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Antimicrobial Activity of Stereoisomers of Butane-Type Lignans

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The relationship between the stereochemistry and antimicrobial activity of butane-type lignans was clarified. All stereoisomers of dihydroguaiaretic acid (DGA) showed both antibacterial and antifungal activity. The (+)- and (−)-7,7′-dioxodihydroguaiaretic acid (ODGA) also showed both antibacterial and antifungal activity, while *meso*-ODGA did not show antibacterial activity, but showed antifungal activity. No activity of any stereoisomer of secoisolariciresinol (SECO) was apparent.

Key words: lignans; butane-type lignan; antimicrobial activity

Dihydroguaiaretic acid (DGA) is a well-known butane type of lignan (Fig. 1) which has three stereoisomers. The isolation of (−)-DGA and antimicrobial *meso*-DGA has been reported,[1–6] but the isolation and antimicrobial activity of (+)-DGA has not been reported. As for the DGA derivative, the 7,7′-dioxodihydroguaiaretic acid (ODGA) type of lignan has been isolated.[7] In our previous research on the antimicrobial activity of 7,7′-dioxo lignan, the antibacterial activity of (−)- and (+)-7,7′-dioxomatairesinol and antifungal activity of (−)-7,7′-dioxo-9,9′-epoxylignan have been reported.[8] In this present article, the relationship between the stereochemistry of butane-type lignans (DGA, ODGA and SECO) and antimicrobial activity is described for the first time, and the effect of the benzylic oxo group of DGA on this activity is clarified. To achieve this study, the first stereoselective syntheses of *meso*-DGA and *meso*-7,7′-dioxo-DGA were accomplished. There is a possibility that the derivative of a stereoisomer having high antimicrobial activity shows higher activity, to give a new lead compound. Research into the effect of natural lignans biosynthesized by the plants on anti-phytopathogenic fungal activity would contribute to knowledge of chemical ecology. The results of this research will also contribute to more effective use of natural plants containing bioactive lignans as industrial material. Food poisoning bacteria and phytopathogenic fungi were selected in this study.

Materials and Methods

Melting point (mp) data are uncorrected. NMR data were measured by a JNM-EX400 spectrometer, using TMS as a standard (0 ppm), MS data were measured with a JMS-MS700V spectrometer, and optical rotation values were evaluated with a Horiba SEPA-200 instrument. Elemental analysis was carried out with Yanako MT-5 CHN coder. The silica gel used was Wakogel C-300 (Wako, 200–300 mesh). (−)- and (+)-DGA, (−)- and (+)-ODGA,[9] and all stereoisomers of SECO[10] were synthesized by the previously reported method.

*(4*S*)-4-Benzyl-3-{(*R*)-2-[(*S*)-(4-benzyloxy-3-methoxyphenyl)(methoxymethoxy)methyl]-4-pentenoyl}-2-oxazolidinone (**2**).* To a mixture of **1** (5.00 g, 9.97 mmol) and *N*,*N*-diisopropylethylamine (13.0 ml, 74.6 mmol) in CH_2Cl_2 (100 ml) was added MOMCl (3.10 ml, 40.8 mmol). The resulting reaction mixture was stirred at room temperature for 4 h before addition of MeOH. The mixture was dissolved in H_2O and CH_2Cl_2. The organic solution was separated, washed with 1 M aq HCl solution, sat. aq. $NaHCO_3$ solution, and brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/3) gave **2** (5.21 g, 9.55 mmol, 96%) as a colorless oil, $[\alpha]^{20}_D$ −76 (*c* 1.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.97 (1H, m, CH_2=CHC*H*H), 2.22 (1H, m, CH_2=CHCH*H*), 2.80 (1H, dd, *J* = 13.4, 9.6 Hz, C*H*HPh), 3.27 (3H, s, OCH_3), 3.35 (1H, dd, *J* = 13.4, 3.3 Hz, CH*H*Ph), 3.93 (3H, s, OCH_3), 4.13 (2H, d, *J* = 4.6 Hz, 5-H_2), 4.41 (1H, d, *J* = 6.7 Hz, OC*H*HOCH$_3$), 4.44 (1H, d, *J* = 6.7 Hz, OCH*H*OCH$_3$), 4.67 (1H, ddd, *J* = 10.2, 10.2, 3.8 Hz, CHC=O), 4.73 (1H, m, 4-H), 4.79 (1H, d, *J* = 10.1 Hz, Ar(MOM)CH), 4.92 (1H, d, *J* = 9.7 Hz, C*H*H=CH), 4.95 (1H, d, *J* = 16.4 Hz, CH*H*=CH), 5.16 (2H, s, OCH_2Ph), 5.63 (1H, m, CHH=C*H*), 6.86 (1H, d, *J* = 8.2 Hz, ArH), 6.90 (1H, dd, *J* = 8.2, 1.7 Hz, ArH), 7.04 (1H, d, *J* = 1.7 Hz, ArH), 7.28–7.39 (8H, m, ArH), 7.45 (2H, d, *J* = 7.6 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 34.1, 37.8, 48.3, 55.8, 55.9, 56.0, 65.6, 70.9, 79.3, 93.4, 111.0, 113.3, 117.2, 121.1, 127.3, 127.8, 128.4, 128.5, 128.9, 129.4, 134.4, 135.5, 137.0, 148.3, 149.8, 153.4, 174.5. EIMS *m*/*z* 545 (M^+, 13), 287 (91), 91 (100); HREIMS *m*/*z* 545.2411 (calcd. for $C_{32}H_{35}O_7N$, 545.2413).

