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Quantitative Structure−Activity Relationship Analysis of Antifungal (+)-Dihydroguaiaretic Acid Using 7-Phenyl Derivatives

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ABSTRACT: The relationship between antifungal activity against *Alternaria alternata* and structure on the 7-phenyl group of (+)-dihydroguaiaretic acid ((+)-DGA) was clarified by employing 38 synthesized (+)-DGA derivatives. The results were identified by quantitative structure−activity relationship (QSAR) analysis employing the Hansch−Fujita method. Some compounds showed higher activity than (+)-DGA. The compound showing highest activity was 3,5-difluorophenyl derivative **37**. It was suggested that the small electron-withdrawing group at the meta-position of the 7-phenyl group is important for the higher activity by antifungal test and Hansch−Fujita analysis. The whitening activity of 3-hydroxy-4-methoxyphenyl derivative **28**, 3-hydroxy-4-ethoxyphenyl derivative **29**, and 3-hydroxy-4-isopropoxyphenyl derivative **30** against *A. alternata* Japanese pear pathotype was also discovered.

KEYWORDS: *lignan, dihydroguaiaretic acid, antifungal activity, Hansch−Fujita analysis, antimelanogenic activity*

INTRODUCTION

Lignan is one of the most popular natural compounds consisting of two phenylpropanoid units. Because many kinds of biological activities of lignans have been reported,[1,2] research on the effects of stereochemistry and structure on biological activity is required to develop new lead compounds for medicine and pesticides. This effort would contribute to bioorganic chemistry and chemical ecology of lignan. As for the antifungal activity of lignan, the activities of tetrasubstituted tetrahydrofuran lignan[3,4] and *meso*-dihydroguaiaretic acid[4] have been known. The cytotoxic lignan, podophyllotoxin, has also been reported as an antifungal lignan.[5] We reported the effect of the stereochemistry of tetrasubstituted tetrahydrofuran lignan,[6] disubstituted tetrahydrofuran lignan,[7] butane type lignan,[8] and sesquineolignan[9] on the antifungal activity. However, quantitative structure−antifungal activity relationship analyses of these lignans have not been done. Dihydroguaiaretic acid (DGA) has a simple butane structure and is expected to be a commercial lead compound (Figure 1). *Alternaria alternata* Japanese pear pathotype is known as a plant pathogen, which causes black spot disease. Controlling this fungus is important to prevent economic loss to agriculture. The purpose of our project is discovery of the lead antifungal compound to natural product-based control agent and collation of natural compound library by showing structure−activity relationship. This paper describes the quantitative structure−activity relationship (QSAR) of (+)-dihydroguaiaretic acid ((+)-DGA) using Hansch−Fujita analysis and design of derivatives showing higher activity than (+)-DGA. We also describe the whitening activity of some (+)-DGA derivatives against black *A. alternata*.

MATERIALS AND METHODS

Chemicals. Compounds **1**−**41** (Figure 1) were synthesized by using a previously reported method.[8,10] NMR data were measured by a JNM-ECS400 spectrometer, using TMS and TFA as standards for ^{1}H NMR (0.00 ppm) and ^{19}F NMR (−76.2 ppm), respectively; MS data were measured with a JMS-MS700 V spectrometer, and optical rotation values were evaluated with a Jasco P-2100 polarimeter. Nomenclature of compounds follows the literature for lignans.[11]

*Derivatives **5**, **6**, **7**, **11**, **12**, **16**, **17**, **22**−**25**, **28**, **32**, **38**, **39**.* The optical rotations were opposite but equal amounts to the corresponding enantiomers in the literature.[12] NMR data agreed with those of the literature.[12]

*(8S,8′S)-1,2,3,4,5,6-Hexahydro-3′-methoxylignan-4′-ol (**4**):* colorless oil; $[\alpha]^{25}_{D}$ −5 (*c* 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.73 (3H, d, *J* = 6.4 Hz), 0.74−0.86 (2H, m), 0.80 (3H, d, *J* = 6.9 Hz), 1.03 (1H, m), 1.10−1.26 (5H, m), 1.56−1.68 (7H, m), 2.31 (1H, dd, *J* = 13.3, 8.7 Hz), 2.55 (1H, dd, *J* = 13.3, 6.0 Hz), 3.86 (3H, s), 5.46 (1H, s), 6.63−6.64 (2H, m), 6.81 (1H, d, *J* = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 14.6, 26.4, 26.5, 26.8, 32.4, 33.3, 34.0, 34.9, 38.9, 41.0, 42.8, 55.8, 111.4, 113.9, 121.6, 134.0, 143.4, 146.2; MS (EI), *m*/*z* 290 (M^+, 57), 137 (100); HRMS (EI) *m*/*z* calcd for $C_{19}H_{30}O_2$ 290.2246, found 290.2228.

*(8S,8′S)-3′-Methoxy-2a-homoligan-4′-ol (**8**):* colorless oil; $[\alpha]^{25}_{D}$ +34 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, d, *J* = 6.9 Hz), 0.88 (3H, d, *J* = 6.4 Hz), 1.70−1.86 (2H, m), 2.17 (3H, s), 2.38 (1H, dd, *J* = 13.5, 4.6 Hz), 2.41 (1H, dd, *J* = 13.5, 5.0 Hz), 2.54 (1H, dd, *J* = 13.5, 7.3 Hz), 2.63 (1H, dd, *J* = 13.5, 5.8 Hz), 3.78 (3H, s), 5.49 (1H, s), 6.54 (1H, d, *J* = 1.4 Hz), 6.58 (1H, dd, *J* = 8.2, 1.4 Hz), 6.79 (1H, d, *J* = 8.2 Hz), 7.02 (1H, m), 7.05−7.10 (3H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 14.2, 19.4, 35.9, 38.5, 38.7, 40.9, 55.7, 111.2, 113.9, 121.5, 125.4, 125.7, 129.9, 130.1, 133.5, 136.2, 139.8, 143.4, 146.2; MS (EI) *m*/*z* 298 (M^+, 71), 137 (100); HRMS (EI) *m*/*z* calcd for $C_{20}H_{26}O_2$ 298.1933, found 298.1930.

*(8S,8′S)-2-Fluoro-3′-methoxylignan-4′-ol (**9**):* colorless oil; $[\alpha]^{25}_{D}$ +32 (*c* 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.83 (3H, d, *J* = 8.7 Hz), 0.84 (3H, d, *J* = 8.7 Hz), 1.75 (1H, m), 1.82 (1H, m), 2.36 (1H, dd, *J* = 13.5, 8.5 Hz), 2.46 (1H, dd, *J* = 13.5, 8.7 Hz), 2.57 (1H, dd, *J* = 13.7, 6.4 Hz), 2.69 (1H, dd, *J* = 13.7, 6.4 Hz), 3.81 (3H, s), 5.48 (1H, s), 6.56

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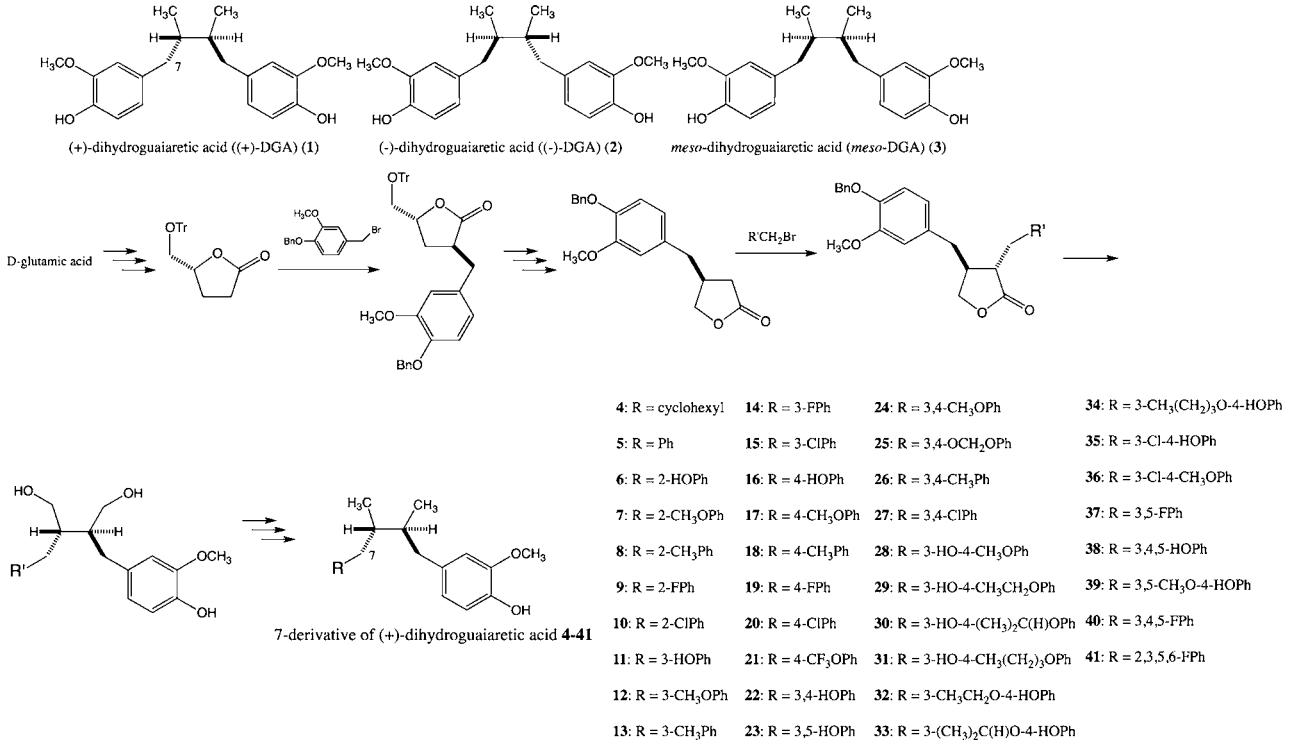

Figure 1. Structures of all stereoisomers of dihydroguaiaretic acid and 7-phenyl derivatives of (+)-dihydroguaiaretic acid.

