Note

Antioxidative Activity of Flavonoids from Thyme (*Thymus vulgaris* L.)

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During the course of our study on the isolation and structural elucidation of antioxidants from the spices of the family Labiatae, we found that the diterpenoids from rosemary^{1,2)} and sage,³⁾ and phenolic acid from oregano⁴⁾ had effective antioxidative activity. Recently, we have reported new antioxidative and deodorant biphenyls from thyme (*Thymus vulgaris* L.).^{5~7)} This paper deals with the isolation, structural elucidation and antioxidative effect of flavonoids from thyme.

The weakly acidic fraction of the non-volatile part was subjected to column chromatography on silica gel, eluting with benzene-acetone mixture to separate eleven fractions, all of which showed antioxidative activity as strong as that of *tert*-butyl-4-hydroxytoluene (BHT), one of the synthetic antioxidants.⁵⁾ Each fraction was further purified by silica gel and Sephadex LH-20 column chromatography to afford six flavonoids (flavones 1~6).

	R ₁	R ₂	R ₃	R ₄	R ₅
Flavone 1	OCH ₃	OCH ₃	OCH ₃	H	OH
Flavone 2	OCH ₃	OCH ₃	OCH ₃	OCH ₃	OH
Flavone 3	OCH ₃	OCH ₃	H	H	OH
Flavone 4	OCH ₃	OCH ₃	H	OCH ₃	OH
Flavone 5	H	OCH ₃	H	H	OH
Flavone 6	H	OCH ₃	H	H	OCH ₃

Flavone 1 was obtained as yellow needles, mp 228°C, *m/z* 344, from the third fraction. This was identified as 5,4'-dihydroxy-6,7,8-trimethoxyflavone by a comparison with the reported data.⁸⁾ From the same fraction, flavone 2 was obtained as yellow needles, mp 165°C. The molecular ion peak was observed at *m/z* 374, suggesting it contained one more methoxy group in the B-ring than in that of flavone 1. This compound was identified as 5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone.⁹⁾ From the fourth fraction, flavone 3 was obtained as pale yellow needles,

mp 256°C, *m/z* 314. Acetylation of 3 led to a diacetate, whose spectral data were in good agreement with those of the diacetate of 5,4'-dihydroxy-6,7-dimethoxyflavone (cirsilineol).^{8,9)} This supported the identification of flavone 3 as cirsilineol. From the fourth fraction, flavone 4 was also obtained as pale yellow crystals, mp 205°C. Its spectral characteristics were also similar to those of flavone 2, except it lacked one methoxy group at the C-8 position. This compound was identified as 5,4'-dihydroxy-6,7,3'-trimethoxyflavone on the basis of spectral data.¹⁰⁾ The third fraction was triturated with benzene-acetone (9:1, v/v) to separate an insoluble compound, which was recrystallized from acetone to afford a yellow powder, flavone 5, mp 270°C, *m/z* 284. Acetylation of 5 led to the diacetate of 5,4'-dihydroxy-7-methoxyflavone (genkwanin). This structure was also supported by the reported spectral data.¹¹⁾ From the first fraction, flavone 6 was

Fig. 1. Antioxidative Activity of Flavonoids from Thyme.

Ferric thiocyanate method, 0.02% concentration: ⊠, flavone 1; □, flavone 2; ⊙, flavone 4; ⊖, flavone 5; □, α-tocopherol; ⊕, BHT; ●, control.

Fig. 2. Antioxidative Activity on the 10th Day of Flavonoids from Thyme (TBA).

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obtained as yellow crystals, mp 173°C. The $^1\text{H-NMR}$ spectrum of **6** was also similar to that of flavone **5**, except for the presence of a methoxy group on the B-ring instead of the hydroxy group in flavone **5**. This compound was identified as 5-hydroxy-7,4'-dimethoxy flavone.^{8,11} Flavones **1**, **4**, **5** and **6** were isolated from thyme for the first time.

