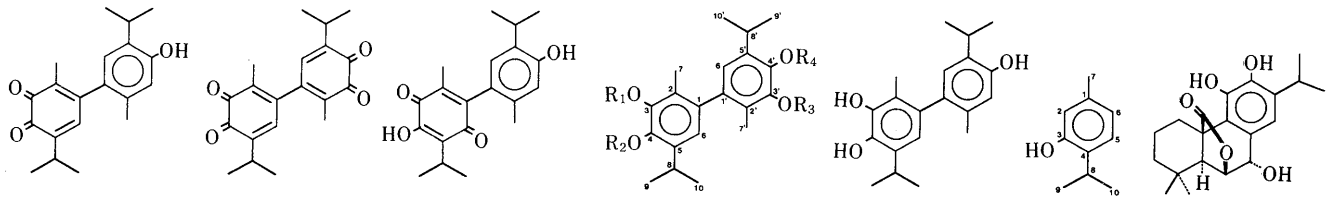
Two new biphenyls, 3,4,3′,4′-tetrahydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl (4a) and 3,4,4′-trihydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl (5), were isolated from the leaves of thyme (*Thymus vulgaris* L.). Their structures were elucidated by spectroscopic methods and chemical transformations. Both biphenyls 4a and 5 showed more effective deodorant activity against methyl mercaptan than did rosmanol, carnosol or sodium copper chlorophylline.

Keywords thyme; deodorant activity; *Thymus vulgaris* L; Labiatae; 3,4,3′,4′-tetrahydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl; 3,4,4′-trihydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl; methyl mercaptan; spice; biphenyl

Thyme (*Thymus vulgaris* L., Labiatae) is a herb with deodorant properties.[1-3] Although the composition of the essential oil[4,5] and flavonoids[6-8] have already been reported, there have been only a few reports so far on the structures of deodorant compounds from thyme. We have previously reported the isolation and structural elucidation of new deodorant biphenyl compounds (**1**—**3**) from thyme.[9] In the present study we describe the isolation of two additional new deodorant biphenyls (**4a** and **5**), which were obtained from the same weakly acidic fraction.

The dried leaves of thyme were extracted with *n*-hexane to remove the non polar components. The residue was further extracted with acetone and the extract was steam-distilled. The non volatile components were fractionated in the usual way. The weakly acidic fraction was subjected to chromatography on a silica gel column using benzene–acetone as an eluent to afford eleven fractions. The second fraction was successively chromatographed on a silica gel column and a Sephadex LH-20 column to give compounds **4a** and **5**. The extraction and isolation procedures are shown in Fig. 1.

