Isolation, Structure Elucidation, and Biological Evaluation of Secondary Metabolites from Indonesian Plants

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学位の種類	博士（学術）
学位記番号	医薬保博甲 306 号
学位授与の日付	令和元年 9 月 26 日
学位授与の要件	課程博士（学位規則第 4 条第 1 項）
学位授与の題目	Isolation, Structure Elucidation, and Biological Evaluation of Secondary Metabolites from Indonesian Plants （インドネシア産植物由来新規天然物の探索と生理活性評価）
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学位論文要旨

The phytochemical studies of two selected Indonesian medicinal plants, *Kleinhovia hospita* and *Melochia umbellata*, have been conducted. The MeOH extract of *K. hospita* led to the isolation of seven novel cycloartanes **12**–**18**, along with four known compounds **8–11**. The kleinhospitine E (**12**) is the first unusual cycloartane alkaloid characterized by γ-lactam with an oxopropylidene side chain. A rare α-oriented cyclopropyl ring was also found in novel cycloartanes **13**, **14**, and **17**. Cycloartanes holding a 21,23-diacetal ring, such as **8** and **14**–**16**, were first isolated from the genus of Malvaceae. The investigation of *M. umbellata* resulted in the isolation of six novel quinoline alkaloids **26**–**32** and a new 4-quinolone alkaloid, waltherione R (**29**), along with eighteen known compounds **21**, **24**, and **33**–**47**. A characteristic 3,4-methylenedioxy quinoline ring fused with oxabicyclo-octane was found in the newly isolated compounds **26**−**28** and **30**–**32**. Most isolated cycloartanes exhibited potent to moderate antiproliferative activities against five human tumor cell lines, while quinolones **21**, **24** and **29** exhibited selective antiproliferative activity against A549 and MCF-7. Furthermore, the cycloartanes **8**, **13**, **15**, and a quinolone **33** showed 2–6-fold greater activity against a MDR tumor cell line than its parent non-MDR cell line. Meanwhile, cycloartanes **14** and **17** from *K. hospita* exhibited potent anti-HIV activity in MT4 cells, although no activity was shown in all isolated compounds from *M. umbellata*.

[Background and purpose]

Indonesia is one of the high-biodiversity countries in the world. The plants inhabited in Indonesian tropical rainforest were a potential source of bioactive compounds. Unfortunately, the biodiversity reduced rapidly year by years and many of tropical plants have not been discovered and well-studied yet. The aim of this study was to discover novel natural products with biological activities through the fine phytochemical investigations using Indonesian plants, *Klienhovia hospita* and *Melochia umbellata* which have not been well-studied. Biological activities are mainly focused on anticancer and anti-HIV. Results would be expected not only to discover novel lead candidates as anticancer or anti-HIV in future but also to contribute the further phytochemical finding of tropical plants.

[Method]

The Indonesian medicinal plants, *K. hospita* and *M. umbellata* were extracted with MeOH. Subsequently, the MeOH extracts were partitioned and fractionated by a combination of various chromatographic methods, either in normal phase or reversed phase silica gel. Structures of isolated compounds were elucidated by based on extensive 1D, 2D NMR, and HRMS analyses, as well as

interpretation of ECD spectra to determine the absolute configurations when necessary. Antiproliferative evaluation was carried out against five human tumor cell lines (HTCLs), A549, MDA-MB-231, MCF-7, KB, and a KB-subline KB-VIN, which is a p-gp overexpressing MDR tumor cell. While anti-HIV assay was performed in MT4 cells.

[Results and Discussions]

The unique cycloartane alkaloids, kleinhospitines A–D (**1**–**4**),[1] four cycloartanes **5**–**10**,[2] and taraxerone (**11**)[3] have been previously reported from *K. hospital* (Figure 1). In the present study, a crude MeOH extract of *K. hospita* led to the isolation of seven novel cycloartanes **12**–**18** (Figure 2) and four known compounds, which were recognized as 21*S*,23*R*-21/23,23/27-diepoxy-21-methoxycycloartan-1,24-diene-3,27-dione (**8**),[2,4] (23*R*)-21,23:23,27-diepoxy-cycloarta-1,24-diene-3,27-dione (**9**), cycloartan-1,24-diene-3,23-dione (**10**), and taraxerone (**11**),[3,4] after comparison with their spectroscopic data described in the literatures.