The antioxidative activity of flavones **1**, **2**, **4** and **5** was evaluated by the ferric thiocyanate and thiobarbituric acid (TBA) methods at the concentration of 0.02% as previously reported.¹⁾ The activity of the flavones **3** and **6** could not be measured because of their low yield. As shown in Fig. 1, the activity of flavones **2**, **4** and **5** was found to be more than that of α -tocopherol, and about the same as that of BHT. Similar results were obtained from the TBA method on the tenth day (Fig. 2). Flavones **2**, **4** and **5** showed antioxidative activity as high as that of BHT.

Experimental

$^1\text{H-NMR}$ (400 MHz) and $^{13}\text{C-NMR}$ (100 MHz) spectra were run on a JEOL GX-400, using TMS as an internal standard. MS data were obtained on a Hitachi M-2000.

Extraction and isolation. After removing the non-polar components with *n*-hexane, the leaves (2 kg) of thyme was extracted with acetone. The acetone extract (100 g) was steam-distilled and fractionated as described in the previous paper.^{6,7)}

Flavone 1 (5,4'-dihydroxy-6,7,8-trimethoxy flavone). Yellow needles (11.12 mg), mp 228°C (recrystallized from MeOH). MS m/z (%): 344.0905 (M^+ , 63), 329 (100), 211 (15), 183 (17), 118 (11). UV λ_{max} (MeOH) nm: 232, 281, 294sh. IR ν_{max} (nujol) cm^{-1} : 3250, 1655, 1605, 1282, 1218, 1178, 1120, 1073. $^1\text{H-NMR}$ (acetone- d_6) δ : 3.88, 3.98, 4.08 (3H each, s), 6.71 (1H, s), 7.06 (2H, dd, $J=1.95$, 8.79 Hz), 8.00 (2H, dd, $J=1.95$, 8.79 Hz), 12.80 (1H, s, D_2O exchangeable).

Flavone 2 (5,4'-dihydroxy-6,7,8,3'-tetramethoxyflavone). Yellow needles (14.9 mg), mp 165°C (isopropyl alcohol). MS m/z (%): 374 (M^+ , 66), 359 (100), 211 (13), 183 (12), 151 (4), 148 (3). UV λ_{max} (MeOH) nm: 253, 281, 344. IR ν_{max} (nujol) cm^{-1} : 3320, 1645, 1620, 1280, 1225, 1200. $^1\text{H-NMR}$ (CDCl_3) δ : 3.96, 3.98, 4.00, 4.12 (each 3H, s), 6.19 (1H, s, D_2O exchangeable), 6.59 (1H, s), 7.05 (1H, d, $J=8.30$ Hz), 7.41 (1H, d, $J=1.95$ Hz), 7.54 (1H, dd, $J=1.95$, 8.30 Hz), 12.55 (1H, s, D_2O exchangeable). $^{13}\text{C-NMR}$ (CDCl_3) δ : 56.03, 61.09, 61.69, 62.04, 103.77, 103.80, 106.95, 108.34, 115.12, 120.76, 123.25, 132.94, 136.58, 145.73, 146.94, 149.48, 152.97, 164.00, 182.96.

Flavone 3 (5,4'-dihydroxy-6,7-dimethoxyflavone). Yellow needles (39 mg), mp 256°C (acetone). MS m/z (%): 314 (M^+ , 100), 299 (70), 284 (11), 271 (51), 181 (30), 121 (19).

UV λ_{max} (MeOH) nm: 208, 284, 334. IR ν_{max} (nujol) cm^{-1} : 3230, 1650, 1630, 1595, 1240, 1200, 1178, 1119. $^1\text{H-NMR}$ (acetone- d_6) δ : 3.80, 3.99 (3H each, s), 6.68 (1H, s), 6.85 (1H, s), 7.03 (2H, d, $J=8.79$ Hz), 7.96 (2H, d, $J=8.79$ Hz), 9.25 (1H, s, D_2O exchangeable), 12.96 (1H, s, D_2O exchangeable).

