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論文題目	<b>Studies on saccharothriolides, phenyl-substituted 10-membered macrolides from a rare actinomycete <i>Saccharothrix</i> sp. and precursor-directed <i>in situ</i> synthesis of saccharothriolide analogs</b> (希少放線菌 <i>Saccharothrix</i> sp. が産生する新規 saccharothriolide 類と PDSS に関する研究)		
<p>Expanding natural chemical diversity is important for drug leads discovery, since natural products can exhibit highly potent and/or selective biological activities. So far, over 10,000 bioactive secondary metabolites have been identified from a variety of actinomycetes. Rare actinomycetes (or non-streptomycete actinomycetes) are attractive resources of secondary metabolites that show biological activities with unprecedented chemical structures. Genus <i>Saccharothrix</i>, one of the rare actinomycetes, was first reported from a soil sample collected in Australia in 1984. A growing number of novel natural products with a structural and biological diversity have been reported from this genus over the past few years. During the chemical screening, we found that the culture extract of <i>Saccharothrix</i> sp. A1506 contains novel metabolites and discovered a variety of phenyl-substituted 10-membered macrolides, designated as saccharothriolides (<b>Chapter 1</b>). Structure analyses implied the presence of common biosynthetic precursors, including precursor A (<b>Chapter 2</b>). Identification of precursor A allowed us to generate new saccharothriolide analogs by precursor-directed <i>in situ</i> synthesis (PDSS) method (<b>Chapter 3</b>). In this thesis, isolation and generation of unique metabolites by exploring rare resources and PDSS method are described (Figure).</p> <p><b>Chapter 1: Isolation, structure elucidation, and biological activities of saccharothriolides A-F, produced by a rare actinomycete <i>Saccharothrix</i> sp. A1506</b></p> <p>We surveyed more than 30,000 microbe cultures by LC-MS analysis to find novel metabolites, and isolated six new 10-membered macrolides, saccharothriolides A-F, from the rare actinomycete <i>Saccharothrix</i> sp. A1506. Their chemical structures were deduced by extensive spectroscopic analyses including advanced universal NMR database method and HR-ESI-MS data. The absolute configurations were determined by the modified Mosher’s method and TDDFT-calculation of ECD spectra. Saccharothriolides A, B, D and E had an aryl amine substituent in the lactone ring through a C-N bond, while saccharothriolide C possessed a hydroxy group at C-7. Saccharothriolides D and E were determined to be C-2 epimers of saccharothriolides A and B, respectively. Saccharothriolide F was identified to be a demethylated congener of saccharothriolides A and D at the C-2 position.</p> <p>Among saccharothriolides A-F, only saccharothriolide B exhibited moderate cytotoxicity against human tumor cell lines HeLa and HT1080 with IC<sub>50</sub> values of 17.9 and 13.9 μM, respectively, and showed weak antibacterial activity against <i>Staphylococcus aureus</i> in a paper disc assay.</p> <p><b>Chapter 2: Plausible biosynthetic pathway of saccharothriolides and isolation of a key precursor “precursor A”</b></p> <p>Saccharothriolides possess unique phenyl-substituted 10-membered macrolide structures that contain a variety of substituents at C-7. As in the case of other macrolides, saccharothriolides seem to be synthesized <i>via</i> polyketide biosynthetic pathway. An aryl starter unit and four units of methyl-malonyl-CoA seem to be conjugated followed by cyclization to yield precursor metabolites. The precursors with an α,β-unsaturated ketone that can function as a Michael acceptor, and are likely attacked by aryl amine groups or a water molecule to furnish metabolites saccharothriolides.</p> <p>Saccharothriolides A-C are likely generated from the precursor metabolite “precursor A”. In fact, the culture broth included a significant amount of anthranilic acid and 2-aminophenol. Both of them could be generated from tryptophan, which was included in the culture media. As we expected, saccharothriolides A and B were not detected when cultured in the absence of tryptophan. Instead, we could detect an ion peak corresponding to precursor A. We prepared an EtOAc extract from a large-scale tryptophan-free culture of <i>Saccharothrix</i> sp. A1506. MS-guided isolation, in which acidic or basic condition, and alcoholic solvents were not used, was carried out, and precursor A was successfully isolated.</p>			

### Chapter 3: Precursor-directed *in situ* synthesis of saccharothriolide analogs and their structure-activity relationship study

As described above, saccharothriolides were generated from precursors possessing  $\alpha,\beta$ -unsaturated ketone via Michael-type addition. This reaction allowed us to explore precursor-directed *in situ* synthesis (PDSS) to obtain further saccharothriolide analogs simply by adding nucleophilic substituents to the culture.