*(*S*)-2-[(*S*)-(4-Benzyloxy-3-methoxyphenyl)(methoxymethoxy)methyl]-4-penten-1-ol (**3**).* To an ice-cooled solution of $LiBH_4$ (24 mg, 1.10 mmol) and MeOH (0.045 ml, 1.10 mmol) in THF (5 ml) was added a solution of **2** (0.29 g, 0.53 mmol) in THF (5 ml). After the reaction solution was stirred at 60 °C for 6 h, sat. aq. NH_4Cl solution was added, and then the mixture was concentrated. The residue was dissolved in EtOAc and H_2O. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/2) gave **3** (0.12 g, 0.32 mmol, 60%) as colorless crystals, mp 54–55 °C (EtOAc/hexane = 1/6), $[\alpha]^{20}_D$ −98 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.87–2.00 (4H, m, 2-H, 3-H_2, OH), 3.41 (3H, s, OCH_2OCH_3), 3.69 (1H, dd, *J* = 11.3, 5.5 Hz, 1-*H*H), 3.85 (1H, dd, *J* = 11.3, 3.0 Hz, 1-H*H*), 3.89 (3H, s, OCH_3), 4.47 (1H, d, *J* = 6.8 Hz, OC*H*HOCH$_3$), 4.49 (1H, d, *J* = 6.8 Hz, OCH*H*OCH$_3$), 4.54 (1H, d, *J* = 7.6 Hz, ArCH(OMOM)), 4.99 (1H, d, *J* = 16.8 Hz, 5-*H*H), 5.00 (1H, d, *J* = 10.3 Hz, 5-H*H*), 5.15 (2H, s, OCH_2Ph), 5.70 (1H, m, 4-H), 6.78 (1H, dd, *J* = 8.2, 1.9 Hz, ArH), 6.84–6.86 (2H, m, ArH), 7.30 (1H, d, *J* = 7.3 Hz, ArH), 7.32–7.39 (2H, m, ArH), 7.44 (2H, d, *J* = 7.3 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 32.8, 46.4, 56.0, 63.7, 71.0, 81.3, 81.4, 93.9, 100.6, 110.6,

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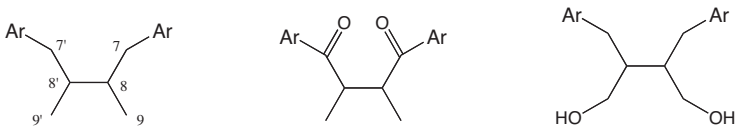


Dihydroguaiaretic acid (DGA) 7,7'-Dioxodihydroguaiaretic acid (ODGA) Secoisolariciresinol (SECO)

Fig. 1. Butane Type of Lignans.
Ar, 4-hydroxy-3-methoxyphenyl

113.5, 116.8, 120.4, 127.3, 127.9, 128.5, 132.8, 136.0, 136.1, 137.1, 148.0, 149.8. Anal. Found: C, 70.67; H, 7.60%. Calcd. for $C_{22}H_{28}O_5$: C, 70.94; H 7.58%.

*(4*S*,5*S*)-5-(4-Benzyloxy-3-methoxyphenyl)-5-methoxymethoxy-4-(*p*-toluenesulfonyloxy)methyl-1-pentene (**4**).* To an ice-cooled solution of **3** (1.40 g, 3.76 mmol) and pyridine (1.20 ml, 14.8 mmol) in CH_2Cl_2 (5 ml) was added *p*-TsCl (1.40 g, 7.34 mmol). The resulting reaction solution was stirred at room temperature for 20 h before additions of H_2O and CH_2Cl_2. The organic solution was separated, washed with 1 M aq. HCl solution, sat. aq. $NaHCO_3$, and brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/3) gave **4** (1.98 g, 3.76 mmol, 100%) as colorless crystals, mp 80–81 °C ((*iso*-Pr)$_2$O), $[\alpha]^{20}_D$ −55 (*c* 1.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.83–1.92 (2H, m, 3-H$_2$), 2.02 (1H, m, 4-H), 2.43 (3H, s, CH_3PhSO_2), 3.27 (3H, s, OCH_2OCH_3), 3.85 (3H, s, OCH_3), 4.11 (1H, dd, *J* = 9.3, 3.8 Hz, TsOC*H*H), 4.35 (1H, dd, *J* = 9.3, 4.6 Hz, TsOCH*H*), 4.40 (1H, d, *J* = 6.6 Hz, OC*H*HOCH$_3$), 4.42 (1H, d, *J* = 6.6 Hz, OCH*H*OCH$_3$), 4.46 (1H, d, *J* = 8.4 Hz, ArCH(OMOM)), 4.80 (1H, d, *J* = 17.0 Hz, 1-*H*H), 4.87 (1H, d, *J* = 10.3 Hz, 1-H*H*), 5.13 (2H, s, OCH_2Ph), 5.48 (1H, m, 2-H), 6.70 (1H, dd, *J* = 8.3, 1.8 Hz, ArH), 6.78 (1H, d, *J* = 1.8 Hz, ArH), 6.81 (1H, d, *J* = 8.3 Hz, ArH), 7.27–7.37 (5H, m, ArH), 7.43 (2H, d, *J* = 7.8 Hz, ArH), 7.78 (2H, d, *J* = 8.3 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 21.5, 31.2, 44.2, 55.8, 55.9, 68.6, 70.9, 76.5, 94.0, 110.6, 113.5, 117.5, 120.1, 127.2, 127.8, 127.9, 128.5, 129.7, 132.1, 132.9, 134.7, 134.8, 137.0, 144.6, 147.9, 149.7. Anal. Found: C, 65.95; H, 7.45%. Calcd. for $C_{29}H_{34}O_7S$: C, 66.13; H 6.51%.

