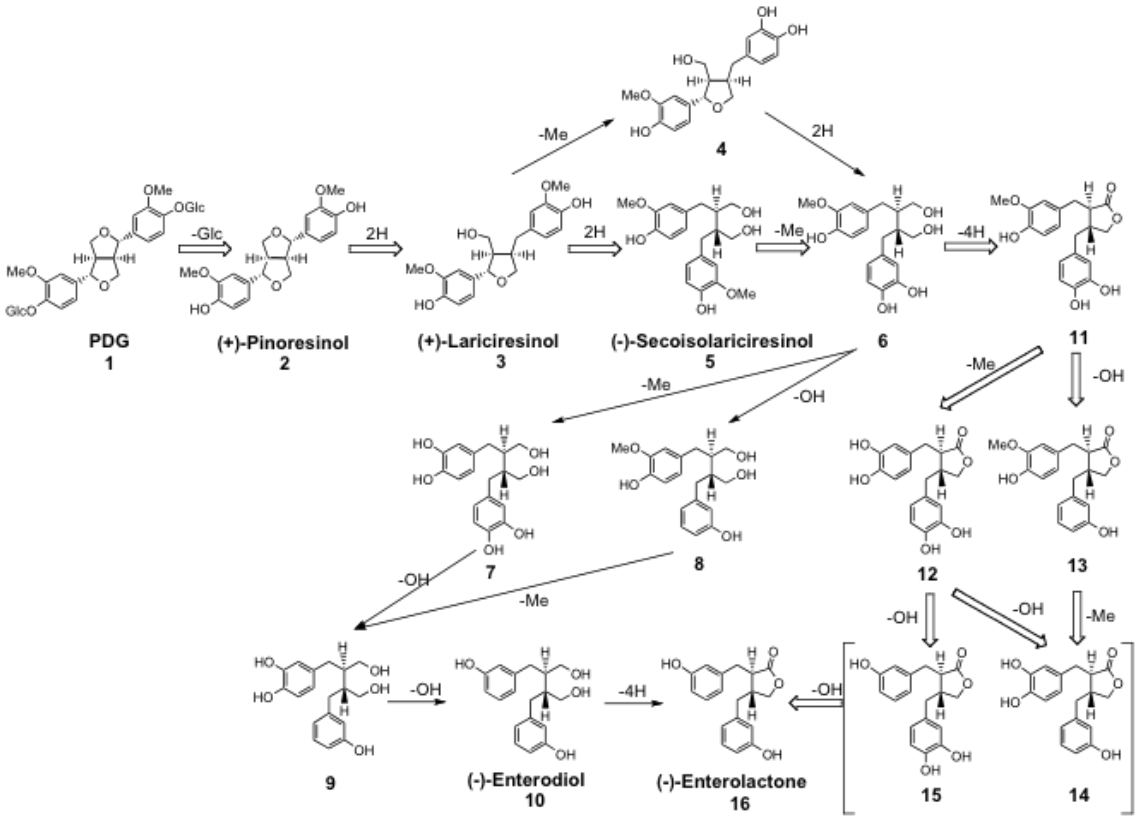
Pinoresinol diglucoside



Metabolic processes of pinoresinol diglucoside (PDG) by human intestinal microflora

An HIB mixture (300 ml) and PDG (**1**, 1.9g) dissolved in 10 ml of MeOH were added to 3.0 l of GAM broth and anaerobically incubated at 37 ^{o}C for seven days. ⟹ Major pathway; → minor pathway; conversions: **7**→ **12**; **8**→ **13**; **9**→ **14** or **15** are also considered to be possible. [Xie et al., *Chem. Pharm. Bull.*, **51**, 508-515 (2003) and Gao *et al*., *J. Trad. Med*., **22**, 213-221 (2005)].

