Study on Biologically Active Compounds from Brazilian Plant Tabebuia avellanedae

学位論文要旨

学位論文題名
Study on Biologically Active Compounds from Brazilian Plant *Tabebuia avellanedae*
(和訳)
ブラジル産植物 *Tabebuia avellanedae* に由来する生物活性化合物の探索

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Tabebuia avellanedae Lorentz ex Griseb (family: *Bignoniaceae*) is distributed throughout the tropical rain forests of Central and South America. Its inner bark, commonly known as “taheebo”, “lapacho”,“pau d’arco”, and “ipe roxo”, is widely used in local and traditional phytomedicine for a long time. Colombians use the bark infusion the bark infusion as stimulant of central nervous system; Bahamians commonly use the bark decoction to prepare an energizing tonic for strength, and Brazilians use this plant to treat malaria, cancer, fever, stomach disorders, bacterial and fungal infections and to relief of a variety of mental and emotional states such as anxiety, poor memory, irritability and depression.

Recent pharmacological study indicated that constituents of the bark of this plant exert a number of activities, such as anti-inflammation, anti-infectious, anti-cancer, antinociceptive, anti-emetogenic , anti-microbial, anti-fungal, anti-trypanosomal, anti-gastric lesions, anti-depressant, antiulcerogenic and anti-angiogenic.

Following its popular use, the chemistry of this plant was extensively studied, and a variety of constituents have been isolated, such as furanonaphthoquinones, naphthoquinones, quinones, lignans, benzoic acid, cyclopentene dialdehyde, flavonoids, iridoids, phenolic glycosides, saponins, and coumarins.

Aim: To find more bioactive natural product skeletons from the inner bark of *Tabebuia avellanedae* as leads to novel agents especially for the treatment of cancer and inflammatory in the future.

Methods: Water extract and methanol extract of inner bark of *Tabebuia avellanedae* were separated, and the chemical structures and relative configurations of the new compounds were determined by 1D, 2D NMR and MS spectroscopic analyses. Cytotoxicity was evaluated by MTT assay on A549, SiHa and MCF-7 cells. Cell cycle analysis was evaluated by PI staining and apoptosis was determined by annnexin-V FITC/PI staining using flow cytometry analysis. Protein levels of cyclin protein family members was assessed by Western blotting and mRNA expressions of P53, BCL 2 and BAX were analyzed by RT-PCR. Inflammatory factors of NO, PGE_2 and TNF- α were checked by ELISA on RAW 264.7 cells and the macrophages from BCG infected mouse.

Results: 22 new compounds together with 10 known compounds were isolated and identified (in Fig. 1.). Among them, compound **30** and compound **31** inhibited the cell viability of all three cell lines; Results showed that compounds **29, 30** and **31** caused dose-dependent decreases in cell viability in all the cell lines; whereas the cells used in this study did not die significantly even at the highest concentration (13.5 μM) of compound **32**. Furthermore, results indicated that the presence of a phenolic hydroxyl group at C-5 seems to play an important role in increasing anti-proliferative effect. Exposure of A549 to compound **30** and compound **31** resulted in significant increased distributions in the G2/M phase and S phase accompanied by a decreased distribution in the G1 phase time-dependently. The cell percentages of G2/M phase and S phase increased by 2.47-fold and 2.84-fold, respectively, after 48h treatment of compound **30**, and increased by 3.33-fold and 1.86-fold, respectively, after 48h treatment of compound **31**, while the cell percentage of G1 phase decreased by 3.31-fold after 48h treatment of compound **30** and decreased by 2.46-fold after 48h treatment of compound **31**. The protein expression of cyclin A and cyclin B1 in A549 cells was significantly down-regulated after 24 h of exposure to **30** and **31** and maintained at an extremely low level after 30 h of exposure to **30** and **31**. In addition, decreased D1 protein level was also observed after 36 h of exposure. Compound **30** and compound **31** induced apoptosis in a time-dependent manner. The quantitative analysis showed the apoptosis rates (early and late) of compound **30** and compound **31** were 16.95% and 5.04% after 36h of treatment, and 22.81% and 26.60% after 48h of treatment, respectively. Compound **30** and compound **31** induced rapid increases in P53 mRNA level from about 6-12 h after treatment, followed by apparent up-regulation of BAX at 36 h after treatment with no effect on BCL-2. Both compound **30** and compound **31** induced the activities of caspase-3 in a time-dependent way. LPS triggered macrophage activation and induced the rise in the production of inflammatory mediators of NO and PGE2, and all of the compounds exclude compound **1** can down-regulate the NO in a dose-dependent manner, while all of the compounds exclude compound **3** and **10** can down-regulate the PGE2 weakly, and 50μg/ml of compound **3** and **10** can down-regulate the PGE2 strongly. The concentrations of TNF-α were markedly increased after treatment with LPS in RAW 264.7 macrophages, but all these

