Food Sci. Technol., Int., **1** (2), 115–120, 1995

Antioxidative Constituents from Dendrobii Herba (Stems of *Dendrobium* spp.)

Masateru ONO,[1] Yasuyuki ITO,[1] Chikako MASUOKA,[2] Hideharu KOGA[2] and Toshihiro NOHARA[3]

[1]*Faculty of Research Institute of General Education, Kyushu Tokai University, Choyo 5435, Aso, Kumamoto 869-14, Japan*
[2]*Faculty of Agriculture, Kyushu Tokai University, Choyo 5435, Aso, Kumamoto 869-14, Japan*
[3]*Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi 5-1, Kumamoto 862, Japan*

Received August 17, 1995

Ten phenolic compounds; gigantol, moscatilin, tristin, crepidatin, 4-hydroxy-3,3′,4′,5-tetramethoxybibenzyl, 2, 4,8-trimethoxyphenanthrene-3,7-diol, confusarin, moscatin, medioresinol and syringaresinol were isolated from Dendrobii Herba (stems of *Dendrobium* spp.), which is used as one of the important crude drugs for tonics, antipyretics and stomach agents, and their structures were elucidated on the basis of spectroscopic data. All of these compounds showed stronger antioxidative activity than BHA by the ferric thiocyanate method.

Keywords: Dendrobii Herba, antioxidative effect, Orchidaceae, *Dendrobium* spp., phenanthrene, bibenzyl, lignan

Lipid peroxidation is known as one of the major factors in rancidity of fats and oils in food, and it is connected with aging and carcinogenesis (Yagi, 1987; Hirai, 1994). Antioxidants are major constituents in protection against lipid peroxidation. Currently, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) and natural antioxidants such as α-tocopherol and ascorbic acid are used as food additives for the purpose of preventing deterioration of foods. However, these synthetic antioxidants are suspected to have toxic side effects (Ito *et al.*, 1986), although α-tocopherol and ascorbic acid are less active than synthetic antioxidants. Therefore, strongly active natural antioxidants which would be safer for human use than synthetic antioxidants are desired.

Dendrobii Herba, the stems of *Dendrobium nobile* and several other *Dendrobium* species (Orchidaceae), is used as one of the important crude drugs for tonics, antipyretics and stomach agents (Namba, 1980).

We have identified a strong antioxidative effect of the AcOEt fraction (fr.), which was prepared from the MeOH extract of this crude drug. Therefore, we have examined the constituents to determine the substances responsible for the antioxidative effect.

Materials and Methods

The Dendrobii Herba was purchased at the Kunming market in southern China. The optical rotations were measured with a JASCO DIP 360 digital polarimeter, and visible absorptions were measured with a Shimadzu UV-140-02 spectrometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on JEOL JNM-GX-400 and JEOL alpha 500 spectrometers; chemical shifts were given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. The MS was obtained on JEOL JMS-DX-303HF instruments. Column chromatography was carried out with silica gel 60 (Merck, Art. 7734 and Art. 9385), Sephadex LH 20 (Pharmacia Fine Chemicals) and Bio-Beads S-X2 (200–400 mesh, Bio Rad Lab.). Preparative thin layer chromatography (TLC) was done with silica gel 60 (Merck, Art. 5715, 20 cm×20 cm). High performance liquid chromatography (HPLC) separation was run on a Micro Pump KPW-20 (Kusano Kagakukikai Co.) with a UV-Detector KU-331 (Kusano Kagakukikai Co.). For HPLC column chromatography, Cosmosil 5C18-Ar (Nacalai Tesque Inc., 6 mm i.d.×250 mm), YMC-pack S-5 120A ODS (YMC, 20 mm i.d.×250 mm and 10 mm i.d.×300 mm) and Kusano C.I.G. prepacked ODS (Kusano Kagakukikai Co., 22 mm i.d.×100 mm) with an MeOH-H_2O system as the developing solvent and Kusano C.I.G. prepacked Si-gel (Kusano Kagakukikai Co., 22 mm i.d.×100 mm) with *n*-hexane-AcOEt, 2:1, as the developing solvent were used.

Extraction and isolation The cut dried stems of Dendrobii Herba (1935 g) were extracted with MeOH (9.0 *l*, 5.4 *l*, 4.9 *l*, 3 *l*) under reflux, and the solvent was removed under reduced pressure to afford a brown syrup (120.8 g), which was suspended in H_2O (500 ml) and then extracted with AcOEt (600 ml, 400 ml×3). Concentration of the AcOEt layer and the H_2O layer furnished an AcOEt soluble fr. (34.83 g) and an H_2O soluble fr. (**H_2O fr.**, 83.1 g), respectively. The AcOEt soluble fr. (30.65 g) was defatted repeatedly by treatment with *n*-hexane (100 ml, 500 ml×2) to give the *n*-hexane soluble fr. (***n*-hexane fr.**, 6.74 g) and a residue (**AcOEt fr.**, 23.74 g). This residue was chromatographed over silica gel [Art. 7734, eluant: *n*-hexane-AcOEt (15:1 → 10:1 → 5:1 → 3:1 → 1:1 → 1:2 → 1:4) → AcOEt → MeOH] to afford fr. 1 (89 mg), fr. 2 (413 mg), fr. 3 (277 mg), fr. 4 (426 mg), fr. 5 (499 mg), fr. 6 (366 mg), fr. 7 (737 mg), fr. 8 (362 mg), fr. 9 (4588 mg), fr. 10 (1144 mg), fr. 11 (4694 mg) and fr. 12 (658 mg). Chromatography of fr. 9 over silica gel [Art. 7734, eluant: $CHCl_3$ → $CHCl_3$-MeOH (30:1 → 15:1 → 10:1) → $CHCl_3$-MeOH-H_2O (14:2:0.1 → 10:2:0.1 → 8:2:0.2 → 7:3:0.5) → MeOH] furnished fr. 13 (73 mg), fr. 14 (122 mg), fr. 15 (171 mg), fr. 16 (922 mg), fr. 17 (662 mg), fr. 18 (85 mg), fr. 19 (787 mg) and fr. 20 (1622 mg). Fr. 16 was chromatographed over Bio-Beads S-X2 (eluant: *n*-hexane-AcOEt, 1:1) to give fr. 21

