薬物代謝工学分野　Division of Metabolic Engineering

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◇研究目的

薬物代謝工学分野は和漢薬の薬効，毒性発現に関与する代謝系の分子生物学的研究を発展させることを設置目的とし，(1) 和漢薬の薬効発現に関与する腸内細菌の役割の解明，(2) LC/MS/MS による和漢薬成分分析と薬物動力学的研究，(3) AIDS，C 型肝炎ウイルスに有効な天然薬物の探索，(4) 霊芝，樟芝などの担子菌類の薬効評価，(5) 内分泌調節作用を有する和漢薬の研究 などを研究テーマとしている。

◇研究概要

Ⅰ）　和漢薬の薬効発現に関与する腸内細菌の役割の解明

紅花種子に含まれるリグナン tracheloside のヒト腸内細菌による代謝を検討した結果，この化合物もまたエストロゲン様作用を有する enterolactone に変換されることを明らかにした。また得られた中間代謝物のエストロゲンレセプターα および β との結合の強さを比較検討した。

Ⅱ）　LC/MS/MS による和漢薬成分分析と薬物動力学的研究

台湾産樟芝菌糸体中に含まれる antrodin D, E の吸収，代謝，分布，排泄等の体内動態を明らかにする目的で動物に経口投与後，血漿，尿，糞中の成分の LC/MS/MS による分析を行い，代謝物の生成を認めた。

Ⅲ）　AIDS，C 型肝炎ウイルスに有効な天然薬物の探索

トリテルペンの A 環の開裂した化合物や化学的に合成した種々の類似化合物の HIV-1 および HCV プロテアーゼに対する阻害作用を検討し，強い阻害活性物質を見出した。

Ⅳ）　霊芝，樟芝などの担子菌類の薬効評価

ベトナム産の黄芝から単離した化合物の HIV-プロテアーゼ阻害活性を調べた結果，プロテアーゼの活性中心を競合的に阻害する ganomicin 誘導体と，酵素の二量体形成を阻害するトリテルペンに大別されることが判明した。

Ⅴ）　内分泌調節作用を有する和漢薬の研究

当帰芍薬散の効果を下垂体摘出ラットを用いて解析した結果、卵巣中 KiSS-1 mRNA 発現、回復を通して機能を回復させていることが示唆された。中医学理論に基づいて創製された春至カプセルの生殖内分泌効果を調べ，そのメカニズムを検討した。

Ⅵ）　α-Glucosidase 阻害活性を有する天然物質とその誘導体の探索

Chlorogenic acid 誘導体、flavonoid と他の phenolic 化合物に強い阻害活性が見出された。

◇著書

1) シャクヤク，ボタンピ．「現代医療における漢方薬」日本生薬学会監修，96，131，南江堂，東京，2008.
2) 7.2 エンテロラクトン．「食品機能性の科学」 西川研次郎監修，1024-1029，株式会社産業技術サービスセンター，東京，2008.

◇原著論文

1) Ma C., Winsor L., Daneshtalab M.: Quantification of spiroether isomers and herniarin of different parts of *Matricaria matricarioides* and flowers of *Chamaemelum nobile*. Phytochem. Anal., 18: 42-49, 2007.

Abstract: A simple HPLC-PAD-MS method was established to quantitatively analyse two spiroether isomers (*cis*-en-yn-dicycloether and *trans*-en-yn-dicycloether) and the main coumarin, herniarin, in chamomile herbs, simultaneously. By using this method, the contents of these three compounds in the flowers of two chamomile species, Roman chamomile (*Chamaemelum nobile*) and pineapple weed (*Matricaria matricarioides*), as well as in different parts of pineapple weed, were investigated. It was found that the flowers of both herbs contained large amounts of *cis*-en-yn-dicycloether and *trans*-en-yn-dicycloether, with the *trans*-form being more abundant than the *cis*-form. The leaves of pineapple weed were found to have the highest concentration of *cis*-en-yn-dicycloether and herniarin than the other parts. HPLC-PAD-MS-guided isolation and identification of other constituents are also discussed.

2) Lee J., Ma C., Hattori M.: Quantification of ergosterol and ergosterol peroxide in several medicinal fungi by high performance liquid chromatography monitored with a diode array detection-atmospheric pressure chemical ionization-ion trap mass spectrometer. J. Trad. Med., 25: 18-23, 2008.