(1H, br s), 6.58 (1H, br d, J = 7.8 Hz), 6.80 (1H, d, J = 7.8 Hz), 6.95−7.03 (2H, m), 7.07 (1H, m), 7.14 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.87, 13.91, 34.2, 37.0, 38.3, 40.9, 55.8, 111.2, 113.9, 115.1 (d, J = 23.0 Hz), 121.6, 123.6 (d, J = 2.7 Hz), 127.3 (d, J = 7.6 Hz), 128.5 (d, J = 15.3 Hz), 131.3 (d, J = 5.7 Hz), 133.4, 143.5, 146.2, 161.3 (d, J = 244.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −118.6; MS (EI) m/z 302 (M^+, 98), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{23}O_2F$ 302.1682, found 302.1685.

*(8S,8′S)-2-Chloro-3′-methoxylignan-4′-ol (**10**):* colorless oil; $[\alpha]^{25}_D$ +34 (c 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, d, J = 6.9 Hz), 0.88 (3H, d, J = 6.9 Hz), 1.79 (1H, m), 1.90 (1H, m), 2.37 (1H, dd, J = 13.7, 8.7 Hz), 2.51 (1H, dd, J = 13.3, 8.7 Hz), 2.58 (1H, dd, J = 13.7, 6.7 Hz), 2.81 (1H, dd, J = 13.3, 6.0 Hz), 3.81 (3H, s), 5.50 (1H, br s), 6.56 (1H, d, J = 1.8 Hz), 6.59 (1H, dd, J = 7.8, 1.8 Hz), 6.80 (1H, d, J = 7.8 Hz), 7.07−7.15 (3H, m), 7.31 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 14.1, 36.3, 38.5, 38.7, 40.9, 55.8, 111.3, 113.9, 121.6, 126.3, 127.0, 129.4, 131.2, 133.4, 134.2, 139.2, 143.5, 146.2; MS (EI) m/z 318 (M^+, 61), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{23}O_2Cl$ 318.1388, found 318.1395.

*(8S,8′S)-3′-Methoxy-3a-homoligan-4′-ol (**13**):* colorless oil; $[\alpha]^{25}_D$ +29 (c 0.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, d, J = 6.9 Hz), 0.83 (3H, d, J = 6.4 Hz), 1.77 (2H, m), 2.30 (3H, s), 2.36 (1H, dd, J = 13.7, 8.2 Hz), 2.39 (1H, dd, J = 13.7, 8.2 Hz), 2.56 (1H, dd, J = 13.7, 6.8 Hz), 2.59 (1H, dd, J = 13.7, 6.4 Hz), 3.80 (3H, s), 5.48 (1H, s), 6.55 (1H, d, J = 1.8 Hz), 6.59 (1H, dd, J = 7.8, 1.8 Hz), 6.80 (1H, d, J = 7.8 Hz), 6.88−6.89 (2H, m), 6.97 (1H, br d, J = 7.8 Hz), 7.13 (1H, dd, J = 8.2, 7.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 21.4, 37.6, 37.9, 41.0, 41.2, 55.7, 111.3, 113.9, 121.6, 126.0, 126.3, 127.9, 129.8, 133.5, 137.6, 141.6, 143.4, 146.2; MS (EI) m/z 298 (M^+, 93), 137 (100); HRMS (EI) m/z calcd for $C_{20}H_{26}O_2$ 298.1933, found 298.1934.

*(8S,8′S)-3-Fluoro-3′-methoxylignan-4′-ol (**14**):* colorless oil; $[\alpha]^{25}_D$ +28 (c 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, d, J = 6.7 Hz), 0.82 (3H, d, J = 6.9 Hz), 1.69−1.81 (2H, m), 2.36 (1H, dd, J = 13.5, 7.8 Hz), 2.42 (1H, dd, J = 13.5, 7.8 Hz), 2.54 (1H, dd, J = 13.5, 6.6 Hz), 2.60 (1H, dd, J = 13.5, 6.8 Hz), 3.79 (3H, s), 5.57 (1H, s), 6.54 (1H, d, J = 1.8 Hz), 6.58 (1H, dd, J = 8.3, 1.8 Hz), 6.76−6.86 (4H, m), 7.17 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 37.5, 37.8, 40.9, 41.0, 55.7, 111.2, 112.4 (d, J = 21.1 Hz), 113.9, 115.6 (d, J = 20.1 Hz), 121.5, 124.6 (d, J = 1.9 Hz), 129.4 (d, J = 8.6 Hz), 133.2, 143.5, 144.2 (d, J = 6.7 Hz), 146.2, 162.7 (d, J = 244.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −114.7; MS (EI) m/z 302 (M^+, 73), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{23}O_2F$ 302.1682, found 302.1688.

*(8S,8′S)-3-Chloro-3′-methoxylignan-4′-ol (**15**):* colorless oil; $[\alpha]^{25}_D$ +30 (c 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, d, J = 6.9 Hz), 0.82 (3H, d, J = 6.9 Hz), 1.71−1.78 (2H, m), 2.36 (1H, dd, J = 13.8, 8.2 Hz), 2.39 (1H, dd, J = 13.8, 8.3 Hz), 2.54 (1H, dd, J = 13.8, 6.8 Hz), 2.58 (1H, dd, J = 13.8, 6.8 Hz), 3.79 (3H, s), 5.55 (1H, s), 6.54 (1H, d, J = 1.4 Hz), 6.57 (1H, dd, J = 8.3, 1.4 Hz), 6.81 (1H, d, J = 8.3 Hz), 6.94 (1H, ddd, J = 5.9, 2.3, 2.3 Hz), 7.07 (1H, br s), 7.12−7.15 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.76, 13.80, 37.5, 37.8, 40.90, 40.92, 55.7, 111.2, 113.9, 121.5, 125.7, 127.2, 128.9, 129.3, 133.2, 133.8, 143.5, 143.7, 146.2; MS (EI) m/z 318 (M^+, 59), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{23}O_2Cl$ 318.1388, found 318.1389.

*(8S,8′S)-3′-Methoxy-4a-homoligan-4′-ol (**18**):* colorless oil; $[\alpha]^{25}_D$ +24 (c 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, d, J = 6.9 Hz), 0.82 (3H, d, J = 6.9 Hz), 1.75 (2H, m), 2.30 (3H, s), 2.36 (1H, dd, J = 13.5, 7.8 Hz), 2.39 (1H, dd, J = 13.5, 8.2 Hz), 2.55 (1H, dd, J = 13.5, 6.8 Hz), 2.59 (1H, dd, J = 13.5, 6.5 Hz), 3.80 (3H, s), 5.48 (1H, s), 6.54 (1H, d, J = 1.8 Hz), 6.58 (1H, dd, J = 8.2, 1.8 Hz), 6.80 (1H, d, J = 8.2 Hz), 6.97 (2H, d, J = 7.8 Hz), 7.06 (2H, d, J = 7.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 13.9, 21.0, 37.7, 37.9, 40.9, 41.0, 55.7, 111.3, 113.9, 121.6, 128.7, 128.9, 133.5, 134.9, 138.5, 143.4, 146.2; MS (EI) m/z 298 (M^+, 66), 137 (100); HRMS (EI) m/z calcd for $C_{20}H_{26}O_2$ 298.1933, found 298.1929.

*(8S,8′S)-4-Fluoro-3′-methoxylignan-4′-ol (**19**):* colorless oil; $[\alpha]^{25}_D$ +38 (c 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, d, J = 6.4 Hz), 0.82 (3H, d, J = 6.8 Hz), 1.73 (2H, m), 2.37 (1H, dd, J = 13.5, 8.2 Hz), 2.40 (1H, dd, J = 13.5, 8.2 Hz), 2.54 (1H, dd, J = 13.5, 6.8 Hz), 2.58 (1H, dd, J = 13.5, 6.4 Hz), 3.81 (3H, s), 5.48 (1H, s), 6.53 (1H, d, J = 1.8 Hz), 6.58 (1H, dd, J = 8.2, 1.8 Hz), 6.80 (1H, d, J = 8.2 Hz), 6.89−6.94 (2H, m), 7.00−7.04 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 37.8, 40.5, 41.0, 55.7, 111.2, 113.9, 114.8 (d, J = 21.1 Hz), 121.6, 130.2 (d, J = 7.6 Hz), 133.4, 137.2 (d, J = 2.9 Hz), 143.5, 146.2, 161.1 (d, J = 243.5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −118.6; MS (EI) m/z 302 (M^+, 60), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{23}O_2F$ 302.1682, found 302.1683.