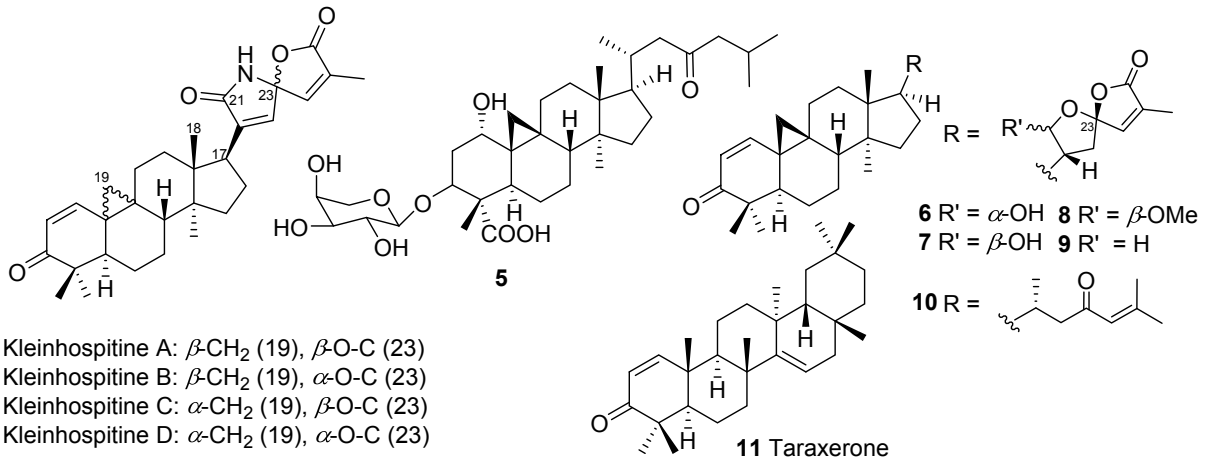

Figure 1. Previously isolated compounds from *K. hospital*

Compound (**12**), kleinhospitine E, was the first representative of unusual cycloartane alkaloid characterized by γ-lactam with an oxopropylidene side chain. While compounds **13**, **14**, and **17** were cycloartanes with a 9α, 10α-cyclopropyl ring rarely occurring naturally. In addition, compounds **8** and **14**–**16** were cycloartanes with a unique 21,23-diacetal, which were first reported from Malvaceae.

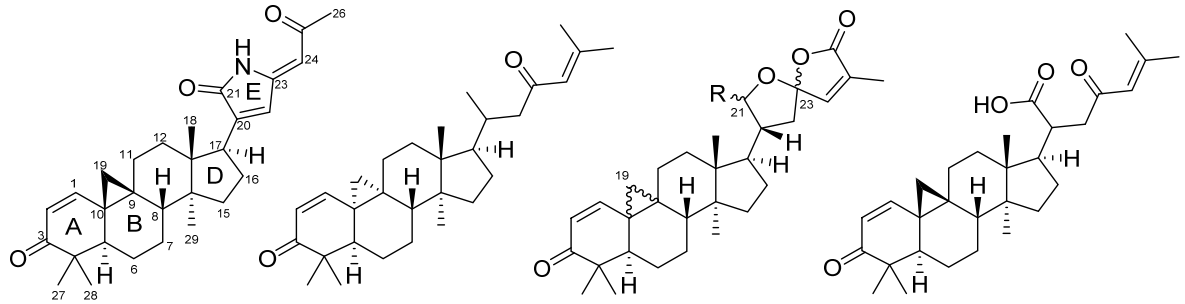

Figure 2. Structures of newly isolated compounds from *K. hospita*

Regarding *M. umbellata*, the only two characteristic natural products, a quinolone alkaloid, waltherione C (**19**) and a coumarou-lignan, cleomiscosin A (**20**), have been isolated so far.[5] In this study, methanol extract of *M. umbellate* resulted in the isolation of six new quinoline alkaloids **26**–**32**, a new 4-quinolone alkaloid, waltherione R (**29**), together with eighteen known compounds **21**, **24**, and **33**–**47** (Figure 3). The uniqueness of basic skeleton of the novel compounds **26**−**28** was a 3,4-methylenedioxy quinoline system fused with oxabicyclo-octane at C-5 and C-6 positions.

All isolated compounds were assayed for antiproliferative with paclitaxel as positive control. Cycloartanes of *K. hospita* displayed potent to mild activity against all tested HTCLs, including MDR tumor cell line, KB-VIN. Cycloartanes **8**, **13**, and **15** displayed 3–6-fold greater activity against a MDR tumor cell line (KB-VIN) than its parent chemosensitive cell line (KB). On the other hand, 4-quinolone **21**, **24,** and **29** from *M. umbellata* exhibited selective and potent activity against A549 and MCF-7 cell lines. In addition, antidesmone (**33**) displayed 2-fold greater activity against a MDR tumor cell line than its parent non MDR. According to these results, the configuration of the cyclopropyl ring and the type of side chain substitution of the cycloartanes could affect the antiproliferative activities. While, the quinolone system is likely important for antiproliferative activity, particularly for A549 and MCF-7 cell lines.

Further biological evaluation for anti-HIV activity in MT4 cells showed a potent activity of compound **14** with an IC_{50} value of 2.4 μM (SI = 5.3), while **17** demonstrated submicromolar inhibitory activity with an IC_{50} value of 0.8 μM (SI = 5.8). Although based on the limited assay results available at present, an α-oriented cyclopropane and spiro-furan ring are likely essential for such antiviral activity. Meanwhile, none of isolated compounds from *M. umbellata* showed anti-HIV activity.

