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# First Stereoselective Synthesis of *meso*-Secoisolariciresinol and Comparison of Its Biological Activity with (+) and (−)-Secoisolariciresinol

Takuya SUGAHARA,[1] Satoshi YAMAUCHI,[1,†] Ai KONDO,[1] Fumi OHNO,[1] Siori TOMINAGA,[1] Yuki NAKASHIMA,[1] Taro KISHIDA,[1] Koichi AKIYAMA,[2] and Masafumi MARUYAMA[1]

[1]*Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan*
[2]*Integrated Center for Sciences, Ehime University, 3-5-7 Tarumi, Matsuyama, Ehime 790-8566, Japan*

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**The first stereoselective synthesis of *meso*-secoisolariciresinol is reported. A comparison of the cytotoxic and immunosuppressive activity between *meso*-secoisolariciresinol and optically active secoisolariciresinols was similarly performed for the first time. Both enantiomers of secoisolariciresinol accelerated IgM production, although *meso*-secoisolariciresinol did not affect IgM production. Only *meso*-secoisolariciresinol showed cytotoxic activity.**

**Key words:** lignan; immunosuppressive activity; cytotoxic activity

The widely distributed lignans[1)] have been target compounds for organic synthesis[2)] and biological research[3,4)] because of their many kinds of bonding of the C6-C3 units, oxidation of the lignan structure, and as important components in plant foods and medicines. Of the many previous studies, only a few have mentioned the relationship between biological activity and stereochemistry, because the lignans used for biological research were isolated from plants and stereoisomers could not be collected in many cases. An investigation of the effect of lignan stereochemistry on biological activity is impossible if only isolated compounds are applied to biological research. The biosynthesis of lignans as an enantiomeric mixture has recently been reported.[5,6)] This means that stereoselective synthetic studies are important to promote research about the relationship between stereochemistry and biological activity. There is a possibility that using stereoselective synthetic lignans will enable the discovery of new functions of lignan. This direction of research will suggest the safe and effective use of natural resources containing lignans.

The stereoselective cytotoxic activity of matairesinol, which is one of the popular lignans, and its related compounds has been reported in our previous study.[7)] In this article, (+)- and (−)-secoisolariciresinol (**SECO-1** and **SECO-2**) and *meso*-secoisolariciresinol (**SECO-3**) were selected as target compounds. (−)-Secoisolariciresinol (**SECO-2**) is also one of the common lignans contained in food plants,[8,9)] various whole-grain cereals (barley, rye and wheat), seeds, nuts, legumes and vegetables, and is biosynthesized as a mixture with (+)-secoisolariciresinol (**SECO-1**) in some plants.[6)] Research on the isolation[10–12)] and synthesis[13,14)] has been continued by organic chemists, and it has been reported by bioorganic researchers that secoisolariciresinol was converted to enterodiol (END) and enterolactone (ENL), which are compounds that have been postulated to have anti-carcinogenic properties, by intestinal microbes to mammalian lignans in the body.[15,16)] However, the relationship between the stereochemistry and biological activity has not been elucidated. On the other hand, *meso*-secoisolariciresinol (**SECO-3**) is not common and there have been only two reports about its isolation.[12,17)] There is no report about the stereoselective synthetic research and biological research on *meso*-secoisolariciresinol (**SECO-3**). In this present study, (+)- and (−)-secoisolariciresinol (**SECO-1** and **SECO-2**) and *meso*-secoisolariciresinol (**SECO-3**) were synthesized, and then their biological activities were compared for the first time.

## Experimental

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. The silica gel used was Wakogel C-300 (Wako, 200–300 mesh). The numbering of compounds follows IUPAC nomenclatural rules.

*(+)-and (−)-Secoisolariciresinol (**SECO-1** and **SECO-2**).* **SECO-1** and **SECO-2** were synthesized by

† To whom correspondence should be addressed. Fax: +81-89-977-4364; E-mail: syamauch@agr.ehime-u.ac.jp

the previously reported method.[18] **SECO-1**: colorless crystals, mp 111–112 °C ($CHCl_3$-(*iso*-Pr)$_2$O), 114–115 °C in the literature,[13] $[\alpha]^{20}_D = +31$ (*c* 0.3, acetone). The NMR data agree with those in the literature.[13] **SECO-2**: mp 111–112 °C ($CHCl_3$-(*iso*-Pr)$_2$O), $[\alpha]^{20}_D = -32$ (*c* 0.3, acetone), lit.,[10] $[\alpha]^{20}_D = -35.5$ (*c* 1.07, acetone).

*Determination of the enantiomeric excess of **SECO-1** and **SECO-2**.* A reaction solution of (+)-secoisolariciresinol (**SECO-1**) (25 mg, 0.069 mmol) in pyridine (0.1 ml) and $Ac_2O$ (0.1 ml) containing DMAP (1 mg) was stood at room temperature for 16 h before addition of ice. After 6 h, the mixture was extracted with EtOAc. The organic solution was separated, washed with $H_2O$, 6 M aq. HCl solution, sat. aq. $NaHCO_3$ solution and brine, and dried ($Na_2SO_4$). Concentration followed by silica gel TLC (EtOAc/hexane = 1/1) gave a tetraacetate (25 mg, 0.047 mmol, 68%) as a colorless oil, $[\alpha]^{20}_D = +13$ (*c* 0.5, $CDCl_3$), 95% ee (HPLC, Daicel AD-H chiral column, detected at 280 nm, 1 ml min$^{-1}$, hexane/*iso*-propyl alcohol = 3/2, $t_R$ 7.8 min. Tetraacetate of (−)-secoisolariciresinol (**SECO-2**): $[\alpha]^{20}_D = -13$ (*c* 0.4, $CDCl_3$), lit.,[11] $[\alpha]^{20}_D = -7.6$ (*c* 0.65, $CDCl_3$), 95% ee (HPLC, Daicel AD-H chiral column, detected at 280 nm, 1 ml min$^{-1}$, hexane/*iso*-propyl alcohol = 3/2, $t_R$ 5.8 min.

