

## The Structure-Activity Relationships of Flaxseed Lignan, Secoisolariciresinol

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**Abstract**—It is well known that the physiological activities of compounds are affected by their molecular structures. Hence, it is very important for the risk assessment of environmental chemicals such as PCDD and PCDF to evaluate structure-activity relationships (SAR). For precise analysis of SAR, the compounds should be stereoselectively synthesized. In this paper, we evaluated SAR of flaxseed lignan, secoisolariciresinol (SECO). SECO has many physiological effects such as immunostimulatory activity, antioxidant activity, and estrogenic activity. In addition, we first achieved the stereoselective synthesis of *meso*-SECO and optically active SECOs. Hence, SECO is a very good example for examining SAR. Optically active (+)- and (–)-SECO, and *meso*-SECO were synthesized and various physiological functions were compared. At first, the immunostimulatory effects were examined. (+)- and (–)-SECO accelerated IgM production of human hybridoma cells, however, *meso*-SECO did not. However *meso*-SECO showed cytotoxic activity against human breast cancer MCF-7 cells, (–)-SECO slightly stimulated the cell. This means that estrogenic activity of SECO derived from only (–)-SECO. Although (–)-SECO stimulated adiponectin production of 3T3-L1 adipose cells, (+)- and *meso*-SECO suppressed the production. These facts suggested that slight difference in the structure of SECO affects the physiological activity.

**Keywords:** lignan, structure-activity relationships, immunostimulatory activity, cytotoxic activity

### INTRODUCTION

The widely distributed lignans have been target compounds for organic synthesis and biological function research because of their many kinds of bonding of the C6–C3 units, oxidation of the lignan structure, and as important components in

foods and medicines from plants. 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. This means that stereoselective synthetic studies are important to promote research about the structure-activity relationships of lignans.

In this article, (+)- and (-)-secoisolariciresinol (SECO) and *meso*-SECO were selected as target compounds of investigation of structure-activity relationships of lignans. (-)-SECO is also one of the common lignans contained in edible plants, various whole-grain cereals (barley, rye and wheat), seeds, nuts, legumes and vegetables, and is biosynthesized as a mixture with (+)-SECO in some plants. Research on the isolation and synthesis of SECO compounds has been continued by organic chemists. However, the relationship between the molecular structure and biological activity has not been elucidated. On the other hand, *meso*-SECO is not common and there have been only two reports about its isolation (Agrawal *et al.*, 1982; Fang *et al.*, 1992). There is no report about the stereoselective synthetic research and biological investigation on *meso*-SECO. In this present study, relationships between structure and biological activity of (+)-, (-)- and *meso*-SECO were revealed.

## MATERIALS AND METHODS

### *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. HB4C5 cell line was a fusion product of a human B lymphocyte from a lung cancer patient and human fusion partner NAT-30 cells (Murakami *et al.*, 1982). HB4C5 cells were cultured in an ERDF medium (Kyokuto Pharmaceutical, Tokyo, Japan) supplemented with 10  $\mu\text{g/ml}$  of insulin, 20  $\mu\text{g/ml}$  of transferrin, 20  $\mu\text{M}$  ethanolamine, and 25 nM selenite (ITES-ERDF) at 37°C in humidified 5% CO<sub>2</sub>-95% air. The IgM production-stimulating activity was examined by measuring the amount of IgM secreted by HB4C5 cells in the culture media. 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, HB4C5 cells being inoculated at a density of  $5 \times 10^4$  cells/ml. After cultivating for 6 h in a CO<sub>2</sub> 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 (Sugahara *et al.*, 2006). Briefly, 1.0  $\mu\text{g/ml}$  of the goat anti-human IgM antibody (Cappel, NC, USA) was added to a 96-well plate at 100  $\mu\text{l/well}$ , 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

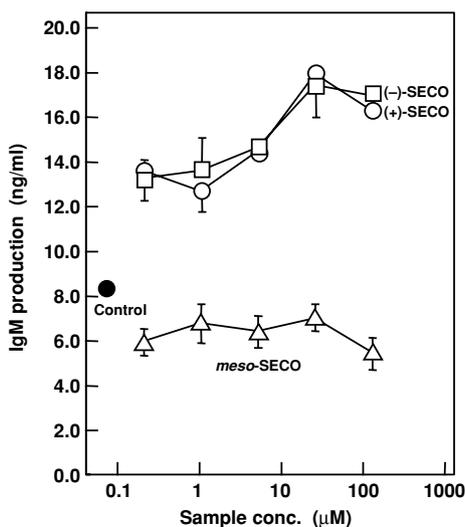


Fig. 1. IgM production stimulating effect of SECOs on human hybridoma HB4C5 cells.

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  $\mu$ l of the culture supernatant for 1 h at 37°C. A 100  $\mu$ l/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% H<sub>2</sub>O<sub>2</sub>-0.05 M citrate buffer (pH 4.0) was added to the wells at 100  $\mu$ l/well, and the absorbance at 415 nm was measured after finally adding 100  $\mu$ l/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<sub>2</sub>. The IgE production-suppressing activity was examined by measuring the amount of IgE secreted by U266 cells in to the culture media. 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<sub>2</sub> incubator at 37°C, the amount of IgE secreted into each culture medium was determined by ELISA that was performed by using an anti-human IgE antibody (Biosource International).