The structure-activity relationship (SAR) study using saccharothriolides A–F had revealed the importance of the substituent at C-2'' on their cytotoxicity; metabolites possessing an alcohol group showed activity, while those possessing a carboxylic acid were less potent. To investigate the necessity of the free hydroxy group, we prepared 2- or 3-methoxyaniline-substituted saccharothriolide analogs G–J by PDSS method, and examined their effect on the cytotoxicity against human fibrosarcoma HT1080 cells. Saccharothriolide G–J exhibited only weak activity, which confirmed the importance of the phenolic hydroxy group at C-2''.

This thesis represents a systematic work on the isolation, structure elucidation, cytotoxicity evaluation of new metabolites saccharothriolides A–F and precursor A, precursor-directed *in situ* synthesis (PDSS) of saccharothriolide analogs G–J, and SAR study of saccharothriolides as potential anticancer drug-leads from actinomycete source. The novel chemical scaffolds and bioactivities of saccharothriolides promote us to discover more potent analogs and investigate their mode of action. This effective PDSS method can provide functional modified analogs which can be obtained difficultly from total synthesis method.

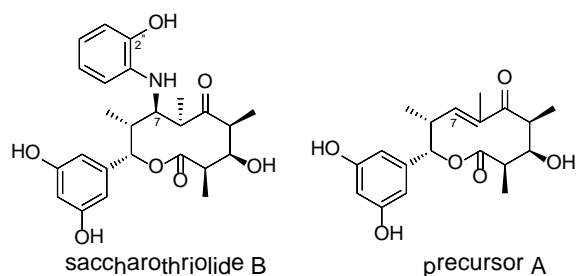


Figure: chemical structures of saccharothriolide B and precursor A.

(論文審査の結果の要旨)

著者は、希少放線菌 *Saccharothrix* sp. が生産する天然有機化合物の化学構造の決定と生合成研究、ならびに天然物前駆体を利用した *in situ* 合成による類縁体・誘導体の創製法について研究を行った。

すなわち、著者は、*Saccharothrix* sp. A1506 が生産する新規天然物を単離し、NMR、MS、CD など各種スペクトル解析により、絶対立体配置を含む化学構造の決定に成功した。見出した *saccharothriolides* A-F のうち、互いに C-2 位がエピマーの関係にある *saccharothriolide* B および E がヒト繊維肉腫細胞株 HT1080 に対する細胞増殖抑制効果を示すことなど、興味深い構造活性相関を明らかにした。また、*saccharothriolide* B は *Staphylococcus aureus* に弱いながらも抗菌活性を示すことを明らかにした。

続いて著者は、見出した *saccharothriolide* 類の構造的特徴から、天然物前駆体 (precursor A) に対する Michael 付加反応が生成物の構造的多様性を付与しているものと考え、各種培養条件および単離・精製条件を検討した結果、天然物前駆体 (precursor A) の単離・精製、構造決定に成功した。さらに、天然物前駆体 (precursor A) が HT1080 細胞に対して *saccharothriolide* B と同程度の細胞増殖抑制効果を示すことを明らかにした。

最後に著者は、*saccharothriolide* 類の生産菌培養液に求核試薬を添加して反応を行う precursor-directed *in situ* synthesis (PDSS) 法により、非天然型類縁化合物 *saccharothriolides* G-J の単離・構造決定に成功した。さらに、HT1080 細胞に対する増殖抑制に対する構造活性相関研究の結果、*saccharothriolide* 類の C-2 位、C-2' 位などに関して興味深い知見を得た。

以上、希少放線菌 *Saccharothrix* sp. が生産する新規 *saccharothriolide* 類の化学構造の決定と生合成研究、ならびに天然物前駆体を利用した *in situ* 合成による類縁体・誘導体の創製法を確立した。さらに、*saccharothriolide* 類の作用機序を解明することで、新規抗がん剤開発に大いに貢献することが期待される。

よって、本論文は博士 (薬科学) の学位論文として価値あるものと認める。また、平成 29 年 2 月 24 日、論文内容とそれに関連した事項について試問を行った結果、合格と認めた。

なお、本論文は、京都大学学位規程第 14 条第 2 項に該当するものと判断し、公表に際しては、当分の間、当該論文の全文に代えてその内容を要約したものとすることを認める。

要旨公表可能日：2017 年 6 月 22 日以降