*(4S)-4-Benzyl-3-{(R)-2-[(S)-(4-benzyloxy-3-methoxyphenyl)(hydroxy)methyl]-4-pentenoyl}-2-oxazolidinone (**1**).* Compound **1** was prepared by the previously described method,[19] no production of the *syn* form being apparent. $^1$H-NMR of *(4S)-3-{(S)-2-[(S)]}*-**1** ($CDCl_3$): 2.55–2.70 (3H, m), 2.73 (1H, d, *J* 1.2 Hz), 3.16 (1H, dd, *J* 13.2, 2.9 Hz), 3.62 (1H, dd, *J* 8.3, 8.3 Hz), 3.87 (3H, s), 3.90 (1H, dd, *J* 8.3, 8.3 Hz), 4.27 (1H, m), 4.49 (1H, m), 4.83 (1H, dd, *J* 6.8 Hz), 5.03 (1H, d, *J* 10.3 Hz), 5.11 (2H, s), 5.12 (1H, d, *J* 10.8 Hz), 5.87 (1H, m), 6.80 (2H, s), 7.00 (1H, s), 7.14–7.16 (2H, m), 7.21–7.32 (6H, m), 7.39–7.41 (2H, m).

*(1S,2S)-1-(4-Benzyloxy-3-methoxyphenyl)-2-trityloxymethyl-4-penten-1-ol (**2**).* To an ice-cooled solution of *anti*-aldol product **1**[19] (15.0 g, 29.9 mmol) and MeOH (2.6 ml) in THF (200 ml) was added a solution of $LiBH_4$ (1.35 g, 62.0 mmol) in THF (80 ml), and then the reaction solution was stirred at 0 °C for 1 h before addition of 1 M aq. NaOH solution (120 ml) and EtOAc. The organic solution was separated, washed with $H_2O$, and brine, dried ($Na_2SO_4$), and concentrated to give a crude diol. A solution of this crude diol and TrCl (8.25 g, 29.6 mmol) in pyridine (20 ml) was stirred at room temperature for 16 h before additions of $H_2O$ and $CHCl_3$. The organic solution was separated, washed with a sat. aq. $CuSO_4$ solution, sat. aq. $NaHCO_3$ solution and brine, and dried ($Na_2SO_4$). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/4) gave trityl ether **2** (8.57 g, 15.0 mmol, 50%, 2 steps) as a colorless oil, $[\alpha]^{20}_D = +15$ (*c* 1.8, $CHCl_3$). NMR $\delta_H$ ($CDCl_3$): 1.91–2.01 (2H, m, $CH_2$=CH-C*H*$_2$), 2.13 (1H, m, 2-H), 3.20 (1H, dd, *J* 9.6, 5.6 Hz, TrOC*H*H), 3.38 (1H, dd, *J* 9.6, 3.3 Hz, TrOCH*H*), 3.47 (1H, d, *J* 3.7 Hz, OH), 3.82 (3H, s, $OCH_3$), 4.65 (1H, br. s, 1-H), 4.87–4.92 (2H, m, C*H*$_2$=CH), 5.11 (2H, s, $OCH_2$Ar), 5.51–5.61 (1H, m, $CH_2$=C*H*), 6.64 (1H, dd, *J* 8.2, 1.7 Hz, ArH), 6.73 (1H, d, *J* 8.2 Hz, ArH), 6.83 (1H, d, *J* 1.7 Hz, ArH), 7.19–7.29 (12H, m, ArH), 7.32–7.36 (2H, m, ArH), 7.41–7.43 (6H, m, ArH). NMR $\delta_C$ ($CDCl_3$): 33.3, 45.8, 56.2, 64.3, 71.3, 76.6, 87.7, 110.3, 113.9, 116.7, 119.0, 127.35, 127.42, 127.5, 128.0, 128.1, 128.2, 128.7, 128.9, 136.6, 137.6, 143.9, 147.5, 149.8. *Anal.* Found: C, 81.81; H, 6.56. Calcd. for $C_{39}H_{38}O_4$: C, 82.08; H, 6.71%.