Abstract: A high performance liquid chromatography-diode array detection-atmospheric pressure chemical ionization-ion trap mass spectrometry (HPLC-DAD-APCI-MS/MS) was applied to determine the contents of ergosterol and ergosterol peroxide (EPO) in seventeen fruiting bodies and five mycelia of medicinal fungi, which are used as important traditional or folk medicines for prevention and treatment of cancer. A comprehensive validation of the method, including sensitivity, lineartity, reproducibility and recovery was conducted using the optimized chromatographic conditions; an HPLC-DAD method was used for determination of ergosterol and an HPLC-APCI-Ion Trap MS method was adopted for determination of EPO. The calibration lines of ergosterol and EPO were obtained with R^2>0.999 (both standard compounds), and the limits of detection (S/N=3) were estimated to be 16.4 and 1.8 ng, respectively. The relative standard deviations of the respective methods were less than 4.81 and 6.30% (n=5) for intraday and interday assays, and the recoveries were 94.6-98.4% and 93.0-99.6% for ergosterol and EPO, respectively. Of the medicinal fungi examined, the content of ergosterol was found to be the highest in the fruiting body of *Grifola frondosa* (0.283%, w/w), while that of EPO was the highest in the fruiting body of *Ganoderma colossum* (0.053%, w/w). The described analytical methods are rapid, accurate and applicable to the quantitative determination of ergosterol and EPO in other fungi and their commercial products.

3) Wang Z., Ma C., Tang S., Xiao H., Kakiuchi N., Kida H., and Hattori M.: Qualitative and quantitative analysis of Swertia herbs by high performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS). Chem. Pharm. Bull., 56: 485-490, 2008.

Abstract: We evaluated the composition of Swertia herbs using high performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS). Eleven peaks of 6 species

were unequivocally identified by comparing their retention times, UV spectra, on-line electrospray ionization mass (ESI-MS) spectra, and collision-induced dissociation mass spectrometry/mass spectromety (CID-MS/MS) data with those of authentic compounds. We adopted wavelengths of 254nm, 340 nm and 230 nm to simultaneously determine these 11 compounds. By comparing the overall DAD and total ion current (TIC) profiles of various samples, the 6 species were differentiated in terms of the occurrence and/or relative concentrations of the eleven compounds. Our novel validated HPLC-DAD-MS method not only facilitates quality control and identification of Swertia herbs, but is also applicable to systematic investigations of the distribution of secoiridoids, flavonoids, and xanthones in the genus Swertia.

4) Lee J., Ma C., Park D., Yoshimi Y., Hatanaka M., and Hattori M.: Transformation of ergosterol peroxide to cytotoxic substances by rat intestinal bacteria. Biol. Pharm. Bull., 31: 949-954, 2008.

Abstract: Ergosterol peroxide (EPO, **1**) is a major antitumor sterol produced by edible or medicinal mushrooms. Following oral administration of **1** to rats or anaerobic *in vitro* incubation of **1** with rat fecal bacteria, three metabolites were detected and their structures were identified to be 5α,6α-epoxy-ergosta-8(14), 22-diene-3β, 7α-diol (M1, **2**), 5α,6α-epoxyergosta-8,22-diene-3β,7α-diol (M2, **3**), and 5α,6α-epoxy-3β-hydroxyergosta-22-ene-7-one (M3, **4**) by spectroscopic analysis. Of these, M2 and M3 showed more potent inhibitory activity than the original compound **1** against proliferation of CACO-2, WiDr, DLD-1 and Colo320 human colorectal adenocarcinoma cells. These findings suggest that bacterial metabolites of EPO play a significant role in its cytotoxic activity against human colorectal cancer cells.

5) El Dine R. S., El Halawany A. M., Nakamura N., Ma C., and Hattori M.: New lanostane triterpene lactones from the Vietnamese mushroom *Ganoderma colossum* (FR.) C. F. BAKER. Chem. Pharm. Bull., 56: 642-646, 2008.

Abstract: Four new lanostane triterpene lactones (colossolactone I, colossolactone II, colossolactone III and colossolactone IV) were isolated from the Vietnamese mushroom *Ganoderma colossum* (FR.) C. F. BAKER along with five known compounds. The structures of the new compounds were determined on the basis of MS, NMR and circular dichroism.