(12 mg), fr. 22 (152 mg), fr. 23 (518 mg) and fr. 24 (119 mg). HPLC (YMC Pack S-5 120A ODS, 20 mm i.d.×250 mm, eluant: 70% MeOH) of fr. 23 gave fr. 25 (28 mg), fr. 26 (323 mg) and fr. 27 (85 mg). Similar HPLC (eluant: 60% MeOH) of fr. 26 to fr. 23 afforded **2** (73 mg) and **7** (15 mg). Fr. 27 was subjected to HPLC (Kusano C.I.G. prepacked Si-gel) to give **4** (37 mg) and **5** (37 mg). Fr. 17 was chromatographed over Bio-Beads S-X2 (eluant: *n*-hexane-AcOEt, 1:1) to give fr. 28 (72 mg), fr. 29 (115 mg), fr. 30 (231 mg) and fr. 31 (200 mg). Fr. 30 was subjected to HPLC under the same conditions as fr. 23 to give fr. 32 (32 mg), fr. 33 (36 mg) and fr. 34 (67 mg). Chromatography of fr. 34 over silica gel (Art. 9385, eluant: *n*-hexane-AcOEt, 1:1) gave **6** (3 mg) and **2** (50 mg). Fr. 18 was subjected to Sephadex LH 20 (eluant: MeOH) to afford fr. 35 (51 mg) and fr. 36 (27 mg). HPLC (Kusano C.I.G. prepacked ODS, eluant: 70% MeOH → 75% MeOH → 80% MeOH → 90% MeOH → MeOH) of fr. 36 furnished fr. 37 (9 mg). HPLC (YMC Pack S-5 120A ODS, 10 mm i.d.×300 mm, eluant: 75% MeOH) of fr. 37 afforded **8** (6 mg). Fr. 19 was chromatographed over Bio-Beads S-X2 (eluant: *n*-hexane-AcOEt, 1:1) to give fr. 38 (171 mg), fr. 39 (154 mg), fr. 40 (120 mg), fr. 41 (269 mg) and fr. 42 (48 mg). HPLC of fr. 41 under the same conditions as for fr. 23 furnished fr. 43 (12 mg), fr. 44 (156 mg) and fr. 45 (5 mg). Similar HPLC (eluant: 60% MeOH) of fr. 44 to fr. 41 gave **1** (131 mg). Fr. 10 was subjected to Sephadex LH 20 (eluant: MeOH) to give fr. 46 (234 mg), fr. 47 (272 mg), fr. 48 (188 mg), fr. 49 (75 mg) and fr. 50 (332 mg). Fr. 48 was subjected repeatedly to preparative TLC [solvent: $CHCl_3$-MeOH-H_2O, 14:2:0.1] and HPLC (YMC pack S-5 120A ODS, 20 mm i.d.×250 mm, eluant: 65% MeOH) to give **3** (21 mg). Fr. 11 was chromatographed over silica gel [Art. 7734, eluant: $CHCl_3$ → $CHCl_3$-MeOH (30:1 → 15:1 → 10:1) → $CHCl_3$-MeOH-H_2O (14:2:0.1 → 10:2:0.1 → 7:3:0.5) → MeOH] to give fr. 51 (166 mg), fr. 52 (495 mg), fr. 53 (303 mg), fr. 54 (458 mg), fr. 55 (636 mg), fr. 56 (378 mg), fr. 57 (632 mg), fr. 58 (213 mg), fr. 59 (163 mg), fr. 60 (61 mg), fr. 61 (44 mg), fr. 62 (69 mg), fr. 63 (158 mg) and fr. 64 (312 mg). Fr. 64 was subjected to HPLC under the same conditions as for fr. 44 to give fr. 65 (71 mg), fr. 66 (61 mg), fr. 67 (59 mg), fr. 68 (20 mg) and fr. 69 (57 mg). HPLC (Cosmosil 5C18Ar, eluant: 60% MeOH) of fr. 69 furnished **9** (7 mg) and **10** (33 mg).

Gigantol (**1**): An amorphous powder. EI-MS *m/z* (%): 274 $[M]^+$ (54), 137 (100). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.83 (1H, d, J=7.9 Hz, 5′-H), 6.68 (1H, dd, J=1.8, 7.9 Hz, 6′-H), 6.62 (1H, d, J=1.8 Hz, 2′-H), 6.31 (1H, dd, J=1.8, 1.8 Hz, 4-H*), ca. 6.25 (2H, 2-H*, 6-H*), 5.48 (1H, br s, OH), 4.92 (1H, br s, OH), 3.84 (3H, s, 3′-OCH_3), 3.75 (3H, s, 3-OCH_3), ca. 2.80 (4H, α-H_2, α'-H_2). *, Assignment may be interchanged. ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 160.8 (5-C), 156.6 (3-C), 146.3 (3′-C), 144.5 (1-C), 143.7 (4′-C), 133.7 (1′-C), 121.0 (6′-C), 114.2 (5′-C), 111.2 (2′-C), 108.1 (2-C), 106.8 (6-C), 99.1 (4-C), 55.9 (OCH_3), 55.3 (OCH_3), 38.2, 37.2 (α-C, α'-C).