*(8S,8′S)-4-Chloro-3′-methoxylignan-4′-ol (**20**):* colorless oil; $[\alpha]^{25}_D$ +23 (c 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (3H, d, J = 6.4

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Hz), 0.82 (3H, d, J = 6.4 Hz), 1.72 (2H, m), 2.36 (1H, dd, J = 13.3, 7.8 Hz), 2.39 (1H, dd, J = 13.8, 8.3 Hz), 2.52 (1H, dd, J = 13.3, 6.9 Hz), 2.57 (1H, dd, J = 13.8, 6.8 Hz), 3.79 (3H, s), 5.51 (1H, s), 6.51 (1H, d, J = 1.4 Hz), 6.57 (1H, dd, J = 8.3, 1.4 Hz), 6.80 (1H, d, J = 8.3 Hz), 6.99 (2H, d, J = 8.3 Hz), 7.19 (2H, d, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 37.5, 37.7, 40.6, 40.9, 55.7, 111.2, 113.9, 121.5, 128.1, 130.3, 131.2, 133.3, 140.1, 143.5, 146.2; MS (EI) m/z 318 (M^+, 80), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{23}O_2Cl$ 318.1388, found 318.1391.

(8S,8′S)-4-Trifluoromethoxy-3′-methoxylignan-4′-ol (**21**): colorless oil; $[\alpha]^{25}_D$ +22 (c 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (3H, d, J = 6.4 Hz), 0.83 (3H, d, J = 6.9 Hz), 1.75 (2H, m), 2.37 (1H, dd, J = 13.7, 7.8 Hz), 2.43 (1H, dd, J = 13.7, 7.8 Hz), 2.53 (1H, dd, J = 13.7, 6.9 Hz), 2.60 (1H, dd, J = 13.7, 6.9 Hz), 3.78 (3H, s), 5.53 (1H, s), 6.52 (1H, d, J = 1.8 Hz), 6.57 (1H, dd, J = 8.2, 1.8 Hz), 6.80 (1H, d, J = 8.2 Hz), 7.06 (2H, d, J = 9.2 Hz), 7.09 (2H, d, J = 9.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 37.6, 37.7, 40.6, 41.0, 55.7, 111.1, 113.9, 120.5 (d, J = 255.9 Hz), 120.6, 121.6, 130.1, 133.3, 140.4, 143.5, 146.3, 147.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −58.5; MS (EI) m/z 368 (M^+, 68), 137 (100); HRMS (EI) m/z calcd for $C_{20}H_{23}O_3F_3$ 368.1597, found 368.1595.

(8S,8′S)-3′-Methoxy-3a,4a-dihomolignan-4′-ol (**26**): colorless oil; $[\alpha]^{25}_D$ +26 (c 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (6H, d, J = 5.0 Hz), 1.76 (2H, m), 2.21 (6H, s), 2.36 (2H, dd, J = 13.3, 8.0 Hz), 2.56 (2H, dd, J = 13.3, 6.2 Hz), 3.81 (3H, s), 5.46 (1H, s), 6.55 (1H, br s), 6.59 (1H, d, J = 7.7 Hz), 6.79−6.85 (3H, m), 7.00 (1H, d, J = 7.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 19.3, 19.7, 37.7, 38.0, 40.9, 41.0, 55.7, 111.3, 113.9, 121.6, 126.4, 129.3, 130.3, 133.5, 133.6, 136.1, 139.0, 143.5, 146.2; MS (EI) m/z 312 (M^+, 96), 137 (100), 119 (47); HRMS (EI) m/z calcd for $C_{21}H_{28}O_2$ 312.2089, found 312.2090.

(8S,8′S)-3,4-Dichloro-3′-methoxylignan-4′-ol (**27**): colorless oil; $[\alpha]^{25}_D$ +23 (c 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (3H, d, J = 6.9 Hz), 0.82 (3H, d, J = 5.0 Hz), 1.72 (2H, m), 2.36 (1H, dd, J = 13.1, 8.2 Hz), 2.38 (1H, dd, J = 13.5, 8.2 Hz), 2.52 (1H, dd, J = 13.1, 6.8 Hz), 2.55 (1H, dd, J = 13.5, 6.8 Hz), 3.81 (3H, s), 5.52 (1H, s), 6.53 (1H, br s), 6.57 (1H, br d, J = 7.8 Hz), 6.81 (1H, d, J = 7.8 Hz), 6.88 (1H, dd, J = 8.2, 1.8 Hz), 7.15 (1H, d, J = 1.4 Hz), 7.28 (1H, d, J = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 37.3, 37.8, 40.4, 40.9, 55.7, 111.1, 113.9, 121.5, 128.4, 129.4, 129.9, 130.7, 131.9, 133.1, 141.9, 143.6, 146.3; MS (EI) m/z 352 (M^+, 34), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{22}O_2Cl_2$ 352.0998, found 352.1001.

(8S,8′S)-4-Ethoxy-3′-methoxylignane-3,4′-diol (**29**): colorless oil; $[\alpha]^{25}_D$ +22 (c 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (6H, t, J = 6.9 Hz), 1.41 (3H, t, J = 6.9 Hz), 1.75 (2H, m), 2.33 (1H, dd, J = 13.7, 7.8 Hz), 2.35 (1H, dd, J = 13.7, 7.8 Hz), 2.53 (1H, dd, J = 13.7, 7.3 Hz), 2.55 (1H, dd, J = 13.7, 7.8 Hz), 3.82 (3H, s), 4.06 (2H, q, J = 6.9 Hz), 5.53 (1H, br s), 5.66 (1H, br s), 6.54 (1H, dd, J = 8.2, 2.0 Hz), 6.57 (1H, br s), 6.59 (1H, dd, J = 7.8, 2.0 Hz), 6.69 (1H, d, J = 2.0 Hz), 6.71 (1H, d, J = 8.2 Hz), 6.80 (1H, d, J = 7.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.81, 13.84, 14.9, 37.78, 37.83, 40.7, 41.0, 55.7, 64.5, 111.3, 113.9, 115.0, 120.3, 121.6, 133.5, 134.9, 143.4, 143.8, 145.3, 146.2; MS (EI) m/z 344 (M^+, 26), 137 (100), 123 (89); HRMS (EI) m/z calcd for $C_{21}H_{28}O_4$ 344.1988, found 344.1985.

(8S,8′S)-4-Isopropoxy-3′-methoxylignane-3,4′-diol (**30**): colorless oil; $[\alpha]^{25}_D$ +19 (c 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (6H, d, J = 6.9 Hz), 1.35 (6H, d, J = 6.0 Hz), 1.69−1.80 (2H, m), 2.33 (1H, dd, J = 13.7, 8.2 Hz), 2.35 (1H, dd, J = 13.7, 8.2 Hz), 2.53 (1H, dd, J = 13.7, 6.9 Hz), 2.55 (1H, dd, J = 13.7, 6.4 Hz), 3.82 (3H, s), 4.51 (1H, m), 5.52 (1H, s), 5.68 (1H, s), 6.53 (1H, dd, J = 8.2, 1.8 Hz), 6.57 (1H, br s), 6.58 (1H, dd, J = 7.7, 1.8 Hz), 6.69 (1H, d, J = 1.8 Hz), 6.73 (1H, d, J = 8.2 Hz), 6.80 (1H, d, J = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 13.9, 22.2, 37.80, 37.82, 40.8, 41.0, 55.7, 71.7, 111.3, 113.2, 113.9, 115.2, 120.3, 121.6, 133.5, 135.1, 142.5, 143.4, 146.2, 146.3; MS (EI) m/z 358 (M^+, 31), 137 (100), 123 (51); HRMS (EI) m/z calcd for $C_{22}H_{30}O_4$ 358.2144, found 358.2138.

(8S,8′S)-4-Butoxy-3′-methoxylignane-3,4′-diol (**31**): colorless oil; $[\alpha]^{25}_D$ +12 (c 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (6H, d, J = 6.9 Hz), 0.98 (3H, t, J = 7.6 Hz), 1.49 (2H, m), 1.72−1.80 (4H, m), 2.33 (1H, dd, J = 13.4, 7.8 Hz), 2.35 (1H, dd, J = 13.4, 7.8 Hz), 2.54 (1H dd, J = 13.4, 8.2 Hz), 2.55 (1H, dd, J = 13.4, 8.2 Hz), 3.83 (6H, s), 4.01 (2H, t, J = 7.6 Hz), 5.47 (1H, s), 5.59 (1H, s), 6.54 (1H, dd, J = 8.3, 1.8 Hz), 6.57 (1H, d, J = 1.8 Hz), 6.59 (1H, dd, J = 8.3, 1.8 Hz), 6.69 (1H, d, J = 1.8 Hz), 6.72 (1H, d, J = 8.3 Hz), 6.80 (1H, d, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 13.9, 19.2, 31.3, 37.8, 37.9, 40.8, 41.1, 55.8, 68.6, 111.2, 111.3, 113.9, 115.0, 120.3, 121.6, 133.5, 134.8, 143.4, 143.9, 145.4, 146.2; MS (EI) m/z 372 (M^+, 29), 137 (100), 123 (69); HRMS (EI) m/z calcd for $C_{23}H_{32}O_4$ 372.2301, found 372.2299.