6) Chung M. H., Suzuki S., Nishihara T., and Hattori M.: Estrogenic effects of a Kampo formula, *Tokishakuyakusan*, in parous ovariectomized rats. Biol. Pharm. Bull., 31: 1145-1149, 2008

Abstract: Female hormone-dependent cancers and other diseases pose a serious health threat for women, and low-risk medicines against such cancers hve not yet been discovered. The present study examines the effects of the traditional Chinese herbal mixture, Tokishakuyakusan (TS) and 17β-estradiol on the uterus of parous ovariectomized rats. Uterine atrophy that causes a reduction in uterine tissue and the uterine cavity area, was induced by ovariectomy, and slightly recovered by the daily oral administration of TS for two weeks (1000 mg/kg body weight). TS restored the decreased plasma estradiol concentration due to ovariectomy. However the yeast two-hybrid assay showed that TS did not bind estrogen receptors α and β and immunohistochemnical staining revealed that 17β-estradiol stimulated the protein expression of estrogen receptor α, progesterone receptor, c-fos and c-jun in the uterus, whereas TS did not. These results suggest that TS might be useful for treating menopausal syndromes among women, as well as for patients when hormone replacement therapy (HRT) with estrogen is contraindicated.

7) Wang Z., Tang S., Ma C. Toyooka N., Kida H., Kawasaki M. and Hattori M.: Determination of novel nitrogen-containing metabolites after oral administration of swertiamarin to rats. J. Trad. Med., 25: 29-34, 2008.

Abstract: We investigated the metabolic fate of swertiamarin (**1**) in Wister rats. Swertiamarin (**1**) is a principal component of Swertia herbs used traditional medicine. Liquid chromatography/ion trap mass spectrometry detected new metabolites (*R*)-gentianol (**4a**) and (*S*)-gentianol (**4b**) in rat plasma, together with the known metabolite gentianine (**2**), all of which contained nitrogen. The structures of the

metabolites were identified by comparing the retention times, as well as MS and MS/MS spectra with those of authentic compounds, which were synthesized from swertiamarin (**1**). We prepared (*S*)-gentianol (**4b**) by stereoselective reduction from gentianone (**3**) which is a new oxidation product of gentianine (**2**), and the absolute configuration was unequivocally determined using an improved Mosher's method.

8) Wei Y., Ma C., Chen D., Hattori M.: Anti-HIV-1 protease triterpenoids from *Stauntonia obovatifoliola* Hayata subsp. *intermedia*. Phytochemistry, 69: 1875-1879, 2008.

Abstract: Three triterpenoids, 16β-hydroxy-2,3-*seco*-lup-20(29)-ene-2,3-dioic acid (**1**), 3β,21β,24-trihydroxy-30-noroleana-12,20(29)-dien-28-oic acid (**2**) and 16β-hydroxylupa-1,20(29)-dien-3-one (**3**), along with eleven known triterpenes were isolated from stems of *Stauntonia obovatifoliola* Hayata subsp. *intermedia* (Y.C. Wu) T. Chen. Their structures were determined by analysis of HR-EI/FAB-MS and 1D and 2D NMR spectroscopic data and comparison with those in the literature. Ten of the compounds showed inhibitory activity against HIV-1 protease.

9) Lee J., Miyashiro H., Nakamura N., and Hattori M.: Two new triterpenes from the rhizome of *Dryopteris crassirhizoma*, and inhibitory activities of its constituents on human immunodeficiency virus-1 protease. Chem. Pharm. Bull., 56: 711-714, 2008.

Abstract: Two new hopane type triterpenes, named dryopteric acids A (**1**) and B (**2**), were isolated from the rhizome of *Dryopteris crassirhizoma* (Aspiadaceae) together with sixteen known compounds (**3-18**). Of isolated compounds, ursolic acid (**15**), and dryopteric acid A (**1**) and B (**2**) showed potent inhibitory activities against HIV-1 protease with IC_{50} values of 8.9-44.5 μM. In addition, acetylated compounds **1** and **2** appreciably increased inhibitory activities with their IC_{50} values of 1.7 and 10.8 μM, respectively.

10) Jin J., Nishihata T., Kakiuchi N., and Hattori M.: Biotransformation of *C*-glucosylisoflavone puerarin to estrogenic (3*S*)-equol in co-culture of two human intestinal bacteria. Biol. Pharm. Bull., 31: 1621-1625, 2008.