代謝実験
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単一化合物　pinoresinol diglucoside

(+)-Pinoresinol (2)

Amorphous powder. $[\alpha]_D^{25}$ +69 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 231 (14000), 280 (5800) nm. IR (KBr) ν_{max}: 3448 (OH), 1511 (arom. C=C) cm^{-1}. EI-MS *m/z*: 358 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 3.12 (2H, m, H-8, 8'), 3.83 (2H, dd, *J*=8.9, 3.6 Hz, H_a-9, 9'), 3.84 (6H, s, -OC$\underline{H}_3$), 4.21 (2H, dd, *J*=8.9, 6.8 Hz, H_b-9, 9'), 4.69 (2H, d, *J*=4.4 Hz, H-7, 7'), 6.75 (2H, d, *J*=8.0 Hz, H-5, 5'), 6.79 (2H, dd, *J*=8.0, 1.7 Hz, H-6, 6'), 6.93 (2H, d, *J*=1.7 Hz, H-2, 2'). CD (MeOH): CD (MeOH): $\Delta\varepsilon_{211}$ +2.67, $\Delta\varepsilon_{221}$ + 1.07, $\Delta\varepsilon_{232}$ +0.64, $\Delta\varepsilon_{282}$ +0.45 (dm^3 $mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(+)-Lariciresinol (3)

Amorphous powder. $[\alpha]_D^{25}$ +30 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 229(13000), 281(5600) nm. IR (KBr) ν_{max}: 3448 (OH), 1516 (arom. C=C) cm^{-1}. EI-MS *m/z*: 360 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.37 (1H, m, H-8), 2.48 (1H, dd, *J* =13.4, 11.1 Hz, H_a-7'), 2.73 (1H, m, H-8'), 2.92 (1H, dd, *J* =13.4, 4.8 Hz, H_b-7'), 3.62 (1H, dd, *J*=10.9, 6.5 Hz, H_a-9), 3.72 (1H, dd, *J*=8.4, 5.8 Hz, H_a-9'), 3.82 (3H, s, -OC$\underline{H}_3$), 3.83 (1H, dd, *J*=10.9, 8.0 Hz, H_b-9), 3.84 (3H, s, -OC$\underline{H}_3$), 3.97 (1H, dd, *J*=8.4, 6.5 Hz, H_b-9'), 4.74 (1H, d, *J*=7.0 Hz, H-7), 6.64 (1H, dd, *J*=8.0, 1.9 Hz, H-6'), 6.71 (1H, d, *J*=8.0 Hz, H-5'), 6.75 (1H, m, H-6), 6.76 (1H, m, H-5), 6.79 (1H, d, *J*=1.9 Hz, H-2'), 6.90 (1H, d, *J*=1.8 Hz, H-2). CD (MeOH): $\Delta\varepsilon_{288}$ -0.26 , $\Delta\varepsilon_{235}$ -1.07 (dm^3 $mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

3'-Demethyl-(+)-Lariciresinol (4)

Amorphous powder. $[\alpha]_D^{25}$ +15 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 221(7800), 281(3400) nm. IR (KBr) ν_{max}: 3448 (OH), 1520 (arom. C=C) cm^{-1}. EI-MS *m/z*: 346 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.34 (1H, m, H-8), 2.43 (1H, dd, *J* =13.7, 12.1 Hz, H_a-7'), 2.70 (1H, m, H-8'), 2.84 (1H, dd, *J* =13.7, 4.9 Hz, H_b-7'), 3.61 (1H, dd, *J*=11.0, 6.5 Hz, H_a-9), 3.70 (1H, dd, *J*=8.0, 6.5 Hz, H_a-9'), 3.82 (1H, m, H_b-9), 3.84 (3H, s, -OC$\underline{H}_3$), 3.97 (1H, dd, *J*=8.0, 6.8 Hz, H_b-9'), 4.75 (1H, d, *J*=6.8 Hz, H-7), 6.52 (1H, dd,

J =8.0, 1.9 Hz, H-6'), 6.63 (1H, d, *J*=1.9 Hz, H-2'), 6.67 (1H, d, *J*=8.0 Hz, H-5'), 6.75 (1H, m, H-6), 6.77 (1H, m, H-5), 6.89 (1H, d, *J*=1.7 Hz, H-2). CD (MeOH): $\Delta\varepsilon_{235}$ -0.4, $\Delta\varepsilon_{287}$ -0.19 ($dm^3\ mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie et al., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(-)-Secoisolariciresinol (5)

