First Stereoselective Synthesis of \textit{meso}-Secoisolariciresinol and Comparison of Its Biological Activity with (+) and (−)-Secoisolariciresinol

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

Key words: lignan; immunosuppressive activity; cytotoxic activity

The widely distributed lignans1) have been target compounds for organic synthesis2) and biological research3,4) 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 \textit{meso}-secoisolariciresinol (SECO-3) were selected as target compounds. (−)-Secoisolariciresinol (SECO-2) is also one of the common lignans contained in food plants,5,9) 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 isolation10–12) and synthesis13,14) 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, \textit{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 \textit{meso}-secoisolariciresinol (SECO-3). In this present study, (+)- and (−)-secoisolariciresinol (SECO-1 and SECO-2) and \textit{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 (0ppm), 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
the previously reported method.  

**SECO-1**: colorless crystals, mp 111–112°C (CHCl3-(iso-Pr)2O), 114–115°C in the literature, \[^{13}\alpha\]D = +31 (c 0.3, acetone).

The NMR data agree with those in the literature.  

**SECO-2**: mp 111–112°C (CHCl3-(iso-Pr)2O), \[^{20}\alpha\]D = −32 (c 0.3, acetone), lit.,  [\[^{10}\alpha\]D] = −35.5 (c 1.07, acetone).

** Determination of the enantiomeric excess of SECO-1 and SECO-2**: A reaction solution of (+)-eco-steroidal esinol (SECO-1) (25 mg, 0.069 mmol) in pyridine (0.1 ml) and Ac2O (0.1 ml) containing DMAP (1 mg) was stirred 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 H2O, 6m aq. HCl solution, sat. aq. NaHCO3 solution and brine, and dried (Na2SO4). Concentration followed by silica gel TLC (EtOAc/hexane = 1/1) gave a tetraacetate (25 mg, 0.047 mmol, 68%) as a colorless oil, \[^{20}\alpha\]D = +13 (c 0.5, CDCl3), 95% ee (HPLC, Daicel AD-H chiral column, detected at 280 nm, 1 ml min\(^{-1}\), hexane/iso-propyl alcohol = 3/2, \(\tau_k\) 7.8 min. Tetraacetate of (−)-eco-steroidal esinol (SECO-2): \[^{20}\alpha\]D = −13 (c 0.4, CDCl3), lit., \[^{10}\alpha\]D = −7.6 (c 0.65, CDCl3), 95% ee (HPLC, Daicel AD-H chiral column, detected at 280 nm, 1 ml min\(^{-1}\), hexane/iso-propyl alcohol = 3/2, \(\tau_k\) 5.8 min.

\((4S)-4\text{-Benzyl-3-\{\text{(R)\-2-\{(S\-4\text{-benzoyloxy-3\-methoxyphenyl\-(hydroxy)methyl\-4\-pentenyl\-2\-oxazolidinone (1)}}}}\) Compound 1 was prepared by the previously described method, \[^{19}\] no production of the syn form being apparent. \(^1\)H-NMR of \((4S)-3\-\{(S\-2\-\{(S\-3\text{-benzoxyl-3\-methoxyphenyl-4\text{-pentenyl-1\-ol (2)}}}}\) To an ice-cooled solution of tosyl chloride (19.0 g, 29.9 mmol) and MeOH (2.6 ml) in THF (200 ml) was added a solution of LiBH4 (13.5 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 ml aq. NaOH solution (120 ml) and EtOAc. The organic solution was separated, washed with H2O and brine, dried (Na2SO4), and concentrated to give a crude diol. A solution of this crude diol and TMS (8.25 g, 29.6 mmol) in pyridine (20 ml) was stirred at room temperature for 16 h before addition of H2O and CHCl3. The organic solution was separated, washed with a sat. aq. CuSO4 solution, sat. aq. NaHCO3 solution and brine, and dried (Na2SO4). 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, \[^{20}\alpha\]D = +15 (c 1.8, CHCl3), NMR \[^{3}\]H (CDCl3): 1.91–2.01 (2H, m, CH2=CH-CH2), 2.13 (1H, m, 2-H), 3.20 (1H, dd, J 9.6, 5.6 Hz, TrOCH3), 3.38 (1H, dd, J 9.6, 3.3 Hz, TrOH), 3.47 (1H, d, J 3.7 Hz, OH), 3.82 (3H, s, OCH3), 4.65 (1H, br. s, 1-H, 4.87–4.92 (2H, m, CH2=CH), 5.11 (2H, s, OCH2Ar), 5.51–5.61 (1H, m, CH=CH2), 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 \[^{5}\]C (CDCl3): 33.3, 34.5, 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 C38H38O4: C, 82.08; H, 6.71%.

