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Regular Article

Effect of further substitutions at 5-, 6-, 7-, or 8-position of 3-[3-(4-methoxyphenyl)-1-hydroxyprop-2-yl]coumarin on phytotoxicity

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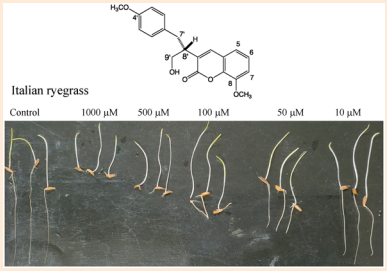
Supplementary material

Derivatives of the coumarin ring in (*R*)-3-[3-(4-methoxyphenyl)-1-hydroxyprop-2-yl]coumarin **2**, which is a lignan structure, were synthesized to clarify their structure–phytotoxicity relationships. The growth-inhibitory activity of the 8-OCH_3 derivative **8** (IC_{50}=228 μM) was more potent against the roots of lettuce seedlings than the compound without substituents **2**. As for the roots of Italian ryegrass seedlings, the presence of the methoxy group at the 7- or 8-position was extremely effective for inhibiting growth (7-OCH_3 **7**: IC_{50}=121 μM, 8-OCH_3 **8**: 56.7 μM). Methyl derivatives at the 5- or 8-position showed activity levels similar to those of the compound without substituents **2** (5-CH_3 **13**: IC_{50}=214 μM, 8-CH_3 **16**: IC_{50}=225 μM). The activities of OH- and F-derivatives were not observed or were lower.



Keywords: lignan, coumarin, 3-substituted coumarin, phytotoxicity, 2*H*-chromen-2-one.

Introduction

Phenprocoumon (**1**) (Fig. 1) is a well-known medicinal compound,[1] which has a coumarin structure with a phenylpropanoid unit, such as a lignan. In our biological research on coumarins bearing phenylpropanoid units, we have synthesized phytotoxic coumarin **2**[2] bearing a lignan structure, which has a bond between the 3-position of the coumarin structure and the 8′-position of the phenylpropanoid unit. It can be assumed that coumarin **2** bearing a lignan structure is transformed from *Z*-α-2-hydroxybenzylidene γ-butyrolactone **3** by *trans*-lactonization in the body of the plant. In our previous study, we observed the plant growth inhibitory activity of both coumarin **2**[2] bearing phenylpropanoid and a *Z*-α-2-hydroxybenzylidene γ-butyrolactone **4** compound.[3] Because of the lower pK_a value of the phenolic hydroxy group, the existence ratio of the coumarin type compound **2** bearing phenylpropanoid should be higher. Since the structure-plant growth inhibitory activity relationship for the 7′-phenyl group has been shown previously,[2] in this research we attempted to clarify the effect on phytotoxicity of substituents to the coumarin ring.

Since the 4-methoxy group, the primary hydroxy group, and the *R*-form in the phenylpropanoid unit are important for higher activity,[2] *R*-derivatives **5**–**20** with substituents at each position from the 5-position to the 8-position were synthesized (Fig. 2) to estimate their phytotoxicities. Electron donating, withdrawing, hydrophobic, and hydrophilic groups were employed as substituents to clarify the structure–activity relationship. Lettuce as a dicotyledon and Italian ryegrass as a monocotyledon were selected for the test plants. In addition to the previously discovered phytotoxic coumarin and isocoumarin compounds,[4–12] this research would provide novel phytotoxic coumarins bearing phenylpropanoids, which are lignan structures.

Materials and methods

Melting points (mp) data are uncorrected. Optical rotations were measured on a JASCO P-2100 instrument (JASCO Corporation, Japan). ^{1}H and ^{13}C NMR data were recorded on a JMS-EX400 spectrometer (JEOL, Tokyo, Japan) $CDCl_3$ as solvent

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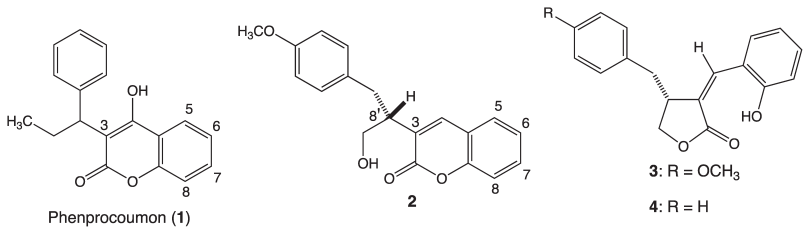


Fig. 1. Phenprocoumon (**1**), phytotoxic coumarin **2** bearing phenylpropanpnoid, *Z*-*α*-2-hydroxybenzylidene *γ*-butyrolactone **3**, which is a *trans*-lactonization compound from **2**, and phytotoxic *Z*-*α*-2-hydroxybenzylidene *γ*-butyrolactone **4**.

with TMS as reference. EIMS data were measured with a ESI-JMS-MS700V (JEOL, Tokyo, Japan). The numbering of compounds follows the IUPAC rule.