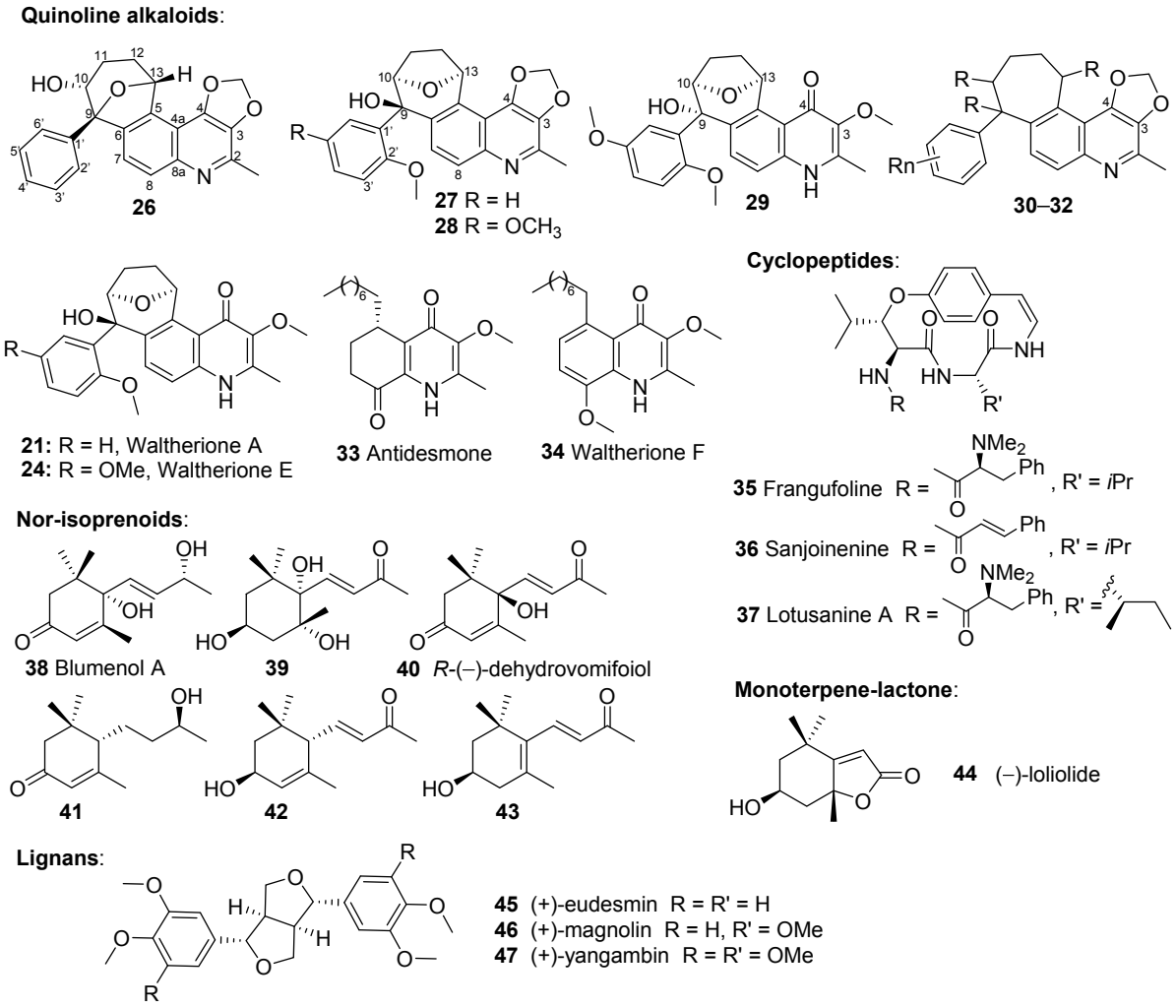

Figure 3. Novel alkaloids **26**–**32** and known compounds isolated from *M. umbellata*

Reference:

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審査結果の要旨

メガダイバーシティ国の 1 つであるインドネシアで，伝統的に薬用植物として使用されている 2 種 *Klienhovia hospita* ならびに *Melochia umbellata* について，植物化学的研究を行った。両者のメタノール抽出液をそれぞれ，各種クロマトグラフィーを駆使して単離精製を繰り返し，*K. hospita* からはユニークな γ-ラクタムを有するシクロアルタンアルカロイド kleinhospitine E を含む 7 種の新規シクロアルタンと 4 種の既知トリテルペンを単離した。一方 *M. umbellata* からは，フェニルオキサビシクロオクタンがキノリンまたはキノロンに縮合した独特の骨格を有する 7 種の新規アルカロイドとともに 18 種の既知化合物を単離した。新規 3 種のキノリンアルカロイドを除き全ての化合物は，各種スペクトルデータを詳細に解析することにより、その構造を決定した。得られた化合物については，多剤耐性がんを含む 5 種のヒトがん細胞に対する増殖抑制効果ならびに抗 HIV 活性を評価し，いくつかの興味深い知見を得ることができた。

以上，本研究では 2 種のインドネシア産薬用植物の化学的精査により，新規天然物の発見と生理活性評価を行い，植物化学界に重要な知見を提供し，学術的に大きな貢献をしたと考えられる。従って，審査委員会は本論文が博士（学術）に値すると判断した。