Abstract: Puerarin and daidzein are the major naturally occurring isoflavones in leguminous plants. These two compounds are metabolized to equol by human intestinal flora. Here we isolated two intestinal bacteria capable of metabolizing puerarin and daidzein, respectively, from human feces. One of them, strain PUE, converted puerarin to daidzein by cleaving a *C*-glucosyl bond, whereas the other, strain DZE, converted daidzein to equol by reducing a double bond in ring C followed by elimination of an oxo group. Based on the 16S ribosomal RNA gene sequence, strain DZE showed 85% similarity with *Eggerthella lenta*. Equol produced by strain DZE was identified as (3*S*)-equol through several analytical methods. Moreover, we obtained (3*S*)-equal from puerarin by coincubation with strain PUE and DZE. In addition, 5-hydroxyequol was obtained from genistein by incubation with strain DZE.

11) El Dine R. S., El Halawany A., Ma C., and Hattori M.: Anti-HIV-1 protease activity of lanostane triterpenes from the Vietnamese mushroom *Ganoderma colossum*. J. Nat. Prod., 71: 1022-1026, 2008.

Abstract: Four new lanostane triterpenes, colossolactone V (**1**), colossolactone VI (**2**), colossolactone VII (**3**), and colossolactone VIII (**4**), were isolated from the fruiting bodies of the Vietnamese mushroom *Ganoderma colossum*, together with the known compound colossolactone E (**5**). The structures of **1-4** were assigned on the basis of spectroscopic evidence, and their absolute configurations were determined by CD spectroscopy and the Mosher ester method. Compounds **1-5**, as well as two previously isolated compounds [schisanlactone A (**6**) and colossolactone G (**7**)] from the same mushroom, were evaluated for inhibition of HIV-1 protease, with IC_{50} values for the most potent compounds ranging from 5 to 13μg/mL.

12) Xu Q., Ma C., Wang W., Fu X., Tang C., and Hattori M.: Study on the nitric oxide generating effects from aristolochic acid *in vitro*. Eur. J. Mass Spectrom., 14: 231-237, 2008.

Abstract: In this study, an *in vitro* nitric oxide [NO]-assay system based on the Griess reaction was used to investigate the [NO]-generating effects of aristolochic acid [AA] for the first time. AA was separated

into its different components, aristolochic acid I [AAI] and aristolochic acid II [AAII], by preparative HPLC. AAI and AAII were incubated with human intestine bacteria [HIB] or rat intestine bacteria [RIB]. A NO mixture generated from AAI and AAII by intestinal bacteria was observed and denitroso metabolites of AAI or AAII were detected *in vitro* by liquid chromatography/tandem mass spectrometry. Therefore, NO generation might be closely related to the metabolic process of AA *in vitro*. It suggested that one possible mechanism for the toxicity of AA may be due to the generation of NO from these compounds by intestinal bacteria.

13) Ismail S. M. N., El Dine S. R., Hattori M., Takahashi K. and Ihara M.: Computer based design, synthesis and biological evaluation of novel indole derivatives as HCV NS3-4A serine protease inhibitors. Bioorg. Med. Chem., 16: 7877-7887, 2008.

Abstract: A series of novel indoles were designed and their molecular modeling simulation study including fitting to a 3D pharmacophore model using CATALYST program and their docking into the NS3 active site was examined as HCV NS3 protease inhibitor. Several compounds showed significant high simulation docking score and fit values. The designed compounds were synthesized and biologically evaluated *in vitro* using an NS3 protease binding assay, where compounds **10a-k** showed significant inhibitory activity (≧67%inhibition at 100 μg/mL). Of these, compounds **10c** and **10f** demonstrated potent HCV NS3 protease inhibitors with IC_{50} values of 15 and 13μM, respectively. Enantio-selective Michael addition of an indole derivative in the presence of catalytic amount of $AlCl_3$ and quinine at room temperature afforded the adduct **7e** in excellent yield with 73% ee. The product was converted into **10l**, which showed lower activity than the mixture of the corresponding diastereoisomers.

14) Tang S., Wang Z., Ma C., and Hattori M.: Simultaneous determination of ten bioactive constituents in *Eucommia ulmoides* leaves and Tochu tea products by high-performance liquid chromatography-diode array detector-mass spectrometry (HPLC-DAD-MS). J. Trad. Med., 25: 112-118, 2008.