(8S,8′S)-3-Isopropoxy-3′-methoxylignane-4,4′-diol (**33**): colorless oil; $[\alpha]^{25}_D$ +27 (c 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (6H, d, J = 6.6 Hz), 1.31 (6H, d, J = 5.2 Hz), 1.72 (2H, m), 2.35 (1H, dd, J = 13.4, 5.6 Hz), 2.37 (1H, dd, J = 13.4, 6.0 Hz), 2.51 (1H, dd, J = 13.4, 6.3 Hz), 2.52 (1H, dd, J = 13.4, 6.3 Hz), 3.79 (3H, s), 4.47 (1H, m), 5.50 (1H, s), 5.59 (1H, s), 6.53−6.58 (4H, m), 6.79 (1H, d, J = 7.7 Hz), 6.80 (1H, d, J = 7.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 22.14, 22.18, 37.5, 37.6, 41.1, 55.8, 71.5, 111.3, 113.9,114.0, 114.1, 121.6, 121.7, 133.4, 133.5, 143.5, 144.2, 144.5, 146.3; MS (EI) m/z 358 (M^+, 45), 137 (100); HRMS (EI) m/z calcd for $C_{22}H_{30}O_4$ 358.2144, found 358.2136.

(8S,8′S)-3-Butoxy-3′-methoxylignane-4,4′-diol (**34**): colorless oil; $[\alpha]^{25}_D$ +27 (c 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (6H, d, J = 6.6 Hz), 0.98 (3H, t, J = 7.5 Hz), 1.49 (2H, m), 1.73 (2H, m), 1.76 (2H, m), 2.35 (1H, dd, J = 13.6, 2.9 Hz), 2.38 (1H, dd, J = 13.5, 2.9 Hz), 2.51 (1H, dd, J = 13.6, 3.9 Hz), 2.52 (1H, dd, J = 13.5, 3.9 Hz), 3.80 (3H, s), 3.94 (2H, m), 5.48 (1H, s), 5.52 (1H, s), 6.52 (2H, d, J = 1.8 Hz), 6.57 (1H, dd, J = 8.0, 1.8 Hz), 6.58 (1H, dd, J = 8.0, 1.8 Hz), 6.79 (1H, d, J = 8.0 Hz), 6.80 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 13.9, 19.2, 31.4, 37.45, 37.51, 41.1, 55.8, 68.5, 111.3, 112.2, 113.8, 113.9, 121.5, 121.6, 133.5, 133.6, 143.5, 143.6, 145.6, 146.2; MS (EI) m/z 372 (M^+, 100), 179 (58), 137 (95); HRMS (EI) m/z calcd for $C_{23}H_{32}O_4$ 372.2301, found 372.2293.

(8S,8′S)-3-Chloro-3′-methoxylignane-4,4′-diol (**35**): colorless oil; $[\alpha]^{25}_D$ +28 (c 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.81 (6H, d, J = 6.0 Hz), 1.72 (2H, m), 2.35 (1H, dd, J = 13.6, 7.8 Hz), 2.36 (1H, dd, J = 13.6, 7.8 Hz), 2.52 (1H, dd, J = 13.6, 4.4 Hz), 2.54 (1H, dd, J = 13.6, 4.4 Hz), 3.83 (3H, s), 5.42 (1H, s), 5.47 (1H, s), 6.55 (1H, d, J = 1.6 Hz), 6.58 (1H, dd, J = 8.0, 1.6 Hz), 6.81 (1H, d, J = 8.0 Hz), 6.87 (1H, d, J = 8.3, 1.6 Hz), 6.90 (1H, d, J = 8.3 Hz), 7.02 (1H, d, J = 1.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.76, 13.81, 37.6, 37.7, 40.2, 41.0, 55.8, 111.2, 113.9, 115.8, 119.4, 121.6, 128.9, 129.0, 133.3, 134.9, 143.5, 146.3, 149.2; MS (EI) m/z 334 (M^+, 49), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{23}O_3Cl$ 334.1336, found 334.1340.

(8S,8′S)-3-Chloro-3′,4-dimethoxylignan-4′-ol (**36**): colorless oil; $[\alpha]^{25}_D$ +26 (c 0.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (3H, d, J = 6.9 Hz), 0.81 (3H, d, J = 6.4 Hz), 1.73 (2H, m), 2.34 (1H, dd, J = 13.5, 5.0 Hz), 2.36 (1H, dd, J = 13.5, 5.0 Hz), 2.52 (1H, dd, J = 13.5, 2.8 Hz), 2.54 (1H, dd, J = 13.5, 2.7 Hz), 3.81 (3H, s), 3.85 (3H, s), 5.53 (1H, s), 6.54 (1H, d, J = 1.8 Hz), 6.58 (1H, dd, J = 8.2, 1.8 Hz), 6.79 (1H, d, J = 8.2 Hz), 6.81 (1H, d, J = 8.2 Hz), 6.91 (1H, dd, J = 8.2, 1.8 Hz), 7.09 (1H, d, J = 1.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 37.6, 37.7, 40.1, 41.0, 55.7, 56.0, 111.2, 111.7, 113.9, 121.5, 121.8, 128.0, 130.4, 133.3, 134.8, 143.5, 146.2, 152.9; MS (EI) m/z 348 (M^+, 46), 137 (100); HRMS (EI) m/z calcd for $C_{20}H_{25}O_3Cl$ 348.1493, found 348.1495.

(8S,8′S)-3,5-Difluoro-3′-methoxylignan-4′-ol (**37**): colorless oil; $[\alpha]^{25}_D$ +24 (c 2.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (6H, d, J = 6.4 Hz), 1.67−1.83 (2H, m), 2.37 (1H, dd, J = 13.7, 8.2 Hz), 2.41 (1H, dd, J = 13.7, 8.2 Hz), 2.54 (1H, dd, J = 13.7, 6.9 Hz), 2.59 (1H, dd, J = 13.7, 6.9 Hz), 3.82 (3H, s), 5.51 (1H, s), 6.56 (1H, d, J = 1.8 Hz), 6.57−6.63 (4H, m), 6.82 (1H, d, J = 8.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.7, 13.8, 37.3, 37.8, 40.9, 41.1, 55.7, 101.1 (dd, J = 24.9, 14.9 Hz), 111.2, 111.6 (J = 17.8, 6.8 Hz), 114.0, 121.6, 133.1, 143.6, 145.7 (dd, J = 8.7, 8.7 Hz), 146.3, 162.8 (dd, J = 247.3, 12.5 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ−111.9; MS (EI) m/z 320 (M^+, 45), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{22}O_2F_2$ 320.1587, found 320.1593.

(8S,8′S)-3,4,5-Trifluoro-3′-methoxylignan-4′-ol (**40**): colorless oil; $[\alpha]^{25}_D$ +25 (c 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (6H, d, J = 7.4 Hz), 1.72 (2H, m), 2.37 (1H, dd, J = 13.7, 8.3 Hz), 2.38 (1H, dd, J = 13.7, 8.2 Hz), 2.53 (1H, dd, J = 13.7, 6.9 Hz), 2.55 (1H, dd, J = 13.7, 6.9 Hz), 3.84 (3H, s), 5.50 (1H, s), 6.56 (1H, d, J = 1.8 Hz), 6.58 (1H, dd, J = 8.3, 1.8 Hz), 6.67 (2H, dd, J = 8.5, 6.7 Hz), 6.82 (1H, d, J = 8.3 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.6, 13.8, 37.2, 37.8, 40.7, 40.9, 55.7, 111.1, 112.6 (dd, J = 15.3, 4.8 Hz), 114.0, 121.5, 133.0, 137.9 (dd, J = 11.5, 6.7 Hz), 137.9 (ddd, J = 249.2, 15.4 15.3 Hz), 143.7, 146.3, 150.8 (ddd, J =