Compound **4a** was obtained as a light brown powder, and the high-resolution mass spectrum (HRMS) showed a molecular ion peak at *m/z* 330.1803, from which the molecular formula $C_{20}H_{26}O_4$ was deduced. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum showed only ten signals, but because of the MS results, it was proposed that a symmetrical carbon skeleton was formed from two C_{10} moieties. The infrared (IR) spectrum showed an absorption band at 3300 cm^{-1} (hydroxyl). In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum hydroxyl protons were observed at δ 5.07 and 5.21 which disappeared upon the addition of D_2O. Two doublets at δ 1.24 and 1.25 (6H each, J=6.8 Hz) coupled with a benzylic septet at δ 3.18 (2H) indicated the presence of two isopropyl groups. The signal at δ 1.94 (6H) was attributed to two methyl groups on an aromatic ring. In addition, only one aromatic singlet at δ 6.57 (2H) was observed in the ^{1}H-NMR spectrum. This required a pentasubstituted phenyl moiety. This was further supported by the ^{13}C-NMR spectrum. Substitution types of all carbons in the ^{13}C-NMR spectrum were identified through insensitive nuclei enhanced by polarization transfer (INEPT) experiments and their relative intensities, on the basis of the results of additivity calculations. These data suggested that compound **4a** is a dimer of a hydroxy thymol derivative. The ^{13}C- and ^{1}H-NMR chemical shifts are summarized in Tables I and II, together with those of related compounds (**4b**—**6**). The ultraviolet (UV) spectrum obtained in MeOH, and the shifts caused by the addition of aluminum chloride revealed the presence of an aromatic *ortho*-dihydroxyl group. The acetylation of **4a** with acetic anhydride in pyridine led to the tetraacetate (**4b**), which exhibited four acetyl methyls at δ 2.32 and 2.34 in the ^{1}H-NMR spectrum. The upfield shifts to δ 2.99 and 1.85 of methine protons of isopropyl groups (H-8 and 8′) and methyl protons (H-7 and 7′), respectively, indicated the presence of acetyl groups *ortho* to the isopropyl and methyl groups. A spin-decoupling experiment was carried out in order to confirm the linkage position of the two *ortho*-hydroxythymols. When the isopropyl methine signal at δ 3.18 was irradiated, the singlet of an aromatic proton at δ 6.57 was sharpened, and *vice versa*. Although the irradiation of the methyl singlet at δ 1.94 showed a slight sharpening of the singlet at δ 6.57, it should cause more considerable sharpening if the methyl group was located *ortho* to the aromatic proton. Therefore these facts indicated the biphenyl linkage to be at the *ortho*-position to the methyl group. Furthermore, the ^{1}H-NMR signal of the methyl groups at δ 1.94 in **4a** was shifted upfield by about 0.3 ppm from that of thymol (**6**), because of the shielding of the ring current induced by the rotating biphenyl group.[10,11] It has already been proposed that bulky *ortho*-substituted biphenyl derivatives are incapable of taking a planar structure in solution. This proposal was



1 **2** **3** **4a** : R_1, R_2, R_3, R_4=H; **4b** : R_1, R_2, R_3, R_4=Ac **5** **6** **7**

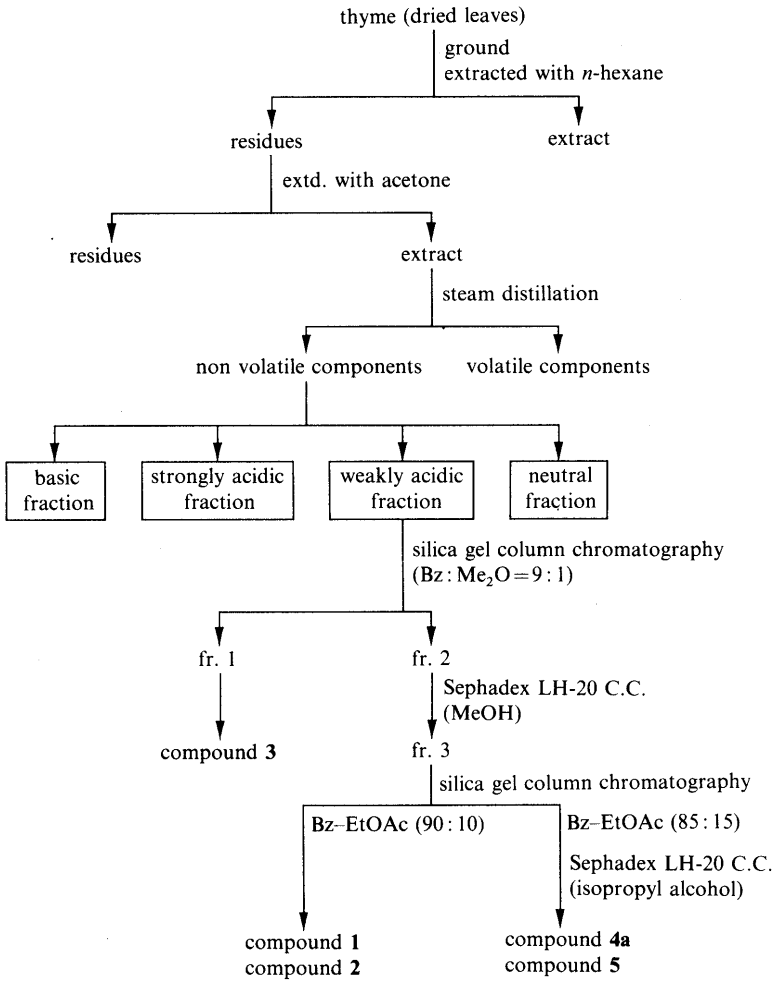


Fig. 1. Extraction and Isolation of Components from Thyme (*Thymus vulgaris* L.)