*(3S,4S)-4-(4-Benzyloxy-3-methoxyphenyl)-3-trityloxymethyl-4-butanolide (**3**).* A reaction solution of olefin **2** (8.57 g, 15.0 mmol), NMO (2.04 g, 17.4 mmol), and $OsO_4$ (2 ml, 2% $H_2O$ solution) in acetone (100 ml), *tert*-BuOH (25 ml), and $H_2O$ (25 ml) was stirred at room temperature for 16 h under $N_2$ gas before addition of a sat. aq. sodium thiosulfate solution. 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 a crude glycol. A reaction mixture of this crude glycol and $NaIO_4$ (3.17 g, 14.8 mmol) in MeOH (150 ml) was stirred at room temperature for 16 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 followed by silica gel column chromatography (EtOAc/hexane = 1/3) gave hemiacetal (6.63 g, 14.8 mmol) as a colorless oil. A reaction mixture of this hemiacetal (6.63 g, 14.8 mmol), PCC (3.00 g, 13.9 mmol), and MS 4A (0.5 g) in $CH_2Cl_2$ (150 ml) was stirred at room temperature for 16 h before addition of ether. After filtration, the filtrate was concentrated. The residue was applied to silica gel column chromatography (EtOAc/hexane = 1/3) to give lactone **3** (5.90 g, 10.3 mmol, 69%) as a colorless oil, $[\alpha]^{20}_D = +5$ (*c* 1.9, $CHCl_3$). NMR $\delta_H$ ($CDCl_3$): 2.48 (1H, dd, *J* 17.3, 4.7 Hz, 2-*H*H), 2.70 (1H, dd, *J* 17.3, 8.2 Hz, 2-H*H*), 2.75 (1H, dd, *J* 9.1, 5.0 Hz, C*H*HOTr), 2.90 (1H, dd, *J* 9.1, 5.4 Hz, CH*H*OTr), 2.91 (1H, m, 3-H), 3.64 (3H, s, $OCH_3$), 5.12 (2H, s, $OCH_2$Ar), 5.61 (1H, d, *J* 6.5 Hz, 4-H), 6.58 (1H, dd, *J* 8.3, 1.9 Hz, ArH), 6.74 (1H, d, *J* 8.3 Hz, ArH), 6.78 (1H, d, *J* 1.9 Hz, ArH), 7.15–7.24 (15H, m, ArH), 7.28–7.38 (3H, m, ArH), 7.42–7.44 (2H, m, ArH). NMR $\delta_C$ ($CDCl_3$): 32.6, 40.7, 55.7, 62.0, 71.0, 82.5, 87.1, 109.4, 113.6, 117.6, 127.0, 127.2, 127.7, 127.9, 128.4, 128.5, 128.7, 137.0, 143.4, 147.7, 149.5, 176.2. *Anal.* Found: C, 79.71; H, 5.97. Calcd. for $C_{38}H_{34}O_5$: C, 79.98; H, 6.01%.

*(1S,2S,3R)-1-(4-Benzyloxy-3-methoxyphenyl)-3-[(S)-(4-benzyloxy-3-methoxyphenyl)(methoxymethoxy)methyl]-2-trityloxymethyl-1,4-butanediol (**6**) and (1S,2S,3R)-*