compounds used in this research had no effect on the TNF-α lever. In addition, all tested compounds showed no cytotoxic effects on RAW 264.7 macrophages. The anti-inflammatory properties of compounds were also investigated in LPS-activated macrophages from BCG infected mouse. We found that LPS triggered macrophage activation and induced the rise in the production of inflammatory mediators of NO and PGE2 and TNF-*α*. In the current investigation, all of the compounds exclude compound **1** can down-regulate the NO in a dose-dependent manner, and all of the compounds can down-regulate the PGE2 in a dose-dependent manner, but almost all these compounds used in this research had no effects on the TNF-α lever. The 12.5μg/ml of compound **20** decreased the lever of TNF-α, but it seems to because of suppression of the cell viability. In addition, 50 μg/ml of **6**, **8** and **13** showed cytotoxic effects on LPS-activated macrophages from BCG infected mouse.

Conclusion: 22 new compounds together with 10 known compounds were isolated and identified (in Fig. 1.). Among them, compound **30** and compound **31** inhibited the cell viability of all three cell lines; compound **30** and compound **31** caused cell cycle arrest at G2/M and S phase and down-regulated cyclins A, B1, D1in a time dependent way; Compound **30** and compound **31** inducted apoptosis and up-regulated of P53 and BAX in a time dependent way. Furanonaphthoquinones (**30** and **31**) isolated from *Tabebuia avellanedae* are promising leads for potential anticancer drugs. Compounds **1**, **3**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, and **13** blocked the production of NO and PGE2 in a dose dependent way but not TNF-*α* without altering cell viability (except for high dose of **6**, **8**, **13** on mcrophages from mouse). Cyclopentenes and iridoids (**1**, **3**, **5**, **6**, **8**, **9**, **10**, **11**, **12**, and **13**) isolated from *Tabebuia avellanedae* may be the main constituents related to the anti-inflammatory effect of *Tabebuia avellanedae*.

