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論　文　名	「Studies on Bioactive Compounds from Indonesian Medicinal Plants and Mushrooms(インドネシア産薬用植物およびキノコに含まれる生理活性物質に関する研究)」
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論文要旨

Chapter 1. Introduction

For thousands of years, natural products derived from plants and microorganisms have played an important role throughout the world in treating and preventing human diseases. The medicinal plants and mushrooms which grow in Indonesian rain forest are a rich source of natural products with a great diversity of molecular structures which can be used as lead compounds for development of new drugs or agrochemicals, but their chemical constituents have not yet been completely recognized. From this viewpoint, the research was conducted to find novel bioactive compounds from Indonesian medicinal plants and mushrooms.

Chapter 2. Screening of Indonesian medicinal plants and mushrooms for biological activity

In order to select the active samples, a total of 278 methanol extracts of wild mushrooms and 150 methanol extracts of medicinal plants were tested against brine shrimp (*Artemia salina*). As a result, 167 samples of mushrooms exhibited the mortality rate of 80-100% and one sample showed immotile activity, and 10 samples of medicinal plants exhibited the mortality rate of 80-100%. The mushroom of *Microporellus subsessilis* showing immotile activity and the medicinal plant of *Phaleria macrocarpa*

(Scheff.) Boerl. were selected for further purification of the active constituents. This study deals with the isolation and structural determination of active principles and their bioactivities are also evaluated.

Chapter 3. 10-Phenyl-[11]-cytochalasans isolated from mushroom, *Microporellus subsessilis*

The methanol extract of *M. subsessilis* was purified by using the immotile activity to follow the separations with combination of column chromatography on Wakogel C-200, Silica G-60, Chromatorex ODS and preparative HPLC on Inertsil ODS-3 to afford four active compounds of 10-phenyl-[11]-cytochalasans (**1**-**3** and **6**) together with two inactive compounds (**4** and **5**). Compounds **1**-**3** were identified as known compounds, while compounds **4** and **5** were determined to be new members of 10-phenyl-[11]-cytochalasans. In compound **4**, the six-membered ring of **3** was modified with a double bond newly introduced between C-6 and C-7 and a hydroxylmethyl was located at C-6. On the other hand, new compound **5** was determined as a 6,7-epoxide of **3**. In new compound **6**, the hydroxyl at C-19 of **1** was acylated with a C_{17} fatty acid containing two double bonds and a branched methyl. The structure of fatty acid moiety was determined as a novel fatty acid. To our best knowledge, compound **6** is the first member of cytochalasin family which contains a long chain fatty acid moiety.

Chapter 4. Biological activities of cytochalasin compounds

The immotile activities of **1**-**6** were evaluated against the brine shrimp. 10-Phenyl-[11]-cytochalasans **1**, **2**, **3** and **6** induced 72%, 78%, 64%, and 63% immotility against the brine shrimp at 10 ppm, respectively. On the other hand, compounds **4** and **5** were inactive. These facts indicated that the hydroxyl group at C-7 and the exomethylene at C-6 were components essential for exhibiting immotility. The fatty acid moiety as well as the substituents at C-19 in compound **6** was not significant because the activities of compounds **1**, **2**, **3,** and **6** were almost equal. These results were in good agreement with structure activity relationship studies of cytochalasins which showed the important role of the hydroxyl group at C-7 in exhibiting biological activity on mammalian cell.

Chapter 5. 29-Norcucurbitacins isolated from medicinal plant, *Phaleria macrocarpa* (Scheff.) Boerl.

The methanol extract of *P. macrocarpa* was purified by a combination of column

chromatography on Wakogel C-200, Silica G-60, and Chromatorex ODS to afford four active compounds of 29-norcucurbitacin derivatives (**7**-**10**). Compounds **7**, **9** and **10** were identified as fevicordin A, fevicordin A glucoside and fevicordin D glucoside, respectively, which have been isolated from *Fevillea cordifolia*. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **8** with those of fevicordin A (**7**) showed that the acetyl signals attached at C-25 of fevicordin A disappeared in **8**, thus all the spectral data were consistent with those of the proposed structure of **8** for fevicordin A derivative. Consequently, compound **8** was determined to be a new member of norcucurbitacin, and was named desacetylfevicordin A.