*1-(4-benzyloxy-3-methoxyphenyl)-3-[(R)-(4-benzyloxy-3-methoxyphenyl)(methoxymethoxy)methyl]-2-trityloxymethyl-1,4-butanediol (**8**).* To a solution of KHMDS (17.2 ml, 0.5 M in toluene, 8.60 mmol) in THF (50 ml) was added a solution of lactone **3** (4.10 g, 7.18 mmol) in THF (20 ml) at −70 °C. After 15 min at −70 °C, a solution of 4-benzyloxy-3-methoxybenzaldehyde (1.74 g, 7.18 mmol) in THF (10 ml) was added. The reaction solution was stirred at −70 °C for 30 min, and then sat. aq. $NH_4Cl$ solution was 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 an aldol product as a mixture of *erythro* and *threo* isomer (4:1) **4** (4.73 g, 5.82 mmol, 81%) as a colorless oil. NMR $\delta_H$ ($CDCl_3$): 2.24–2.28 (1H, m, 2-H), 2.61 (0.2H, dd, *J* 9.6, 5.8 Hz, C*H*HOTr), 2.72 (0.2H, dd, *J* 9.6, 7.9 Hz, CH*H*OTr), 2.81 (0.8H, d, *J* 5.0 Hz, OH), 2.87–2.89 (1.6H, m, $CH_2OTr$), 2.90 (0.2H, m, 3-H), 3.03 (0.8H, m, 3-H), 3.44 (0.6H, s, $OCH_3$), 3.56 (2.4H, s, $OCH_3$), 3.74 (0.6H, s, $OCH_3$), 3.81 (2.4H, s, $OCH_3$), 4.00 (0.2H, d, *J* 2.0 Hz, OH), 4.88 (0.2H, dd, *J* 7.8, 2.0 Hz, ArC*H*OH)), 5.04 (0.8H, s, $OCH_2Ph$), 5.10 (3.2H, s, $OCH_2Ph$), 5.33 (0.8H, dd, *J* 5.0, 3.2 Hz, ArC*H*OH), 5.59 (0.2H, d, *J* 7.8 Hz, 4-H), 5.70 (0.8H, d, *J* 7.6 Hz, 4-H), 6.48–6.56 (2H, m, ArH), 6.66–6.75 (3.2H, m, ArH), 6.83 (0.8H, d, *J* = 1.8 Hz, ArH), 7.02–7.18 (15H, m, ArH), 7.28–7.43 (10H, m, ArH). *Anal.* Found: C, 78.30; H, 5.59. Calcd. for $C_{53}H_{48}O_8$: C, 78.09; H, 5.81%. A reaction mixture of aldol product **4** (2.00 g, 2.46 mmol), MOMCl (6.00 ml, 79.0 mmol), and *iso*-$Pr_2NEt$ (27.5 ml, 158 mmol) in $CH_2Cl_2$ (10 ml) was stirred at room temperature for 16 h before addition of MeOH and $CH_2Cl_2$. The organic solution was separated, washed with 1 M aq. HCl solution, sat. aq. $NaHCO_3$ solution, and brine, and then dried ($Na_2SO_4$). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/3) gave MOM ether **5** (1.94 g, 2.26 g, 92%) as a colorless oil and diastereomeric mixture. *Anal.* Found: C, 76.72; H, 6.27. Calcd. for $C_{55}H_{52}O_9$: C, 77.08; H, 6.12%. To an ice-cooled suspension of $LiAlH_4$ (0.10 g, 2.64 mmol) in THF (10 ml) was added a solution of lactone **5** (2.60 g, 3.03 mmol) in THF (20 ml). After the reaction mixture had been stirred at room temperature for 1 h, sat. aq. $MgSO_4$ solution and $K_2CO_3$ were added. After filtration, the filtrate was concentrated. The resulting residue was applied to silica gel column chromatography (EtOAc/toluene = 1/4) to give 3-[(3*S*)] isomer **6** (*R*f 0.16, 2.08 g, 2.42 mmol, 80%) as a colorless oil, $[\alpha]^{20}_D$ = −45 (*c* 2.2, $CHCl_3$) and 3-[(3*R*)] isomer **8** (*R*f 0.10, 0.49 g, 0.57 mmol, 19%) as a colorless oil, $[\alpha]^{20}_D$ = +30 (*c* 1.2, $CHCl_3$). 3-[(*S*)] isomer **6**: NMR $\delta_H$ ($CDCl_3$): 2.26 (1H, m, CH), 2.32 (1H, s, OH), 2.65 (1H, m, CH), 3.18 (3H, s, $OCH_3$), 3.22 (1H, dd, *J* 9.0, 9.0 Hz, C*H*HOTr), 3.45–3.56 (3H, m, CH*H*OTr, $CH_2OH$), 3.77 (3H, s, $OCH_3$), 3.81 (3H, s, $OCH_3$), 4.33 (1H, d, *J* 6.0 Hz, OC*H*HOCH$_3$), 4.37 (1H, d, *J* 6.0 Hz, OCH*H*OCH$_3$), 4.54 (1H, br. s, OH), 4.60 (1H, d, *J* 9.3 Hz, 1-H), 5.05 (1H, s, ArCH(OMOM)), 5.13 (2H, s, $ArCH_2O$), 6.10 (2H, s, $ArCH_2O$), 6.76–6.82 (4H, m, ArH), 6.85 (1H, s, ArH), 6.91 (1H, s, ArH), 7.14–7.46 (25H, m, ArH). NMR $\delta_C$ ($CDCl_3$): 47.2, 48.4, 55.75, 55.80, 56.4, 56.5, 60.3, 62.0, 70.9, 71.0, 77.7, 87.3, 94.7, 109.7, 110.2, 113.3, 113.5, 117.8, 120.1, 125.2, 126.8, 127.1, 127.6, 128.1, 128.3, 128.4, 128.9, 133.1, 136.9, 137.0, 137.3, 137.7, 143.5, 146.7, 147.8, 149.2, 149.6. FABMS *m/z* (%): 861 ($(M+H)^+$, 0.2), 243 (100). HRFABMS $(M+H)^+$: Calcd. for $C_{55}H_{57}O_9$, 861.4002. Found, 861.4001. 3-[(*R*)] isomer **8**: NMR $\delta_H$ ($CDCl_3$): 1.83 (1H, m, CH), 2.33 (1H, s, OH), 2.58 (1H, m, CH), 3.12 (1H, dd, *J* 10.0, 3.4 Hz, C*H*HOTr), 3.25 (1H, dd, *J* 10.0, 9.4 Hz, CH*H*OTr), 3.32 (3H, s, $OCH_3$), 3.61 (3H, s, $OCH_3$), 3.71 (1H, m, C*H*HOH), 3.78 (3H, s, $OCH_3$), 4.12 (1H, m, CH*H*OH), 4.49 (2H, s, OC$H_2$OCH$_3$), 4.65 (1H, br. s, OH), 4.79 (1H, d, *J* 10.0 Hz, 1-H), 4.83 (1H, s, ArCHOMOM), 5.07 (2H, s, $ArCH_2O$), 5.14 (2H, s, $ArCH_2O$), 6.31–6.33 (2H, m, ArH), 6.59 (1H, d, *J* 8.2 Hz, ArH), 6.83 (1H, d, *J* 8.2 Hz, ArH), 6.88 (1H, d, *J* 8.1 Hz, ArH), 6.95 (1H, s, ArH), 7.11–7.46 (25H, m, ArH). NMR $\delta_C$ ($CDCl_3$): 44.4, 45.6, 55.6, 55.78, 55.80, 57.9, 59.4, 69.8, 70.9, 77.9, 86.7, 94.7, 108.8, 110.4, 113.1, 113.3, 116.9, 120.9, 125.2, 126.7, 127.1, 127.2, 127.5, 127.7, 127.9, 128.1, 128.3, 128.4, 128.5, 128.9, 132.6, 136.4, 136.8, 137.3, 137.7, 143.8, 146.4, 148.2, 149.0, 150.2. FABMS *m/z* (%): 861 ($(M+H)^+$, 2), 243 (100). HRFABMS $(M+H)^+$: Calcd. for $C_{55}H_{57}O_9$, 861.4002. Found, 861.3996.