The derivatives **5**–**20** were synthesized by the previously reported synthetic method[2] with modification. The general synthetic method is described in supporting information.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-5-methoxy-2*H*-chromen-2-one 5. Colorless oil, $[\alpha]_D^{25}$–70 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 1.88 (1H, br s), 2.98 (1H, dd, *J*=13.8, 7.6 Hz), 3.02 (1H, dd, *J*=13.8, 7.6 Hz), 3.31 (1H, m), 3.77 (3H, s), 3.84 (2H, d, *J*=4.7 Hz), 3.92 (3H, s), 6.69 (1H, d, *J*=8.2 Hz), 6.80 (2H, d, *J*=8.3 Hz), 6.90 (1H, d, *J*=8.4 Hz), 7.13 (2H, d, *J*=8.3 Hz), 7.39 (1H, dd, *J*=8.4, 8.3 Hz), 7.92 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 34.9, 45.2, 55.2, 55.9, 63.7, 105.0, 108.8, 109.9, 2×113.8, 127.5, 2×130.0, 2×131.4, 135.1, 154.0, 155.8, 158.0, 162.0. MS (EI) *m*/*z* (%): 340 (15) M^+, 322 (10) $[M–H_2O]^+$, 219 (6) $[M–CH_3OPhCH_2]^+$, 202 (8) $[M–CH_3OPhCH_2OH]^+$, 174 (2) $[CH_3C(H)Coumarin]^+$, 138 (3) $[CH_3OPhCH_2OH]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{20}H_{20}O_5$: 340.1311, Found: 340.1316.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-6-methoxy-2*H*-chromen-2-one 6. Colorless crystals, mp 136–137°C (EtOH-hexane), $[\alpha]_D^{25}$–67 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 3.00 (2H, d, *J*=7.5 Hz), 3.29 (1H, m), 3.76 (3H, s), 3.83 (3H, s), 3.85 (2H, br s), 6.79 (2H, d, *J*=8.1 Hz), 6.85 (1H, s), 7.05 (1H, d, *J*=8.9 Hz), 7.11 (2H, d, *J*=8.1 Hz), 7.23 (1H, d, *J*=8.9 Hz), 7.46 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 34.8, 45.1, 55.2, 55.8, 63.5, 109.6, 2×113.8, 117.4, 118.7, 119.6, 129.8, 2×130.0, 131.2, 140.1, 147.4, 156.0, 158.0, 161.9; MS (EI) *m*/*z* (%): 340 (14) M^+, 322 (7) $[M–H_2O]^+$, 219 (2) $[M–CH_3OPhCH_2]^+$, 202 (6) $[M–CH_3OPhCH_2OH]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{20}H_{20}O_5$: 340.1311, Found: 340.1317.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-7-methoxy-2*H*-chromen-2-one 7. Colorless crystals, mp 108–110°C (EtOH), $[\alpha]_D^{25}$–95 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.06 (1H, br s), 2.98 (2H, d, *J*=7.6 Hz), 3.24 (1H, m), 3.75 (3H, s), 3.84 (2H, br s), 3.85 (3H, s), 6.77–6.82 (2H, m), 6.78 (2H, d, *J*=8.3 Hz), 7.10 (2H, d, *J*=8.3 Hz), 7.30 (1H, d, *J*=8.5 Hz), 7.44 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 34.9, 45.1, 55.2, 55.8, 63.6, 100.3, 112.6, 112.9, 2×113.8, 125.6, 128.4, 2×130.0, 131.4, 140.4, 154.7, 158.0, 2×162.2; MS (EI) *m*/*z* (%): 340 (19) M^+, 322 (10) $[M–H_2O]^+$, 309 (3) $[M–CH_3O]^+$, 219 (40) $[M–CH_3OPhCH_2]^+$, 202 (18) $[M–CH_3OPhCH_2OH]^+$, 191 (3) $[M–CH_3OPhCH_2CHCH_2]^+$, 161 (4) $[CH_3OPhCH_2CHCOH]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{20}H_{20}O_5$: 340.1311, Found: 340.1318.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-8-methoxy-2*H*-chromen-2-one 8. Colorless crystals, mp 42–45°C, $[\alpha]_D^{25}$–80 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 2.03 (1H, br s), 3.00 (2H, d, *J*=7.6 Hz), 3.30 (1H, m), 3.75 (3H, s), 3.84 (2H, br s), 3.94 (3H, s), 6.78 (2H, d, *J*=8.3 Hz), 6.98 (1H, d, *J*=8.0 Hz), 7.00 (1H, d, *J*=7.9 Hz), 7.10 (2H, d, *J*=8.3 Hz), 7.16 (1H, dd, *J*=8.0, 7.9 Hz), 7.47 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) *δ* 34.7, 45.2, 55.2, 56.2, 63.5, 112.8, 2×113.8, 119.0, 119.9, 124.2, 129.6, 2×130.0, 131.3, 140.4, 142.6, 146.9, 158.0, 161.2; MS (EI) *m*/*z* (%): 340 (14) M^+, 322 (8) $[M–H_2O]^+$, 219 (2) $[M–CH_3OPhCH_2]^+$, 202 (3) $[M–CH_3OPhCH_2OH]^+$, 138 (3) $[CH_3OPhCH_2OH]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{20}H_{20}O_5$: 340.1311, Found: 340.1320.