Amorphous powder. $[\alpha]_D^{25}$ -16 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 226 (10000), 281 (4600) nm. IR (KBr) ν_{max}: 3425 (OH), 1516 (arom. C=C) cm^{-1}. EI-MS *m/z*: 362 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 1.86 (2H, m, H-2, 3), 2.50 (2H, dd, *J* =13.8, 7.7 Hz, H_a-7', 7"), 2.61 (2H, dd, *J*=13.8, 7.0 Hz, H_b-7', 7"), 3.54 (4H, m, H-1, 4), 3.68 (3H, s, -OC$\underline{H}_3$), 6.49 (2H, dd, *J*=8.0, 1.9 Hz, H-6', 6"), 6.54 (2H, d, *J* =1.9 Hz, H-2', 2"), 6.61 (2H, d, *J*=8.0 Hz, H-5', 5"). CD (MeOH): $\Delta\varepsilon_{289}$ -0.29, $\Delta\varepsilon_{228}$ -1.05 ($dm^3\ mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al.*, *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(-)-3-(3",4"-Dihydroxybenzyl)-2-(4'-hydroxy-3'-methoxybenzyl) butane-1,4-diol (6)

Amorphous powder. $[\alpha]_D^{25}$ -10 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 221(12000), 282(5300) nm. IR (KBr) ν_{max}: 3425 (OH), 1516 (arom. C=C) cm^{-1}. EI-MS *m/z*: 348 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 1.90 (2H, m, H-2, 3), 2.59 (4H, m, H-7', 7"), 3.56 (4H, m, H-1, 4), 3.76 (3H, s, -OC$\underline{H}_3$), 6.43 (1H, dd, *J*=8.0, 2.2 Hz, H-6"), 6.55 (1H, dd, *J*=8.0, 1.9 Hz, H-6'), 6.56 (1H, d, *J*=2.2 Hz, H-2"), 6.63 (1H, d, *J* =1.9 Hz, H-2'), 6.63 (1H, d, *J* =8.0 Hz, H-5"), 6.66 (1H, d, *J*=8.0 Hz, H-5'). CD (MeOH): $\Delta\varepsilon_{229}$ -1.26, $\Delta\varepsilon_{280}$ -0.13 ($dm^3\ mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al.*, *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

2-(3',4'-Dihydroxybenzyl)-3-(3",4"-dihydroxybenzyl)butane-1,4-diol (7)

Amorphous powder. UV λ_{max}^{MeOH} (ε): 220 (12000), 283 (4600) nm. IR (KBr) ν_{max}: 3448 (OH), 1523 (arom. C=C) cm^{-1}. EI-MS *m/z*: 334 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 1.90 (2H, m, H-2, 3), 2.55 (4H, m, H-7', 7"), 3.54 (4H, m, H-1, 4), 6.44 (2H, dd, *J*=8.0, 2.1 Hz, H-6', 6"), 6.58 (2H, d, *J* =2.1 Hz, H-2', 2"), 6.63 (2H, d, *J*=8.0 Hz, H-5', 5").

^{13}C-NMR: see the reference [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

3-(3''-Hydroxybenzyl)-2-(4'-hydroxy-3'-methoxybenzyl)butane-1,4-diol (8)

Amorphous powder. UV λ_{max}^{MeOH} (ε): 220 (13000), 275 (6800) nm. IR (KBr) ν_{max}: 3394 (OH), 1520 (arom. C=C) cm^{-1}. EI-MS *m/z*: 332 [M]$^{+}$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 1.94 (2H, m, H-2, 3), 2.61 (4H, m, H-7', 7''), 3.56 (4H, m, H-1, 4), 3.76 (3H, s, -OC<u>H</u>$_3$), 6.56 (1H, m, H-6'), 6.56 (1H, dd, *J*=8.1, 1.8 Hz, H-4''), 6.58 (1H, m, H-6''), 6.58 (1H, d, *J*=1.8 Hz, H-2''), 6.65 (1H, d, *J* =1.9 Hz, H-2'), 6.66 (1H, d, *J* =8.0 Hz, H-5'), 7.03 (1H, t, *J*=8.1 Hz, H-5''). ^{13}C-NMR: see the reference [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