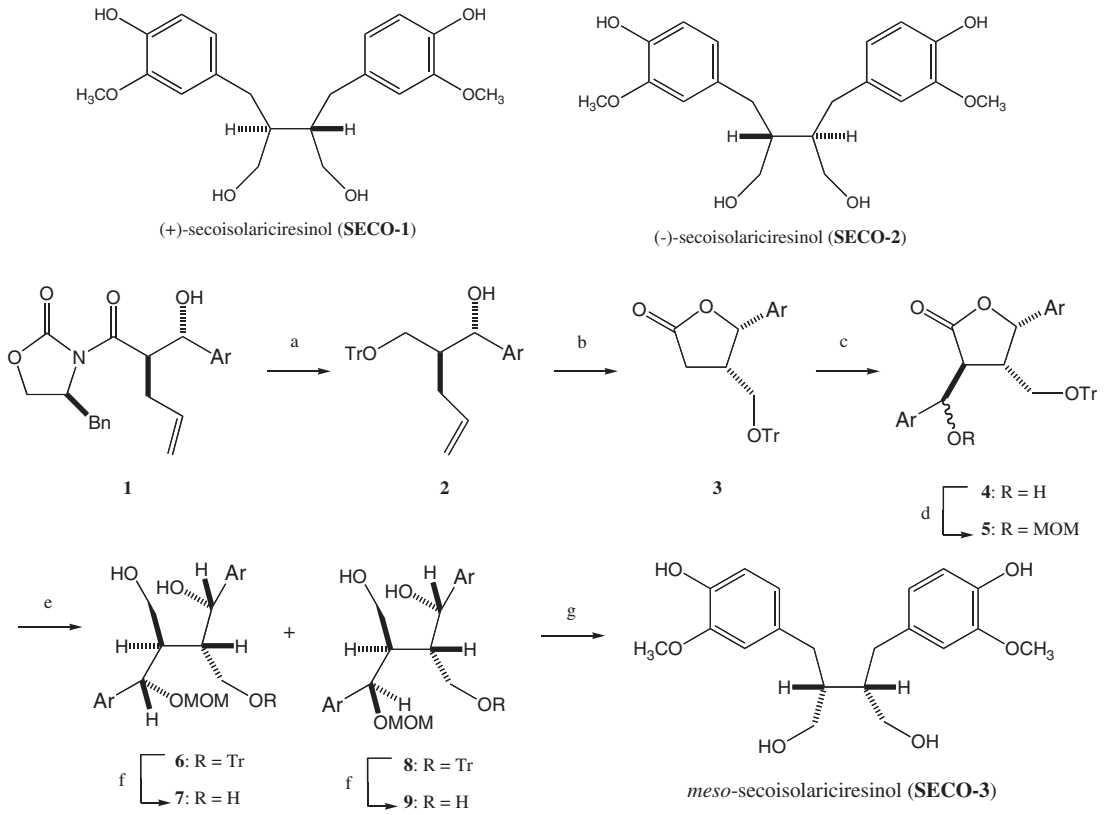
*(1S,2S,3R)-1-(4-Benzyloxy-3-methoxyphenyl)-3-[(R)-(4-benzyloxy-3-methoxyphenyl)(methoxymethoxy)methyl]-2-hydroxymethyl-1,4-butanediol (**9**).* To a solution of 3-[(*R*)]-trityl ether **8** (0.78 g, 0.91 mmol) in ether (40 ml) was added formic acid (40 ml) at −10 °C. After stirring at −10 °C for 10 min, $CHCl_3$ and $H_2O$ were added. The organic solution was separated, washed with sat. aq. $NaHCO_3$ solution, and dried ($Na_2SO_4$). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/1 and EtOAc/hexane = 4/1) gave 3-[(*R*)]-triol **9** (0.30 g, 0.48 mmol, 53%) as a colorless oil, $[\alpha]^{20}_D$ = +65 (*c* 1.2, $CHCl_3$). The trityl ether (0.10 g, 0.16 mmol, 18%) was recovered. NMR $\delta_H$ ($CDCl_3$): 1.80 (1H, m, CH), 2.36 (1H, m, CH), 2.61 (1H, br. s, OH), 3.35 (3H, s, $OCH_3$), 3.56 (1H, m, C*H*HOH), 3.67 (1H, m, CH*H*OH), 3.74 (3H, s, $OCH_3$), 3.85 (3H, s, $OCH_3$), 4.03 (1H, m, C*H*HOH), 4.18 (1H, m, CH*H*OH), 4.51 (2H, s, OC$H_2$OCH$_3$), 4.56 (1H, br. s, OH), 4.92 (1H, d, *J* 7.9 Hz, 1-H), 5.04 (1H, s, ArCH(OMOM)), 5.09 (2H, s, $ArCH_2O$), 5.13 (2H, s, $ArCH_2O$), 6.62–6.65 (2H, m, ArH), 6.76 (1H, d, *J* 8.3 Hz, ArH), 6.83–6.89 (3H, m, ArH), 7.25–7.44 (10H, m, ArH). NMR $\delta_C$ ($CDCl_3$): 45.7, 45.8, 55.8, 56.0, 59.4, 61.2, 71.0, 72.2, 78.2, 94.7, 109.1, 110.7, 113.5, 113.7, 117.3, 120.2, 127.2, 127.8, 127.9, 128.5, 128.6, 132.7, 136.7, 136.9, 137.1, 146.9, 148.1, 149.4, 150.0. FABMS *m/z* (%): 619 ($(M+H)^+$, 1), 154 (100). HRFABMS $(M+H)^+$: Calcd. for $C_{36}H_{43}O_9$, 619.2907. Found, 619.2909.