\((3S,4S)-4\text{-\{(4\text{-Benzoyloxy-3\-methoxyphenyl\-3\-tritylomethyl-4\-butanediol (3)}}\) A reaction solution of olefin 2 (8.57 g, 15.0 mmol), NMO (2.04 g, 17.4 mmol), and OsO4 (2 ml, 2% H2O solution) in acetone (100 ml), tert-BuOH (25 ml), and H2O (25 ml) was stirred at room temperature for 16 h under N2 gas before addition of a sat. aq. sodium thiosulfate solution. After concentration of the mixture, the residue was dissolved in EtOAc and H2O. The organic solution was separated, washed with brine, and dried (Na2SO4). Concentration gave crude glycol. A reaction mixture of this crude glycol and NaO4 (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 H2O. The organic solution was separated, washed with brine, and dried (Na2SO4). 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 CH2Cl2 (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, \[^{20}\alpha\]D = +5 (c 1.9, CHCl3). NMR \[^{3}\]H (CDCl3): 2.48 (1H, dd, J 17.3, 4.7 Hz, 2-HH), 2.70 (1H, dd, J 17.3, 8.2 Hz, 2-HH), 2.75 (1H, dd, J 9.1, 5.0 Hz, CHHOTr), 2.90 (1H, dd, J 9.1, 5.4 Hz, CHHOTr), 2.91 (1H, m, 3-H), 3.64 (3H, s, OCH3), 5.12 (2H, s, OCH2Ar), 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 \[^{5}\]C (CDCl3): 32.6, 40.7, 55.7, 62.0, 71.0, 82.5, 87.1, 109.4, 113.6, 117.6, 127.0, 127.2, 127.9, 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 C38H38O4: C, 79.79; H, 6.01%.