(*R*)-5-Hydroxy-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 9. Colorless crystals, mp 189–192°C;

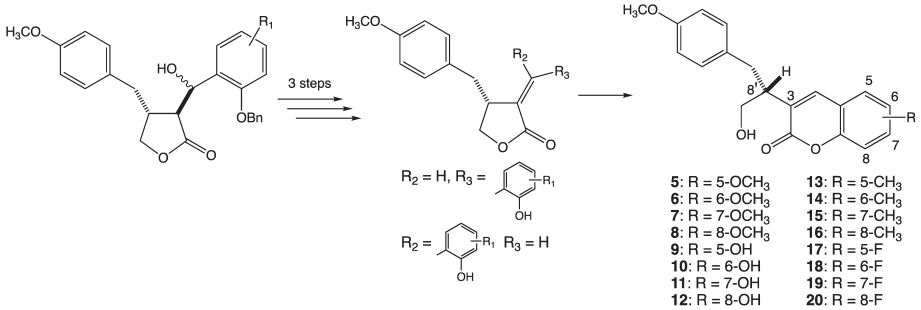


Fig. 2. Synthesized Derivatives of (*R*)-3-[7′-(4-Methoxyphenyl)-9′-hydroxyprop-8′-yl]coumarin **5**–**20**.

$[\alpha]_D^{25}$–82 (*c* 0.3, acetone); ^{1}H NMR (400 MHz, pyridine-d_5) δ 3.29 (1H, dd, *J*=13.6, 7.8 Hz), 3.38 (1H, dd, *J*=13.6, 7.4 Hz), 3.58 (3H, s), 3.83 (1H, m), 4.24 (1H, dd, *J*=10.6, 4.8 Hz), 4.31 (1H, dd, *J*=10.6, 6.2 Hz), 6.50–6.70 (1H, br), 6.81 (1H, d, *J*=8.3 Hz), 6.86 (1H, d, *J*=8,3 Hz), 6.89 (2H, d, *J*=8.2 Hz), 7.27 (1H, dd, *J*=8.3, 8.3 Hz), 7.37 (2H, d, *J*=8.2 Hz), 8.57 (1H, s), 12.0–14.0 (1H, br); ^{13}C NMR (100 MHz, acetone-d_6) δ 35.5, 46.5, 55.3, 63.3, 107.7, 109.9, 110.4, 2×114.4, 128.0, 2×130.8, 132.1, 132.9, 135.5, 154.9, 155.1, 158.9, 161.6; MS (EI) *m*/*z* (%): 326 (11) M^+, 308 (8) $[M–H_2O]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{19}H_{18}O_5$: 326.1154, Found: 326.1147.

(*R*)-6-Hydroxy-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 10. Colorless crystals, mp 206–208°C (washed with $CHCl_3$); $[\alpha]_D^{25}$–86 (*c* 0.7, acetone); ^{1}H NMR (400 MHz, pyridine-d_5) δ 3.25 (1H, dd, *J*=13.6, 7.9 Hz), 3.34 (1H, dd, *J*=13.6, 7.3 Hz), 3.60 (3H, s), 3.79 (1H, m), 4.21 (1H, dd, *J*=10.6, 4.7 Hz), 4.28 (1H, dd, *J*=10.6, 6.0 Hz), 6.57 (1H, br s), 6.90 (2H, d, *J*=7.9 Hz), 7.19–7.25 (3H, m), 7.35 (2H, d, *J*=7.9 Hz), 7.96 (1H, s), 11.9 (1H, br s); ^{13}C NMR (100 MHz, acetone-d_6) δ 35.4, 46.2, 55.3, 63.3, 112.9, 2×114.3, 117.5, 119.4, 121.0, 130.5, 2×130.8, 132.8, 140.5, 147.4, 154.5, 158.8, 161.7; MS (EI) *m*/*z* (%): 326 (13) M^+, 308 (7) $[M–H_2O]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{19}H_{18}O_5$: 326.1154, Found: 326.1153.