*meso-Secoisolariciresinol (**SECO-3**).* To a solution of 3-[(*S*)]-trityl ether **6** (2.08 g, 2.42 mmol) in ether (100 ml) was added formic acid (100 ml) at −10 °C. After 10 min at −10 °C, $CHCl_3$ and $H_2O$ were added. The organic solution was separated, washed with $NaHCO_3$, and dried ($Na_2SO_4$). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/1 and 4/1) gave 3-[(*S*)]-triol **7** (0.85 g) as a mixture with impurities. HRFABMS $(M+H)^+$: Calcd. for $C_{36}H_{43}O_9$, 619.2907. Found, 619.2908. Trityl ether **6** (0.30 g, 0.45 mmol, 19%) was recovered. A reaction mixture of unpurified 3-[(*S*)]-triol **7** (0.85 g) and 20% $Pd(OH)_2$ in EtOAc (20 ml) was stirred under $H_2$ gas at ambient temperature for 30 h before filtration. The filtrate was concentrated, and then the residue was applied to silica gel column chromatography (EtOAc/hexane = 1/1) to give **SECO-3** (0.35 g, 0.97 mmol, 40%, 2 steps) as a colorless oil. NMR $\delta_H$ ($CDCl_3$): 2.01 (2H, m, 2-H, 3-H), 2.57 (2H, dd, *J* 13.6, 6.2 Hz, ArC*H*H), 2.64 (2H, dd, *J* 13.6, 9.2 Hz, ArCH*H*), 3.49 (2H, dd, *J* 11.0, 2.8 Hz, C*H*HOH), 3.59 (2H, dd, *J* 11.0, 6.9 Hz, CH*H*OH), 3.82 (6H, s, $OCH_3$), 5.72 (2H, s, ArOH), 6.64–6.67 (4H, m, ArH), 6.80 (2H, d, *J* 7.9 Hz, ArH). NMR $\delta_C$ ($CDCl_3$): 33.3, 45.0, 55.8, 63.1, 111.4, 114.2, 121.6, 132.2, 143.9, 146.6. FABMS *m/z* (%): 363 ($(M+H)^+$, 5), 137 (100). HRFABMS $(M+H)^+$: Calcd. for $C_{20}H_{27}O_6$, 363.1808. Found, 363.1799. **SECO-3** (0.10 g, 0.28 mmol) was also obtained from 3-[(*R*)]-triol **9** (0.25 g, 0.40 mmol) by the same method as that just described in 69% yield. The NMR spectral data were identical with those for the compound prepared from **6**.

*Assay of the IgM production-stimulating activity.* Human-human hybridoma HB4C5 cells producing monoclonal IgM were used for an assay of the IgM production-stimulating activity. The HB4C5 cell line was a fusion product of a human B lymphocyte from a lung cancer patient and human fusion partner NAT-30 cells.[20] The HB4C5 cells were cultured in an ERDF medium (Kyokuto Pharmaceutical, Tokyo, Japan) supplemented with 10 μg/ml of insulin (Sigma), 20 μg/ml of transferrin (Sigma), 20 μM ethanolamine (Sigma), and 25 nM selenite (Sigma; ITES-ERDF) at 37 °C in humidified 5% $CO_2$-95% air.[21] The IgM production-stimulating activity was examined by measuring the amount of IgM secreted by the HB4C5 cells in the culture media. The HB4C5 cells were inoculated in to the ITES-ERDF medium supplemented with SECO compounds. The assay of the IgM production-stimulating activity was performed in a 96-well culture plate, the HB4C5 cells being inoculated at a density of $5 \times 10^4$ cells/ml. After cultivating for 6 h in a $CO_2$ incubator at 37 °C, the amount of IgM secreted into each culture medium was determined by an enzyme-linked immunosorbent assay (ELISA) which was performed by using an anti-human IgM antibody (Biosource International) as described in a previous report.[22] Briefly, 1.0 μg/ml of the goat anti-human IgM antibody (Cappel, NC, USA) was added to a 96-well plate at 100 μl/well, and the culture incubated for 2 h at 37 °C. After washing with 0.05% Tween 20-PBS (T-PBS) three times, each well was blocked with a 1.0% bovine serum albumin (BSA)-PBS solution for 2 h at 37 °C. Following this blocking reaction, each well was treated with 50 μl of the culture supernatant for 1 h at 37 °C. A 100 μl/well amount of horseradish peroxidase (HRP)-conjugated anti-human IgM antibody (Biosource International, Camarillo, CA, USA) that had been diluted 1000 times by 1.0% BSA-PBS was then added, and the culture incubated for 1 h at 37 °C. Following this, 0.6 mg/ml of 2,2′-azino-bis(ethylbenzothazoline-6-sulfonic acid diammonium salt) (ABTS) dissolved in a 0.03% $H_2O_2$-0.05 M citrate buffer (pH 4.0) was added to the wells at 100 μl/well, and the absorbance at 415 nm was measured after finally adding 100 μl/well of 1.5% oxalic acid to terminate the coloring reaction. This Ig production assay was repeated three times.

*Assay of the IgE production-suppressing activity.* Human myeloma cell line U266 cells producing IgE were obtained from ATCC, and subcultured in an ERDF medium supplemented with 5% of FBS at 37 °C in a humidified atmosphere of 5% $CO_2$. The IgE production-suppressing activity was examined by measuring the amount of IgE secreted by the U266 cells in to the culture media. The U266 cells were inoculated in to the ITES-ERDF medium supplemented with SECO compounds. The assay of the IgE production-suppressing activity was performed in a 96-well culture plate, and the U266 cells were inoculated at a density of $5 \times 10^4$ cells/ml. After cultivating for 24 h in a $CO_2$ incubator at 37 °C, the amount of IgE secreted into each culture medium was determined by an enzyme-linked immunosorbent assay (ELISA) that was performed by using an anti-human IgE antibody (Biosource International).

