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論文題目	Discovery of Novel Bioactive Compounds from a Rare Actinomycete *Amycolatopsis* sp.26-4		

（論文内容の要旨）

Natural products have played an indispensable role in the drug development process. It is estimated that ca. 35% of small-molecule drugs is either natural products or their semisynthetic derivatives. The compounds with novel skeletons and biological activities have always been the hotspot of natural products research.

Based on preliminary screenings for structurally unique and biologically interesting natural products, I isolated and identified a series of novel sulfur-containing heterocyclic lipopeptides named thioamycolamides from the culture broth of *Amycolatopsis* sp. 26-4 (Chapter 1-1). A bioinspired concise total synthesis of thioamycolamide A has been accomplished (Chapter 1-2). Moreover, two novel nonapeptides, amycolapeptins A and B, which were not produced by monoculture of *Amycolatopsis* sp. 26-4, were isolated from combined-culture broth of *Amycolatopsis* sp. 26-4 and *Tsukamurella pulmonis* TP-B0596 (Chapter 2).

In this thesis, discovery of novel sulfur-containing cyclic lipopeptides thioamycolamides, strategy for the absolute configuration determination, total synthesis of thioamycolamide A, and discovery of novel nonapeptides amycolapeptins by combined-culture method are described.

Chapter 1. Studies on Thioamycolamides Produced by *Amycolatopsis* sp. 26-4

Chapter 1-1. Isolation, Structure Determination, and Cytotoxicity of Sulfur-Containing Cyclic Lipopeptides Thioamycolamides

Through my screenings by using physicochemical properties including both mass spectrometry and UV spectroscopy, thioamycolamide A and its analogues were successfully isolated. The planar structural elucidation was accomplished by HRMS and 1D/2D NMR spectroscopic data analyses. The absolute configurations were determined by Marfey's method, CD spectroscopy, and synthesis of partial structures. The side chain at lipid moiety of each analogue varied in terms of the number of carbon atoms and the branching pattern of the methyl groups. The carbon numbers found in the side chains of thioamycolamides A–E were three, four and five, and the terminal structure of the side chains was normal- (without branched methyl) or iso-type. These structural moieties were confirmed by HRMS and NMR analyses.

The cytotoxicities of all isolated compounds were evaluated with two human cancer cell lines, HT1080 and HeLa S3. Thioamycolamides A and D with a four carbon-chain length at side chain exhibited moderate cytotoxicity with IC_{50} values of 6.53–21.22 μM, whereas thioamycolamide E with a 2,2-dimethyl butanol side chain had an IC_{50} value of greater than 100 μM, indicating the importance of the aliphatic property for their biological activities. Moreover, thioamycolamides B and C, both of which have a three carbon-chain length at side chain, showed weak (IC_{50}, 78.57, 67.88 μM, respectively) or no cytotoxicity even at 100 μM, respectively. These structure-activity relationships (SAR) suggest that both hydrophobicity and the length of the side chain at side chain are crucial for exhibiting the cytotoxicity presumably based on the difference of membrane permeability.

Chapter 1-2. Bioinspired Concise Total Synthesis of Thioamycolamide A

The sulfur-containing lipopeptides, thioamycolamides A–E, exhibited varying cytotoxicities toward the growth of two human cancer cells. The differences in the activities seemed to be related to the length and methylation/hydroxylation patterns of the side chain, which suggests that an expanded SAR study would be helpful for possible anticancer development of this class of compounds.

Thioamicolamide A is a biosynthetically unique cytotoxic cyclic lipopeptide, which bears a D-configured thiazoline, a thioether bridge, a fatty acid side chain, and a reduced C-terminus. To get more amount and different derivatives of thioamycolamides for further biological activity research, a concise total synthesis of thioamycolamide A has been accomplished based on its putative biosynthesis. The peptide chain was elongated by an isomerization-suppressed procedure, and the thioether linkage was constructed by thio-Michael addition as a key reaction in the final step.

Furthermore, the structural identity between natural and synthetic thioamicolamide A was confirmed by the LC-MS experiments with a new highly sensitive labeling reagent. In the LC-MS experiments, stereochemical purity of chemically constructed thioamicolamide A was surely verified, which corroborates the efficiency of our bioinspired synthesis and strongly supports the proposed thio-Michael addition pathway for its thioether biosynthesis.