(*R*)-7-Hydroxy-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 11. Colorless crystals, mp 170–171°C (washed with 20% EtOAc/toluene); $[\alpha]_D^{25}$–101 (*c* 0.26, acetone); ^{1}H NMR (400 MHz, pyridine-d_5) δ 3.27 (1H, dd, *J*=13.6, 7.9 Hz), 3.37 (1H, dd, *J*=13.6, 7.2 Hz), 3.60 (3H, s), 3,75 (1H, m), 4.22 (1H, dd, *J*=10.6, 5.1 Hz), 4.29 (1H, dd, *J*=10.6, 6.2 Hz), 6.40–6.80 (1H, br), 6.91 (2H, d, *J*=8.4 Hz), 6.98 (1H, s), 7.02 (1H, d, *J*=8.4 Hz), 7.37 (2H, d, *J*=8.4 Hz), 7.42 (1H, d, *J*=8.4 Hz), 7.91 (1H, s), 12.0–14.0 (1H, br); ^{13}C NMR (100 MHz, acetone-d_6) δ 35.5, 46.1, 55.3, 63.4, 102.7, 113.3, 113.6, 2×114.3, 125.9, 129.8, 2×130.8, 133.0, 141.0, 155.6, 158.8, 161.1, 161.9; MS (EI) *m*/*z* (%): 326 (11) M^+, 308 (6) $[M–H_2O]^+$, 205 (7) $[M–CH_3OPhCH_2]^+$, 188 (7) $[M–CH_3OPhCH_2OH]^+$, 121 (100) $[CH_3OPhCH_2)]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{19}H_{18}O_5$: 326.1154, Found: 326.1148.

(*R*)-8-Hydroxy-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 12. Colorless crystals, mp 148–149°C; $[\alpha]_D^{25}$–129 (*c* 0.14, acetone); ^{1}H NMR (400 MHz, pyridine-d_5) δ 3.28 (1H, dd, *J*=13.6, 6.8 Hz), 3.36 (1H, dd, *J*=13.6, 7.3 Hz), 3.60 (3H, s), 3.78 (1H, m), 4.23 (1H, dd, *J*=10.7, 4.9 Hz), 4.30 (1H, dd, *J*=10.7, 6.2 Hz), 6.60 (1H, br s), 6.90 (2H, d, *J*=8.5 Hz), 7.01 (1H, dd, *J*=7.8, 1.4 Hz), 7.14 (1H, dd, *J*=7.8, 7.8 Hz), 7.30 (1H, dd, *J*=7.8, 1.4 Hz), 7.36 (2H, d, *J*=8.5 Hz), 8.74 (1H, s), 12.0–14.0 (1H, br); ^{13}C NMR (100 MHz, acetone-d_6) δ 35.4, 46.3, 55.3, 63.3, 2×114.4, 118.0, 119.1, 121.2, 125.1, 130.3, 2×130.8, 132.8, 141.1, 142.3, 145.0, 158.9, 161.1; MS (EI) *m*/*z* (%): 326 (12) M^+, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{19}H_{18}O_5$: 326.1154, Found: 326.1149.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-5-methyl-2*H*-chromen-2-one 13. Colorless oil, $[\alpha]_D^{25}$–84 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.96 (1H, br s), 2.47 (3H, s), 3.00 (1H, dd, *J*=14.1, 7.9 Hz), 3.04 (1H, dd, *J*=14.1, 7.6 Hz), 3.32 (1H, m), 3.76 (3H, s), 3.84–3.94 (2H, m), 6.80 (2H, d, *J*=8.2 Hz), 7.06 (1H, d, *J*=7.4 Hz), 7.12 (2H, d, *J*=8.2 Hz), 7.15 (1H, d, *J*=7.4 Hz), 7.34 (1H, dd, *J*=7.4, 7.4 Hz), 7.65 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.4, 34.9, 45.5, 55.2, 63.6, 2×113.8, 114.4, 118.0, 125.6, 128.5, 2×130.0, 130.7, 131.3, 135.6, 137.3, 153.4, 158.0, 161.8; MS (EI) *m*/*z* (%): 324 (10) M^+, 306 (8) $[M–H_2O]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{20}H_{20}O_4$ 324.1362, Found: 324.1362.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-6-methyl-2*H*-chromen-2-one 14. Colorless crystals, mp 118–119°C (EtOH); $[\alpha]_D^{25}$–83 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.02 (1H, br t, *J*=5.3 Hz), 2.38 (3H, s), 2.99 (2H, d, *J*=7.3 Hz), 3.28 (1H, m), 3.76 (3H, s), 3.84 (2H, dd, *J*=5.3, 4.5 Hz), 6.78 (2H, d, *J*=7.8 Hz), 7.11 (2H, d, *J*=7.8 Hz), 7.16–7.20 (2H, m), 7.27 (1H, s), 7.44 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 34.8, 45.1, 55.2, 63.5, 2×113.8, 116.0, 118.9, 127.3, 129.1, 2×129.9, 131.3, 131.9, 134.0, 140.2, 151.1, 158.0, 162.0; MS (EI) *m*/*z* (%): 324 (10) M^+, 306 (8) $[M–H_2O]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{20}H_{20}O_4$: 324.1362, Found: 324.1358.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-7-methyl-2*H*-chromen-2-one 15. Colorless crystals, mp 138°C; $[\alpha]_D^{25}$–87 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.35 (1H, br s), 2.41 (3H, s), 2.95 (1H, dd, *J*=14.5, 7.6 Hz), 2.99 (1H, dd, *J*=14.5, 7.6 Hz), 3.26 (1H, m), 3.74 (3H, s), 3.81 (1H, dd, *J*=11.1, 4.2 Hz), 3.84 (1H, dd, *J*=11.1, 4.2 Hz), 6.77 (2H, d, *J*=8.5 Hz), 7.03 (1H, d, *J*=7.9 Hz), 7.05 (1H, s), 7.09 (2H, d, *J*=8.5 Hz), 7.27 (1H, d, *J*=7.9 Hz), 7.46 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.7, 34.7, 45.0, 55.1, 63.3, 2×113.7, 116.4, 116.8, 125.4, 127.1, 127.9, 2×129.9, 131.3, 140.2, 142.0, 152.9, 157.9, 162.0; MS (EI) *m*/*z* (%) 324 (13) M^+, 306 (8) $[M–H_2O]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): Calcd. for $C_{20}H_{20}O_4$: 324.1362, Found: 324.1360.