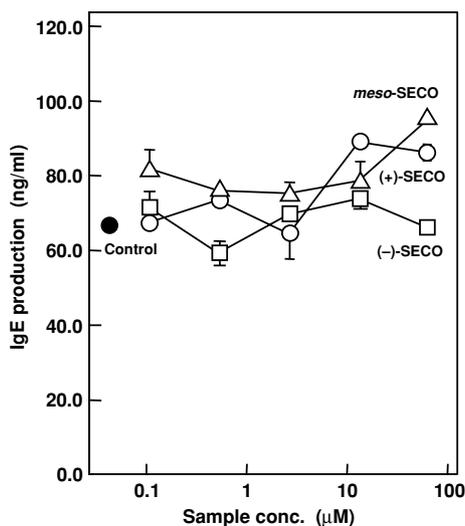


Fig. 2. IgE production suppression effect of SECOs on human myeloma U266 cells.

#### *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 ERDF medium supplemented with 5% of fetal bovine serum (FBS) at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. 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 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 (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 the measurement of absorbance at 450 nm.

#### *Assay of adiponectin production of 3T3-L1 adipocytes*

Mouse embryo fibroblast 3T3-L1 pre-adipocyte cells ( $5.0 \times 10^5$  cells/ml) were treated with insulin, dexamethasone, and isobutyl-methylxanthine at Day 0 for induction, and used for experiment between Day 12 and 16 after induction at which time >95% of the cells expressed the adipocyte phenotype. 3T3-L1 adipocytes were cultured in 10% FBS-ERDF medium supplemented with SECO compounds. Two days after, the amount of adiponectin secreted in culture medium was determined by mouse adiponectin ELISA kit (Cyclex, Nagano, Japan).

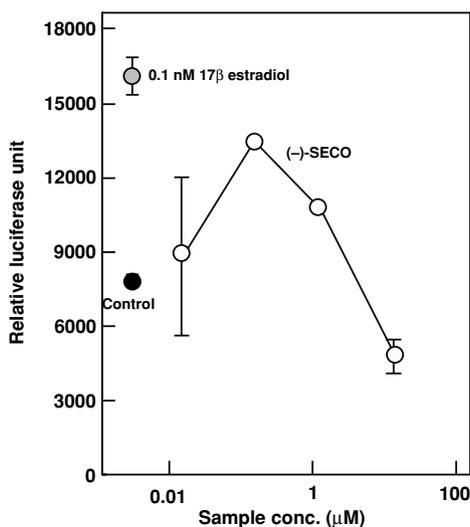


Fig. 3. Estrogen-like activity of (-)-SECO.

## RESULTS AND DISCUSSION

### *IgM production-stimulating activity of SECOs*

The bio-functional activities of three kinds of SECO compounds were compared. The immunostimulatory 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, (+)-SECO and (-)-SECO, stimulated the IgM production of HB4C5 cells. These two compounds accelerated IgM production by about 2-fold. However, *meso*-SECO, a diastereomer of (+)- and (-)-SECO, had no effect on IgM production of HB4C5 cells. This result means that the molecular configuration of the SECO 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 production-stimulating activity was observed between the optically active compounds and the *meso* compound.

### *IgE production-suppressing activity of SECOs*

The anti-allergy activity was evaluated by the IgE production-suppressing activity toward U266 cells. As indicated in Fig. 2, none of the three SECO compounds suppressed the IgE production by U266 cells.

### *Growth-inhibitory effect of SECOs 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. On the other hand, SECO-2 stimulated cell growth at 5  $\mu\text{M}$  and suppressed it at 100  $\mu\text{M}$ , while (+)-SECO did not demonstrate the any cell growth-inhibitory effect toward either cell line. The growth-inhibitory effect of *meso*-SECO was much higher than that of (+)-SECO or (-)-SECO. 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 (Pajari *et al.*, 2006). MCF-7 cells have an estrogen receptor, and the growth is stimulated by 17 $\beta$ -estradiol or estrogen-mimicking compounds. It is presumed from these facts that (-)-SECO associated with estrogen receptors on the MCF-7 cells and stimulated cell growth, and that (+)-SECO did not associate or did not have estrogen-like activity. In fact, it is revealed by the estrogen-responsible reporter gene assay using T47D-KBluc cells (Wilson *et al.*, 2004) that (-)-SECO had estrogen activity (Fig. 3). These findings suggest that it is critically important to distinguish between (+) and (-)-isomers when the biological function of SECO is being discussed. A bioassay using a racemate is thus insufficient for precisely evaluating the biological functions of secoisolariciresinol.

#### *Effect of SECOs on adiponectin production by 3T3-L1 mouse adipocytes*

3T3-L1 adipocytes were stimulated by SECO compounds in 10% FBS-ERDF medium for two days. As a result, only when the adipocytes stimulated by (-)-SECO, the adiponectin production was increased about 1.6-fold. However, treatment with (+)- or *meso*-SECO showed no stimulatory effects on adiponectin production. Thus, these facts also indicated that the difference in molecular structure of SECO contribute to biological functions.

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