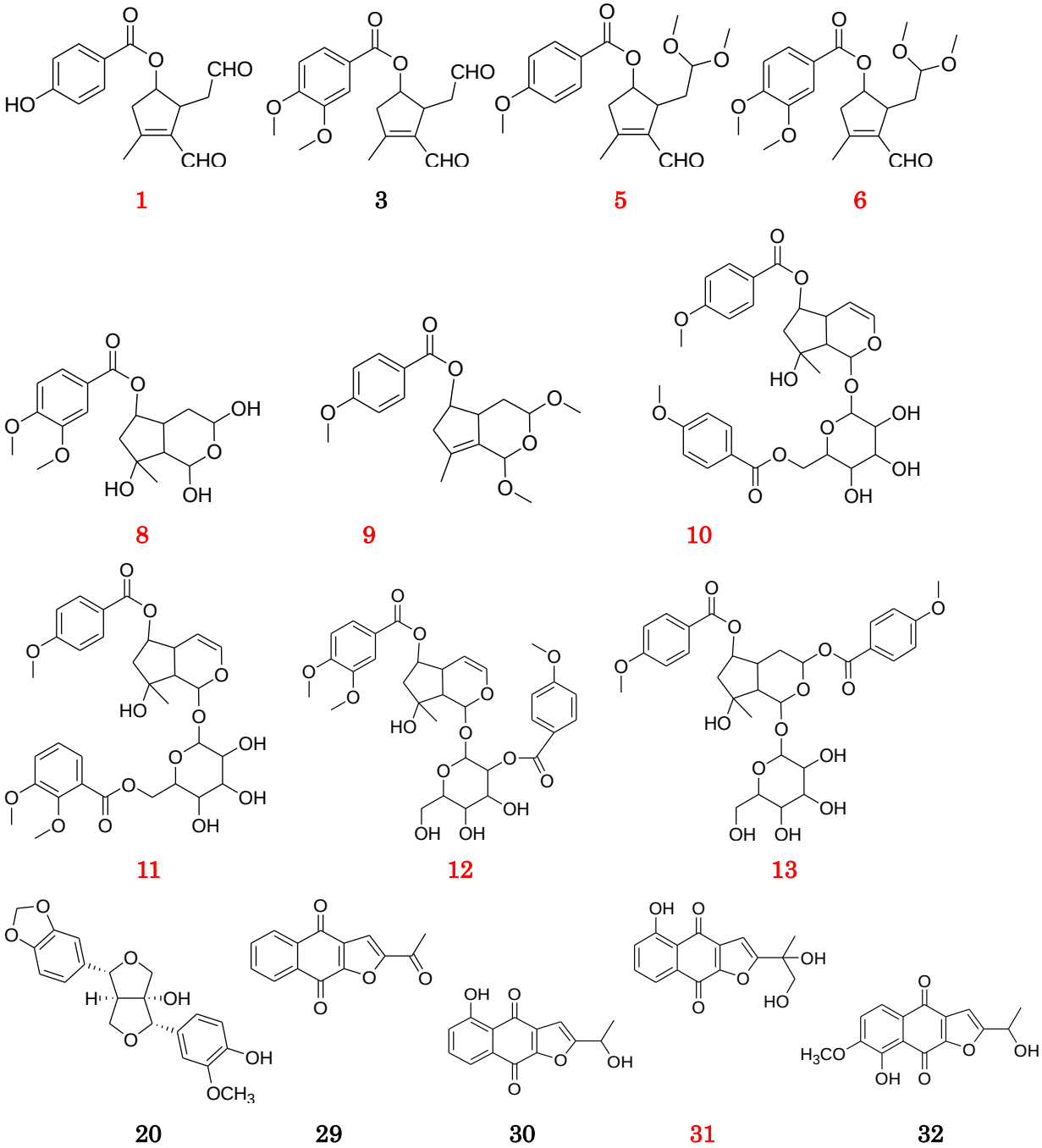

Fig. 1. Anti-inflammatory and anti-cancer compounds from *Tabebuia avellanedae*.

(■: New compounds ■: known compounds)

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学位論文審査報告書（甲）

1．学位論文題目（外国語の場合は和訳を付けること。）

Study on Biologically Active Compounds from Brazilian Plant *Tabebuia avellanedae*

（ブラジル産植物 *Tabebuia avellanedae* に由来する生物活性化合物の探索）

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3．審査結果の要旨（600～650 字）

Tabebuia avellanedae は中南米地域に広く分布するノウゼンカズラ科の植物である。この植物の内部樹皮は“tahebo（タヒボ）”などと称され、健康を維持する薬物として中南米を中心に伝統的に使用されてきた。本研究ではブラジル産タヒボから抗腫瘍活性および抗炎症作用を示す化合物を単離し、医薬品シードを探索することを目的とした。化合物の単離は、熱水抽出物から得た AcOEt 可溶部と MeOH 抽出物から得た $CHCl_3$ 可溶部について各種カラムクロマトグラフィーおよび HPLC により行った。2D-NMR を中心に構造解析を行い 10 種類の既知化合物とともに 22 種類は新規化合物を得た。抗腫瘍活性成分は、細胞株 A549, MCF-7, SiHa を使用した細胞毒性試験、細胞周期解析およびアポトーシス誘導試験などにより 2 種類の furanonaphthoquinone 類（1 種類は新規）を明らかにした。抗炎症活性成分は、マクロファージ細胞などを使用した NO、PGE_2、TNF-*a* の誘導阻害試験により cyclopentene 類および iridoid 類 10 種類（9 種類は新規）を明らかにした。以上、本研究結果はブラジル産薬用植物から多数の新規化合物を単離しただけでなく、抗腫瘍活性および抗炎症活性を示す化合物を明らかにした。これらの化合物は医薬品シードとして創薬研究につながることが充分期待できることからも、審査委員会は本論文が博士（薬学）に値すると判断した。

4．審査結果　(1) 判　　定（いずれかに○印）　（合　格）・　不合格

(2) 授与学位　　博　士（薬学）