(*R*)-3-[1-Hydroxy-3-(4-methoxyphenyl)prop-2-yl]-8-methyl-2*H*-chromen-2-one 16. Colorless oil; $[\alpha]_D^{25}$–77 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.32 (1H, br s), 2.42 (3H, s), 2.97 (1H, dd, *J*=14.9, 7.8 Hz), 3.00 (1H, dd, *J*=14.9, 7.8 Hz), 3.30 (1H, m), 3.74 (3H, s), 3.82 (1H, dd, *J*=11.5, 4.8 Hz), 3.85 (1H, dd, *J*=11.5, 5.5 Hz), 6.77 (2H, d, *J*=8.5 Hz), 7.10 (2H, d, *J*=8.5 Hz), 7.13 (1H, dd, *J*=7.5, 7.4 Hz), 7.23 (1H, d, *J*=7.5 Hz), 7.29 (1H, d, *J*=7.4 Hz), 7.48 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.3, 34.7, 44.9, 55.1, 63.4, 2×113.7, 118.9, 123.9, 125.2, 125.7, 128.8, 2×129.9, 131.3, 132.2, 140.6, 151.2, 157.9, 162.0; MS (EI) *m*/*z* (%) 324 (19) M^+, 306 (14) $[M–H_2O]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) *m*/*z* (M^+): calcd. for $C_{20}H_{20}O_4$: 324.1362, Found: 324.1361.

(*R*)-5-Fluoro-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 17. Colorless oil; $[\alpha]_D^{25}$–58 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.97 (1H, br s), 2.97 (1H, dd, *J*=14.1, 7.7 Hz), 3.02 (1H, dd, *J*=14.1, 7.6 H), 3.34 (1H, m), 3.76 (3H, s), 3.85 (2H, d, *J*=5.0 Hz), 6.80 (2H, d, *J*=8.5 Hz), 6.95 (1H, dd, *J*=8.7, 8.7 Hz), 7.08–7.13 (1H, overlapped), 7.12 (2H,

d, J=8.5 Hz), 7.41 (1H, m), 7.75 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.9, 45.1, 55.2, 63.4, 109.4 (d, J=18.9 Hz), 110.2 (d, J=19.8 Hz), 112.2, 2×113.9, 129.8, 2×130.0, 131.1, 131.2 (d, J=9.3 Hz), 132.8, 153.5 (d, J=5.3 Hz), 158.1, 158.2 (d, J=254.7 Hz), 161.1; MS (EI) m/z (%): 328 (12) M^+, 310 (5) $[M–H_2O]^+$, 149 (3) $[CH_3OPhCH_2CHCH_3]^+$, 133 (2) $[CH_3OPhCH_2CO]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) m/z (M^+): Calcd. for $C_{19}H_{17}FO_4$: 328.1111, Found: 328.1113.