Abstract: Tochu tea products generated from the leaves of *Eucommia ulmoides* OLIVER are habitually consumed as a health beverage in Japan, China and Korea. Therefore, quality control of these products is important for safety and validity evaluation. A method using high-performance liquid chromatography-diode array detector/electrospray ionization mass spectrometry (HPLC-DAD-/ESI-MS) was established to simultaneously determine ten bioactive constituents including 2 iridoids, 2 phenolic acids and 6 flavonoids in *E. ulmoides* leaves and Tochu tea products. Peaks were unequivocally identified by comparing retention times, on-line electrospray ionization mass (ESI-MS) spectra and UV data with those of reference compounds isolated from *E. ulmoides* leaves and Tochu tea products. All calibration curves of the ten compounds showed good linear regression ($R > 0.9994$) within test ranges. The new method provided satisfactory presicision and accuracy with overall intra-day and inter-day variations of 1.8 - 3.0% and 3.5-5.9%, respectively, and overall recovery ranging from 95.4 - 106.1% with relative standard deviations (RSDs) within 3.6% ($n = 3$) for the ten compounds analyzed. The exclusive selecitivity and excellent sensitivity of HPLC-DAD/ESI-MS provided comprehensive chemical information about the constituents of Tochu tea products and *E. ulmoides* leaves.

15) Ma C., Hattori M., Daneshtalab M., and Wang L.: Chlorogenic acid derivatives with alkyl chains of different lengths and orientations: Potent α-glucosidase inhibitors. J. Med. Chem., 51: 6188-6194, 2008.

Abstract: α-Glucosidases play important roles in the digestion of carbohydrates and biosynthesis of viral envelope glycoproteins. Inhibitors of α-glucosidase are promising candidates for the development of antitype II diabetics and anti-AIDS drug. Here, we report the synthesis and α-glucosidase inhibitory activity of mono- and diketal/acetal derivatives of chlorogenic acid. The diketal drivatives showed more potent inhibitory activity than the monoketals. The 1,7-(5-nonanone) 3,4-(5-nonanone)-chlorogenic acid diketal showed remarkable inhibitory activity against α-glucosidases with potency better than that of 1-deoxynojirimycin hydrochloride. Four diastereomers of pelargonaldehyde diacetal and two of monoacetal derivatives of chlorogenic acid were synthesized in this study. They showed significant potent inhibition similar to or more potent than the ketal counterparts. Acetals with the alkyl chain oriented toward position 2 of chlorogenic acid showed more potent activity than those oriented toward position 6.

16) **Lee J., Hattori M., Kim J.: Inhibition of HIV-1 protease and RNase H of HIV-1 reverse transcriptase activities by long chain phenols from the sarcotestas of *Ginkgo biloba*. Planta Med., 74: 532-534, 2008.**

Abstract: Nine long-chain phenols: four cardanols (**1**-**4**), two bilobols (**5**, **6**) and three alkylsalicylic acids (**7**-**9**) were isolated from the CH_2Cl_2 extracts of the sarcotestas of *Ginkgo biloba* as HIV-1 protease (PR) inhibitors. From these phenols, the bilobols (IC_{50}, 2.6-5.8 μM) and alkylsalicylic acids (IC_{50}, 10.2-24.9 μM) exhibited dose-dependent potent inhibitory activities on HIV-1 PR, while the cardanols did not. On the other hand, only the alkylsalicylic acids (IC_{50}, 33.7-170.3 μM) inhibited the activities of RNase H of HIV-1 reverse transcriptase (RT), while all of the compounds failed to affect the RNA dependent DNA polymerase (RDDP) of HIV-1 RT. Therefore, we regard bilobols as a new class and selective inhibitors of HIV-1 PR; in addition, alkylsalicylic acids are elucidated as a new class of inhibitors against HIV-1 PR and RNase H of HIV-1 RT.