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Table 1. Antifungal Activities of All Stereoisomers of DGA and 7-Derivatives of (+)-DGA[a]

compd no.	compd (*R*)	*Alternaria alternata* Japanese pear pathotype		*Bipolaris oryzae*	*Colletotrichum lagenarium*	*Fusarium solani*
1	(+)-DGA (3-CH_3O-4-HOPh)	62.0 ± 4.19%	EC_{50}: 71.8 ± 5.10 μM	86.5 ± 4.33%	77.1 ± 4.06%	85.2 ± 0.71%
2	(−)-DGA	50.9 ± 7.80%	EC_{50}: 51.5 ± 0.99 μM	83.8 ± 1.93%	73.7 ± 7.65%	80.2 ± 1.65%
3	*meso*-DGA	53.5 ± 6.54%	EC_{50}: 67.7 ± 20.9 μM	85.2 ± 5.66%	71.9 ± 5.41%	85.1 ± 1.99%
4	cyclohexyl	82.1 ± 5.01%	>1000 μM (59.2 ± 1.71%)[b]	88.0 ± 6.78%	79.2 ± 2.53%	96.5 ± 0.72%
5	Ph	32.1 ± 5.47%	EC_{50}: 33.6 ± 4.42 μM	90.0 ± 4.78%	58.2 ± 5.43%	89.8 ± 1.10%
6	2-HOPh	44.1 ± 2.47%	EC_{50}: 50.2 ± 1.15 μM	71.5 ± 5.72%	42.7 ± 4.12%	69.6 ± 1.94%
7	2-CH_3OPh	67.4 ± 2.92%	EC_{50}: 92.6 ± 18.4 μM	82.3 ± 5.06%	75.6 ± 0.92%	87.6 ± 1.65%
8	2-CH_3Ph	70.6 ± 3.64%	EC_{50}: 146 ± 40.8 μM	69.7 ± 1.93%	69.9 ± 3.96%	89.2 ± 1.91%
9	2-FPh	27.6 ± 0.52%	EC_{50}: 31.1 ± 3.39 μM	83.1 ± 7.62%	71.4 ± 2.16%	90.8 ± 2.22%
10	2-ClPh	30.4 ± 0.84%	EC_{50}: 44.5 ± 11.4 μM	74.7 ± 3.26%	73.1 ± 2.68%	92.6 ± 0.69%
11	3-HOPh	52.7 ± 3.23%	EC_{50}: 58.0 ± 2.96 μM	76.3 ± 6.49%	75.0 ± 5.65%	84.7 ± 3.43%
12	3-CH_3OPh	51.5 ± 4.49%	EC_{50}: 48.7 ± 6.97 μM	90.7 ± 7.36%	66.6 ± 4.23%	92.5 ± 1.39%
13	3-CH_3Ph	68.3 ± 4.70%	EC_{50}: 131 ± 31.5 μM	72.8 ± 3.13%	77.2 ± 3.78%	92.0 ± 1.81%
14	3-FPh	28.5 ± 0.92%	EC_{50}: 20.4 ± 1.32 μM	74.5 ± 4.13%	71.1 ± 3.35%	86.6 ± 1.94%
15	3-ClPh	30.1 ± 1.64%	EC_{50}: 24.6 ± 1.82 μM	74.3 ± 2.75%	75.8 ± 1.66%	88.2 ± 0.73%
16	4-HOPh	51.6 ± 3.33%	EC_{50}: 63.5 + 3.13 μM	80.2 ± 8.24%	64.0 ± 10.9%	85.8 ± 2.08%
17	4-CH_3OPh	60.8 ± 3.20%	EC_{50}: 79.1 ± 22.4 μM	79.5 ± 2.31%	76.0 ± 7.29%	94.2 ± 1.98%
18	4-CH_3Ph	57.6 ± 8.05%	EC_{50}: 125 ± 38.7 μM	79.3 ± 5.26%	79.1 ± 3.05%	91.7 ± 0.16%
19	4-FPh	28.0 ± 0.20%	EC_{50}: 27.9 ± 2.79 μM	78.0 ± 3.95%	80.7 ± 1.96%	90.8 ± 2.49%
20	4-ClPh	37.0 ± 4.75%	EC_{50}: 30.3 ± 3.16 μM	74.6 ± 6.62%	73.0 ± 5.63%	92.7 ± 0.88%
21	4-CF_3OPh	71.2 ± 2.44%,	EC_{50}: 135 ± 26.5 μM	82.8 ± 6.39%	79.8 ± 0.13%	95.5 ± 1.98%
22	3,4-HOPh	58.5 ± 6.70%	EC_{50}: 48.6 ± 5.99 μM	117 ± 4.47%	75.5 ± 2.67%	96.0 ± 1.18%
23	3,5-HOPh	82.6 ± 3.64%	EC_{50}: 79.0 ± 6.01 μM	76.0 ± 7.40%	93.5 ± 5.08%	91.9 ± 1.53%
24	3,4-CH_3OPh	62.5 ± 2.68%,	EC_{50}: 89.2 ± 17.5 μM	80.9 ± 5.07%	79.2 ± 10.3%	84.1 ± 2.60%
25	3,4-OCH_2OPh	63.0 ± 4.86%	EC_{50}: 125 ± 34.6 μM	88.4 ± 6.17%	71.8 ± 7.66%	92.6 ± 0.78%
26	3,4-CH_3Ph	70.4 ± 5.59%	EC_{50}: 235 ± 33.1 μM	88.3 ± 4.53%	74.7 ± 3.32%	90.2 ± 0.33%
27	3,4-ClPh	39.2 ± 5.44%	EC_{50}: 28.0 ± 4.81 μM	84.6 ± 7.17%	73.8 ± 1.26%	90.6 ± 0.33%
28	3-HO-4-CH_3OPh	51.8 ± 3.11%	EC_{50}: 106 ± 11.7 μM	82.8 ± 6.39%	69.1 ± 7.82%	84.2 ± 2.65%
29	3-HO-4-CH_3CH_2OPh	44.2 ± 8.16%	EC_{50}: 89.8 ± 27.7 μM	72.4 ± 6.57%	56.5 ± 10.7%	79.7 ± 1.04%
30	3-HO-4-$(CH_3)_2C(H)O$Ph	50.5 ± 2.47%	EC_{50}: 76.4 ± 14.5 μM	83.6 ± 7.36%	58.1 ± 6.74%	83.1 ± 2.58%
31	3-HO-4-$CH_3(CH_2)_3O$Ph	51.0 ± 4.29%	EC_{50}: 79.5 ± 15.1 μM	80.0 ± 6.99%	75.2 ± 2.68%	93.5 ± 1.36%
32	3-CH_3CH_2O-4-HOPh	42.6 ± 1.32%	EC_{50}: 51.2 ± 11.8 μM	74.9 ± 3.07%	62.8 ± 5.84%	77.4 ± 2.61%
33	3-$(CH_3)_2C(H)O$-4-HOPh	54.9 ± 3.11%	EC_{50}: 80.2 ± 6.50 μM	80.8 ± 6.73%	53.4 ± 1.23%	86.6 ± 1.25%
34	3-$CH_3(CH_2)_3O$-4-HOPh	47.0 ± 5.47%	EC_{50}: 48.6 ± 14.9 μM	76.9 ± 4.48%	66.7 ± 3.77%	81.7 ± 1.15%
35	3-Cl-4-HOPh	22.6 ± 6.25%	EC_{50}: 26.3 ± 3.30 μM	72.8 ± 6.88%	55.3 ± 9.15%	70.0 ± 1.07%
36	3-Cl-4-CH_3OPh	43.7 ± 2.98%	EC_{50}: 63.4 ± 10.0 μM	86.0 ± 2.89%	80.6 ± 1.75%	91.2 ± 2.17%
37	3,5-FPh	23.9 ± 1.98%	EC_{50}: 15.5 ± 3.23 μM	80.1 ± 1.93%	66.2 ± 3.48%	80.3 ± 1.47%
38	3,4,5-HOPh	87.3 ± 3.80%	>1000 μM (52.3 + 0.75%)[b]	103 ± 11.2%	90.2 ± 6.13%	96.6 ± 1.10%
39	3,5-CH_3O-4-HOPh	60.7 ± 3.68%	EC_{50}: 68.5 ± 18.5 μM	79.1 ± 5.75%	81.8 ± 7.37%	82.6 ± 2.15%
40	3,4,5-FPh	29.4 ± 1.13%	EC_{50}: 21.9 ± 0.27 μM	72.9 ± 5.11%	70.8 ± 3.01%	79.2 ± 1.83%
41	2,3,5,6-FPh	25.3 ± 1.38%	EC_{50}: 18.7 ± 3.53 μM	63.2 ± 3.48%	59.1 ± 5.25%	82.4 ± 1.63%

[a]Growth percent from control ± SE (n = 3) at 62.5 mM against each fungus and EC_{50} values against *Alternaria alternata* Japanese pear pathotype. Thiabendazol as positive control: EC_{50} = 5.5 μM against *A. alternata* Japanese pear pathotype. [b]Growth rate at 1000 μM.

248.3, 9.6, 4.8 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ −136.2, −166.5; MS (EI) m/z 338 (M^+, 42), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{21}O_2F_3$ 338.1492, found 338.151.

*(8S,8′S)-2,3,5,6-Tetrafluoro-3′-methoxylignan-4′-ol (**41**):* colorless oil; $[\alpha]^{25}_D$ + (*c* 0.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.85 (3H, d, *J* = 7.3 Hz), 0.87 (3H, d, *J* = 6.9 Hz), 1.76 (1H, m), 1.85 (1H, m), 2.38 (1H, dd, *J* = 13.7, 8.7 Hz), 2.56−2.63 (2H, m), 2.74 (1H, dd, *J* = 13.3, 6.0 Hz), 3.84 (3H, s), 5.51 (1H, s), 6.59 (1H, dd, *J* = 8.7, 1.8 Hz), 6.60 (1H, d, *J* = 1.8 Hz), 6.80 (1H, d, *J* = 8.7 Hz), 6.89 (1H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.8, 14.0, 28.4, 36.6, 38.9, 40.8, 55.8, 103.4 (dd, *J* = 23.0, 23.0 Hz), 111.2, 114.0, 120.9 (dd, *J* = 18.3, 18.3 Hz), 121.5, 133.0, 143.6, 144.9 (dddd, *J* = 243.3, 13.5, 6.7, 3.9 Hz), 145.7 (dddd, *J* = 246.4, 15.4, 10.6, 2.8 Hz), 146.3; ^{19}F NMR (376 MHz, $CDCl_3$) δ −140.5, −144.0; MS (EI) m/z 356 (M^+, 42), 137 (100); HRMS (EI) m/z calcd for $C_{19}H_{20}O_2F_4$ 356.1396, found 356.1397.