Diacetate of flavone 3. IR ν_{max} (film) cm^{-1} : 1755, 1635, 1610, 1290, 1260, 1165, 1110, 1089. $^1\text{H-NMR}$ (CDCl_3) δ : 2.35 (3H, s), 2.49 (3H, s), 3.87, 4.00 (3H, each s), 6.58 (1H, s), 6.92 (1H, s), 7.25 (2H, d, $J=9.03$ Hz), 7.88 (2H, d, $J=9.03$ Hz).

Flavone 4 (5,4'-dihydroxy-6,7,3'-trimethoxyflavone). Pale yellow crystals, mp 205°C (MeOH). MS m/z (%): 344 (M^+ , 100), 329, 315, 314, 181, 153, 151, 149. UV λ_{max} (MeOH) nm: 274, 340. IR ν_{max} (nujol) cm^{-1} : 3250, 1655, 1600, 1280, 1265, 1215, 1130. $^1\text{H-NMR}$ (acetone- d_6) δ : 3.78, 3.97, 3.98 (3H each, s), 6.73 (1H, s), 6.84 (1H, s), 7.00 (1H, d, $J=8.30$ Hz), 7.62 (1H, dd, $J=1.95$, 8.30 Hz), 7.64 (1H, d, $J=1.95$ Hz), 8.53 (1H, s, D_2O exchangeable), 12.96 (1H, s, D_2O exchangeable). $^{13}\text{C-NMR}$ (acetone- d_6) δ : 56.64, 56.77, 60.49, 91.92, 104.22, 106.50, 110.59, 115.58, 116.38, 121.39, 123.57, 133.51, 148.88, 151.51, 153.95, 160.06, 165.20, 183.52.

Flavone 5 (5,4'-dihydroxy-7-methoxyflavone). Yellow amorphous powder (86 mg), mp 270°C (acetone). MS m/z (%): 284 (M^+ , 100), 255 (36), 241 (16), 167 (13), 166 (14), 138 (14), 128 (16), 121 (7), 118 (10). UV λ_{max} (MeOH) nm: 271, 300, 328. IR ν_{max} (nujol) cm^{-1} : 3220, 1660, 1600, 1290, 1215, 1175. $^1\text{H-NMR}$ ($\text{DMSO}-d_6$) δ : 3.89 (3H, s), 6.40 (1H, d, $J=2.3$ Hz), 6.79 (1H, d, $J=2.3$ Hz), 6.85 (1H, s), 6.96 (2H, d, $J=9.3$ Hz), 7.98 (2H, d, $J=9.3$ Hz), 12.95 (1H, s, D_2O exchangeable).

Diacetate of flavone 5. MS m/z (%): 368 (M^+ , 3), 326 (53), 284 (100), 255 (22). IR ν_{max} (nujol) cm^{-1} : 1765, 1750, 1638, 1610, 1580, 1295, 1225, 1210, 1195, 1173, 1110. $^1\text{H-NMR}$ (CDCl_3) δ : 2.27 (3H, s), 2.36 (3H, s), 3.85 (3H, s), 6.52 (1H, s), 6.58 (1H, d, $J=2.4$ Hz), 6.82 (1H, d, $J=2.4$ Hz), 7.20 (1H, dd, $J=2.4$, 8.4 Hz), 7.84 (1H, dd, $J=2.4$, 8.4 Hz).

Flavone 6 (5-hydroxy-7,4'-dimethoxyflavone). Yellow crystals (2.17 mg), mp 173°C. (acetone) MS m/z : 298, 269, 255, 166, 138, 135, 132. UV λ_{max} (MeOH): 273, 301, 324. IR ν_{max} (nujol) cm^{-1} : 1665, 1605, 1585, 1270, 1165. $^1\text{H-NMR}$ (CDCl_3) δ : 3.89, 3.90 (3H each, s), 6.37 (1H, d, $J=2.4$ Hz), 6.49 (1H, d, $J=2.4$ Hz), 6.58 (1H, s), 7.02 (2H, d, $J=9.3$ Hz), 7.85 (2H, d, $J=9.3$ Hz), 12.80 (1H, s).

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