TABLE I. ^{13}C-NMR Spectral Data for Compounds **4**—**6**

Carbon no.	4a	4b	5	Carbon no.	6
1	134.11	139.21	134.14		—
2	119.88	127.81	119.74		—
3	139.79	141.16	139.70		—
4	141.34	139.34	141.37		—
5	131.50	138.91	131.48		—
6	119.34	125.16	119.24		—
7	12.62	13.52	12.62		—
8	27.25	27.57	27.23		—
9[a)]	22.63	20.37	22.68		—
10[a)]	22.72	20.41	22.75		—
1′	134.11	139.21	134.14	(6)	121.68
2′	119.88	127.81	134.75	(1)	136.58
3′	139.79	141.16	116.36	(2)	116.03
4′	141.34	139.34	151.51	(3)	152.52
5′	131.50	138.91	131.28	(4)	131.34
6′	119.34	125.16	128.11	(5)	126.23
7′	12.62	13.52	19.47	(7)	20.79
8′	27.25	27.57	26.82	(8)	26.73
9′[b)]	22.63	20.37	22.68	(9)	22.65
10′[b)]	22.72	20.41	22.75	(10)	22.65
OAc		168.03			
		168.51			
		23.04			
		23.04			

δ-Values (ppm), $CDCl_3$, TMS as internal standard. *a,b*) Assignments interchangeable. Carbon numbering of **6** in parenthesis.

supported by the observation of the optical rotation of **4a**. Thus, the structure of compound **4a** was determined to be 3,4,3′,4′-tetrahydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl.

Compound **5** was obtained as pale brown needles from benzene, and its molecular formula was determined to be $C_{20}H_{26}O_3$ on the basis of HRMS (M^+ at m/z 314.1847). This formula suggested loss of one hydroxyl group from the structure of **4a**. Spectral characteristics of **5** were similar to those of **4a**. The IR absorptions (3600 and 3200 cm^{-1}) and UV shift properties showed the presence of *ortho*-dihydroxyl groups. As shown in Table I, the ^{1}H-NMR spectrum showed signals of two isopropyl and two methyl groups attached to an aromatic ring. Further, three singlets at δ 6.59, 6.65 and 6.92 were assigned to aromatic protons, H-6, H-3′ and H-6′, respectively. Three hydroxyl protons at δ 4.69, 5.04 and 5.18 were confirmed by the addition of D_2O. The ^{1}H-NMR spectrum of **5** showed signals of an *ortho*-hydroxythymol moiety, which were in close agreement with those of **4a**, while the remaining signals were attributable to a thymol moiety. The ^{13}C-NMR spectrum also supported the deduced structure of **5**. A satisfactory agreement could be observed between the chemical shifts of **5** and those of *ortho*-hydoxythymol and thymol moiety except at C-1′. This indicated that the *ortho*-hydroxythymol moiety was linked with C-1′, with reference to calculations of the predicted substituent effects on the chemical shifts of

TABLE II. ^{1}H-NMR Spectral Data for Compounds **4**—**6**

Proton no.	4a	4b	5	Proton no.	6
H-6	6.57, s	7.03, s	6.59, s		—
H-7	1.94, s	1.85, s	1.94, s		—
H-8	3.18, sept	2.99, sept	3.18, sept		—
H-9[a]	1.24, d	1.19, d	1.24, d		—
H-10[a]	1.25, d	1.21, d	1.25, d		—
OH-3[b]	5.07, br s	—	5.04, br s		—
OH-4[b]	5.21, br s	—	5.18, br s		—
	—	—	—	(H-6)	6.72, d
H-3′	—	—	6.65, s	(H-2)	6.56, br s
H-6′	6.57, s	7.03, s	6.92, s	(H-5)	7.07, d
H-7′	1.94, s	1.85, s	1.98, s	(H-7)	2.26, s
H-8′	3.18, sept	2.99, sept	3.19, sept	(H-8)	3.16, sept
H-9′[c]	1.24, d	1.19, d	1.25, d	(H-9)	1.23, d
H-10′[c]	1.25, d	1.21, d	1.26, d	(H-10)	1.23, d
OH-3′[d]	5.07, br s	—	—		—
OH-4′[d]	5.21, br s	—	4.69, br s	(OH-3)	4.65, br s
OAc	—	2.32, s	—		—
		2.34, s	—		—