Chapter 2. Studies on Combined-Culture of *Amycolatopsis* sp. 26-4 and *Tsukamurella pulmonis* TP-B0596

In our previously studies, combined-culture method have been proved as an effective method to dig potential metabolites. *Amycolatopsis* sp. 26-4 exhibited the ability of producing structurally unique secondary metabolites in the thioamicolamides research.

As a continuation of combined-culture research, this method was applied to strain *Amycolatopsis* sp. 26-4 with the mycolic acid-containing bacterium (MACB) *Tsukamurella pulmonis* TP-B0596. Two novel nonapeptides, amycolapeptins A and B, were isolated from the combined-culture broth, with 22-membered depsipeptide, unusual ß-hydro-tyrosine, and highly modified tryptophan moieties. The chemical structures including the absolute configuration were elucidated by spectroscopic analysis, chemical synthesis, a highly-sensitive Marfey's method and CD spectroscopy.

Conclusions

This thesis summarized the complete research of novel sulfur-containing lipopeptides and thioamycolamides, including isolation, structure elucidation, absolute configuration determination, total synthesis, biological evaluation and plausible biosynthetic pathway. Their biological activity study suggests that structural modification in the side chain would be helpful for possible drug development of this class of compounds. Combined-culture research and discovery of amycolapeptins proved the potential of actinomycete *Amycolatopsis* sp. 26-4 for producing other unprecedented structures. The biosynthetic mechanisms of these compounds in the producing organism are also of considerable interest.

（論文審査の結果の要旨）

著者は、創薬リード化合物の開拓研究として、希少放線菌*Amycolatopsis* sp. 26-4の純粋培養抽出物及び複合培養抽出物より、化学的に非常に興味深い新規化合物を単離精製し、化学的手法により構造解析決定を行うとともに全合成研究を行った。また、一部の化合物に関しては、抗腫瘍活性に関する構造活性相関を明らかにしている。

すなわち、著者は、第一章第一節において、放線菌 *Amycolatopsis* sp. 24-6 の純粋培養抽出物から新規リポペプチド thioamycolamides A-E を単離し、NMR 及び MS などの各種スペクトルデータに加えて、分解反応及び部分合成などにより化学構造を決定した。Thioamycolamides A-E は、特異なチオエーテル、チアゾリンを含む環状リポペプチド構造を有するが、構成アミノ酸や縮合の状況などから判断して、非リボソームペプチド（NRPS）系の合理的な生合成経路が提唱している。また、thioamycolamides A-E のうち 3 種が中程度の抗腫瘍活性を示すことを明らかにし、その構造活性相関に関しても考察がなされている。第一章第二節においては、著者は thioamycolamide A の提唱生合成経路を模倣した化学的全合成を達成している。チオールの分子内マクロ環化反応が目的の立体選択性で進行するか否かが鍵であるが、生合成経路から推定している通り、この反応が目的の選択性で反応が進行することを証明した。また、チアゾリン構造の存在によるα位エピメリ化の抑制にも成功し、効率的な全合成を達成している。さらに、合成した thioamicolamide A の立体化学の慎重な確認を新規高感度ラベル化剤を用いた Marfey 法により行っている。

続いて、著者は、第二章において、複合培養法による新たな天然物の生産・単離・構造解析を達成している。すなわち、第一章と同じ菌株 *Amycolatopsis* sp. 24-6 に対して、*Tsukamurella pulmonis* TP-B0596 を用いた複合培養液から 2 種の環状リポペプチド amycolapeptins A 及び B を単離した。続いて、第一章と同様に NMR 及び MS などの各種スペクトルデータの解析に加えて、部分合成と分解反応を駆使することで全構造決定を行った。Amycolapeptins A 及び B の各構成アミノ酸の立体化学の決定には、新規高感度ラベル化剤を用いた Marfey 法を駆使して行っている。

中分子創薬に注目が集まっている現在、新たな創薬シードを提供する新規ペプチド性天然物の生産・単離・構造決定・全合成研究は、これまで以上に重要性を増しており、本論文で見出された thioamycolamides 及び amycolapeptins の創薬シードとしての可能性が期待される。また、複合培養で生産される amycolapeptins に関しては、生産メカニズムなどにも非常に興味がもたれる。

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、令和2年8月20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、（令和5年9月22日までの間）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。

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