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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Studies on anti-cancer activity of a new dimer sesquiterpene lactone from yacon leaves
(ヤーコン葉に含まれる新規ダイマーセスキテルペンラクトンの抗がん活性に関する研究)

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Yacon (*Smallanthus sonchifolius*), belongs to a perennial plant in the Asteraceae family, is native to the Andes or Andean Mountains. It has been cultivated in many countries in Europe, Americas, and Asia countries such as China and Japan. Yacon tubers contain fructo-oligosaccharide that has potential for prebiotic effect. Instead, the leaf contains a various kind of metabolites such as polyphenols, kaurene-type diterpenenoides, and melampolide-type sesquiterpene lactones (SLs). In this study, we isolated a novel dimer SL, uvedafolin (**1**) which has the great cytotoxicity against HeLa (cervical cancer cells) cells, being potent anticancer drug. Finally, anticancer activity of uvedafolin (**1**) was clearly attributed to the apoptosis through mitochondria pathway.

In Chapter 2, dimer SLs, uvedafolin (**1**) and enhydrofolin (**2**) which are composed of enhydrin (**4**) + sonchifolin (**6**) for **1**, and uvedalin (**5**) + sonchifolin (**6**) for **2** were isolated from yacon leaf and elucidated the chemical structures by spectroscopic methods such as IR, NMR and MS. After that we further carried out quantitation analysis of the valuable dimer SLs in eight varieties of yacon leaves that cultivated and hybridized in the Western Region Agricultural Research Center at Zentsuji city (National Agriculture and Food Research Organization). It was found that the newly identified dimer, **1**, was found in only 'Sarada otome' (0.064 mg/g fresh weight) and 'Sarada okame' (0.045 mg/g fresh weight) cultivars and the related varieties. The cytotoxicity of two dimer SLs was determined against typical cancer cell lines such as HeLa (cervical cancer), HL-60 (leukemia) and B16-F10 melanoma (skin melanoma) cells. The dimers, **1** and **2**, had the most effective IC_{50} values against the three tumor cell lines in a range from 0.2 to 1.9 μM, compared with those of monomers, **3**-**6** (0.7 - 9.9 μM). Moreover, 1:1 mixtures of enhydrin (**4**) + sonchifolin (**6**), and uvedalin (**5**) + sonchifolin (**6**) did not show high cytotoxicity as **1** and **2** did. Therefore, a cross-linkage of both monomer sesquiterpene

lactones can be concluded as an important factor for the cytotoxicity, which may be due to higher lipophilicity and disorder of functional structure of the cell membranes.

In Chapter 3, *in vitro* cytotoxic mechanism of dimer SL, uvedafolin (**1**) and the related monomer SLs was studied on the HeLa cells by referring to the cytotoxicity on NIH/3T3 cell line (used as a normal cell line). Apoptotic pathway by compound **1** was investigated by various kinds of assay systems such as cell cycle analysis, DAPI stain, caspase activity, JC-1 stain and cytochrome c release. IC_{50} of compound **1** against NIH/3T3 cell line at 24 h was 4.98 μM, meaning that it is 1.68-times safer than that of HeLa cells. Cell cycle analysis after treatment of compound **1** showed a significant increase in the percentage of G_2/M phase time-dependently, increasing the percentage of sub-G_1 peak, a parameter of fragmented DNA caused by apoptosis. Morphological change in nuclear caused by apoptosis was observed by using DAPI. The increases of caspase activities by compound **1**, were observed in the following order, caspase-9 > caspase-3/7 > caspase-8, meaning mitochondria pathway was expected to be the major apoptotic pathway. Mitochondria membrane potential change and cytochrome c releasing were observed after treatment of **1**. Therefore, **1** has the potential to induce apoptosis via mitochondria pathway more than cell death receptor pathway.

In Chapter 4, sonchifolinic acid (**7**) was isolated from the yacon leaf and then esterified in order to understand the effect of lipophilicity of the esterified derivatives. Sonchifolinic acid (**7**) and its synthetic ester derivatives such as propyl, butyl and pentyl esters were examined for the cytotoxicity and structure-activity relationship (SAR). Consequently, sonchifolinic acid (**7**) did not show great activity (IC_{50} 47.12 μM) whereas, alkylation of the carboxylic acid group at C-10 position in **7** was enhanced the cytotoxic activity more than 2-5 times (**7a** - **7d**, IC_{50} 5.29 - 21.07 μM). This result shows sonchifolinic acid (**7**) could be valuable for the modification of active substances and making potent lead compounds through semi-synthetic strategy.

In conclusion, a novel dimer SL, uvedafolin (**1**) newly isolated from the yacon leaf could have potential for anti-cancer drug and lead compound for further investigation, contributing to cancer drug designing.