*(4*R*,5*S*)-5-[(4-Benzyloxy-3-methoxyphenyl)(methoxymethoxy)methyl]-4-methyl-1-pentene (**5**).* To an ice-cooled suspension of $LiAlH_4$ (0.77 g, 0.020 mol) in THF (40 ml) was added a solution of **4** (5.40 g, 0.010 mol) in THF (10 ml). The reaction mixture was stirred at room temperature for 20 h. After additions of sat. aq. $MgSO_4$ and K_2CO_3, the mixture was stirred at room temperature for 30 min, and then the mixture was filtered. The filtrate was concentrated, and then the residue was applied to silica gel column chromatography (EtOAc/hexane = 1/9) to give **5** (3.12 g, 0.0088 mol, 88%) as colorless crystals, mp 43–44 °C (hexane), $[\alpha]^{20}_D$ −80 (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.00 (3H, d, *J* = 6.5 Hz, CH_3), 1.75 (1H, m, 4-H), 1.85 (1H, m, 3-*H*H), 2.07 (1H, m, 3-H*H*), 3.37 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.31 (1H, d, *J* = 7.0 Hz, 5-H), 4.48 (1H, d, *J* = 6.7 Hz, CH_3OCHHO), 4.51 (1H, d, *J* = 6.7 Hz, CH_3OCHHO), 4.96 (1H, d, *J* = 16.7 Hz, 1-*H*H), 4.98 (1H, dd, *J* = 10.2 Hz, 1-H*H*), 5.14 (2H, s, OCH_2Ph), 5.73 (1H, m, 2-H), 6.74 (1H, dd, *J* = 8.2, 1.8 Hz, ArH), 6.82–6.84 (2H, m, ArH), 7.29 (1H, d, *J* = 7.3 Hz, ArH), 7.32–7.38 (2H, m, ArH), 7.44 (2H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 15.3, 37.6, 39.6, 55.7, 56.0, 71.1, 81.5, 94.2, 110.8, 113.4, 116.1, 120.1, 127.3, 127.8, 128.5, 133.9, 137.0, 137.2, 147.6, 149.5. Anal. Found: C, 73.94; H, 7.88%. Calcd. for $C_{22}H_{28}O_4$: C, 74.12; H 7.92%.

*(3*R*,4*S*)-4-(4-Benzyloxy-3-methoxyphenyl)-3-methyl-4-butanolide (**6**).* A reaction solution of **5** (3.10 g, 8.70 mmol), NMO (1.20 g, 10.2 mmol), and OsO_4 (2% aq. solution, 1.00 ml) in acetone (80 ml), *tert*-BuOH (20 ml), and H_2O (20 ml) was stirred at room temperature for 18 h under N_2 gas, and then sat. aq. $Na_2S_2O_3$ solution was added. After concentration of the mixture, the residue was dissolved in EtOAc and H_2O. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration gave crude glycol. A reaction mixture of this crude glycol and $NaIO_4$ (2.20 g, 10.3 mmol) in MeOH (50 ml) was stirred at room temperature for 1 h before concentration. The residue was dissolved in EtOAc and H_2O. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration gave crude aldehyde. To an ice-cooled mixture of this crude aldehyde, 2-methyl-2-butene (3.60 ml, 34.0 mmol), and $NaH_2PO_4.2H_2O$ (1.30 g, 8.33 mmol) in *tert*-BuOH (50 ml) and H_2O (1.5 ml) was added $NaClO_2$ (2.30 g, 25.4 mmol). The reaction mixture was stirred at room temperature for 1 h, and then 6 M aq. HCl solution and $CHCl_3$ were added. The organic solution was separated, washed with H_2O and brine, and dried (Na_2SO_4). After concentration, the residue was dissolved in THF (30 ml) and 6 M aq. HCl solution (30 ml). The reaction solution was stirred at 50 °C for 20 min, and then EtOAc and brine were added. The organic solution was separated, washed with sat. aq. $NaHCO_3$ solution and brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/2) gave **6** (2.64 g, 8.45 mmol, 97%) as a colorless oil, $[\alpha]^{20}_D$ −17 (*c* 0.4, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.16 (3H, d, *J* = 6.5 Hz, CH_3), 2.33 (1H, dd, *J* = 16.8, 10.9 Hz, 2-*H*H), 2.47 (1H, m, 3-H), 2.78 (1H, dd, *J* = 16.8, 7.6 Hz, 2-H*H*), 3.90 (3H, s, OCH_3), 4.86 (1H, d, *J* = 8.7 Hz, 4-H), 5.16 (2H, s, OCH_2Ph), 6.79 (1H, dd, *J* = 8.2, 2.0 Hz, ArH), 6.86–6.88 (2H, m, ArH), 7.31 (1H, d, *J* = 7.1 Hz, ArH), 7.35–7.39 (2H, m, ArH), 7.43 (2H, d, *J* = 7.1 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 16.3, 37.4, 39.7, 56.1, 71.0, 88.3, 109.4, 113.6, 118.7, 127.17, 127.21, 127.9, 128.6, 130.6, 136.9, 148.6, 150.0, 176.1. EIMS *m*/*z* 312 (M^+, 100), 91 (99); HREIMS *m*/*z* 312.1361 (calcd for $C_{19}H_{20}O_4$, 312.1362).