Moscatilin (**2**): An amorphous powder. EI-MS *m/z* (%): 304 $[M]^+$(44), 167 (100), 137 (35). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.83 (1H, d, J=8.5 Hz, 5′-H), 6.67 (1H, dd, J=1.8, 8.5 Hz, 6′-H), 6.61 (1H, d, J=1.8 Hz, 2′-H), 6.35 (2H, s, 2-H, 6-H), 5.59 (1H, br s, OH), 5.46 (1H, br s, OH), 3.83 (6H, s, 3-OCH_3 and 5-OCH_3), 3.82 (3H, s, 3′-OCH_3), ca. 2.81 (4H, α-H_2, α'-H_2). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 146.8 (3-C, 5-C), 146.3 (3′-C), 143.8 (4′-C), 133.6 (1′-C), 132.9 (1-C, 4-C), 121.1 (6′-C), 114.2 (5′-C), 111.3 (2′-C), 105.5 (2-C, 6-C), 56.3 (3-OCH_3, 5-OCH_3), 55.9 (3′-OCH_3), 38.4, 37.9 (α-C, α'-C).

Tristin (**3**): An amorphous powder. negative FAB-MS *m/z* (%): 259 $[M-H]^-$ (100), 123 (29). Positive FAB-MS *m/z* (%): 261 $[M+H]^+$ (15), 137 (100). EI-MS *m/z* (%): 260 $[M]^+$ (16), 137 (100). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.83 (1H, d, J=7.9 Hz, 5′-H), 6.67 (1H, dd, J=1.8, 7.9 Hz, 6′-H), 6.61 (1H, d, J=1.8 Hz, 2′-H), 6.22 (2H, d, J=0.2 Hz, 2-H, 6-H), 6.19 (1H, t, J=0.2 Hz, 4-H), 5.46 (1H, s, OH), 4.79 (2H, br s, OH), 3.84 (3H, s, 3′-OCH_3), ca. 2.81 (2H, α-H_2 or α'-H_2), ca. 2.79 (2H, α-H_2 or α'-H_2). ^{13}C-NMR (in $CDCl_3$ + CD_3OD, 100 MHz) δ: 157.3 (3-C, 5-C), 146.5 (3′-C), 144.2 (1-C), 143.5 (4′-C), 133.7 (1′-C), 120.7 (6′-C), 114.3 (5′-C), 111.5 (2′-C), 107.2 (2-C, 6-C), 100.0 (4-C), 55.6 (OCH_3), 37.9, 37.0 (α-C, α'-C).

Crepidatin (**4**): Colorless needles (*n*-hexane-AcOEt), mp 99–100°C. EI-MS *m/z* (%): 318 $[M]^+$(27), 181 (100), 137 (33). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.84 (1H, d, J=7.9 Hz, 5′-H), 6.69 (1H, dd, J=1.8, 7.9 Hz, 6′-H), 6.61 (1H, d, J=1.8 Hz, 2′-H), 6.36 (2H, s, 2-H, 6-H), 5.55 (1H, br s, OH), 3.83 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.82 (6H, s, 3-OCH_3, 5-OCH_3), ca. 2.83 (4H, α-H_2, α'-H_2). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 153.0 (3-C, 5-C), 146.3 (3′-C), 143.8 (4′-C), 137.6 (1-C), 136.2 (4-C), 133.5 (1′-C), 121.0 (6′-C), 114.2 (5′-C), 111.2 (2′-C), 105.5 (2-C, 6-C), 60.8 (4-OCH_3), 56.0 (3-OCH_3, 5-OCH_3), 55.9 (3-OCH_3), 38.6, 37.7 (α-C, α'-C).

5: An amorphous powder, EI-MS *m/z* (%): 318 $[M]^+$(35), 167 (87), 151 (100). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.79 (1H, d, J=8.1 Hz, 5′-H), 6.70 (1H, dd, J=1.8, 8.1 Hz, 6′-H), 6.66 (1H, d, J=1.8 Hz, 2′-H), 6.36 (2H, s, 2-H, 6-H), 5.40 (1H, br s, OH), 3.85 (3H, s, OCH_3), 3.84 (9H, s, OCH_3×3), ca. 2.83 (4H, α-H_2 and α'-H_2). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 148.7 (4′-C), 147.2 (3′-C), 146.8 (3-C, 5-C), 134.3 (1′-C), 132.8 (1-C, 4-C), 120.4 (6′-C), 111.9, 111.2 (2′-C, 5′-C), 105.2 (2-C, 6-C), 56.2 (3-OCH_3, 5-OCH_3), 55.9, 55.8 (3′-OCH_3, 4′-OCH_3), 38.3, 37.8 (α-C, α'-C).

6: An amorphous powder, EI-MS *m/z* (%): 300 $[M]^+$ (100), 285 (54), 253 (21). ^{1}H-NMR (in acetone-d_6, 500 MHz) δ: 9.16 (1H, d, J=9.2 Hz, 5-H), 7.85 (1H, d, J=9.2 Hz, 9-H), 7.67 (1H, d, J=9.2 Hz, 10-H), 7.26 (1H, s, 1-H), 7.24 (1H, d, J=9.2 Hz, 6-H), 4.00 (3H, s, OCH_3), 3.93 (3H, s, OCH_3), 3.92 (3H, s, OCH_3). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 9.16 (1H, d, J=9.2 Hz, 5-H), 7.82 (1H, d, J=9.2 Hz, 9-H), 7.63 (1H, d, J=9.2 Hz, 10-H), 7.30 (1H, d, J=9.2 Hz, 6-H), 7.09 (1H, s, 1-H), 6.01 (1H, s, OH), 5.79 (1H, s, OH), 4.05 (3H, s, 2-OCH_3), 3.98 (3H, s, 8-OCH_3), 3.94 (3H, s, 4-OCH_3). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 146.8 (2-C), 145.6 (7-C), 144.0 (4-C), 140.8 (8-C), 139.4 (3-C), 127.5 (10-C), 126.6 (10a-C), 125.7 (8a-C), 124.2 (4b-C), 124.0 (5-C), 119.2 (4a-C), 117.9 (9-C), 116.1 (6-C), 104.9 (1-C), 61.9 (OCH_3), 59.8 (OCH_3), 56.1 (OCH_3).

