

氏 名	ARIEFTA, Nanang Rudianto
本籍（国籍）	インドネシア共和国
学 位 の 種 類	博士（農学）
学 位 記 番 号	連研第 766 号
学位授与年月日	令和 2 年 3 月 2 3 日
学位授与の要件	学位規則第 5 条第 1 項該当課程博士
研究科及び専攻	連合農学研究科 生物資源科学専攻
学位論文題目	Study of New Metabolites Produced by Endophytic Fungi from Merapi Volcano Area in Java Island, Indonesia（インドネシア・ジャワ島のメラピ火山の麓で採取した植物内生菌類が生産する新規二次代謝産物に関する研究）
学位審査委員	主査 山形大学教授 塩野 義人 副査 小関 卓也(山形 教授), 木村 賢一(岩手 教授), 橋本 勝(弘前 教授)

論 文 の 内 容 の 要 旨

〇〇〇〇〇（2,000 字程度。英文の場合は 1,200 語程度とし，和訳を添付する）

Natural products have played a major role in the search for new drugs or drug candidates throughout the course of many years. Because of their enormous structural diversity and complexity, they are a continuing and inspiring source for researchers. Fungi are one important source of pharmacologically active and structurally diverse natural products. This thesis describes investigations of new compounds from endophytic fungi, through fermentation process. The isolation of secondary metabolites from the fermentation products have done utilizing several steps of chromatography. The structures of the isolated compounds were established by extensive spectroscopic analyses of 1D/2D-NMR and HRESITOFMS. The absolute configurations were determined using a combination of the modified Mosher's ester method, X-ray crystallography, experimental and calculated ECD analysis, X-ray crystalline sponge method, and/or comparison with reported data.

Mount Merapi in Java island, Indonesia, is known as the most active and hazardous volcano in the world. Merapi is 25–30 km north of Yogyakarta City and is home to approximately 1.6 million people. One of the most feared aspects of Merapi is the pyroclastic flow eruption type, which consists of revolving clouds of superheated gases. A recent large eruption in 2010 caused many changes to the environment, including plants and microorganisms. It was assumed that during the post-eruption in this area, fungal endophytes were not investigated as metabolite sources. We explored new metabolite sources from the damaged area, which led to the isolation of several fungal strains. Three of them, *Nectria pseudotrichia* 120-1NP, *Fusarium solani* B-18, and *Colletotrichum boninense* AM-12-2, were described in this thesis.

N. pseudotrichia 120-1NP is an endophytic fungus isolated from the stem of *Gliricidia sepium*. Seven new compounds, nectrianolins A–C (1–3); 6,8-dihydroxy-3,4,7-trimethylisocoumarin (5); 8-hydroxy-6-methoxy-3,4,7-trimethylisocoumarin (6); nectriaquinone B (8); and zythiostromic acid C (11), were isolated from the brown rice culture of this strain, along with four known compounds (4, 7, 9, and

10). To the best of our knowledge, this is the first isolation of **7** from a natural source. Compounds **1** and **2** have a rearranged monocyclo-farnesyl skeleton (which is uncommon to sesquiterpene-epoxycyclohexane conjugates) instead of a bicyclo-farnesyl skeleton. Compounds **1–3** were evaluated for their in vitro cytotoxicity against HL-60 and HeLa cell lines by the MTT method. Compounds **1–3** exhibited cytotoxic activity against the HL-60 cell lines with IC₅₀ values of 1.7, 1.5 and 10.1 μ M, respectively. Compound **1–3** also exhibited cytotoxicity against the HeLa cell lines with IC₅₀ values of 34.7, 16.6 and 52.1 μ M, respectively. Additionally, compounds **4–11** were evaluated for their antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, and *Aspergillus clavatus*, phytotoxicity, and in vitro cytotoxicity. Unlike the other compounds, **8** and **9** exhibited antibacterial activity against *S. aureus* and *P. aeruginosa* with MIC values (μ g/mL) of >50 and 6.25, and of >50 and 3.125, respectively. The phytotoxicity was tested using lettuce seeds; notably, only **9** induced significant seedling growth inhibition compared to control. Moreover, **7–10** exhibited cytotoxicity against HL60 cells with IC₅₀ values (μ M) of 11.9, 1.33, 1.93, and 11.6, respectively. The higher cytotoxicity of **8** and **9** compared to that of the related compounds **7** and **10** was attributed to their increased cell membrane permeability due to the presence of the hydroxyl group.