Fungal Strains. *A. alternata* Japanese pear pathotype, *Bipolaris oryzae*, *Colletotrichum lagenarium*, and *Fusarium solani* are stored strains at Ehime University. *A. alternata* apple pathotype (MAFF305016) and *Alternaria citri* (MAFF242828) were purchased from NIAS Genebank. Each fungal strain was cultured on potato dextrose agar (PDA, Sigma-Aldrich, Canada).

Antifungal Assay. Thirty microliters of dimethyl sulfoxide solution containing each test compound was added to 3 mL of PDA at 50 °C, followed by rapid mixing, and the resultant mixture was poured into a Petri dish (diameter = 50 mm) to prepare the PDA agar plate containing the test compound. Dimethyl sulfoxide only (without any test compound) served as the control. After inoculation of each strain on the center of the PDA agar plate and incubation at 28 °C for 3 days for *A. alternata*, *A. citri*, *B. oryzae*, and *F. solani* and for 5 days for *C. lagenarium*, respectively, the diameter of the mycelial colony was measured with a caliper. For screening process, the growth ratio (%) of the mycelial colony applied with the test compound at the appropriate concentration (250 or 62.5 μM) compared with the control was calculated. For QSAR analysis, the value of EC_{50} (effective concentration for inducing 50% growth ratio compared with the control) was calculated using PRISM software ver. 5.0 (GraphPad Software Inc., San Diego, CA, USA) as

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biological activity and reciprocal logarithm of the EC_{50} value (pEC_{50}) was used. All of the assays were performed in triplicate.

QSAR Analysis Using the Hansch–Fujita Method.[13,14] The relationship between the structure of the test compounds and their antifungal activity was quantitatively analyzed using QREG ver. 2.05.[15] For the Hansch–Fujita method, σ_I, which is the inductive component of Hammett σ, was employed as the electrostatic parameter.[16] The higher the values of σ_I, the more electron-withdrawing feature the substituent, for example, because the values of σ_I for CH_3 and F groups are −0.01 and 0.54, respectively (Table 2), the F group is more electron-withdrawing.

Table 2. Parameter Values of Each Substituent

	parameters	
substituent	σ_I	ΔB_5
H	0.00	0.00
OH	0.24	0.93
OCH_3	0.30	2.07
OCH_2O	0.60	4.14
OCH_2CH_3	0.28	2.36
$OC(H)(CH_3)_2$	0.26	3.10
$O(CH_2)_3CH_3$	0.28	3.79
CH_3	−0.01	1.04
F	0.54	0.35
OCF_3	0.39	2.61
Cl	0.47	0.80

As the steric parameter, B_5, which is the maximum width parameter of Verloop's STERIMOL parameters,[16] was used, and ΔB_5 was calculated by subtracting the B_5 value of hydrogen, 1, from the intact B_5 value of each substituent. The values of the physicochemical parameters employed are listed in Table 2, and the correlation among the parameters is shown in Table 3.

RESULTS AND DISCUSSION

The antifungal activities of (+)-DGA, (−)-DGA, and *meso*-DGA (growth ratio (%) at 250 μM compared with the control) were

Table 3. Parameter Correlation (Upper Right, γ; Lower Left, γ^2)

	σ_I	σ_I^3	σ_I^4	ΔB_5^o	ΔB_5^m	ΔB_5^p
σ_I^2		−0.148	−0.364	0.618	−0.286	−0.344
σ_I^3	0.022		0.078	−0.298	0.374	0.013
σ_I^4	0.132	0.006		−0.368	0.123	0.475
ΔB_5^o	0.382	0.089	0.135		−0.324	−0.348
ΔB_5^m	0.082	0.140	0.015	0.105		0.122
ΔB_5^p	0.118	0.000	0.225	0.121	0.015	

38.3 ± 3.64, 32.2 ± 1.40, and 29.8 ± 6.14% against *A. alternata* Japanese pear pathotype, 49.9 ± 0.45, 49.9 ± 1.31, and 53.7 ± 1.13% against *B. oryzae*, 19.9 ± 2.35, 33.8 ± 4.34, and 26.0 ± 6.00% against *C. lagenarium*, and 34.5 ± 2.61, 36.5 ± 3.54, and 45.8 ± 6.57% against *F. solani*, respectively. Significant difference was not observed in three stereoisomers. To examine whether any fungal strain should be proper for further QSAR analysis of DGA derivatives, 38 (+)-DGA derivatives were synthesized, and their antifungal activities against four phytopathogenic fungi were evaluated at the lower concentration, 62.5 μM (Table 1). Three stereoisomers of DGA showed higher activity against *A. alternata* than the other three phytopathogenic fungi. Also, most of the derivatives suppressed the growth ratio of *A. alternata* below 70%, and the effect of the substituents on the activity against *A. alternata* was more apparent than against the other fungal strains. These results prompted us to select *A. alternata* in further research. The EC_{50} values of three stereoisomers of DGA and synthesized (+)-DGA derivatives are also shown in Table 1. The lesser potency of 7-cyclohexyl derivative **4** indicates the importance of the benzene ring for the activity. Derivative **5**, bearing no substituent on the 7-phenyl group, showed about 2-fold higher activity than three stereoisomers. By comparison of derivatives **6**–**21** bearing one substituent on the phenyl group with derivative **5**, the activity of the hydroxyphenyl (**6**, **11**, **16**) and methoxyphenyl (**7**, **12**, **17**) derivatives was 1.5–2.8-fold lower than that of unsubstituted phenyl derivative **5**, and the activity of methylphenyl derivatives (**8**, **13**, **18**) was 4-fold lower. On the other hand, the activity of fluorophenyl (**9**, **14**, **19**) and chlorophenyl (**10**, **15**, **20**) derivatives was comparable to or higher than that of phenyl derivative **5**. It could be assumed that the presence of an electron-withdrawing group is favorable for the higher activity. The EC_{50} values of 3-fluorophenyl derivative **14** (20.4 μM) and 3-chlorophenyl derivative **15** (24.6 μM) were a little smaller than those of the other mono fluorophenyl or chlorophenyl derivatives, the presence of an electron-withdrawing group at the 3-position being important for the higher activity. At every position, the derivatives bearing a fluorine atom displayed higher activity than the corresponding chlorophenyl derivatives. The larger 4-trifluoromethoxyphenyl derivative **21** was less potent, even though the substituent is a moderately electron-withdrawing group. By comparison of the activities of methoxyphenyl derivatives **7**, **12**, and **17**, the 3-methoxyphenyl derivative **12** showed highest activity. Among the disubstituted phenyl derivatives **22**–**37**, the 3,5-difluorophenyl derivative **37**, which had the smallest electron-withdrawing group on both meta-positions, showed the highest activity (EC_{50} = 15.5 μM), whereas the 3,4-dimethylphenyl derivative **26**, which had two electron-donating groups on meta- and para-positions, showed the lowest activity (EC_{50} = 235 μM). Comparison among the derivatives **22**-**26** showed little potent activity of the dihydroxy derivatives (**22**, **23**), whereas the dimethoxy and methylenedioxy derivatives (**24**, **25**) were less potent than derivatives **22** and **23**, and the 3,4-dimethylphenyl derivative **26** had the lowest potency. The antifungal activities of 3,4-dichlorophenyl derivative **27** and 3-chloro-4-hydroxyphenyl derivative **35** were almost the same as that of 3-clorophenyl derivative **15**, demonstrating that an additional substituent such as a hydroxy or chloro group at the 4-position of the 3-chlorophenyl moiety did not change the activity. The activity of 3-hydroxy-4-methoxyphenyl derivative **28**, having positions of substituents that were exchanged from that of (+)-DGA, was less potent, suggesting the positions of the substituents influence the activity and that the bulky substituent is more tolerable on the 3-position than on the 4-position. It seems compatible for the derivatives **29**–**34**, because derivatives **29** and **31** (4-ethoxy-3-hydroxyphenyl and 4-butoxy-3-hydroxyphenyl derivatives) were less potent than the corresponding derivatives **32** and **34** (3-ethoxy-4-hydroxyphenyl and 3-butoxy-4-hydroxyphenyl derivatives). Derivatives **29**–**34** bearing a bulkier or longer substituent at the 3- or 4-position were found to be less potent than both phenyl derivative **5** and 3-halophenyl derivatives **14** and **15**. The bulkier and longer group seems disadvantageous for the higher activity. Comparing the activity of 3-chloro-4-hydroxyphenyl derivative **35** with that of 3-chloro-4-methoxyphenyl derivative **36** revealed that 3-chloro-4-hydroxyphenyl derivative **35** was more potent, which might be due to the steric effect of the methoxy group. Among the tri- and tetrasubstituted compounds **38**–**41**, 3,4,5-trifluorophenyl derivative **40** and 2,3,5,6-tetrafluorophenyl derivative **41** were more