\((1S,2S,3R)-1-(\text{4\-Benzyloxy-3\-methoxyphenyl\-3\-\{(S\-4\-benzyloxy-3\-methoxyphenyl\-(methoxymethoxymethyl\-2\-trityloxyethyl-1,4-butanediol (6)}}\) and \((1S,2S,3R)-")
I-(4-benzyloxy-3-methoxyphenyl)-3-[(R)-(4-benzyloxy-3-methoxyphenyl)methoxymethoxy)methyl]-2-trityloxyethyl-1,4-butanediol (8). To a solution of KHDS (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. NH₄Cl solution was added. The organic solution was separated, washed with brine, and dried (Na₂SO₄). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/2) gave an aldol product as a mixture of erthro and threo isomer (4:1) 4 (4.73 g, 5.82 mmol, 81%) as a colorless oil. NMR δ(CDCl₃): 2.24–2.28 (1H, m, H-2), 2.61 (0.2H, dd, J 9.6, 5.8 Hz, CHJOH), 2.72 (0.2H, dd, J 9.6, 7.9 Hz, CHJOH), 2.81 (0.8H, d, J 5.0 Hz, OH), 2.87–2.89 (1.6H, m, CH₂OT₃), 2.90 (0.2H, m, 3-H), 3.03 (0.8H, m, 3-H), 3.44 (0.6H, s, OCH₃), 3.56 (2.4H, s, OCH₃), 3.79 (0.6H, s, OCH₃), 3.81 (2.4H, s, OCH₃), 4.00 (0.2H, d, J 2.0 Hz, OH), 4.88 (0.2H, dd, J 7.8, 2.0 Hz, ArCHOH), 5.04 (0.8H, s, OCH₂Ph), 5.10 (3.2H, s, OCH₂Ph), 5.33 (0.8H, dd, J 5.0, 3.2 Hz, ArCHOH), 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 = 10 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 C₃₅H₄₅O₇: 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-Pr₂NEt (27.5 ml, 158 mmol) in CHCl₃ (10 ml) was stirred at room temperature for 16 h before addition of MeOH and CH₂Cl₂. The organic solution was separated, washed with 1 m aq. HCl solution, sat. aq. NaHCO₃ solution, and brine, and then dried (Na₂SO₄). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/3) gave the diastereomeric mixture. NMR δ(CDCl₃): 2.24–2.28 (1H, m, H-2), 2.61 (0.2H, dd, J 9.6, 5.8 Hz, CHJOH), 2.72 (0.2H, dd, J 9.6, 7.9 Hz, CHJOH), 2.81 (0.8H, d, J 5.0 Hz, OH), 2.87–2.89 (1.6H, m, CH₂OT₃), 2.90 (0.2H, m, 3-H), 3.03 (0.8H, m, 3-H), 3.44 (0.6H, s, OCH₃), 3.56 (2.4H, s, OCH₃), 3.79 (0.6H, s, OCH₃), 3.81 (2.4H, s, OCH₃), 4.00 (0.2H, d, J 2.0 Hz, OH), 4.88 (0.2H, dd, J 7.8, 2.0 Hz, ArCHOH), 5.04 (0.8H, s, OCH₂Ph), 5.10 (3.2H, s, OCH₂Ph), 5.33 (0.8H, dd, J 5.0, 3.2 Hz, ArCHOH), 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 = 10 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 C₃₅H₄₅O₇: 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-Pr₂NEt (27.5 ml, 158 mmol) in CHCl₃ (10 ml) was stirred at room temperature for 16 h before addition of MeOH and CH₂Cl₂. The organic solution was separated, washed with 1 m aq. HCl solution, sat. aq. NaHCO₃ solution, and brine, and then dried (Na₂SO₄). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/3) gave the diastereomeric mixture. NMR δ(CDCl₃): 2.24–2.28 (1H, m, H-2), 2.61 (0.2H, dd, J 9.6, 5.8 Hz, CHJOH), 2.72 (0.2H, dd, J 9.6, 7.9 Hz, CHJOH), 2.81 (0.8H, d, J 5.0 Hz, OH), 2.87–2.89 (1.6H, m, CH₂OT₃), 2.90 (0.2H, m, 3-H), 3.03 (0.8H, m, 3-H), 3.44 (0.6H, s, OCH₃), 3.56 (2.4H, s, OCH₃), 3.79 (0.6H, s, OCH₃), 3.81 (2.4H, s, OCH₃), 4.00 (0.2H, d, J 2.0 Hz, OH), 4.88 (0.2H, dd, J 7.8, 2.0 Hz, ArCHOH), 5.04 (0.8H, s, OCH₂Ph), 5.10 (3.2H, s, OCH₂Ph), 5.33 (0.8H, dd, J 5.0, 3.2 Hz, ArCHOH), 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 = 10 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 C₃₅H₄₅O₇: 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-Pr₂NEt (27.5 ml, 158 mmol) in CHCl₃ (10 ml) was stirred at room temperature for 16 h before addition of MeOH and CH₂Cl₂. The organic solution was separated, washed with 1 m aq. HCl solution, sat. aq. NaHCO₃ solution, and brine, and then dried (Na₂SO₄).
*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, CHCl₃ and H₂O were added. The organic solution was separated, washed with NaHCO₃, and dried (Na₂SO₄). 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 C₄₂H₃₃O₁₃, 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 H₂ 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 (CDCl₃): 2.01 (2H, m, 2-H, 3-H), 2.57 (2H, dd, J 13.6, 6.2 Hz, ArCHH), 3.64 (2H, dd, J 13.6, 9.2 Hz, ArCHH), 3.49 (2H, dd, J 11.0, 2.8 Hz, CHHOH), 3.59 (2H, dd, J 11.0, 6.9 Hz, CHHOH), 3.82 (6H, s, OCH₃), 5.72 (2H, s, ArOH), 6.64–6.67 (4H, m, ArH), 6.80 (2H, d, J 7.9 Hz, ArH), NMR (CDCl₃): 33.3, 45.0, 55.8, 63.1, 111.4, 114.2, 121.6, 132.2, 143.9, 146.6. FABMS (M + H)⁺: 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 IgE production-stimulating 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% CO₂. The IgE production-stimulating 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-stimulating activity was performed in a 96-well culture plate, and the U266 cells were inoculated at a density of 5 × 10⁴ cells/ml. After cultivating for 24 h in a CO₂ 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).

**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(ethylbenzothiazoline-6-sulfonic acid diammonium salt) (ABTS) dissolved in a 0.03% H₂O₂-0.05 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 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). 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% CO₂. The colon-26 cells and MCF-7 cells were inoculated into an ERDF medium with 5% FBS and various concentrations of SECO compounds. After culturing 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.
**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 11 by using LiBH₄, the resulting primary hydroxy group was protected as a trityl ether to give 2. Oxidative cleavage of the olefin by treating with OsO₄ and NaIO₄, this being followed by pyridinium chlorochromate oxidation of the resulting hemiacetal, gave lactone 3. Aldol condensation using potassium bis(tri-methylsilyl)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 (LiAlH₄, LiBH₄, iso-But₂AlH-NaBH₄) was not effective. Protection of the benzyl hydroxyl group as a trisopropylsilyl ether gave the desired diol in a low yield. The methoxymethyl ether for protecting the benzyl group for reduction. The reduction of lactone 5 by LiAlH₄ and subsequent separation of the diastereomers gave diols 6 and 8. Detritylation in an HCO₂H-ether system, followed by reduction and hydrogenolysis by employing Pd(OH)₂ and H₂ gas gave *meso*-secoisolariciresinol (SECO-3). The treatment of 7 and 9 with Pd-black under H₂ 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-
**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 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. MCF-7 cells have an estrogen receptor, and the growth is stimulated by beta-estradiol or estrogen-mimicking compounds. It is presumed from these facts that (−)-secoisolariciresinol (SECO-2) associated with estrogen receptors on the...
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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