Confusarin (**7**): An amorphous powder, EI-MS *m/z* (%): 300 $[M]^+$(100), 285 (46), 253 (27), 242 (11), 227 (11), 167 (23), 137 (16). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 9.19 (1H, d, J=9.2 Hz, 5-H), 7.85 (1H, d, J=9.2 Hz, 9-H), 7.59 (1H, d, J=9.2 Hz, 10-H), 7.29 (1H, d, J=9.2 Hz, 6-H), 7.19 (1H, s, 1-H), 6.01 (1H, br s, OH), 5.82 (1H, br s, OH), 4.11 (3H, s, 3-OCH_3), 3.97 (6H, s, 4-OCH_3, 8-OCH_3). ^{1}H-NMR (in CD_3OD, 400 MHz)

δ: 9.09 (1H, d, J=9.2 Hz, 5-H), 7.86 (1H, d, J=9.2 Hz, 9-H), 7.50 (1H, d, J=9.2 Hz, 10-H), 7.18 (1H, d, J=9.2 Hz, 6-H), 7.08 (1H, s, 1-H), 4.00 (3H, s, 4-OCH_3), 3.93 (3H, s, OCH_3), 3.92 (3H, s, OCH_3). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 150.7 (4-C), 147.7 (2-C), 145.5 (7-C), 141.0 (3-C, 8-C), 129.3 (10a-C), 127.4 (10-C), 126.3 (8a-C), 124.8 (4b-C), 123.9 (5-C), 119.4 (9-C), 119.0 (4a-C), 116.2 (6-C), 108.2 (1-C), 62.0 (OCH_3), 61.3 (OCH_3), 59.8 (OCH_3). ^{13}C-NMR (in CD_3OD, 100 MHz) δ: 152.9, 150.4 (4-C, 7-C), 147.5 (2-C), 143.4, 142.7 (3-C, 8-C), 130.8, 128.4 (4b-C, 10a-C), 127.8 (10-C), 125.6 (8a-C), 124.4 (5-C), 120.5 (9-C), 119.6 (4a-C), 118.3 (6-C), 110.2 (1-C), 61.5 (OCH_3×2), 60.4 (OCH_3).

Moscatin (**8**): An amorphous powder, EI-MS m/z (%): 240 $[M]^+$(100), 225 (42), 197 (27). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 7.61 (1H, d, J=9.2 Hz, 9-H), 7.48 (1H, dd, J=8.0, 8.0 Hz, 7-H), 7.41 (1H, dd, J=1.2, 8.0 Hz, 8-H), 7.41 (1H, d, J=9.2 Hz, 10-H), 7.23 (1H, dd, J=1.2, 8.0 Hz, 6-H), 6.96 (1H, d, J=2.5 Hz, 1-H), 6.82 (1H, d, J=2.5 Hz, 3-H), 4.04 (3H, s, 4-OCH_3). ^{13}C-NMR (in $CDCl_3$, 100 MHz) δ: 155.4 (4-C), 154.4, 153.8 (2-C, 5-C), 136.1 (10a-C), 134.1 (8a-C), 129.4 (7-C), 127.0 (9-C), 125.9 (10-C), 120.8 (8-C), 118.8 (4b-C), 116.6 (6-C), 114.2 (4a-C), 107.4 (1-C), 101.7 (3-C), 58.4 (4-OCH_3).

Medioresinol (**9**): An amorphous powder, $[\alpha]_D^{27}$−6.6° (c=1.0, MeOH). EI-MS m/z: 388 $[M]^+$ (100), 181 (40), 167 (25), 151 (40), 137 (29). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.89 (1H, d, J=7.9 Hz, 5′-H), 6.88 (1H, s, 2′-H), 6.82 (1H, d, J=7.9 Hz, 6′-H), 6.58 (2H, s, 2-H, 6-H), 5.60 (1H, br s, OH), 5.49 (1H, br s, OH), 4.75 (1H, d, J=4.3 Hz, 7-H or 7′-H), 4.72 (1H, d, J=4.9 Hz, 7-H or 7′-H), ca. 4.27 (2H, 9-Ha, 9′-Ha), 3.91 (3H, s, OCH_3), 3.90 (6H, s, 3-OCH_3, 5-OCH_3), ca. 3.89 (2H, 9-Hb, 9′-Hb), ca. 3.10 (2H, 8-H, 8′-H). ^{13}C-NMR (in $CDCl_3$, 125 MHz) δ: 147.2 (3-C, 5-C), 146.7 (3′-C), 145.3 (4′-C), 134.4 (4-C), 132.9, 132.2 (1-C, 1′-C), 119.0 (6′-C), 114.3 (5′-C), 108.6 (2′-C), 102.8 (2-C, 6-C), 86.1, 85.8 (7-C, 7′-C), 71.9, 71.6 (9-C, 9′-C), 56.4 (3-OCH_3, 5-OCH_3), 56.0 (3′-OCH_3), 54.4, 54.1 (8-C, 8′-C). ^{13}C-NMR (in pyridine-d_5, 100 MHz) δ: 149.3 (3-C, 5-C), 148.8 (3′-C), 147.9 (4′-C), 137.3 (4-C), 133.2 (1′-C), 132.2 (1-C), 119.7 (6′-C), 116.5 (5′-C), 111.0 (2′-C), 104.8 (2-C, 6-C), 86.7, 86.4 (7-C, 7′-C), 72.1, 71.9 (9-C, 9′-C), 56.5 (3-OCH_3, 5-OCH_3), 56.0 (3′-OCH_3), 55.0, 54.8 (8-C, 8′-C).