Chapter 6. Biological activities of norcucurbitacin compounds

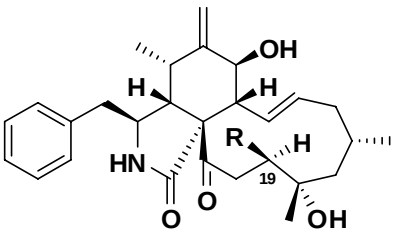
The bioactivity of the compounds **7**, **8**, **9**, and **10** was evaluated using lethality bioassay against the brine shrimp. After 24h observation, compounds **7**, **8**, **9**, and **10** exhibited cytotoxic activity with LD_{50} values of 5 ppm, 3 ppm, 12 ppm and 6 ppm, respectively. The different functional groups attached at C-25 in compounds **7** and **8** showed no significant effect on their LD_{50} values indicating that both of acetyl and hydroxyl groups were not important in exhibiting activity.

Compounds **9** and **10** have the different functional groups at C-25 as well as in **7** and **8**, and compound **10** was also different with compound **9** in the absent of (*E*) olefinic protons H-23 and H-24. Comparison of their LD_{50} values indicated that compound **10** was more active than **9**. This fact suggested that the methylene groups at CH_2-23 and CH_2-24 in compound **10** increased the level of activity against the brine shrimp.

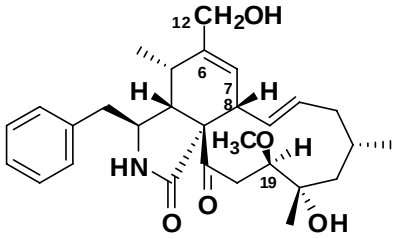
Chapter 7. Conclusion

In the course of our search for novel bioactive compounds from Indonesian medicinal plants and mushrooms, their methanol extracts were tested using primary screening system with the brine shrimp (*Artemia salina*) to detect their bioactive constituents. During the present bioassay-guided investigation of methanol extracts, six cytocahalasins (**1**-**6**) and four 29-norcucurbitacin family (**7**-**10)** have been isolated from mushroom of *M. subsessillis* and medicinal plant of *P. macrocarpa*, respectively.

The important result in the current study suggested that the primary screening system using the brine shrimp is a useful tool to guide a separation and purification process of the active constituents from pharmaceutically valuable natural sources, and that the Indonesian biodiversity including medicinal plants and fungi is an important and promising natural sources in the discovery programs of drugs and agrochemicals.



(1) R = OH
(2) R = H
(3) R = OCH_3



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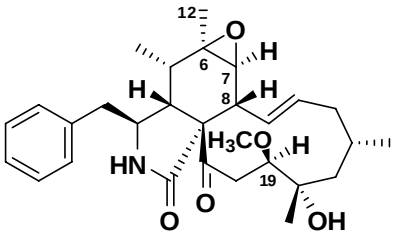

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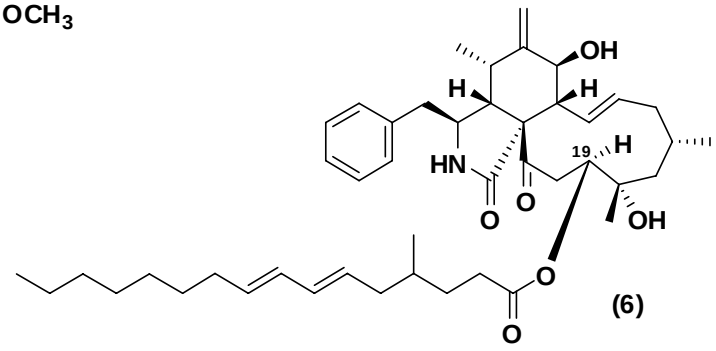

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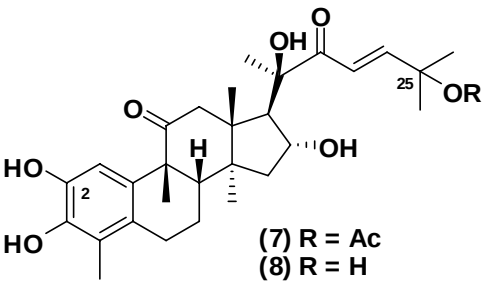
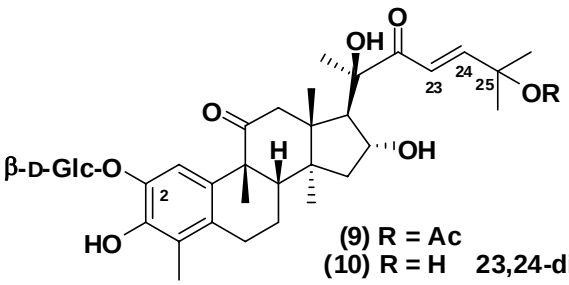