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Table 4. Observed and Expected pEC_{50} Values by Analyzed Equation

compd no.	compd (*R*)	obsd pEC_{50}	calcd pEC_{50}	Δ	compd no.	compd (*R*)	obsd pEC_{50}	calcd pEC_{50}	Δ
1	(+)-DGA (3-CH_3O-4-HOPh)	4.14	4.26	−0.12	**23**	3,5-HOPh	4.10	4.23	−0.13
5	Ph	4.47	4.19	0.28	**24**	3,4-CH_3OPh	4.05	4.11	−0.06
6	2-HOPh	4.30	4.16	0.14	**25**	3,4-OCH_2OPh	3.90	4.11	−0.21
7	2-CH_3OPh	4.03	3.98	0.05	**26**	3,4-CH_3Ph	3.63	3.91	−0.28
8	2-CH_3Ph	3.83	3.98	−0.15	**27**	3,4-ClPh	4.55	4.68	−0.14
9	2-FPh	4.51	4.47	0.05	**28**	3-HO-4-CH_3OPh	3.97	4.16	−0.19
10	2-ClPh	4.35	4.33	0.02	**29**	3-HO-4-CH_3CH_2OPh	4.05	4.10	−0.05
11	3-HOPh	4.24	4.31	−0.07	**30**	3-HO-4-$(CH_3)_2C(H)O$Ph	4.12	3.97	0.15
12	3-CH_3OPh	4.31	4.26	0.05	**31**	3-HO-4-$CH_3(CH_2)_3O$Ph	4.10	3.87	0.23
13	3-CH_3Ph	3.88	4.08	−0.20	**32**	3-CH_3CH_2O-4-HOPh	4.29	4.21	0.08
14	3-FPh	4.69	4.63	0.06	**33**	3-$(CH_3)_2C(H)O$-4-HOPh	4.10	4.12	−0.03
15	3-ClPh	4.61	4.53	0.08	**34**	3-$CH_3(CH_2)_3O$-4-HOPh	4.31	4.08	0.23
16	4-HOPh	4.20	4.18	0.02	**35**	3-Cl-4-HOPh	4.58	4.53	0.05
17	4-CH_3OPh	4.10	4.04	0.07	**36**	3-Cl-4-CH_3OPh	4.20	4.19	0.01
18	4-CH_3Ph	3.90	4.01	−0.11	**37**	3,5-FPh	4.83	4.60	0.23
19	4-FPh	4.56	4.46	0.10	**39**	3,5-CH_3O-4-HOPh	4.16	4.06	0.10
20	4-ClPh	4.52	4.35	0.17	**40**	3,4,5-FPh	4.74	4.87	−0.14
21	4-CF_3OPh	3.87	4.00	−0.13	**41**	2,3,5,6-FPh	4.66	4.81	−0.15
22	3,4-HOPh	4.31	4.31	0.00					

Table 5. Antifungal Activity against *Alternaria alternata* Apple Pathotype and *Alternaria citri*

compd no.	compd (*R*)	*Alternaria alternata* apple pathotype (EC_{50}, μM)	*Alternaria citri* (EC_{50}, μM)
1	(+)-DGA	56.3 ± 1.77	62.1 ± 5.88
2	(−)-DGA	50.7 ± 1.07	55.3 ± 5.27
3	*meso*-DGA	49.7 ± 2.07	50.7 ± 2.65
5	Ph	87.2 ± 12.9	75.1 ± 5.09
6	2-HOPh	50.1 ± 2.49	nt[a]
9	2-FPh	84.0 ± 3.05	62.0 ± 0.79
10	2-ClPh	93.9 ± 10.3	65.9 ± 2.60
11	3-HOPh	62.0 ± 6.62	nt
14	3-FPh	64.9 ± 7.48	47.3 ± 3.11
15	3-ClPh	85.8 ± 6.03	59.3 ± 2.85
16	4-HOPh	52.7 ± 8.08	nt
19	4-FPh	75.5 ± 9.20	60.3 ± 4.71
20	4-ClPh	>1000 (61.3 ± 3.75)[b]	134 ± 10.9
22	3,4-HOPh	135 ± 11.7	nt
27	3,4-ClPh	>1000 (81.6 ± 0.54)[b]	>1000 (61.7 ± 1.02)[b]
35	3-Cl-4-HOPh	36.9 ± 1.81	50.0 ± 5.46
37	3,5-F	46.8 ± 5.64	36.4 ± 3.01
40	3,4,5-F	46.0 ± 3.93	38.8 ± 5.13
41	2,3,5,6-F	45.5 ± 6.58	50.7 ± 9.08

[a]nt, not tested. [b]Growth rate at 1000 μM.

potent than 3,4,5-trihydroxyphenyl derivative **38** and 3,5-dimethoxy-4-hydroxyphenyl derivative **39**, suggesting that the electron-withdrawing group should be favorable to the activity. However, the activities of 3,4,5-trifluorophenyl derivative **40** and 2,3,5,6-tetrafluorophenyl derivative **41** are comparable to those of 3-fluorophenyl derivative **14** and 3,5-difluorophenyl derivative **37**, meaning that the increase of the electron-withdrawing group does not achieve much higher activity. The EC_{50} value of derivative **38** bearing three hydroxy groups was more >1000 μM, which was >50-fold less potent than that of the most potent 3-fluorophenyl derivative **14**.

The relationship between the structure and antifungal activity was quantitatively analyzed using the Hansch−Fujita method[13,14] shown as the following equation:

$$pEC_{50} = 4.188(\pm 0.125) + 0.639(\pm 0.453)\sigma_I^{\,2} + 0.888(\pm 0.297)\sigma_I^{\,3} + 0.613(\pm 0.348)\sigma_I^{\,4} - 0.193(\pm 0.168)\Delta B_5^{\,o} - 0.093(\pm 0.054)\Delta B_5^{\,m} - 0.162(\pm 0.061)\Delta B_5^{\,p}$$

$$n = 37,\ \mathrm{SD} = 0.154,\ r = 0.873,\ F(6, 30) = 16.067,\ q^2 = 0.625$$

In this equation, $\sigma_I^{\,2}$, $\sigma_I^{\,3}$, and $\sigma_I^{\,4}$ were employed as the electrostatic parameter on the 2-, 3-, and 4-positions, respectively, and $B_5^{\,o}$, $B_5^{\,m}$, and $B_5^{\,p}$ as steric parameter on the ortho, meta, and para positions, respectively. The term Δ*B* represents the value relative to that of the hydrogen atom. The figures of *n*, SD, and *r* are the number of compounds, standard

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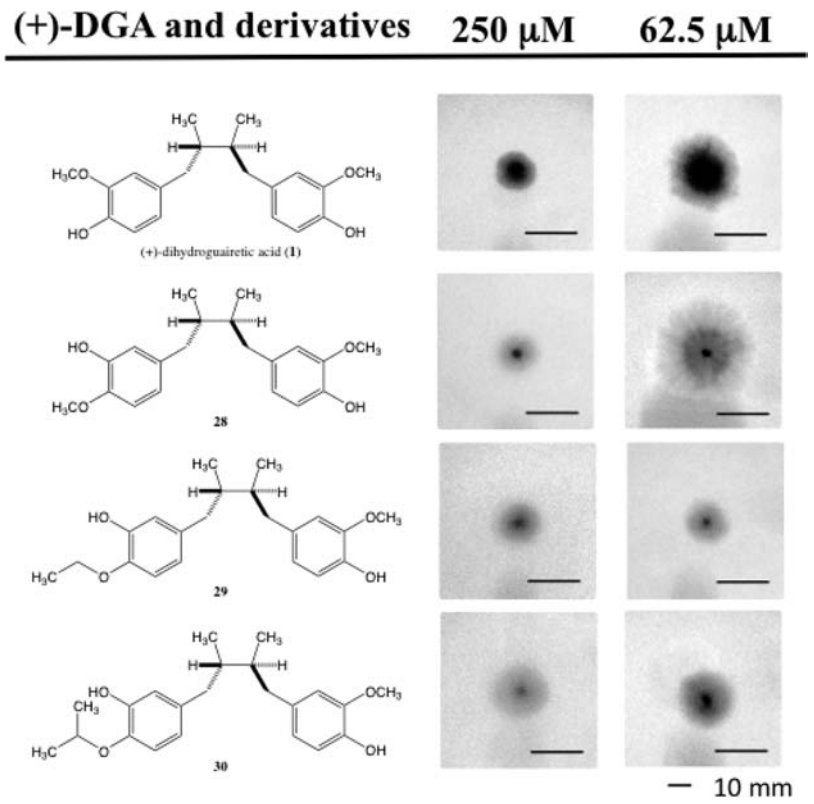

Figure 2. Whitening activity of 3-HO-4-CH_3OPh derivative **28**, 3-HO-4-CH_3CH_2OPh derivative **29**, and 3-HO-4-$(CH_3)_2$C(H)OPh derivative **30** against *Alternaria alternata* Japanese pear pathotype.