Syringaresinol (**10**): An amorphous powder, $[\alpha]_D^{27}$+13.4° (c=1.0, MeOH). EI-MS m/z: 418 $[M]^+$ (100), 181 (64), 167 (52). ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.59 (4H, s, 2-H, 6-H, 2′-H, 6′-H), 5.55 (2H, s, 4-OH, 4′-OH), 4.73 (2H, d, J=4.3 Hz, 7-H, 7′-H), 4.28 (2H, dd like, J=8.8, 6.4 Hz, 9-Ha, 9′-Ha), ca. 3.92 (2H, 9-Hb, 9′-Hb), 3.89 (12H, s, 3-OCH_3, 5-OCH_3, 3′-OCH_3, 5′-OCH_3), 3.10 (2H, m, 8-H, 8′-H). ^{13}C-NMR (in $CDCl_3$, 125 MHz) δ: 147.2 (3-C, 5-C, 3′-C, 5′-C), 134.4 (4-C, 4′-C), 132.1 (1-C, 1′-C), 102.8 (2-C, 6-C, 2′-C, 6′-C), 86.0 (7-C, 7′-C), 71.8 (9-C, 9′-C), 56.4 (3-OCH_3, 5-OCH_3, 3′-OCH_3, 5′-OCH_3), 54.3 (8-C, 8′-C). ^{13}C-NMR (in pyridine-d_5, 100 MHz) δ: 149.3 (3-C, 5-C, 3′-C, 5′-C), 137.3 (4-C, 4′-C), 132.2 (1-C, 1′-C), 104.8 (2-C, 6-C, 2′-C, 6′-C), 86.6 (7-C, 7′-C), 72.1 (9-C, 9′-C), 56.5 (3-OCH_3, 5-OCH_3, 3′-OCH_3, 5′-OCH_3), 55.0 (8-C, 8′-C).

Acetylation of **3** Compound **3** (2 mg) in Ac_2O-pyridine (1:1, 0.2 ml) was allowed to stand at room temperature overnight. After removal of the reagent under a stream of N_2, the residue was chromatographed over silica gel (Art. 9385, eluant: *n*-hexane-AcOEt, 1:1) to give acetate of **3** (2.5 mg).

Acetate of **3**: ^{1}H-NMR (in $CDCl_3$, 500 MHz) δ: 6.93 (1H, d, J=7.9 Hz, 5′-H), 6.80 (2H, s like, 2-H, 6-H), 6.76 (1H, s like, 4-H), 6.74 (1H, d like, J=7.9 Hz, 6′-H), 6.70 (1H, s like, 2′-H), 3.77 (3H, s, 3′-OCH_3), ca. 2.90 (4H, α-H_2, α'-H_2), 2.30 (3H, s, 4′-OAc), 2.28 (6H, s, 3-OAc, 5-OAc).

Antioxidative assay We applied the method of Kikuzaki and Nakatani (1993) slightly modified. A mixture of 2.51% linoleic acid EtOH solution (1.03 ml), 0.05 M phosphate buffer (pH 7.0, 2.00 ml) and H_2O (0.97 ml) were added to 0.1% EtOH solution (1.00 ml) of each sample in a vial with a cap and placed in darkness at 40°C to accelerate oxidation. At intervals during incubation, this assay solution (0.10 ml) was diluted with 75% EtOH (9.70 ml), which was followed by adding 30% ammonium thiocyanate (0.10 ml). Precisely 3 min after the addition of 0.02 M ferrous chloride in 3.5% hydrochloric acid (0.10 ml) to the reaction mixture, the absorbance due to the red color developed was measured at 500 nm.

Results and Discussion

The stems of Dendrobii Herba were extracted with MeOH under reflux. This extract was partitioned between H_2O and AcOEt, and the AcOEt soluble fr. was defatted with *n*-hexane. The residue (AcOEt fr.), which showed a stronger antioxidative effect than α-tocopherol (Figs. 1 and 2), was subjected successively to silica gel column chromatography, Bio-Beads S-X2 column chromatography, preparative TLC and HPLC to give ten compounds (**1**–**10**).

Structure elucidation of ***1***–***10*** Compound **1** was obtained as an amorphous powder, and it exhibited the signals of two aromatic methoxyl groups (δ 3.84, 3.75), two hydroxyl protons (δ 5.48, 4.92), four benzylic methylene protons (δ 2.80) and six aromatic protons in the proton nuclear magnetic resonance (^{1}H-NMR) spectrum. The

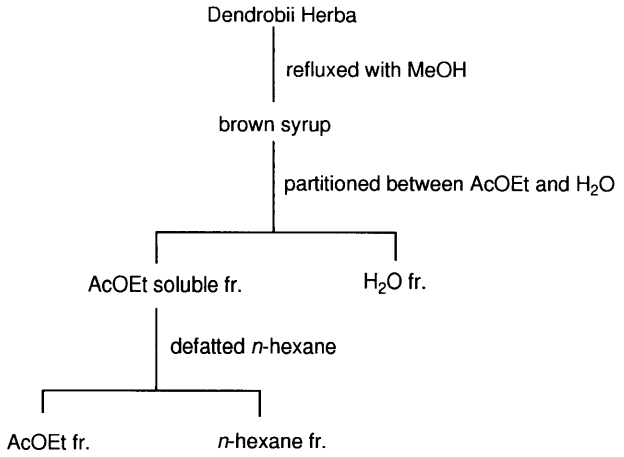

Fig. 1. Separation of MeOH extract of Dendrobii Herba.