(7) R = Ac
(8) R = H



(9) R = Ac
(10) R = H　23,24-dihydro

審査結果の要旨

インドネシアの熱帯雨林地帯は植物やキノコ類の多様性に恵まれており，そこでは多くの植物が薬用として種々の疾病の治療や予防のために用いられている．しかしながらそれらの植物に含まれる有効成分の化学的解明はほとんど進んでいないのが現状である．そこで，本申請者はインドネシア産植物および野生キノコから新規な生理活性物質あるいは新たな薬剤の開発におけるリード化合物となる物質を見出すことを目的として研究を行い，以下の成果を得た．

まず，インドネシア産野生キノコ 278 種および薬用植物 150 種から得られたメタノール抽出物について，ブラインシュリンプ（*Artemia salina*）幼生に対する生理活性を検索し

た．その結果，167 種のキノコおよび 10 種の植物が 1 mg/ml の濃度で 80% 以上の致死作用を示した．さらに興味あることに，キノコの 1 種（*Microporellus subsessilis*）はブラインシュリンプに対し、運動停止活性を示した．そこで，本研究では *Microporellus subsessilis* および顕著な致死活性の認められた植物 *Phaleria macrocarpa* に含まれる活性物質の化学構造の解明を行った．

M. subsessilis のメタノール抽出物をヘキサンおよび酢酸エチルで順次抽出した．活性の認められた酢酸エチル抽出画分を各種クロマトグラフィーを用いて精製し，4 種の運動停止活性サイトカラシン類（**1**，**2**，**3**，**6**）および 2 種の関連化合物（**4**、**5**）を単離した．化合物 **1** は 7,18,19-trihydroxy-16,18-dimethyl-10-phenyl-[11]-cytocharasa-6(12),13(*E*)-diene-1,2-dione であり，化合物 **2** と **3** はいずれも既知の 10-phenyl-[11]- cytocharasan 類であった．また，新規化合物 **4** および **5** は各種スペクトルデータの解析からそれぞれの構造を 12,18-dihydroxy-19-methoxy-16,18-dimethyl-10-phenyl-[11]-cytocharasa-6(7),13(*E*)-diene-1,21-dione および 6,7-epoxy-18-hydroxy-19-methoxy-16,18-dimethyl-10-phenyl-[11]-cytocharasa-13(*E*)- ene-1,21-dione と決定した．さらに、化合物 **6** はスペクトルデータから化合物 **1** の 19 位水酸基が長鎖脂肪酸でアシル化された化合物であることが推定された．そこで化合物 **6** から側鎖脂肪酸のメチルエステルを誘導し，構造解析を試みた．しかしながら，得られるメチルエステルが極微量であったため，同じ脂肪酸を *M. subsessilis* メタノール抽出物のヘキサン相から単離し，これを用いて構造解析を行った．その結果，側鎖脂肪酸は 4-methyl- 6(*E*),8(*E*)-hexadecadienoic acid であることが明らかとなった．

化合物 **1** および **2**，**3**，**6** は 10 μg/ml の濃度でブラインシュリンプの運動をそれぞれ 72% および 78%，64%，63% 停止した．一方，化合物 **4** と **5** は活性を示さなかった．これらのことから活性発現には 6(12) 位の二重結合と 7 位の水酸基が重要であることが示唆された．

次に，薬用植物である *P. macrocarpa* に含まれる活性物質の解明を行った．種子粉末をメタノールで抽出し，得られた抽出物からブラインシュリンプに対する致死作用を指標として活性物質の精製を行い，4 種の 29-ノルククルビタシン類（**7**，**8**，**9**，**10**）を単離した．各種スペクトルデータを解析し，化合物 **7** および **9**，**10** をそれぞれ fevicordin A および fevicordin A glucoside，fevicordin D glucoside と同定した．化合物 **8** はスペクトルデータから新規物質である desacetylfevicordin A であることが推定され，化合物 **7** のデスアセチル体を誘導することにより化合物 **8** の構造を確認した．

化合物 **7** および **8**，**9**，**10** のブラインシュリンプに対する半数致死濃度はそれぞれ 5 μg/ml および 3 μg/ml，12 μg/ml，6 μg/ml であった．すなわち，化合物 **9** と **10** に存在するグルコース部分および化合物 **7** と **8** に存在するアセチル基は活性発現に関与していないことが示唆された．

以上のように，インドネシア産薬用植物およびキノコから多くの生理活性物質を明らか

にしたことから，熱帯産植物が有用天然物の供給源として多いに期待されることが示された．また，ブラインシュリンプを用いる簡便な生物検定系が生理活性物質を検索する際の指標として用い得ることが示された．これらの成果は天然物化学および生物有機化学の両分野に多大の貢献をするものと考えられ、最終試験の結果と併せて、博士（応用生命科学）の学位を授与することを適当と認める．