2-(3',4'-Dihydroxybenzyl)-3-(3''-hydroxybenzyl)butane-1,4-diol (9)

Amorphous powder. UV λ_{max}^{MeOH} (ε): 220(7900), 280(2300) nm. IR (KBr) ν_{max}: 3444 (OH), 1523 (arom. C=C) cm^{-1}. EI-MS *m/z*: 318 [M]$^{+}$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 1.94 (2H, m, H-2, 3), 2.55 (2H, m, H-7''), 2.62 (2H, m, H-7'), 3.50 (2H, m, H-1), 3.60 (2H, m, H-4), 6.45 (1H, dd, *J*=8.1, 1.9 Hz, H-6'), 6.57 (1H, m, 4''), 6.58 (1H, d, *J* =1.9 Hz, H-2'), 6.60 (1H, d, *J* =1.9 Hz, H-2''), 6.61 (1H, m, H-6''), 6.63 (2H, d, *J*=8.1 Hz, H-5'), 7.04 (1H, t, *J*=8.0 Hz, H-5''). ^{13}C-NMR: see the reference [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(-)-Enterodiol (10)

Amorphous powder. $[\alpha]_D^{25}$ -12 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 216(13000), 274(3700) nm. IR (KBr) ν_{max}: 3448 (OH), 1512 (arom. C=C) cm^{-1}. EI-MS *m/z*: 302 [M]$^{+}$. ^{1}H-NMR (CD_3OD, 400 MHz): δ1.97 (2H, m, H-2, 3), 2.63 (4H, m, H-7', 7''), 3.52 (2H, dd, *J*=10.9, 4.6 Hz, H_a-1, 4), 3.60 (2H, dd, *J*=10.9, 3.4 Hz, H_b-1, 4), 6.59 (6H, m, *m*, H-2', 4', 6', 2'', 4'', 6''), 7.03 (2H, t, *J* =8.0 Hz, H-5', 5''). ^{13}C-NMR: see the reference [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(-)-(2*R*,3*R*)-3-(3",4"-Dihydroxybenzyl)-2-(4'-hydroxy-3'-methoxybenzyl)butyrolactone (11)

Amorphous powder. $[\alpha]_D^{25}$ -20 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 221 (12000), 282 (5500) nm. IR (KBr) ν_{max}: 3448 (OH), 1751 (γ-lactone CO), 1519 (arom. C=C) cm^{-1}. EI-MS *m/z*: 344 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.41-2.51 (3H, m, H-3, 7"), 2.64 (1H, m, H-2), 2.84 (2H, d, *J*=5.8, H-7'), 3.80 (3H, s, -OC$\underline{H}_3$), 3.88 (1H, m, H_a-4), 4.09 (1H, dd, *J*=8.7, 7.2 Hz, H_b-4), 6.39 (1H, dd, *J*=8.2, 2.0 Hz, H-6"), 6.52 (1H, d, *J*=2.0 Hz, H-2"), 6.58 (1H, dd, *J*=8.0, 1.9 Hz, H-6'), 6.65 (1H, d, *J*=8.2 Hz, H-5"), 6.67 (1H, d, *J*=1.9 Hz, H-2'), 6.70 (1H, d, *J*=8.0 Hz, H-5'). CD (MeOH): $\Delta\varepsilon_{232}$ -2.93 (dm^3 $mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al.*, *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(-)-(2*R*,3*R*)-2-(3',4'-Dihydroxybenzyl)-3-(3",4"-dihydroxybenzyl) butyrolactone