17) **Chuanasa T., Phromjai J., Lipipun V., Suzuki M., Pramyothin P., Hattori M., and Shiraki K.: Anti-herpes simplex virus (HSV-1) activity of oxyresveratrol derived from Thai medicinal plant: Mechanism of action and therapeutic efficacy on cutaneous HSV-1 infection in mice. Antiviral Res., 80: 62-70, 2008.**

Abstract: Oxyresveratrol, a major compound purified from *Artocarpus lakoocha*, a Thai traditional medicinal plant, was evaluated for its mechanism of action and therapeutic efficacy on cutaneous herpes simplex virus (HSV) infection in mice. The inhibitory concentrations for 50% HSV-1 plaque formation of oxyresveratrol, three clinical isolates, thymidine kinase (TK)–deficient and phosphonoacetic acid (PAA)-resistant HSV-1 were 19.8, 23.3, 23.5, 24.8, 25.5 and 21.7 μg/ml, respectively. Oxyresveratrol exhibited the inhibitory activity at the early and late phase of viral replication and inhibited the viral replication with pretreatment in one-step growth assay of HSV-1 and HSV-2. Oxyresveratrol inhibited late protein synthesis at 30 μg/ml. The combination of oxyresveratrol and acyclovir (ACV) produced synergistic anti-HSV-1 effect, as characterized by the isobologram of plaque inhibition. Mice orally treated with oxyresveratrol (500 mg/kg/dose) dose at 8 h before and three times daily had significant delay in herpetic skin lesion development ($P<0.05$). Topical application of 30% oxyresveratrol ointment five times daily significantly delayed the development of skin lesions and protected mice from death ($P<0.0001$).

18) **Ngoc T., Hung T., Thuong P., Kim J., Choi J., Bae K., Hattori M., Choi C., Lee J., and Min B.: Antioxidative activities of galloyl glucopyranosides from the stem-bark of *Juglans mandshurica*. Biosci. Biotechnol. Biochem., 72: 2158-2163, 2008.**

Abstract: Two phenolics, 1,2,6-trigalloylglucose (**1**) and 1,2,3,6-tetragalloylglucose (**2**), isolated from the stem-bark of *Juglans mandshurica* were evaluated for their antioxidative activities. The results showed that compounds **1** and **2** exhibited strong scavenging activities against 1,1′-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis-(3-ethylbenzenthiazoline-6-sulphonic) acid, and superoxide radicals, and also had a significant inhibitory effect on lipid peroxidation and low-density lipoprotein (LDL) oxidation. The strong superoxide radical scavenging of **1** and **2** resulted from the potential competitive inhibition with xanthine at the active site of xanthine oxidase (OX). In addition, compounds **1** and **2** displayed significant lipoxygenase inhibitory activity, the mode of inhibition also being identified as competitive. In comparison, the antioxidative activities of compounds **1** and **2**, together with gallic acid, indicated that the number of galloyl moieties could play an important role in the antioxidative activity.

19) **Ma C., Hattori M., Chen H., Cai S., and Daneshtalab M.: Profiling the phenolic compounds of *Artemisia pectinata* by HPLC-PAD-MS^n. Phytochem. Anal., 19: 294-300, 2008.**

Abstract: An HPLC-PADMS^n method was employed to profile the phenolic compounds of the aerial part of *Artemisia pectinata* (*Neopallasia pectinata*), a plant with no previous reports concerning its phenolic constituents. Three isomers of *trans*-caffeoylquinic acid accompanied by *cis*-5-caffeoylquinic acid, six isomers of *trans*-dicaffeoylquinic acid, two isomers of methyl *trans*-dicaffeoylquinate (including one new somer), a *trans*-caffeoylferuloylquinic acid and three flavanoids were identified unambiguously by analysis of their UV and MS^n spectra in comparison with standard compounds that were isolated from natural sources, or synthesised, or were surrogate standards (green coffee extract). Other compounds were identified by analysis or their UV and MS^n data in comparison with those reported in the literature. MS^n experiments also suggested the presence of groups of dicaffeoylquinic acid glycosides, caffeoylquinic acid diglycosides, caffeoylquinic acid glycosides and quinic acid diglycosides.

20) Yang X., Zhao J., Hattori M.: Three new triterpenoid saponins from the seeds of *Aesculus turbinata*. J. Asian Nat. Prod. Res., 10: 259-265, 2008.