F. solani B-18 is an endophytic fungus isolated from an unidentified forest litter. Four new compounds with γ -methylidene-spirobutanolide core, fusaspirols A-D (**12–15**), were isolated from the brown rice culture of *Fusarium solani* B-18. Oxaspirol analogues have been reported to possess various biological activities, **12–15** and its derivatives did not show cytotoxicity against murine macrophage derived RAW264.7 cells. Thus, they were tested for osteoclastic differentiation activity using the same cells. After four days of the osteoclastic induction, mature osteoclasts of multinucleated-TRAP (tartrate-resistant acid phosphatase)-positive cells were counted. Compounds **12** and **12a** significantly increased the number of mature osteoclasts at the comparable levels to the positive control (kenpaullone) and the negative control (DMSO), suggesting that **12** and **12a** activated a signaling pathway in osteoclastic differentiation. In the scale-up fermentation based on this strain, with the same method, additional four new polyketides, fusopoltides B–E (**20–23**), were isolated along with four known compounds (**16–19**). Fusopoltide B (**20**) is a diastereomer of its co-isolated known compound, fusopoltide A (**19**), featuring a pentaleno[1,2-*c*]pyran ring system. Fusopoltide C (**21**) and fusopoltides D–E (**22–23**) are incorporated the first natural polyketides featured decahydro-pentaleno[1,2-*c*]pyran and pentaleno[1,2-*c*]furan ring systems, respectively.

C. boninense AM-12-2 is an endophytic fungus isolated from the stem of *Acacia mangium*. Seven new compounds, 3-(3-hydroxy-2-(hydroxymethyl)phenyl)propanoic acid (**24**), 2-hydroxymethyl-3-hydroxy-(*E*)-cinnamic acid (**25**), and colletofurans A–E (**26–30**), were isolated from brown-rice culture of *Colletotrichum boninense* AM-12-2. Colletofurans A–E (**26–30**) are first natural compounds featured an unprecedented 1-octyl-1,3-dihydroisobenzofuran core. Additionally, colletofuran A (**26**) contained a unique 1,6-dioxaspiro[4.4]nonane ring system. Compounds **24–30** were evaluated for their anti-aphicidal activity. Aphids are a serious insect pest in agriculture worldwide. This assay was performed to test the susceptibility of *Aphis gossypii* (Glover) and *Myzus persicae* (Sulzer), against **26–30**. The commercial insecticide milbemectin was tested as the positive control. Compound **26** and **29** exhibited strong aphicidal activities against *A. gossypii* with the LC₅₀ (μ g/mL) values of 1 and 0.5, respectively. Furthermore, **27**, **29**, and **30** showed moderate activity against *M. persicae* with the LC₅₀ (μ g/mL) values

of 169, 118, and 26, respectively. Notably, **29** exhibited strong aphicidal activity ($LC_{50} = 0.5 \mu\text{g/mL}$) against *A. gossypii* and weak activity ($LC_{50} = 118 \mu\text{g/mL}$) against *M. persicae*. In contrast, **30** exhibited moderate aphicidal activity against *M. persicae* ($LC_{50} = 26 \mu\text{g/mL}$), but inactive against *A. gossypii*. Other compounds displayed either weak or no aphicidal activity ($LC_{50} > 200 \mu\text{g/mL}$) against tested aphids. In general, the data showed dose dependent responses and *M. persicae* was more resistant than *A. gossypii* to the treatments.

This thesis describes the chemical investigation of 30 fungal metabolites, including 22 new compounds and two compounds firstly isolated from fungal culture. Complex interactions with their host may involve the presence of these fungi in volcanic areas. The interactions possibly related to their chemistry in some respects and have been shown to produce or elicit secondary metabolites that are new, attractive, and biologically active. Since endophytic fungi from Merapi volcano area are unexplored, there is a high likelihood that new metabolites will be discovered. Although certain new compounds exhibited no activity in bioassay, the new chemistry faced difficulties in the determination of the structure that had to be addressed. These results also disclosed that endophytic fungi from Merapi volcano area harbor an enormous reservoir of new compounds for drug discovery.