*(2*S*,3*R*,4*S*)-4-(4-Benzyloxy-3-methoxyphenyl)-2-[(*R*)-(4-benzyloxy-3-methoxyphenyl)(triisopropylsilyloxy)methyl]-3-methyl-4-butanolide (**7**).* To a solution of KHMDS (25.0 ml, 0.5 M toluene solution, 12.5 mmol) in THF (10 ml) was added **6** (2.60 g, 8.32 mmol) in THF (10 ml) at −70 °C. After stirring at −70 °C for 10 min, a solution of 4-benzyloxy-3-methoxybenzaldehyde (2.00 g, 8.26 mmol) in THF (10 ml) was added. After the resulting reaction solution was stirred at −70 °C for 1 h, sat. aq. NH_4Cl solution and EtOAc were added. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/2) gave a mixture of *erythro* and *threo* aldol product (1/3, 4.49 g, 8.10 mmol, 97%). FABMS *m*/*z* 555 ($(M + H)^+$, 4), 154 (100); HRFABMS *m*/*z* 555.2383 (calcd for $C_{34}H_{35}O_7$, 555.2382). To an ice-cooled solution of a mixture of *erythro* and *threo* aldol product (5.00 g, 9.01 mmol) and 2,6-lutidine (1.50 ml, 12.9 mmol) in CH_2Cl_2 (50 ml) was added TIPSOTf (2.90 ml, 10.8 mmol). After the reaction solution was stirred at room temperature for 1 h, sat. aq. $NaHCO_3$ solution was added. The organic solution was separated, washed with sat. aq. $CuSO_4$ solution and sat. aq. $NaHCO_3$ solution, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtAOc/hexane = 1/7) gave *threo* **7** (2.54 g, 3.57 mmol, 40%) as a colorless oil, $[\alpha]^{20}_D$ +7 (*c* 1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.01–1.09 (18H, m, $CH(CH_3)_2$), 1.11 (3H, d, *J* = 7.1 Hz, CH_3), 1.31 (3H, m, $CH(CH_3)_2$), 2.52 (1H, m, 3-H), 3.20 (1H, dd, *J* = 9.8, 6.6 Hz, 2-H), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.64 (1H, d, *J* = 8.4 Hz, 4-H), 5.15 (2H, s, OCH_2Ph), 5.16 (2H, s, OCH_2Ph), 5.29 (1H, d, *J* = 6.6 Hz, ArCHOSi), 6.86–6.92 (3H, m, ArH), 6.94 (1H, dd, *J* = 8.3, 1.9 Hz, ArH), 6.99 (1H, s, ArH), 7.00 (1H, s, ArH), 7.27–7.31 (2H, m, ArH), 7.33–7.38 (4H, m, ArH), 7.42–7.44 (4H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 11.9, 13.0, 17.8, 44.6, 55.9, 56.0, 58.3, 71.1, 82.1, 87.3, 109.98, 110.01, 110.4, 113.9, 114.1, 118.1, 118.9, 127.19, 127.22, 127.8, 128.5, 133.5, 134.8, 137.2, 147.6, 148.0, 149.6, 149.7, 172.6. Anal. Found: C, 72.91; H, 7.64%. Calcd. for $C_{43}H_{54}O_7Si$: C, 72.64; H 7.66%.

*(1*R*,2*R*,3*R*,4*S*)-1,4-Bis(4-benzyloxy-3-methoxyphenyl)-2-hydroxymethyl-3-methyl-1,4-butanediol (**8**).* To a solution of *threo* **7** (0.58 g, 0.82 mmol) in toluene (10 ml) was added DIBAL-H (1.20 ml, 1.0 M in toluene, 1.20 mmol) at −70 °C. After the reaction solution was stirred

at −70 °C for 3 h before additions of 6 M aq. HCl solution and EtOAc. The organic solution was separated, washed with sat. aq. $NaHCO_3$ solution and brine, and dried (Na_2SO_4). After concentration, the residue was dissolved in EtOH (10 ml), and then $NaBH_4$ (46 mg, 1.22 mmol) was added at 0 °C. The reaction mixture was stirred at room temperature for 1.5 h before additions of 6 M aq. HCl. The mixture was neutralized with sat. aq. $NaHCO_3$ solution and concentrated. The residue was dissolved in H_2O an EtOAc. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/1) gave **8** (0.35 g, 0.63 mmol, 77%) as a colorless oil, $[\alpha]^{20}_D$ −12 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.08 (3H, d, J = 6.7 Hz, CH_3), 1.57 (2H, br. s, OH), 2.38–2.50 (2H, m, 2,3-H), 3.79 (1H, dd, J = 10.6, 6.4 Hz, C*H*HOH), 3.82 (1H, br. s, OH), 3.86 (6H, s, OCH_3), 3.89 (1H, dd, J = 10.6, 6.0 Hz, CH*H*OH), 4.52 (1H, d, J = 7.6 Hz, 4-H), 4.85 (1H, d, J = 5.5 Hz, 1-H), 5.15 (4H, s, OCH_2Ph), 6.84–6.91 (3H, m, ArH), 6.93 (1H, dd, J = 8.3, 1.8 Hz, ArH), 6.98 (1H, d, J = 1.7 Hz, ArH), 7.03 (1H, d, J = 1.8 Hz, ArH), 7.29 (2H, d, J = 7.3 Hz, ArH), 7.31–7.38 (4H, m, ArH), 7.43 (4H, d, J = 7.4 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 11.9, 43.2, 52.1, 55.9, 61.5, 71.0, 82.7, 87.5, 110.3, 110.4, 113.76, 113.84, 118.4, 118.8, 127.2, 127.8, 128.5, 134.4, 135.9, 137.2, 147.5, 147.7, 149.59, 149.62. EIMS m/z 540 ($M^+ - H_2O$, 15), 91 (100); HREIMS m/z 540.2515 (Calcd. for $C_{34}H_{36}O_6$, 540.2512).