Abstract: Three new triterpenoid saponins, named isoescins VIIa (**1**), VIa (**2**), and VIIIa (**3**), were isolated from the seeds of *Aesculus turbinata* and identified by spectroscopic analysis and chemical hydrolysis. Their structures were established as 21β-*O*-tigloyl-28-*O*-acetylprotoaescigenin 3β-*O*-[β-D-galactopyranosyl(1→2)][β-D-glucopyranosyl(1→4)]-β-D-glucopyranosiduronic acid (Isoescin VIIa, **1**), 21β-*O*-(2-methylbutyryl)-28-*O*-acetylprotoaescigenin 3β-*O*-[β-D-glucopyranosyl(1 → 2)][β-D-glucopyranosyl(1 → 4)]-β-D-glucopyranosiduronic acid (Isoescin VIa, **2**), and 21β-*O*-angeloyl-28-*O*-acetylbarringtogenol C 3β-O-[β-D-glucopyranosyl(1 → 2)][β-D-glucopyranosyl(1 → 4)]-β-D-glucopyranosiduronic acid (Isoescin VIIIa, **3**).

学会報告 （∗：特別講演，シンポジウム，ワークショップ等）

1) Jin J., Nishihata T., Kakiuchi N., Hattori M.: Biotransformation of *C*-glucosylisoflavone puerarin to estrogenic (3*S*)-equol in co-culture of two human intestinal bacteria. The 4th KSP-JSP-CCTCNM Joint Symposium on Pharmacognosy KOREA-JAPAN-CHINA Joint Symposium 2008, 2008, 6, 18-21, Gangneung, Korea.
2) Chung M. H., Suzuki S., and Hattori M.: The mechanism differences between Tokishakuyakusan and 17beta-estradiol using parous ovariectomized rats. The 7th International Symposium on Natural Medicine and Microflora, 2008, 8, 2-4, Toyama.
3) El Halawany A. M., Chung M. H., Ma C., and Hattori M.: Estorogenic activity of hydroxyl-phenylalkanes and diarylheptanoids from *Aframomum melegueta*. The 7th International Symposium on Natural Medicine and Microflora, 2008, 8, 2-4, Toyama.
4) Tobo T., Jin J., Chung M. H., Ma C., Hattori M.: Synthesis of enantiomeric enterolactone and enterodiol, and their estrogenic potency. The 7th International Symposium on Natural Medicine and Microflora, 2008, 8, 2-4, Toyama.
5) Furuhashi K., Chung M. H., Hattori M.: Effect of Tokishakuyakusan on ovary KiSS-1 mRNA expression in hypophysectomized (HPX) rats. The 7th International Symposium on Natural Medicine and Microflora, 2008, 8, 2-4, Toyama.
6) El Halawany A. M.: Estrogenic activity of some naturally occurring phenolics. The 7th International Symposium on Natural Medicine and Microflora, 2008, 8, 2-4, Toyama.
7) Ma C.: Chemistry and bioactivity of caffeoylquinic acid derivatives. The 7th International Symposium on Natural Medicine and Microflora, 2008, 8, 2-4, Toyama.
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学内

1) 腸内嫌気性菌による生薬成分の代謝　　富山大学薬学部　赤尾光昭
2) 抗 HSV 薬の開発研究　　富山大学医学部　白木公康
3) 体内女性ホルモンに与える和漢薬の影響に関する研究　　富山大学和漢医薬学総合研究所　松本欣三

国内

1) 抗 HCV 薬の開発研究　　慶応大学医学部　下遠野邦忠
　　慶応大学薬学部　下遠野久美子
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海外

1) 抗 HSV 薬開発研究　　チュラロンコン大学　Pornpen Pramyothin

◇研究費取得状況

1) 「ほくりく健康創造クラスター」（財）北陸産業活性化センター（服部　分担）100 万円.
2) 「ヒト早発性老化症候群モデルを用いた和漢薬の生殖器・血管へ与える影響」平成 20 年度学長裁量経費（鄭　代表）100 万円.
3) 「伝統薬物による生体機能の革新的解析および創薬戦略」平成 20 年度学長裁量経費（馬分担）（鄭　分担）
4) 「ヒト腸内細菌による lipoaconitine 代謝機構の化学的解明」平成 20 年度笹川科学研究助成金（王 偉）55 万円.
5) 「人参加水分解産物トリテルペン誘導体の抗 HIV protease および抗 HCV protease 作用に関する研究」2008 年度日中医学協会共同研究等助成金（危 英）60 万円.

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◇学位（修士，博士）取得者

修士論文：

中村 賢一：ヒト腸内細菌による Puerarin *C*-配糖体開裂反応に関する研究（3 月）

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