δ-Values (ppm), $CDCl_3$, TMS as internal standard. **4a**: H-8, H-9 or 10, J=6.8 Hz; H-8′, H-9′ or 10′, J=6.8 Hz. **4b**: H-8, H-9 or 10, J=6.8 Hz; H-8′, H-9′ or 10′, J=6.8 Hz. **5**: H-8, H-9 or 10, J=6.8 Hz; H-8′, H-9′ or 10′, J=6.8 Hz. **6**: H-5, H-6, J=7.8 Hz; H-8, H-9 or 10, J=7.1 Hz. *a,d*) Assignments interchangeable. Proton numbering of **6** in parenthesis.

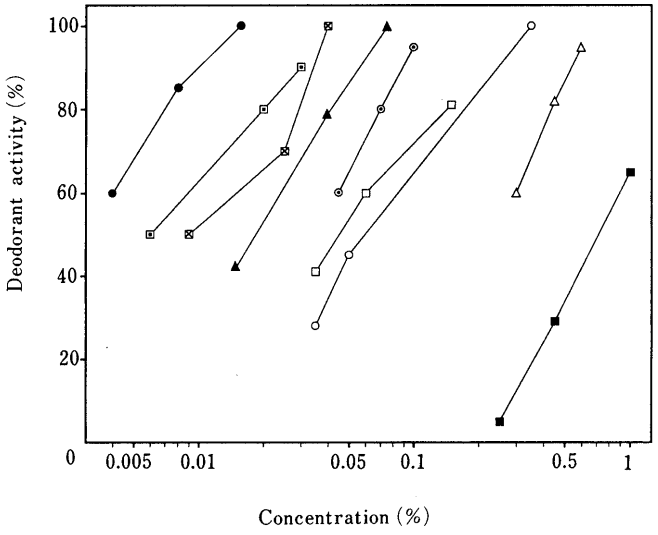


Fig. 2. Deodorant Activity (%) against Methyl Mercaptan (70 ppm)

●—●, compound **1**; ○—○, compound **3**; ⊠—⊠, compound **4**; ⊡—⊡, compound **5**; □—□, weakly acidic fraction; △—△, acetone extract; ▲—▲, rosmanol; ⊙—⊙, carnosol; ■—■, sodium copper chlorophylline.

benzene carbons. In addition, the lincage position was confirmed by a spin-decoupling experiment. Furthermore, Hasegawa *et al.* have reported that the chemical shifts of methyl carbons at the *ortho*-position in *ortho*-substituted biphenyls showed an upfield shift by about 1 ppm in the ^{13}C-NMR spectra.[10] The chemical shift of the 7′-methyl carbon in **5** showed an upfield shift by about 1.3 ppm as compared with that of **6**, while the chemical shifts of the isopropyl carbons were in agreement with those of **6**. These facts suggested that the dimer units must be linked at the *ortho*-positions to the methyl groups. On the basis of these data, the structure of **5** was determined to be 3,4,4′-trihydroxy-5,5′-diisopropyl-2,2′-dimethylbiphenyl.

The deodorant activity of the constituents of thyme against methyl mercaptan was measured by the use of a gas analyzer (Gastec) (Fig. 2). There was no activity in the *n*-hexane extract, while the acetone extract was more effective than that of sodium copper chlorophylline, which is commonly used as an oral deodorizer. The weakly acidic fraction of the acetone extract exhibited a stronger deodorant effect.