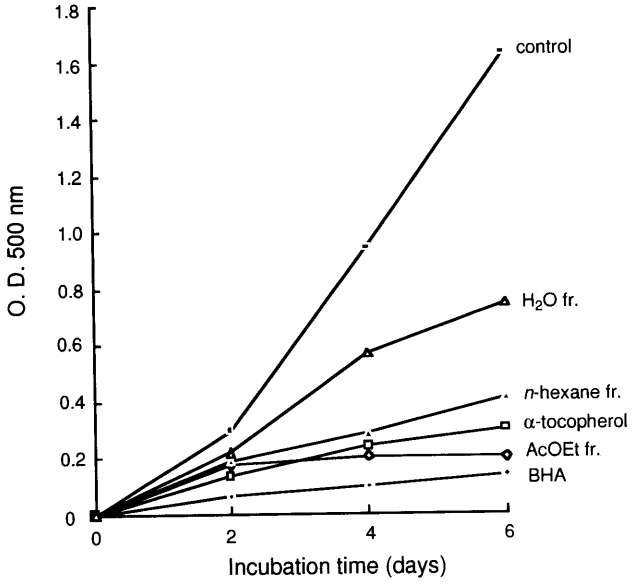

Fig. 2. Antioxidative activities of AcOEt fr., *n*-hexane fr. and H_2O fr.

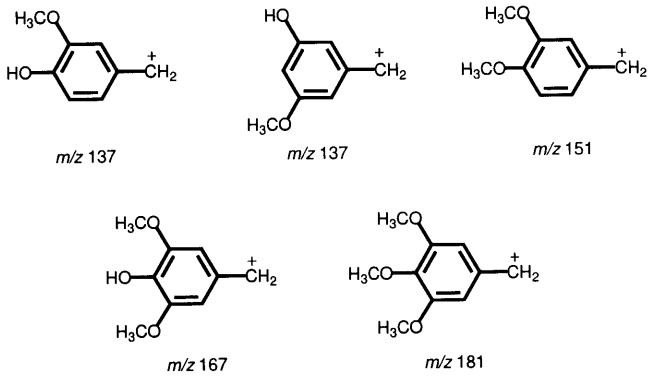

Fig. 3. Mass fragment ions.

carbon-13 (^{13}C)-NMR spectrum of **1** indicated the signals of two methoxyl carbons (δ 55.9, 55.3), two methylene carbons (δ 38.2, 37.2) and twelve aromatic carbons (δ 160.8, 156.6, 146.3, 144.5, 143.7, 133.7, 121.0, 114.2, 111.2, 108.1, 106.8, 99.1). The electron-impact mass spectrum (EI-MS) of **1** showed an $[M]^+$ ion peak at m/z 274 and an intense fragment ion peak at m/z 137. These data indicated **1** to be a bibenzyl derivative, composed of two moles of hydroxymethoxybenzyl groups (Fig. 3). The assignments of the substitution location for the methoxyl groups and hydroxyl groups were based on the chemical shifts and splitting patterns of the aromatic protons in the ^1H-NMR spectrum and the nuclear Overhauser effect (NOE) difference spectra. Irradiation of the signal at δ 3.84 and δ 3.75 gave NOE enhancements of the signals at δ 6.62 (1H, d, J=1.8 Hz) and at δ 6.31 (1H, dd, J=1.8, 1.8 Hz)/δ ca. 6.25 (2H), respectively.

From the above evidence, **1** was found to be identical with gigantol (Juneja *et al.*, 1985, 1987) (Fig. 4).

Compound **2** was obtained as an amorphous powder. The ^1H-NMR spectrum of **2** showed the signals of three aromatic methoxyl groups (δ 3.83×2, 3.82), two hydroxyl protons (δ 5.59, 5.46), four benzylic methylene protons (δ ca. 2.81) and five aromatic protons. Two of the aromatic protons appeared as an equivalent singlet signal (δ 6.35), and the remaining three aromatic protons at δ 6.83 (1H, d, J=8.5 Hz), 6.67 (1H, dd, J=1.8, 8.5 Hz) and 6.61 (1H, d, J=1.8 Hz) were assigned to a 4-hydroxy-3-methoxy-benzyl moiety. The EI-MS of **2** indicated $[M]^+$ and fragment ion peaks at m/z 304, 167 and 137, respectively, which indicated that hydroxydimethoxybenzyl and hydroxymethoxybenzyl moieties were present in **2** (Fig. 3). In the NOE difference spectrum, irradiation of the signal at δ 6.35 showed NOEs of the signals at δ 3.83 and ca. 2.81.

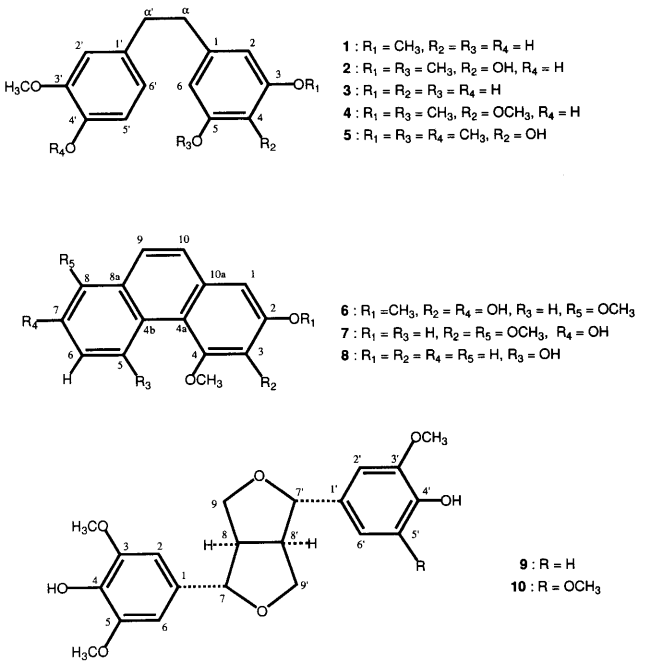

Fig. 4. Structures of **1**–**10**.

From these data, **2** was identified as moscatilin (Majumder & Sen, 1987b) (Fig. 4).