(*R*)-6-Fluoro-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 18. Colorless crystals, mp 130–131°C (toluene-hexane); $[\alpha]_D^{25}$–72 (c 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.91 (1H, br s), 2.99 (2H, d, J=7.6 Hz), 3.31 (1H, m), 3.76 (3H, s), 3.85 (2H, br s), 6.79 (2H, d, J=8.4 Hz), 7.09–7.11 (1H, overlapped), 7.10 (2H, d, J=8.4 Hz), 7.18 (1H, m), 7.27 (1H, m), 7.46 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8, 45.0, 55.2, 63.2, 112.8 (d, J=23.7 Hz), 2×113.9, 117.9 (d, J=8.5 Hz), 118.3 (d, J=24.6 Hz), 119.9 (d, J=8.7 Hz), 2×129.9, 130.7, 131.0, 139.2, 149.1, 158.1, 158.7 (d, J=244.2 Hz), 161.4; MS (EI) m/z (%): 328 (12) M^+, 310 (4) $[M–H_2O]^+$, 149 (2) $[CH_3OPhCH_2CHCH_3]^+$, 133 (2) $[CH_3OPhCH_2CO]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) m/z (M^+): Calcd. for $C_{19}H_{17}FO_4$: 328.1111, Found: 328.1117.

(*R*)-7-Fluoro-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 19. Colorless crystals, mp 134–136°C (EtOH-*iso*-Pr_2O); $[\alpha]_D^{25}$–71 (c 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.83 (1H, br s), 2.98 (2H, d, J=7.6 Hz), 3.27 (1H, m), 3.76 (3H, s), 3.82–3.86 (2H, m), 6.79 (2H, d, J=8.5 Hz), 6.95–7.03 (2H, m), 7.10 (2H, d, J=8.5 Hz), 7.39 (1H, m), 7.48 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.8, 45.0, 55.2, 63.3, 104.0 (d, J=25.5 Hz), 112.5 (d, J=22.8 Hz), 2×113.9, 116.0, 128.1, 129.0 (d, J=10.1 Hz), 2×130.0, 131.1, 139.7, 154.0 (d, J=12.8 Hz), 158.1, 161.3, 163.9 (d, J=252.9 Hz); MS (EI) m/z 328 (90) M^+, 310 (27) $[M–H_2O]^+$, 297 (7) $[M–OCH_3]^+$, 207 (11) $[M–CH_3OPhCH_2]^+$, 189 (9) $[M–CH_3OPhCH_2OH_2]^+$, 149 (14) $[CH_3OPhCH_2CHCH_3]^+$, 138 (7) $[CH_3OPhCH_2OH]^+$, 133 (12) $[CH_3OPhCH_2CO]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) m/z (M^+): Calcd. for $C_{19}H_{17}FO_4$: 328.1111, Found: 328.1119.

(*R*)-8-Fluoro-3-[1-hydroxy-3-(4-methoxyphenyl)prop-2-yl]-2*H*-chromen-2-one 20. Colorless crystals, mp 111–114°C (toluene-hexane); $[\alpha]_D^{25}$–72 (c 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.84 (1H, br s), 2.99 (2H, d, J=7.6 Hz), 3.31 (1H, m), 3.76 (3H, s), 3.86 (2H, br s), 6.79 (2H, d, J=8.4 Hz), 7.11 (2H, d, J=8.4 Hz), 7.16–7.20 (2H, m), 7.24 (1H, m), 7.52 (1H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 34.7, 45.1, 55.2, 63.2, 2×113.9, 117.3 (d, J=16.9 Hz), 121.1, 122.6, 124.2 (d, J=6.6 Hz), 2×129.9, 130.5, 131.0, 139.7, 141.1 (d, J=12.1 Hz), 149.2 (d, J=252.0 Hz), 158.1, 160.3; MS (EI) m/z (%): 328 (64) M^+, 310 (17) $[M–H_2O]^+$, 207 (6) $[M–CH_3OPhCH_2]^+$, 189 (7) $[M–CH_3OPhCH_2OH_2]^+$, 149 (9) $[CH_3OPhCH_2CHCH_3]^+$, 133 (8) $[CH_3OPhCH_2CO)]^+$, 121 (100) $[CH_3OPhCH_2]^+$; HRMS (EI) m/z (M^+): Calcd. for $C_{19}H_{17}FO_4$: 328.1111, Found: 328.1116.