(12) Amorphous powder. $[\alpha]_D^{25}$ -14 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 220 (11000), 283 (5300) nm. IR (KBr) ν_{max}: 3444 (OH), 1747 (γ-lactone CO), 1523 (arom. C=C) cm^{-1}. EI-MS *m/z*: 330 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ2.36-2.39 (1H, m, H_a-7"), 2.42-2.54 (2H, m, H-2, H_b-7"), 2.57-2.62 (1H, m, H-3), 2.78 (1H, dd, *J*=14.0, 6.5 Hz, H_a-7'), 2.83 (1H, dd, *J*=14.0, 5.5 Hz, H_b-7'), 3.86 (1H, t, *J*=8.8 Hz, H_a-4), 4.03 (1H, dd, *J*=8.8, 7.5 Hz, H_b-4), 6.38 (1H, dd, *J*=8.0, 1.9 Hz, H-6"), 6.48 (1H, dd, *J*=8.1, 2.2 Hz, H-6'), 6.51 (1H, d, *J*=1.9 Hz, H-2"), 6.65 (1H, d, *J*=8.0 Hz, H-5"), 6.65 (1H, d, *J*=2.2 Hz, H-2'), 6.69 (1H, d, *J*=8.1 Hz, H-5'). CD (MeOH): $\Delta\varepsilon_{230}$ -2.27, $\Delta\varepsilon_{280}$ -0.14 (dm^3 $mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al.*, *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(-)-(2*R*,3*R*)-3-(3"-Hydroxybenzyl)-2-(4'-hydroxy-3'-methoxybenzyl)butyrolactone (13)

Amorphous powder. $[\alpha]_D^{25}$ -32 ^{o} (*c*=0.10, MeOH). UV λ_{max}^{MeOH} (ε): 220 (11000), 279 (4400) nm. IR (KBr) ν_{max}: 3444 (OH), 1747 (γ-lactone CO), 1519 (arom. C=C) cm^{-1}.

EI-MS *m/z*: 328 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.48-2.58 (3H, m, H-3, 7''), 2.66 (1H, m, H-2), 2.84 (2H, d, *J*=6.0, H-7'), 3.80 (3H, s, -OC$\underline{H}_3$), 3.88 (1H, m, H_a-4), 4.10 (1H, dd, *J*=8.7, 7.0 Hz, H_b-4), 6.53 (1H, d, *J*=1.1 Hz, H-2''), 6.54 (1H, m, H-6''), 6.59 (1H, dd, *J*=8.0, 2.0 Hz, H-6'), 6.62 (1H, ddd, *J*=8.0, 2.5, 1.1 Hz, H-4''), 6.69 (1H, d, *J*=2.0 Hz, H-2'), 6.71 (1H, d, *J*=8.0 Hz, H-5'), 7.06 (1H, t, *J*=8.0 Hz, H-5''). CD (MeOH): $\Delta\varepsilon_{230}$ -2.50 ($dm^3\ mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al.*, *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

2-(3',4'-Dihydroxybenzyl)-3-(3''-hydroxybenzyl)butyrolactone (14)

EI-MS *m/z*: 314 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.44 (1H, m, H_a-7''), 2.57 (1H, m, H-3), 2.60 (2H, m, H-2, H_b-7''), 2.78 (1H, dd, *J*=14.0, 6.5 Hz, H_a-7'), 2.85 (1H, dd, *J*=14.0, 5.3 Hz, H_b-7'), 3.87 (1H, t, *J*=8.9 Hz, H_a-4), 4.06 (1H, dd, *J*=8.9, 7.5 Hz, H_b-4), 6.48 (1H, dd, *J*=8.0, 2.2 Hz, H-6'), 6.52 (1H, d, *J*=2.4 Hz, H-2''), 6.52 (1H, dt, *J*=8.2, 2.4 Hz, H-6''), 6.61 (1H, ddd, *J*=8.2, 2.4, 1.0 Hz, H-4''), 6.65 (1H, d, *J*=2.2 Hz, H-2'), 6.68 (1H, d, *J*=8.0 Hz, H-5'), 7.06 (1H, t, *J*=8.2 Hz, H-5''). ^{13}C-NMR: see the reference [Xie *et al.*, *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

2-(3'-Hydroxybenzyl)-3-(3'',4''-dihydroxybenzyl) butyrolactone (15)