*Assay of the growth inhibitory effect on cancer cells.* Colon-26 colon adenocarcinoma cells derived from a BALB/c mouse were kindly provided by Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan).[23,24] Human breast cancer cell line MCF-7 cells were obtained from ATCC. The colon-26 cells and MCF-7 cells were subcultured in an ERDF medium supplemented with 5% of FBS at 37 °C in a humidified atmosphere of 5% $CO_2$. The colon-26 cells and MCF-7 cells were inoculated into a 96-well culture plate at $1.0 \times 10^5$ cells/ml suspended in the ERDF medium with 5% FBS and various concentrations of SECO compounds. After cultivating for 48 h, the cell viability was assessed by a WST-8 reduction assay kit (Dojin Laboratories, Japan). The WST-8 reduction activity of the cells was represented by the ratio of cell activity. Briefly, a WST-8 solution was added to the culture medium at 10% and incubated for 3 h at 37 °C prior to colorimetry at 450 nm.




**Scheme 1.** Synthesis of *meso*-Secoisolariciresinol (**SECO-3**).

(a) (1) $LiBH_4$, MeOH, THF, 0 °C, 1 h; (2) TrCl, Pyr., r.t., 16 h (50% yield, 2 steps); (b) (1) $OsO_4$, NMO, aq. acetone, *tert*-BuOH, r.t., 16 h; (2) $NaIO_4$, MeOH, r.t., 16 h; (3) PCC, MS 4A, $CH_2Cl_2$, r.t., 16 h (69% yield, 3 steps); (c) 4-benzyloxy-3-methoxybenzaldehyde, KHMDS, THF, −70 °C, 30 min (81% yield); (d) MOMCl, *iso*-$Pr_2NEt$, $CH_2Cl_2$, r.t., 16 h (92% yield); (e) LAH, THF, r.t., 1 h (**6**: 80% yield, **8**: 19% yield); (f) $HCO_2H$, ether, −10 °C, 10 min (**7**: mixture with impurities, **9**: 53% yield); (g) $H_2$, 20% $Pd(OH)_2/C$, EtOAc, 30 h (from **6**: 40% yield, 2 steps, from **9**: 69% yield).

## Results and Discussion

*Synthesis of **SECO-3***

*meso*-Secoisolariciresinol (**SECO-3**) would be converted from benzyl alcohols **7** and **9** by reduction. The stereochemistry of these benzyl alcohols might be obtained from lactone **4**, which could be transformed from **1** (Scheme 1). After reductive removal of the chiral auxiliary of **1**[19] by using $LiBH_4$, the resulting primary hydroxy group was protected as a trityl ether to give **2**. Oxidative cleavage of the olefin by treating with $OsO_4$ and $NaIO_4$, this being followed by pyridinium chlorochromate oxidation of the resulting hemiacetal, gave lactone **3**. Aldol condensation using potassium bis(trimethylsilyl)amide gave aldol product **4** as a mixture of *erythro* and *threo* (4:1) isomers. The reduction of lactone **4** to a diol by employing hydride reagents ($LiAlH_4$, $LiBH_4$, *iso*-$Bu_2AlH$-$NaBH_4$) was not effective. Protection of the benzylic hydroxyl group as a triisopropylsilyl ether gave the desired diol in a low yield. The methoxymethyl ether for protecting the benzyl hydroxy group of **4** was selected as a suitable protective group for reduction. The reduction of lactone **5** by $LiAlH_4$ and subsequent separation of the diastereomers gave diols **6** and **8**. Detritylation in an $HCO_2H$-ether system, followed by reduction and hydrogenolysis by employing $Pd(OH)_2$ and $H_2$ gas gave *meso*-secoisolariciresinol (**SECO-3**). The treatment of **7** and **9** with Pd-black under $H_2$ gas gave many decomposed products. The first synthesis of *meso*-secoisolariciresinol (**SECO-3**) was thus achieved.

*IgM production-stimulating activity*

The bio-functional activities of three kinds of SECO compounds were compared. The immunostimulation activity of the SECO compounds was first examined, and then the IgM production-stimulating activity toward hybridoma HB4C5 cells was assessed. As indicated in Fig. 1, the optically active compounds, (+)-secoisolariciresinol (**SECO-1**) and (−)-secoisolariciresinol (**SECO-2**), stimulated the IgM production of HB4C5 cells. These two compounds accelerated IgM production by about 2-fold. However, **SECO-3**, a diastereomer of **SECO-1** and **SECO-2**, had no effect of IgM production-stimulating activity toward the HB4C5 cells. This result means that the configuration of the SECO-related compounds was important for IgM production-stimulating activity. Moreover, a stereoselective synthesis of the SECO-related compounds was important for precisely evaluating their bio-functions. Different IgM produc-