meso-*1,4-Bis(4-benzyloxy-3-methoxyphenyl)-2,3-dimethyl-1,4-butanediol (**9**).* A reaction solution of **8** (1.40 g, 2.51 mmol), *p*-TsCl (0.57 g, 2.99 mmol), and pyridine (0.30 ml, 3.71 mmol) in CH_2Cl_2 (20 ml) was stirred at room temperature for 18 h before additions of H_2O and CH_2Cl_2. The organic solution was separated, washed with 1 M aq. HCl solution, sat. aq. $NaHCO_3$ solution, and brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/3) gave an unstable tosylate (1.55 g, 2.17 mmol, 86%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, d, J = 7.1 Hz, CH_3), 2.38 (1H, m, 3-H), 2.44 (3H, s, $ArCH_3$), 2.53 (1H, m, 2-H), 3.83 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 4.13 (1H, dd, J = 9.8, 6.6 Hz, C*H*HOTs), 4.23 (1H, dd, J = 9.8, 6.3 Hz, CH*H*OTs), 4.43 (1H, d, J = 8.6 Hz, 4-H), 4.70 (1H, d, J = 6.2 Hz, 1-H), 5.15 (4H, s, OCH_2Ph), 6.78–6.81 (2H, m, ArH), 6.84 (2H, s, ArH), 6.91 (1H, s, ArH), 6.93 (1H, s, ArH), 7.29–7.38 (8H, m, ArH), 7.42–7.43 (4H, m, ArH), 7.76 (2H, d, J = 8.3 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 11.6, 21.6, 42.9, 49.1, 55.9, 56.0, 68.6, 71.0, 82.0, 87.3, 110.1, 110.4, 113.8, 118.2, 118.7, 127.2, 127.8, 127.9, 128.50, 128.52, 129.9, 132.7, 133.5, 134.6, 137.1, 145.0, 147.7, 147.9, 149.7. To an ice-cooled solution of the unstable tosylate (1.55 g, 2.17 mmol) in HMPA (6 ml) was added $NaBH_4$ (0.16 g, 4.23 mmol). The resulting reaction mixture was stirred at room temperature for 13 h before additions of 1 M aq. HCl solution and EtOAc. The organic solution was separated, washed with sat. aq. $NaHCO_3$ solution and brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/4) gave **9** (1.13 g, 2.08 mmol, 96%) as colorless crystals, mp 91–92 °C (MeOH). ^{1}H NMR ($CDCl_3$) δ 1.02 (6H, d, J = 6.5 Hz, CH_3), 2.31 (2H, m, 2,3-H), 2.59 (2H, s, OH), 3.86 (6H, s, OCH_3), 4.49 (2H, d, J = 6.2 Hz, 1,4-H), 5.15 (4H, s, OCH_2Ph), 6.88 (2H, d, J = 8.2 Hz, ArH), 6.89 (2H, dd, J = 8.2, 1.8 Hz, ArH), 7.00 (2H, d, J = 1.8 Hz, ArH), 7.28 (2H, d, J = 7.1 Hz, ArH), 7.31–7.37 (4H, m, ArH), 7.42–7.44 (4H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 12.9, 44.3, 56.0, 71.1, 87.2, 110.3, 113.8, 118.5, 127.2, 127.6, 127.7, 128.5, 135.4, 137.2, 147.6, 149.6. Anal. Found: C, 75.42; H, 6.86%. Calcd. for $C_{34}H_{38}O_6$: C, 75.25; H 7.06%.

meso-*1,4-Bis(4-benzyloxy-3-methoxyphenyl)-2,3-dimethyl-1,4-butanedione (**10**).* To a solution of **9** (0.12 g, 0.22 mmol) in acetone (5 ml) was added a CrO_3 solution (35 ml, CrO_3 (6.68 g) dissolved in H_2SO_4 (5.75 ml), and then filled up to 25 ml with H_2O). The resulting reaction mixture was stirred at room temperature for 5 h before additions of $NaHCO_3$ and CH_2Cl_2. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/1) gave **10** (44 mg, 0.082 mmol, 37%) as colorless crystals, mp 154–155 °C. ^{1}H NMR ($CDCl_3$) δ 1.09 (6H, d, J = 6.2 Hz, CH_3), 3.96 (2H, m, 2,3-H), 3.97 (6H, s, OCH_3), 5.25 (4H, s, OCH_2Ph), 6.94 (2H, d, J = 8.4 Hz, ArH), 7.33 (2H, d, J = 7.2 Hz, ArH), 7.37–7.40 (4H, m, ArH), 7.44–7.46 (4H, m, ArH), 7.61 (2H, d, J = 1.9 Hz, ArH), 7.65 (2H, dd, J = 8.4, 1.9 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 17.7, 42.9, 56.0, 70.8, 110.8, 112.1, 123.0, 127.2, 128.1, 128.7, 130.3, 136.2, 149.6, 152.7, 202.4. EIMS m/z 538 (M^+, 25), 241 (32), 91 (100); HREIMS m/z 538.2357 (calcd. for $C_{34}H_{34}O_6$, 538.2356).