EI-MS *m/z*: 314 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.36 (1H, m, H_a-7''), 2.44 (1H, m, H-3), 2.50 (1H, m, H_b-7''), 2.66 (1H, m, H-2), 2.83 (1H, dd, *J*=13.8, 6.5 Hz, H_a-7'), 2.92 (1H, dd, *J*=13.8, 5.5, H_b-7'), 3.86 (1H, t, *J*=8.9 Hz, H_a-4), 4.03 (1H, dd, *J*=8.9, 7.2 Hz, H_b-4), 6.38 (1H, dt, *J*=8.0, 2.2 Hz, H-6''), 6.50 (1H, d, *J*=2.2 Hz, H-2''), 6.63-6.67 (4H, m, H-2', H-4', H-6', H-5''), 7.10 (1H, t, *J*=8.0 Hz, H-5'). ^{13}C-NMR: see the reference [Xie *et al.*, *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

(-)-(2*R*,3*R*)-Enterolactone (16)

Amorphous powder. $[\alpha]_D^{25}$ –39° (*c*=0.15, MeOH). λ_{max}^{MeOH} (ε): 216(11000), 274 (3100), 280 (sh, 2800) nm. IR (KBr) ν_{max}: 3421 (-OH), 1747 (γ-lactone CO). EI-MS *m/z*: 298 $[M]^+$. ^{1}H-NMR (CD_3OD, 400 MHz): δ 2.43 (1H, m, H_a-7''), 2.49 (1H, m, H-3), 2.56 (1H, m, H_b-7''), 2.67 (1H, m, H-2), 2.83 (1H, dd, *J*=13.8, 7.0 Hz, H_a-7'), 2.93 (1H, dd, *J*=13.8, 5.3 Hz, H_b-7'), 3.88 (1H, t, *J*=8.9 Hz, H_a-4), 4.06 (1H, dd, *J*=8.9, 7.2 Hz, H_b-4), 6.51 (1H, d, *J*=2.2 Hz, H-2''), 6.53 (1H, d, *J*=8.0 Hz, H-6''), 6.61 (1H, ddd, *J*=8.0, 2.2,

1.0 Hz, H-4''), 6.64-6.67 (3H, m, H-2', 4', 6'), 7.05 (1H, t, *J*=8.0 Hz, H-5''), 7.10 (1H, t, *J*=8.2 Hz, H-5'). CD (MeOH): $\Delta\varepsilon_{277}$ -0.24, $\Delta\varepsilon_{220}$ -3.62 ($dm^3\ mol^{-1}cm^{-1}$). ^{13}C-NMR: see the reference [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)].

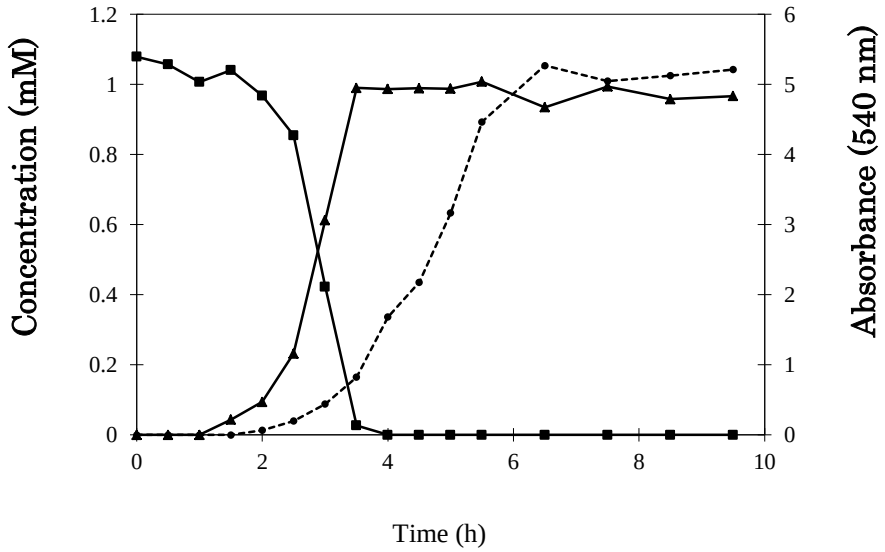

Fig. 1.

Time course of the transformation of (+)-pinoresinol (**2**, ■) to (+)-lariciresinol (**3**, ▲) by *Enterococcus faecalis* strain PDG-1

Bacterial growth (……) was monitored by measuring turbidity at 540 nm. [Xie *et al*., *Chem. Pharm. Bull.*, **51**, 508-515 (2003)]

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