Compound **3** was obtained as an amorphous powder, and it was considered to be a bibenzyl according to the ^1H- and ^{13}C-NMR spectra. The EI-MS of **3** showed $[M]^+$ and fragment ion peaks at m/z 260 and 137, respectively. The ^1H-NMR spectrum of **3** showed six aromatic protons [δ 6.83 (1H, d, J=7.9 Hz), 6.67 (1H, dd, J=0.2, 7.9 Hz), 6.61 (1H, d, J=0.2 Hz), 6.22 (2H, d, J=0.2 Hz), 6.19 (1H, t, J=0.2 Hz), four benzylic methylene protons [δ ca. 2.81 (2H), ca. 2.79 (2H)], three hydroxyl protons [δ 5.46 (1H, s), 4.79 (2H, br s)] and one methoxyl group [δ 3.84 (3H, s)].

Based on these data and the ^1H-NMR data of the acetate of **3**, the structure of **3** was identified as tristin (Majumder & Pal, 1993) (Fig. 4).

Compound **4** was obtained as colorless needles. The ^1H-NMR spectrum of **4** was closely analogous to that of **2**, except for the appearance of the signal due to one methoxyl group and the loss of that due to one hydroxyl proton. The EI-MS of **4** showed an $[M]^+$ ion peak at m/z 318, which was 14 mass units (CH_2) larger than that of **2**, and fragment ion peaks at m/z 181 and 137 (Fig. 3). Moreover, in the NOE difference spectrum, irradiation of the two equivalent singlet

proton signals at δ 6.36 indicated NOEs of the signals of methoxyl group at δ 3.82 and benzylic methylene protons at δ ca. 2.83.

Based on these data, **4** was identified as crepidatin (Majumder & Chatterjee, 1989) (Fig. 4).

Compound **5** was obtained as an amorphous powder. The ^{1}H-NMR spectrum of **5** was quite similar to that of **4**. The EI-MS of **5** exhibited an $[M]^+$ ion peak at m/z 318, the same as that of **4**, and fragment ion peaks at m/z 167 and 151, suggesting hydroxydimethoxybenzyl and dimethoxybenzyl moieties to be present in **5** (Fig. 3). From these spectral data, **5** was considered to be a structural isomer of **4**, in which the methoxyl group and hydroxyl group interchanged between C-4 and C′-4. This was finally confirmed by the ^{13}C-NMR data comparison with those of **4** and NOE difference spectra. Irradiation of the signal of aromatic protons at δ 6.36 (2H, s) showed NOEs of the signals of benzylic methylene protons at δ ca. 2.83 and methoxyl groups at δ 3.84 (9H, s). On the other hand, each irradiation of the signals at δ 6.66 (1H, d, J=1.8 Hz) and 6.70 (1H, dd, J=1.8, 8.1 Hz) indicated NOEs of the signals of benzylic protons at δ ca. 2.83/methoxyl groups at δ 3.84 and benzylic protons/an aromatic proton at δ 6.79 (1H, d, J=8.1 Hz), respectively.

Consequently, the structure of **5** was concluded to be 4-hydroxy-3,3′,4′,5-tetramethoxybibenzyl (Fig. 4).

Compound **6** was obtained as an amorphous powder, and it showed the signals of fourteen aromatic carbons and three methoxyl carbons in the ^{13}C-NMR spectrum. The EI-MS of **6** indicated an $[M]^+$ ion peak at m/z 300. These spectral data suggested **6** to be a phenanthrene derivative. In the ^{1}H-NMR spectrum, **6** showed the signals of three methoxyl groups (δ 4.05, 3.98, 3.94) and five aromatic protons, in which a pair of the signals at δ 7.82 (1H, d, J=9.2 Hz) and δ 7.63 (1H, d, J=9.2 Hz) was assigned to the 9-H and 10-H of the phenanthrene derivative, respectively, and the signal at δ 9.16 (1H, d, J=9.2 Hz) was assigned to H-4 or H-5 (Letcher & Wong, 1978; Letcher & Nhamo, 1971). In the NOE difference spectra, irradiation of the signal at δ 7.09 (1H, s) indicated NOEs of the signals at δ 7.63 and at δ 4.05, and each irradiation of the signals at δ 3.98 and 3.94 gave NOE enhancements of the signals at δ 7.82 and 9.16, respectively.

From these data, **6** was identified as 2,4,8-trimethoxyphenanthrene-3,7-diol which was confirmed by the ^{1}H-NMR data in comparison with those of an authentic sample (Tuchinda *et al.*, 1988) (Fig. 4).

Compound **7** was obtained as an amorphous powder. The ^{1}H- and ^{13}C-NMR spectra of **7** were quite similar to those of **6**, and the EI-MS showed the same $[M]^+$ ion peak at m/z 300 as that of **6**. From these spectral data, **7** was considered to be a structural isomer of **6**. In the NOE difference spectra (in CD_3OD), irradiation of the signal at δ 7.08 (1H, s) indicated an NOE of the signal at δ 7.50 (1H, d, J=9.2 Hz), and irradiation of the signal of methoxyl group at δ 4.00 gave an NOE enhancement of the signal at δ 9.09 (1H, d, J=9.2 Hz). Further, irradiation of the signal of the methoxyl groups at δ 3.97 (6H, s) indicated NOEs of the signals at δ 7.85 (1H, d, J=9.2 Hz), 9.19 (1H, d, J=9.2 Hz) and a methoxyl group at δ 4.11 in the NOE difference spectrum (in $CDCl_3$).

From these data, **7** was suggested to be confusarin, and finally, this was confirmed by the ^{13}C-NMR data in comparison with those of an authentic sample (Majumder & Kar, 1987; Ma *et al.*, 1994) (Fig. 4).