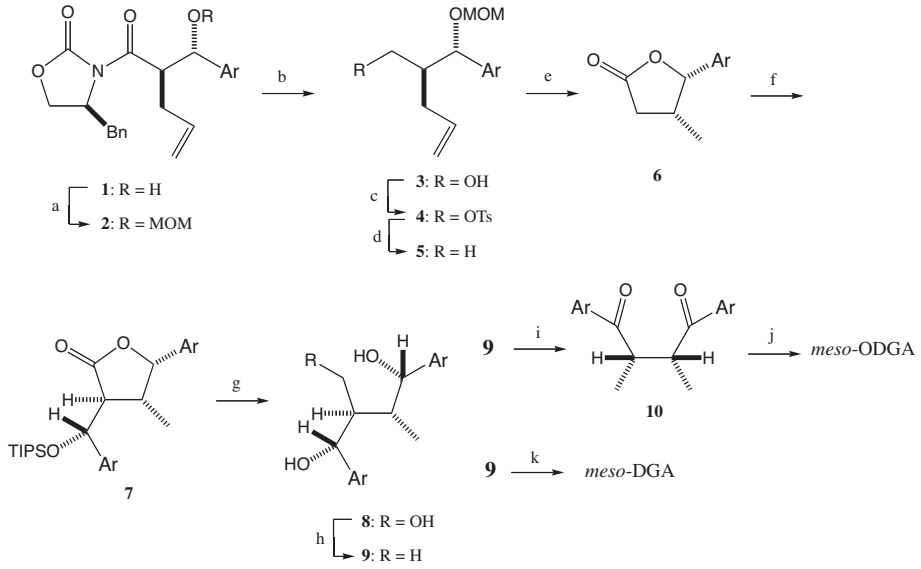
meso-*4,4′-Dihydroxy-3,3′-dimethoxylignane (*meso-*DGA).* A reaction mixture of **9** (0.14 g, 0.26 mmol) and 10% $Pd(OH)_2/C$ (0.22 g) in THF (10 ml) was stirred at ambient temperature under H_2 gas for 70 h before filtration. The filtrate was concentrated. The residue was applied to silica gel column chromatography (EtOAc/hexane = 1/3) to give *meso*-DGA (35 mg, 0.12 mmol, 46%). The NMR data agree with those in the literature.[11)]

meso-*4,4′-Dihydroxy-3,3′-dimethoxylignane-7,7′-dione (*meso-*ODGA).* A reaction mixture of **10** (66 mg, 0.12 mmol) and 5% Pd/C (0.12 g) in EtOAc (20 ml) was stirred at ambient temperature under H_2 gas for 2 h before filtration. The filtrate was concentrated, and then the residue was applied to silica gel column chromatography (EtOAc/hexane = 1/1) to give *meso*-ODGA (40 mg, 0.11 mmol, 92%) as colorless crystals, mp 175–177 °C. ^{1}H NMR ($CDCl_3$) δ 1.11 (6H, d, J = 6.1 Hz, CH_3), 3.92 (2H, m, 8,8′-H), 3.98 (6H, s, OCH_3), 6.15 (2H, br. s, ArOH), 6.99 (2H, d, J = 8.3 Hz, ArH), 7.60 (2H, d, J = 1.8 Hz, ArH), 7.69 (2H, dd, J = 8.3, 1.8 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 17.7, 42.9, 56.1, 110.2, 114.0, 123.9, 129.8, 146.8, 150.7, 202.4. EIMS m/z 358 (M^+, 17), 151 (100); HREIMS m/z 358.1417 (calcd. for $C_{20}H_{22}O_6$, 358.1416).

Organisms. Bacillus subtilis subsp. *subtilis* NBRC 13719^T, *Pseudomonas fluorescens* NBRC 14160^T, and *Staphylococcus aureus* subsp. *aureus* NBRC 14462 were purchased from the National Institute of Technology and Evaluation (NITE), Biological Resource Center, Japan. *Escherichia coli* JCM 1649, *Listeria denitrificans* JCM 11481, *Salmonella choleraesuis* subsp. *choleraesuis* JCM 6977 and *Yersinia intermedia* JCM 7579 were obtained from RIKEN, Japan. The phytopathogenic fungi, *Colletotrichum lagenarium*, *Bipolaris oryzae*, *Fusarium solani*, and *Alternaria alternata*, had been isolated from a farm of Ehime University and kindly presented by Dr. Ohguchi. Each fungus was cultured on potato dextrose agar (PDA, Sigma-Aldrich, Canada).