論文審査の結果の要旨

インドネシアのジャワ島のメラピ火山の麓より、分離した三種の植物内生菌類 (*Nectria pseudotrichia* 120-1NP, *Fusarium solani* B-18, *Colletotrichum boninense* AM-12-2) の生産する生理活性物質について、精査し、新規な化学構造を有する生理活性物質を明らかにした。

Nectria pseudotrichia 120-1NP 株の培養抽出物をカラムクロマトグラフィーにより精査し、11 種の化合物を単離した。それぞれについて、NMR スペクトルデータを中心とした化学解析を行った結果、新規物質として、nectrianolins A–C (**1-3**), 6,8-dihydroxy-3,4,7- trimethyl - isocoumarin (**5**), 8-hydroxy-6-methoxy-3,4,7- trimethyl- lisocoumarin (**6**), nectriaquinone B (**8**), zythiostromic acid C (**11**) を明らかにした。一方、化合物(**4, 7, 9, 10**)は既知物質であった。新規物質 (**1-3**) は HL-60 と HeLa 細胞株に対して、細胞毒性活性を示した [IC_{50} (μM); HL-60, **1**: 1.7, **2**: 1.5, **3**: 10.1; HeLa, **1**: 34.7, **2**: 16.6, **3**: 52.1]。

糸状菌 *Fusarium solani* B-18 株の培養物からは、12 種の生理活性物質が単離された (化

合物 **12-23**)。NMR を中心に、それらの化学構造を解析した。分子内にスピロ結合を有する fusaspirol A (**12**) とそのアセチル誘導体 (**12a**) は前破骨細胞株 RAW264.7 細胞に対して分化誘導活性を示した。また、fusopoltides C, D, E (**21-23**) は、decahydro-pentaleno[1,2-*c*]pyran や pentaleno[1,2-*c*]furan 骨格を有する新規物質であった。

糸状菌 *Colletotrichum boninense* AM-12-2 株の培養物より、7 種の物質が単離された。続いて、化学構造解析の結果、新規物質として、3-(3-hydroxy-2-(hydroxymethyl)phenyl)propanoic acid (**24**), 2-hydroxy methyl-3-hydroxy-(*E*)-cinnamic acid (**25**), colletofurans A-E (**26-30**)を明らかにした。

本博士論文では、30 個の二次代謝産物を明らかにした。そのうち、22 個は新規物質であり、2 個は天然物としては初めての物質であった。メラピ火山の麓において、宿主植物と菌類との共生関係の詳細については明らかになっていないが、今後も多くの生理活性物質を生産する微生物のスクリーニング源として期待できることを示した。

以上の結果より、本審査委員会は、「岩手大学大学院連合農学研究科博士学位論文審査基準」に則り審査した結果、本論文を博士（農学）の学位論文として十分価値のあるものと認めた。

学位論文の基礎となる学術論文

Main Papers

1. **Ariefta NR**, Kristiana P, Nurjanto HH, Momma H, Kwon E, Ashitani T, Tawaraya K, Murayama T, Koseki T, Furuno H, Usukhbayar N, Kimura KI, Shiono Y. Nectrianolins A, B, and C, new metabolites produced by endophytic fungus *Nectria pseudotrichia* 120-1NP. *Tetrahedron Lett.*

58: 4082–4086. October, 2017.

2. **Ariefta NR**, Kristiana P, Aboshi T, Murayama T, Tawaraya K, Koseki T, Kurisawa N, Kimura KI, Shiono Y. New isocoumarins, naphthoquinones, and a cleistanthane-type diterpene from *Nectria pseudotrichia* 120-1NP. *Fitoterapia*. 127: 356–361. June, 2018.
3. **Ariefta NR**, Nikmawahda HT, Aboshi T, Murayama T, Tawaraya K, Koseki T, Katagi G, Kakihara Y, Shiono Y. Fusaspirols A-D, novel oxaspirol derivatives isolated from *Fusarium solani* B-18. *Tetrahedron*. 75: 1371–1377. March, 2019.
4. **Ariefta NR**, Nikmawahda HT, Koseki T, Shiono Y. Fusopoltides B–E, new polyketides isolated from *Fusarium solani* B-18. *Tetrahedron Lett.* in press, <https://doi.org/10.1016/j.tetlet.2019.151361>, November, 2019.

○○○○○