deviation, and correlation coefficient, respectively. F is the ratio between the regression and residual variances. Cross-validated q^2 is the correlation coefficient obtained from the leave-one-out cross-validation. The number in parentheses is the 95% confidence interval. The 3,4-methylenedioxyphenyl derivative **25** was calculated as 3,4-dimethoxyphenyl. In this equation, the coefficient of the σ_I term is positive, demonstrating that the higher value of σ_I caused by the electron-withdrawing group is advantageous for higher activity. The negative values of the coefficient of ΔB_5^o, ΔB_5^m, and ΔB_5^p terms demonstrate that the presence of any substitute gave a smaller value of pEC_{50}, showing lower activity. Even if some values of ΔB_5^o, ΔB_5^m, and ΔB_5^p are given, a larger value of σ_I could contribute to the larger value of pEC_{50}, the higher activity being observed. The largest coefficient of σ_I^3 term among the electrostatic parameters in the equation suggests that the presence of electron-withdrawing group at the 3-position should contribute to the higher activity. Also, the coefficient of ΔB_5 for the meta position is the smallest among the steric parameters, suggesting that the higher activity would be achieved by introduction of a small electron-withdrawing group to the meta position. As the electrostatics parameter, Hammett σ and σ^0 did not improve the equation (data not shown), suggesting that the resonance effect through the benzene ring of the DGA derivatives might not be related to their active site and that localized potential just at the substituent might be important, although the active site of these DGA derivatives should be cleared in the future. The difference between the observed and calculated pEC_{50} values is shown in Table 4.

The effective fluorophenyl and chlorophenyl compounds **9**, **10**, **14**, **15**, **19**, **20**, **27**, **35**, **37**, **40**, **41**, phenyl derivative **5**, and all stereoisomers of DGA (**1**−**3**) were applied to *A. alternata* apple pathotype and *A. citri* (Table 5). To compare with hydroxyphenyl derivatives, derivatives **6**, **11**, **16**, and **22** were also examined. All stereoisomers of DGA showed almost the same level of activity as against Japanese pear pathotype. The 3-chloro-4-hydroxyphenyl derivative **35** showed the highest activity against *A. alternata* apple pathotype; however, 3-chlorophenyl derivative **15**, 4-hydroxyphenyl derivative **16**, 3,4-dihydroxyphenyl derivative **22**, and 3,4-dichlorophenyl derivative **27** were less effective. The activities of all fluorophenyl derivatives, which exhibited higher activity against Japanese pear pathotype, were less potent than that of **35**, suggesting the combination of 3-chlorine and 4-hydroxy groups was important for the higher activity against *A. alternata* apple pathotype. On the other hand, the 3,5-difluorophenyl derivative **37** showed the highest activity against *A. citri*, and 3-fluorophenyl derivative **14** was most potent among the mono halo derivatives, the presence of fluorine on the *m*-position being potent against *A. citri*. However, the activities of all compounds were weaker than against *A. alternata* Japanese pear pathotype.

In this experiment, 3-hydroxy-4-methoxyphenyl derivative **28**, 3-hydroxy-4-ethoxyphenyl **29**, and 3-hydroxy-4-isopropoxyphenyl derivative **30** showed whitening activity, antimelanogenic activity, against *A. alternata* Japanese pear pathotype at 250 and 62.5 μM (Figure 2). This activity was also observed after addition of 5−500 μM 1,8-dihydroxynaphthalene (DHN)[17] to the culture containing **28**−**30**, assuming that the polymerization process of DHN in the melanin biosynthesis might be inhibited by these compounds. The feature of the structure showing whitening activity is not 3-alkoxy-4-hydroxyphenyl found in DGA, but 3-hydroxy-4-alkoxyphenyl bearing a shorter alkoxy substituent on the 4-position. This antimelanogenic activity did not affect the antifungal activity. Although the antimelanogenic activity of some other lignans has been reported,[18] this is the first report of

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the antimelanogenic activity of dihydroguaiaretic acid derivatives.

In summary, the quantitative structure−antifungal activity relationship analysis of traditional lignan, which is widely distributed in food plants, was performed by using the Hansch−Fujita method. The 3,5-difluorophenyl derivative **37** showed 4.5-fold greater activity than that of natural (+)-DGA (**1**). It was suggested that the smaller electron-withdrawing group at the 3-position caused higher activity. This is the first report of quantitative structure−activity relationship analysis of simple lignan, which is linked by only the β,β'-bond of two phenylpropanoid units.[11] This result would contribute to the design and synthesis of more active compounds based on the widely distributed simple natural lignan structure. The establishment of an assay system using leaves is a future project.

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REFERENCES

(1) Ayres, D. C.; Loike, J. D. Aregistry of natural lignans. In *Lignans*; Cambridge University Press: Cambridge, UK, 1990; pp 12−84.

(2) Ward, S. Lignans, neolignan and related compounds. *Nat. Prod. Rep.* **1999**, *16*, 75−96.

(3) Satricia, P.; Young, M. C. M.; Kato, M. J. Antifungal lignans from the arils of *Virola oleifera*. *Phytochemistry* **1998**, *47*, 1003−1006.

(4) Cho, J. Y.; Choi, G. J.; Son, S. W.; Jang, K. S.; Lim, H. K.; Lee, S. O.; Sung, N. D.; Cho, K. Y.; Kim, J.-C. Isolation and antifungal activity of lignans from *Myristica fragrans* against various plant pathogenic fungi. *Pest Manag. Sci.* **2007**, *63*, 935−940.

(5) Kumar, K. A.; Singh, S. K.; Kumar, B. S.; Doble, M. Synthesis, antifungal activity evaluation and QSAR studies on podophyllotoxin derivatives. *Cent. Eur. J. Chem.* **2007**, *5*, 880−897.

(6) Akiyama, K.; Yamauchi, S.; Nakato, T.; Maruyama, M.; Sugahara, T.; Kishida, T. Antifungal activity of tetra-substituted tetrahydrofuran lignan, (−)-virgatusin, and its structure-activity relationship. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 1028−1035.

(7) Nishiwaki, H.; Ouchi, M.; Matsugi, J.; Akiyama, K.; Sugahara, T.; Kishida, T.; Yamauchi, S. Effect of substituents at phenyl group of 7,7′-dioxo-9,9′-epoxylignane on antifungal activity. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6740−6744.

(8) Kawaguchi, Y.; Yamauchi, S.; Masuda, K.; Nishiwaki, H.; Akiyama, K.; Maruyama, M.; Sugahara, T.; Kishida, T.; Koba, Y. Antimicrobial activity of stereoisomers of butane-type lignans. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 1806−1810.

(9) Akiyama, K.; Yamauchi, S.; Maruyma, M.; Sugahara, T.; Kishida, T.; Koba, Y. Antimicrobial activity of stereoisomers of morinols A and B, tetrahydropyran sesquineolignans. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 129−133.

(10) Yamauchi, S.; Masuda, T.; Sugahara, T.; Kawaguchi, Y.; Ohuchi, M.; Someya, T.; Akiyama, J.; Tominaga, S. *Biosci., Biotechnol., Biochem.* **2008**, *72*, 2981−2986.

(11) Moss, G. P. Nomenclature of lignans and neolignans. *Pure Appl. Chem.* **2000**, *72*, 1493−1523.

(12) Nishiwaki, H.; Hasebe, A.; Kawaguchi, Y.; Akamatsu, M.; Shuto, Y.; Yamauchi, S. Larvicidal activity of (−)-dihydroguaiaretic acid derivatives against *Culex pipiens*. *Biosci., Biotechnol., Biochem.* **2011**, *75*, 1735−1739.

(13) Fujita, T.; Iwasa, J.; Hansch, C. A new substituent, π, derived from partition coefficients. *J. Am. Chem. Soc.* **1964**, *86*, 5175−5180.

(14) Okimoto, H.; Lim, C.-H.; Nishimura, K.; Ueno, T. Structure-activity analysis of insecticidal organosila pyrethroid-like ethers against American cockroaches. *J. Pestic. Sci.* **1995**, *20*, 9−15.

(15) Asao, M.; Shimizu, R.; Nakao, K.; Fujita, T. *QREG 2.05. Japan Chemistry Program Exchange*; Society of Computer Chemistry, Japan, 1997.

(16) Hansch, C.; Leo, A. *Exploring QSAR Fundamentals and Applications in Chemistry and Biology*; ACS Professional Reference Book; American Chemical Society: Washington, DC, 1995.

(17) Tanabe, K.; Park, P.; Tsuge, T.; Kohmoto, K.; Nishimura, S. Characterization of the mutants of *Alternaria alternata* Japanese pear pathotype deficient in melanin production and their pathogenicity. *Ann. Phytopathol. Soc. Jpn.* **1995**, *61*, 27−33.

(18) Kim, K. H.; Moon, E.; Kim, S. Y.; Lee, K. R. Lignans from tuber-barks of *Colocasia antiquorum* var. *esculenta* and their antimelanogenic activity. *J. Agric. Food Chem.* **2010**, *58*, 4779−4785.