Antibiotic spectra. The ability of a compound to inhibit the growth of a variety of Gram-positive and Gram-negative bacterial strains was assessed by the paper disc method (Advantec Toyo, Japan, 6 mm thin paper disc), and the minimum inhibitory concentration (MIC) was determined for those strains that showed sensitivity to the tested compounds. The paper disc test used agar plates containing "Nissui" nutrient broth (Nissui Pharmaceutical Co.) One hundred microliter of an exponential culture of each respective strain was made into molten agar containing the nutrient broth. After the agar had solidified, a paper disc containing 15 µl of 50 mM of the tested compound was put on to the agar plate. The plate was incubated for 24 h at 30 °C (*L. denitrificans* and *P. fluorescens*) or at 37 °C (*B. subtilis*, *S. aureus*, *E. coli*, *S. choleraesuis* and *Y. intermedia*), and the diameter of any halo of inhibition around the paper disc was measured. The MIC value was determined from a two-fold dilution series for any strain that showed a halo of inhibition.

Antifungal assay. The paper disc method was adopted for the first screening. Briefly, fungal mycelia were spotted on the center of the PDA plate (φ100 mm dishes) and incubated at 28 °C until the colony diameter had become 4–5 cm. A paper disc soaked in dimethyl sulfoxide containing a test chemical was placed at the edge of the colony. Growth inhibition was observed after culturing at 28 °C for 7–10 d. A further inhibition test was performed with the active compounds. Each compound was added to three aliquots each containing 3 ml of PDA at 50 °C, mixed rapidly and poured into the PDA plate (φ50 mm dishes). Dimethyl sulfoxide only without a test compound served as the control. After incubating at 28 °C for 7–10 d, the area of the mycelial colony was measured with a caliper, the assays being triplicated.



Scheme 1. Synthesis of *meso*-DGA and *meso*-ODGA.

Ar, 4-hydroxy-3-methoxyphenyl. (a) MOMCl, (*iso*-Pr)$_2$NEt, CH_2Cl_2, r.t., 4 h (96%); (b) $LiBH_4$, MeOH, THF, 60 °C, 6 h (60%); (c) *p*-TsCl, Pyr., CH_2Cl_2, r.t., 20 h (100%); (d) $LiAlH_4$, THF, r.t., 20 h (88%); (e) (1) OsO_4, NMO, aq. acetone, *tert*-BuOH, r.t., 18 h; (2) $NaIO_4$, MeOH, r.t., 1 h; (3) 2-methyl-2-butene, $NaH_2PO_4.2H_2O$, $NaClO_2$, aq. *tert*-BuOH; (4) 6 M aq. HCl, THF, 50 °C, 20 min (97%, 4 steps); (f) (1) KHMDS, 4-benzyloxy-3-methoxybenzaldehyde, THF, −70 °C, 1 h (97%, *erythro*/*threo* = 1/3); (2) TIPSOTf, 2,6-lutidine, CH_2Cl_2, r.t., 1 h (40%); (g) (1) DIBAL-H, toluene, −70 °C, 3 h; (2) $NaBH_4$, EtOH, r.t., 1.5 h; (3) 6 M aq. HCl (77%, 3 steps); (h) (1) TsCl, Pyr., CH_2Cl_2, r.t., 18 h (86%); (2) $NaBH_4$, HMPA, r.t., 13 h (96%); (i) CrO_3, conc. H_2SO_4, acetone, r.t., 5 h (37%); (j) H_2, 5% Pd/C, EtOAc, r.t., 2 h (92%); (k) H_2, 10% $Pd(OH)_2$/C, THF, r.t., 70 h (46%).

Results and Discussion

Syntheses of meso-*DGA and* meso-*ODGA*

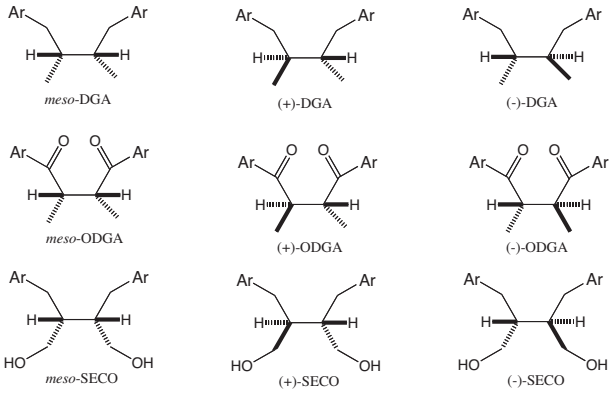
The syntheses of *meso*-DGA and *meso*-ODGA are shown in Scheme 1. The hydroxy group of *anti*-Evans's aldol product **1**[12] was protected as a methoxymethyl ether to give **2**. After $LiBH_4$ reduction of **2**, resulting primary alcohol **3** was converted to **5** by tosylation and subsequent $LiAlH_4$ reduction. Oxidative cleavage of the olefin, followed by transformation to carboxylic acid and cleavage of the methoxymethyl ether under acidic conditions gave lactone **6**. Aldol condensation between **6** and 4-benzyloxy-3-methoxybenzaldehyde, using potassium bis(trimethylsilyl)amide, gave a mixture of *erythro*/*threo* from (1/3) of the aldol product. The *erythro* and *threo* forms were separated after their conversion to triisopropyl silyl ether **7**. The diisobutyl aluminum hydride reduction of **7**, followed by $NaBH_4$ reduction and treatment with an acid gave triol **8**. After the primary hydroxy group was converted to tosylate, $LiAlH_4$ reduction was employed to give *meso*-form **9**. Hydrogenolysis and reduction of two benzylic hydroxy groups of **9** were successful at the same time by using $Pd(OH)_2$ under H_2 gas, giving *meso*-DGA. CrO_3 oxidation of **9** followed by hydrogenolysis employing Pd/C under H_2 gas gave *meso*-ODGA.