Plant growth assay

The plant growth effects of our synthesized compounds were estimated by employing lettuce (*Lactuca sativa* L. Green-wave, Takii Seed Co. Ltd., Kyoto, Japan) and Italian ryegrass (*Lolium multiflorum* Lam. Wase-fudo, Takii Seed Co. Ltd.) seedlings. A sheet of filter paper (diameter=90 mm) was put in a 90 mm Petri dish and wetted with 500 μL of test sample solution dissolved in acetone. After the filter paper had dried, 3 mL of water was poured into the dish to adjust the final concentration from 1000 to 10 μM. Thirty seeds of each plant were placed on the filter paper, and the Petri dishes were sealed with parafilm. The Petri dishes were then incubated in the dark at 20°C. The lengths of roots and shoots were measured after 3 days for lettuce seedlings and after 5 days for Italian ryegrass seedlings by using an ordinary ruler. The shoot and root lengths of the control were 1 and 2 cm for lettuce seedlings and 2 and 3 cm for Italian ryegrass seedlings, respectively. The germination was checked (lettuce: 24 hr, rye grass: 72 hr). All test compounds did not show the germination inhibitory activity. Statistical analyses were conducted one-way ANOVA followed by Tukey's multiple-comparison test by using PRISM software ver. 5.0 (GraphPad software Inc., San Diego, CA, USA), and the values of p were considered to be statistically significant. The IC_{50} values were calculated using a standard dose-response curve by non-linear regression analysis fitting by employing PRISM software ver. 5.0 (GraphPad software Inc., San Diego, CA, USA). The plant growth data at six different concentrations of each compound were analyzed by this method. These analyses were performed in triplicate to obtain IC_{50} value of each compound.

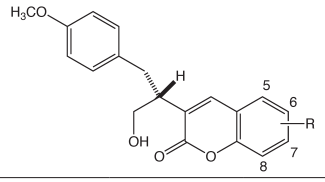
Results and discussion

The synthesized derivatives **5**–**20** (Fig. 1) were applied to plant growth regulatory assays (Table 1), in which the plant growth inhibitory activity of the roots under dark conditions was observed.

The lettuce shoots were not sensitive to all the derivatives. In the lettuce roots experiment, all the hydroxy derivatives **9**–**11** were inactive. The 8-OCH_3 derivative **8** (IC_{50}=228 μM) was 1.6-fold more potent than the compound without substituents **2**. Considering the less active 8-CH_3 derivative **16** (52% inhibition at 1000 μM) and 8-F derivative **20** (IC_{50}=650 μM), the bulkier and lower hydrophobicity substituent at the 8-position was advantageous. Because the activity of the 8-F derivative **20** was more potent than the 8-CH_3 derivative **16** and less potent than the 8-OCH_3 derivative **8**, the activity level did not depend on the electronic effect at the 8-position. Although the activities of all the 7-substituted derivatives are not shown, the activity of the 7-OMe derivative **7** (50% inhibition at 1000 μM) was higher than the other 7-substituted derivatives **11**, **15**, **19**. The lettuce roots were not sensitive to the derivatives bearing substituents at the 5- or 6-positons.

The remarkable activity against Italian ryegrass shoots was not observed in all the derivatives. As observed in the lettuce experiment, all the hydroxy derivatives **9**–**12** were inactive against both shoots and roots, suggesting the higher hydrophilic group was disadvantageous at all positions. Comparing the

Table 1. Plant growth inhibitory activities (IC_{50}±S.E.M. μM, one-way ANOVA, Tukey post-test, $P<0.05$. a, b, c, d: The same letters between different columns do not show significant difference, on the other hand, the different letters between different columns show significant difference). In the case of $IC_{50}>1000\,\mu$M, growth % from control at 1000 μM is shown.



Compound R	Lettuce Seedlings		Italian Ryegrass Seedlings	
	Shoots	Roots	Shoots	Roots
2: H	659±20.1 μM	360±20.3 μM^{b}	65%	221±13.4 μM^{ab}
5: 5-OCH_3	75%	95%	86%	467±75.3 μM^{cd}
6: 6-OCH_3	88%	95%	96%	83%
7: 7-OCH_3	73%	50%	79%	121±21.7 μM^{a}
8: 8-OCH_3	69%	228±10.8 μM^{a}	54%	56.7±5.47 μM^{a}
9: 5-OH	83%	90%	107%	100%
10: 6-OH	88%	95%	85%	73%
11: 7-OH	88%	89%	115%	94%
12: 8-OH	85%	88%	105%	104%
13: 5-CH_3	68%	89%	59%	214±15.2 μM^{ab}
14: 6-CH_3	108%	90%	96%	99%
15: 7-CH_3	80%	79%	75%	54%
16: 8-CH_3	59%	52%	57%	225±11.0 μM^{ab}
17: 5-F	79%	79%	81%	424±62.9 μM^{bc}
18: 6-F	78%	59%	92%	93%
19: 7-F	93%	84%	66%	65%
20: 8-F	73%	650±39.9 μM^{c}	72%	696±90.4 μM^{d}