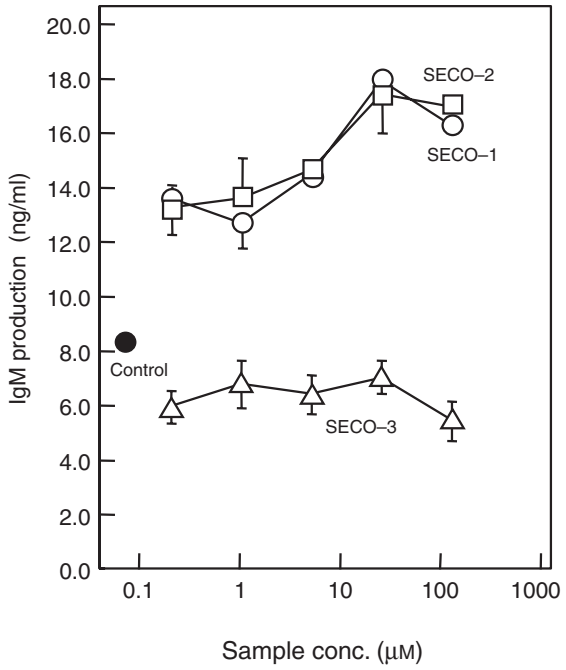



**Fig. 1.** IgM Production-Stimulating Activity.

HB4C5 cells were inoculated in ITES-ERDF medium supplemented with several concentrations of SECOs at $5 \times 10^4$ cells/ml, and cultured for 6 h. The amount of IgM in each culture medium was determined by ELISA. Each result is expressed as the mean ± SD (n = 3).

tion-stimulating activity was observed between the optically active compounds and the *meso* compound.

*IgE production-suppressing activity*

The anti-allergy activity was evaluated by the IgE production-suppressing activity toward U266 cells. None of the three SECO compounds suppressed the IgE production by U266 cells.

*Growth-inhibitory effect of the SECO compounds on cancer cells*

The growth-inhibitory effects of SECOs on cancer cell lines were examined. **SECO-3** weakly suppressed the cell growth of mouse colon cancer colon-26 cells and human breast cancer MCF-7 cells in a dose-dependent manner (Fig. 2). On the other hand, **SECO-2** stimulated cell growth at 5 μM and suppressed it at 100 μM, while **SECO-1** did not demonstrate the any cell growth-inhibitory effect toward either cell line. The growth-inhibitory effect of **SECO-3** was much higher than that of **SECO-1** or **SECO-2**. This means that the effect did not originate from the type of substituent, but instead from its configuration.

Lignans have estrogen-like effects *in vitro*, and their action is at least partly attributable to their capability to bind to estrogen receptors.[25] MCF-7 cells have an estrogen receptor, and the growth is stimulated by beta-estradiol or estrogen-mimicking compounds.[26,27] It is presumed from these facts that (−)-secoisolariciresinol (**SECO-2**) associated with estrogen receptors on the

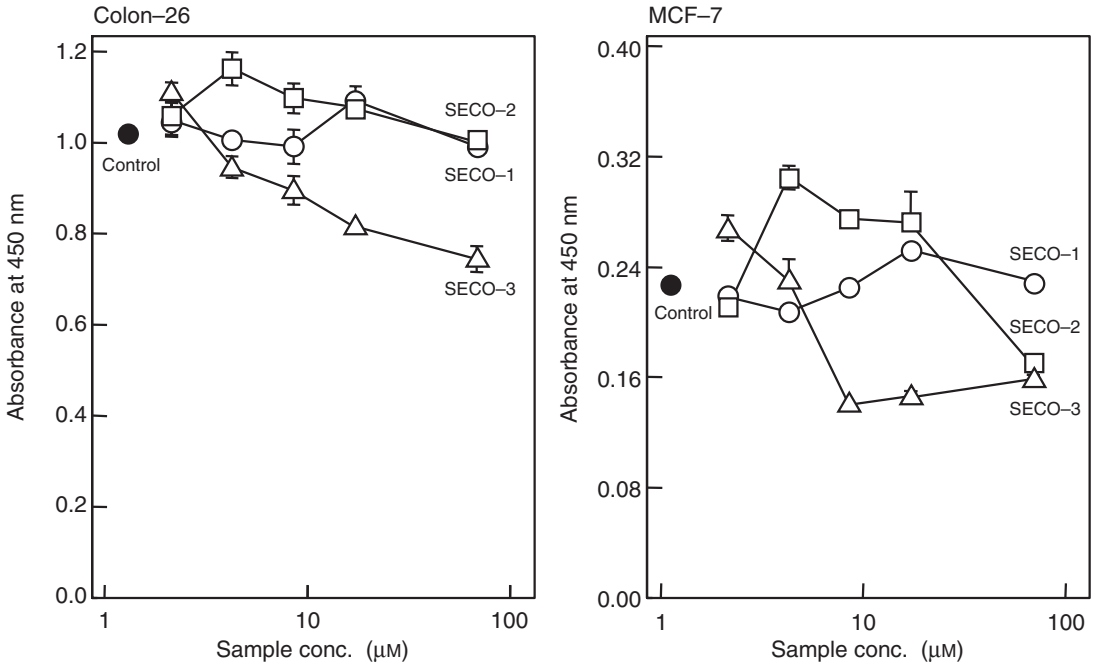



**Fig. 2.** Growth-Inhibitory Effect of SECO Compounds on Cancer Cells.

Colon-26 cells and MCF-7 cells were inoculated into a 5% FBS-ERDF medium supplemented with several concentrations of SECOs at $1 \times 10^5$ cells/ml, and cultured for 48 h. Cell viability was evaluated by a WST-8 assay. Each result is expressed as the mean ± SD (n = 3).

MCF-7 cells and stimulated cell growth, and that (+)-secoisolariciresinol (**SECO-1**) did not associate or did not have estrogen-like activity. These findings suggest that it is critically important to distinguish between the (+) and (−)-isomers when the bio-function of secoisolariciresinol is being discussed. A bioassay using a racemate is thus insufficient for precisely evaluating the bio-functions of secoisolariciresinol.

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