Antimicrobial activity of the butane-type of lignans

The antimicrobial activities of all stereoisomers of DGA, ODGA, and secoisolariciresinol (SECO) were examined. The antibacterial activity is shown in Table 1. All stereoisomers of DGA showed activity. The activity of (+)- and (−)-DGA against *L. denitrificans* was higher than that of *meso*-DGA, and the activity against *S. aureus* of *meso*-ODGA was higher than that of (+)- and (−)-DGA. Against *B. subtilis*, all stereoisomers showed the same level of activity. Only (+)-DGA showed activity against the Gram-negative bacterium, *S. choleraesuis*. The antibacterial activities of all stereoisomers of DGA were higher than those of previously described tetrahydrofuran[13] and butyrolactone[8] types of lignans. As for the stereoisomers of ODGA, *meso*-ODGA did not show any activity. (+)-ODGA showed activity against all Gram-positive bacteria, especially strong activity being observed against *L. denitrificans*. Activity was not shown against Gram-negative bacteria. (−)-ODGA showed activity against only *L. denitrificans*, this activity being stronger than that of (+)-ODGA. It became clear that the antibacterial activity of DGA was generally stronger than that of ODGA. Although oxidation at the 7 and 7′ positions of matairesinol, which is a butyrolactone type of lignan, increased the activity,[8] oxidation at the 7 and 7′-positions of DGA decreased the activity. No stereoisomers of SECO showed any activity. This fact suggests that the presence of 9 and 9′-hydroxy groups was not effective for the antibacterial activity.

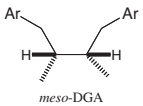
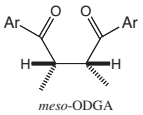
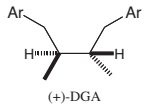
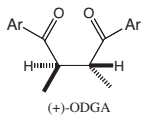
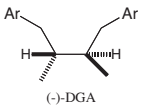
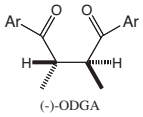
The antifungal activity test was also performed by using phytopathogenic fungi. In this test, DGA and ODGA showed activity against *Alternaria alternata* at 0.25 mM (Table 2). None of the stereoisomers of SECO was showed activity. It could be assumed that the presence of 9 and 9′-hydroxy groups was not effective for the antifungal activity, as well as for the antibacterial activity. In the case of DGA, the activity level of all stereoisomers was almost same. On the other hand, the antifungal activity of *meso*-ODGA was higher than that of (+)- and (−)-ODGA. In the comparing DGA with ODGA, the activity of (+)- and (−)-DGA was higher than that of (+)- and (−)-ODGA. The activity levels of both *meso*-DGA and *meso*-ODGA were almost same. In our previous study on the antifungal activity of lignan,

Table 1. Antibacterial Activity of DGA, ODGA, and SECO (MIC, mM)
Ar, 4-hydroxy-3-methoxyphenyl



	DGA			ODGA			SECO		
	meso	(+)	(−)	*meso*	(+)	(−)	*meso*	(+)	(−)
Bacillus subtilis	1.6	1.6	1.6	>50	13	>50	>50	>50	>50
Staphylococcus aureus	1.6	3.1	3.1	>50	25	>50	>50	>50	>50
Listeria denitrificans	1.6	0.4	0.4	>50	6.3	1.6	>50	>50	>50
Salmonella choleraesuis	>50	3.1	>50	>50	>50	>50	>50	>50	>50

Table 2. Growth Rate (% $\pm\sigma$, n = 3) of *Alternaria alternata* at 0.25 mM of DGA and ODGA
Ar, 4-hydroxy-3-methoxyphenyl

Structure	Growth %	Structure	Growth %
 *meso*-DGA	30 ± 11	 *meso*-ODGA	39 ± 1.6
 (+)-DGA	38 ± 6.3	 (+)-ODGA	66 ± 2.4
 (-)-DGA	32 ± 2.4	 (-)-ODGA	51 ± 7.8

Growth (%), (colony diameter of sample/colony diameter of control) × 100. No stereoisomers of SECO showed activity at 1.0 mM by the paper disc test.

the activity against *Colletotrichum lagenarium*[14] and *Bipolaris oryzae*[8] of tetrahydrofuran lignan has been reported. The butane type of lignan showed activity against *Alternaria alternata* in this research.

The relationship between the stereochemistry of the butane type of lignan and antimicrobial activity was shown for the first time. No large effect of the stereochemistry of DGA on the activity was apparent, except for the effect of (+)-DGA on *S. choleraesuis*, while the stereochemistry of ODGA did affect its activity. As for the effect of oxidation of the structure, benzylic oxidation of DGA decreased the activity, and the activity was disappeared with the primary hydroxy groups. These results are new information to chemical ecology. Synthetic research of (−)- or (+)-DGA derivatives and their antimicrobial testing raises the possibility of discovering compounds having higher activity. The development of new antimicrobial material containing DGA or ODGA, which can be applied as wall, sheet, or fiber, can be expected.

Acknowledgments

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