The deodorant effects of compounds **1**, **3**, **4a** and **5** isolated from this fraction were determined, as well as those of rosmanol (**7**)[12,13] and carnosol,[14] both of which were isolated from rosemary (*Rosmalinus officinalis* L.) and sage (*Salvia officinalis* L.) and are known to be effective deodorant compounds.[15] As shown in Fig. 2, although the deodorant activity of **4a** and **5** was not as potent as that of **1**, both compounds were more effective than rosmanol and carnosol. In a comparison of the deodorant activity at the sample concentration of 60%, compound **4a** and **5** exhibited activities about 60 and 100 times stronger than that of sodium copper chlorophylline, respectively.

Experimental

Melting points were measured with a Yanagimoto micro melting point apparatus and are uncorrected. UV absorption spectra were determined on a Hitachi 220 spectrophotometer and IR spectra were recorded with a Jasco IR-S. ^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were run on a JEOL GX-400 using tetramethylsilane (TMS) as an internal standard. MS were obtained on a Hitachi M-2000. Optical rotation was measured with a Union PM-101. Column chromatographies were performed using Merck silica gel 60 (70—230 mesh) and Pharmacia Sephadex LH-20, and thin layer chromatography (TLC) was done using Silica gel GF-254.

Deodorizing Assay The methods of Tokita *et al.*[3] was slightly modified as previously reported.[9]

Extraction and Isolation As shown in Fig. 1, dried ground leaves of thyme (2 kg) cultivated in Spain were extracted with *n*-hexane (3 l) to remove the non-polar components. The acetone extract (100 g) of the residue was steam-distilled to afford the non-volatile fraction, which was separated by pH fractionation in the usual way. The weakly acidic components (14.6% based on the acetone extract) were subjected to chromatography on a silica gel column with benzene–acetone as an elution solvent system to give eleven fractions. The second fraction (2.16 g) was dissolved in MeOH, and the soluble part (1.28 g) was rechromatographed on a Sephadex LH-20 column with MeOH to separate five fractions. The third fraction (1.05 g) was rechromatographed on a silica gel column with benzene–ethyl acetate (85:15, v/v), and finally on a Sephadex LH-20 column with isopropyl alcohol as an eluent to give a pure light brown powder (**4a**, 70.5 mg) and pale blown needles (**5**, 2.1 mg). The latter product was recrystallized from benzene.

Compound **4a**: mp 104 °C. $[\alpha]_D^{25}$: −8.8° (c=0.51, $CHCl_3$). MS m/z (%): 330 (M^+, 100), 315 (53), 310 (40), 285 (14), 257 (14), 165 (9). HRMS m/z: 330.1803 (M^+, Calcd for $C_{20}H_{26}O_4$: 330.1832). UV λ_{max}^{MeOH} nm (log ε): 215.0 (4.52), 274.5 (3.59). IR ν_{max}^{Nujol} cm^{-1}: 3300, 1330, 1250, 1210, 1160, 1100, 1080, 1030, 960, 940.

Tetraacetate of **4a** (**4b**): Acetic anhydride (1 ml) was added to a solution of 18 mg of **4a** in pyridine (1 ml), and the mixture was allowed to stand overnight at 25 °C. The reaction mixture was worked up in the usual manner to afford a tetraacetate (**4b**, 21.7 mg). IR ν_{max}^{film} cm^{-1}: 1765, 1410, 1370, 1205, 1180, 1085, 1045, 1010, 960, 890.

Compound **5**: mp 75 °C (benzene). $[\alpha]_D^{25}$: −4.8° (c=0.21, $CHCl_3$). MS m/z (%): 314 (M^+, 100), 299 (67.6), 284 (20.3), 269 (10.8). HRMS m/z: 314.1847 (M^+, Calcd for $C_{20}H_{26}O_3$: 314.1883). UV λ_{max}^{MeOH} nm (log ε): 213.5 (4.45), 248.5 sh (3.77), 281.5 (3.60). IR ν_{max}^{film} cm^{-1}: 3600, 3200, 1495, 1250, 1175, 1050, 945.

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