activities against Italian ryegrass roots of 5-OCH_3, 5-CH_3, and 5-F derivatives **5**, **13**, **17** with the compound without substituents **2**, similar activity levels for 5-CH_3 (IC_{50}=214 μM) and 5-F (IC_{50}=424 μM) to **2** and 2-fold less potent activity of 5-OCH_3 (IC_{50}=467 μM) than **2** with a significant difference were shown. Considering these results and the inactivity of the 5-OH derivative **9**, the higher hydrophobic group at 5-position is favored for the activity. As for the 6-position, all the derivatives were inactive, suggesting the presence of a substituent at the 6-position distributes the interaction between the target and the compounds. In comparing the activities of the 7-substituted derivatives, it seems that the bulkier and electron donating methoxy group is tolerable for growth inhibition against Italian ryegrass roots, with the 7-OCH_3 derivative **7** (IC_{50}=121 μM) showing almost the same level of activity as the compound without substituents **2**. As for the 8-position, except for the 8-OH derivative **12**, the 8-OCH_3, 8-CH_3, and 8-F derivatives **8**, **16**, **20** exhibited activities against Italian ryegrass roots. Of these, the 8-F derivative **20** (IC_{50}=696 μM) was 12-fold less potent than the 8-OCH_3 derivative **8** and the activities of the 8-OCH_3 derivative **8** (IC_{50}=56.7 μM) and the 8-CH_3 derivative **16** (IC_{50}=225 μM) were higher than or similar to the compound without substituents **2**. These results suggest that electron withdrawing groups and higher hydrophilic groups at the 8-position are disadvantageous. Among the methoxy derivatives **5**–**8**, the 5-OCH_3 derivative **5**, whose activity was 2-fold less than the compound without substituents **2**, was 4-fold and 8-fold less potent than the 7-OCH_3 and 8-OCH_3 derivatives **7**, **8**, respectively. No significant difference in activity between the 5-CH_3 derivative **13** and the 8-CH_3 derivative **8**, which have the same levels of activity as the compound without substituents **2**, was observed; on the other hand, the 5-F derivative **17** was 1.6-fold more potent than the 8-F derivative **20**. These results also show the advantage of the hydrophobic group at the 5-position and the disadvantage of a higher electron withdrawing group at the 8-position. We found that different factors at each position expressed higher activity.

In our previous work on lettuce and Italian ryegrass, Z-2-hydroxy-α-benzylidene γ-butyrolactone type lignan **4** showed growth inhibition against both shoots and roots.[3] The compound without substituents **2** with a phenylpropanoid unit, which is formed by *trans*-lactonization from *Z*-**3**, was potent against the shoots and roots of lettuce and only the roots of Italian ryegrass,[2] whereas there was no remarkable growth inhibition of the shoots of either plant by the introduction of a substituent to the coumarin ring. A similar pattern of activity has been observed in tri-substituted tetrahydrofuran lignans.[13,14] Selective toxicity to plants was also shown in this experiment. Most of the synthesized derivatives were effective only against monocotyledon (Italian ryegrass). Similar results were also obtained in the experiments using tri-substituted tetrahydrofuran lignans.[14] Only the 8-OCH_3 derivative **8** was potent against both dicotyledon (lettuce) and monocotyledon (Italian ryegrass).

Conclusion

Derivatives of a lignan type coumarin bearing a phenylpropanoid unit at the 3-position **5**–**20** were synthesized to clarify the effect of each substituent in the coumarin ring on the plant growth inhibitory activity. No remarkable growth inhibitory activity against the shoots of either lettuce or Italian ryegrass seedlings was observed. For lettuce roots, the growth inhibitory activity of the 8-OCH_3 derivative **8** was 1.6-times higher than the compound without substituents **2**. The activities of the other derivatives were lower against lettuce roots. For Italian ryegrass roots, the 5-CH_3 derivative **13** had the highest activity among the 5-substituted derivatives, suggesting that a hydrophobic group at the 5-position is advantageous. No activity was found for the 6-substituted derivatives. Of the 7-substituted derivatives, only the 7-OCH_3 derivative **7** was potent, displaying the same level of activity as the compound without substituents **2**. As for the 8-position, the hydrophobic electron donating group was suggested, thus the 8-OCH_3, 8-CH_3 derivatives **8**, **16** were more effective. This is the first report on the effect of substituents at each position from the 5-position to the 8-position on a coumarin ring with phenylpropanoid unit.

Electronic supplementary materials

The online version of this article contains supplementary materials (Synthetic method and ^{1}H-, ^{13}C-NMR of derivatives **5–20**) which are available at https://www.jstage.jst.go.jp/browse/jpestics/.

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