Compound **8** was obtained as an amorphous powder, and it was revealed to a phenanthrene derivative by the ^{1}H- and ^{13}C-NMR spectra. The EI-MS of **8** indicated an $[M]^+$ ion peak at m/z 240, and the ^{1}H-NMR spectrum showed the signals of seven aromatic protons [δ 7.61 (1H, d, J=9.2 Hz), 7.48 (1H, dd, J=8.0, 8.0 Hz), 7.41 (1H, dd, J=1.2, 8.0 Hz), 7.41 (1H, d, J=9.2 Hz), 7.23 (1H, dd, J=1.2, 8.0 Hz), 6.96 (1H, d, J=2.5 Hz), 6.82 (1H, d, J=2.5 Hz)] and one methoxyl group (δ 4.04). The chemical shifts and the splitting patterns of these protons and NOE difference spectra [irradiation of the signal at δ 4.04 showed an NOE of the signal at δ 6.82, and each irradiation at δ 6.96 and 7.61 gave NOE enhancements of the signal at δ 7.41, respectively.] suggested **8** to be moscatin, which was confirmed by the ^{13}C-NMR data in comparison with those of an authentic sample (Majumder & Sen, 1987a) (Fig. 4).

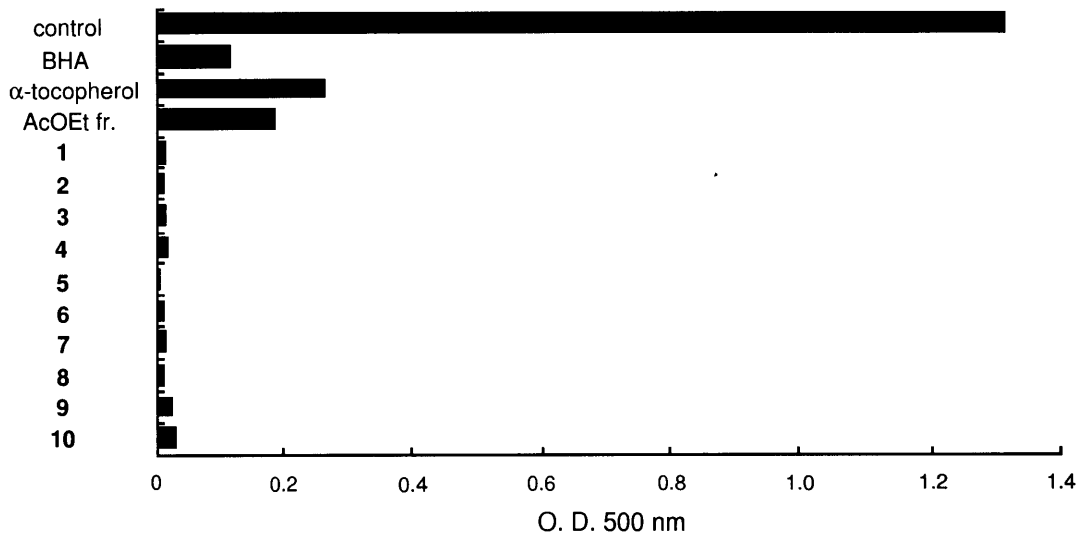

Fig. 5. Antioxidative activities on the 5th day of the lipid peroxidation.

Compound **9** was obtained as an amorphous powder, and it showed the signals of five aromatic protons [δ 6.89 (1H, d, J=7.9 Hz), 6.88 (1H, s), 6.82 (1H, d, J=9.2 Hz), 6.58 (2H, s)] and three methoxyl groups (δ 3.91, 3.90×2), two oxymethine protons [δ 4.75 (1H, d, J=4.3 Hz), 4.72 (1H, d, J=4.9 Hz)], four oxymethylene protons [δ ca. 4.27 (2H), ca. 3.89 (2H)] and two methine protons [ca. 3.10 (2H)] in the ^{1}H-NMR spectrum. The EI-MS indicated an $[M]^+$ ion peak at m/z 388. Therefore, **9** was considered to be a lignan derivative, and further, it was identified as medioresinol by the ^{13}C-NMR data in comparison with those of an authentic sample (Abe & Yamauchi, 1988) (Fig. 4).

Compound **10** was obtained as an amorphous powder. The EI-MS indicated an $[M]^+$ ion peak at m/z 418, and ^{1}H-NMR spectrum showed the signals of four aromatic protons [δ 6.59 (4H, s)], four methoxyl groups (δ 3.89×4), two oxymethine protons [δ 4.73 (2H, d, J=4.3 Hz), four oxymethylene protons [δ 4.28 (2H, dd, J=8.8, 6.4 Hz) and ca. 3.92 (2H)] and two methine protons [δ 3.10 (2H, m)]. These data suggested **10** to be a symmetrical structure.

Finally, **10** was identified as syringaresinol by the comparison of the ^{13}C-NMR data with those of an authentic sample (Abe & Yamauchi, 1988)(Fig. 4).

As far as we know, this is the first example of the isolation of **1**, **3**, **5**, **6**, **9** and **10** from *Dendrobium* species, and further, **5** is believed to be a new compound.

Antioxidative effect of compound 1–10 The antioxidative activities of **1**–**10** were evaluated by the ferric thiocyanate method (Kikuzaki & Nakatani, 1993). All of these compounds showed stronger antioxidative activity than BHA (Fig. 5).

Accordingly, **1**–**10** were considered to be the antioxidative principles of Dendrobii Herba. Kikuzaki and Nakatani (1993) reported that the antioxidative activity depended on the substituents on the benzene ring. Comparisons of the antioxidative activities of each group of bibenzyl, phenanthrene and lignan in this study also supported their report, in particular, the position and number of hydroxyl and methoxyl groups on the benzene ring were important for this activity.

Recently, Chen *et al.* reported that **2** and **8** showed antiplatelet aggregation activities (Chen *et al.*, 1994), but this is the first report on the antioxidative activities of **1**–**10**.

Acknowledgments We express our appreciation to Mr. K. Takeda of Kumamoto University for measurement of the NMR spectra.

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