薬物代謝工学分野　Division of Metabolic Engineering

教　授	服部　征雄	Professor	Masao Hattori (Ph.D.)
助教授	横澤　隆子(3月まで)	Associate Professor	Takako Yokozawa (Ph.D.)
助教授	中村　憲夫(8月より)	Associate Professor	Norio Nakamura (Ph.D.)
助　手	宮代　博継	Assistant Professor	Hirotsugu Miyashiro (Ph.D.)
機関研究員	高　江静 (COE)	Postdoctral Fellow	Gao Jiangjing (Ph.D.)
機関研究員	左　風	Postdoctral Fellow	Feng Zuo (Ph.D.)
事務補佐員	黒岩　純子	Clerical Employee	Junko Kuroiwa

◇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 酵素免疫測定法や LC/MS/MS による和漢薬活性成分の薬物動力学的研究、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

1) ゴボウシに多量に含まれる arctiin の腸内細菌による enterolactone への代謝をヒトを用いて検討し、血中、尿中 enterolactone の濃度には非常に個人差があることが判明した。

2) キサンチン *C* 配糖体 mangiferin の C-C 結合を開裂するヒト腸内細菌 *Bacteroides* sp. BAR から酵素を得、精製することに成功した。

3) Arctiin, arctigenin の脱メチル化反応に関与する *Fusobacterium* sp. strain ARC-2 を単離し、その基質特異性を検討した。

Ⅱ）　酵素免疫測定法や LC/MS/MS による和漢薬活性成分の薬物動力学的研究

Aconitine, mesaconitine および それらの脱アセチル体 (benzoylaconine, benzoylmesaconine) の酵素免疫測定法を開発し、経口投与後の両アルカロイド、代謝物の血中濃度、脊髄中の濃度の測定を行なった。また 樟芝に含まれるスクシイミド誘導体の薬物動態の研究を行なった。

Ⅲ）　AIDS、C 型肝炎ウイルスに有効な天然薬物の探索

タイ薬用植物、中国少数民族薬物の C 型肝炎ウイルスポリメラーゼ阻害作用を検討し、数十種の薬物エキスに顕著な阻害活性を認めた。

Ⅳ）　霊芝、樟芝などの担子菌類の薬効評価

霊芝、樟芝の多糖類を単離し、霊芝酸性多糖、樟芝中性多糖が劇症肝炎モデル動物の肝障害を抑制することを見出した。

◇著書

1) 服部征雄：漢方薬・生薬薬剤師講座テキストIII第2版（分担），日本薬剤師研修センター.

◇原著論文

1) **Park C.H., Kim S.C., Choi M.R., Song S.H., Yoo E.J., Kim S.H., Miyashiro H., and Hattori M.: Anti-HIV protease activity from Rosa family plant extracts and rosamultin from *Rosa rugosa*. *J. Med. Food*, 8: 107-109, 2005.**

Abstract: To identify substances with anti-human immunodeficiency virus (HIV) activity from plant sources, 12 extracts of Rosa family plants were screened for their inhibitory effects against HIV-1 protease. Of the extracts tested, the strongest inhibitory effects were observed in the root of *Rosa rugosa* and the leaves of *Prunus sargentii*, at a concentration of 100μg/mL. Rosamultin isolated from the root of *R. rugosa* inhibited HIV-1 protease by 53% at a concentration of 100μM.

2) **Ma C.M., Cai S.Q., Cui J.R., Wang R.Q., Tu P.F., Hattori M., and Daneshtalab M.: The cytotoxic activity of ursolic acid derivatives. *Eur. J. Med. Chem.*, 40: 582-589, 2005.**

Abstract: Ursolic acid and 2α-hydroxyursolic acid isolated from apple peels were found to show growth inhibitory activity against four tumor cell lines, HL-60, BGC, Bel-7402 and Hela. Structural modifications were performed on the C-3, C-28 and C-11 positions of ursolic scid and the cytotoxicity of the derivatives was evaluated. The SAR revealed that the triterpenes possessing two hydrogen-bond forming groups (an H-donor and a carbonyl group) at positions 3 and 28 exhibit cytotoxic activity. The configuration at C-3 was found to be important for the activity. Introduction of an amino group increased the cytotoxicity greatly. A 3β-amino derivative was 20 times more potent than the parent ursolic acid. The 28-aminoalkyl dimer compounds showed selective cytotoxicity.

3) **Solis P.N., Olmedo D., Nakamura N., Carderon A., Hattori M., and Gupta M.P.: A new lavicidal lignan from *Piper fimbriulatum*. *Pharmaceutical Biology*, 43: 378-381, 2005.**

Abstract: A new lignan, 3,4,5'-trimethoxy-3',4'-methylenedioxy-7,9':7',9-diepoxylignan (**1**) (6-[4-(3,4-dimethoxyphenyl)-tetrahydro-furo[3,4-*c*]furan-l-yl]-4-methoxy-benzo[1,3]dioxole) together with two known lignans, 7'-*epi*-sesartemin (**2**) and diayangambin (**3**), and a known flavonoid, 5-hydroxy-7,4'-dimethoxyflavone (**4**), were isolated from the leaves of *Piper fimbriulatum* C. DC. Their structures were assigned by a combination of one- and two-dimensional NMR techniques. 7'-*epi*-Sesartemin (**2**) showed the highest larvicidal activity against *Aedes aegypti* (LC_{100} 17.6μg/ml) and weak antiplasmodial (IC_{50} 7.0μg/ml) and antitrypanosomal (IC_{50} 39.0μg/ml) activities. None of the compounds was active against *Leishmania mexicana.*

4) **Qiu M.H., Nakamura N., Min B.S., and Hattori M.: Two new pregnanone derivatives with strong cytotoxic activity from *Pachysandra axillaris*. *Chemistry and Biodiversity*, 2: 866-871, 2005.**

Abstract: Two new, bioactive, pregnane-based natural products, pachysanonin (=3β,11α,12β)-12-acetoxy-3-(dimethylamino)-11-[(3,4-dimethylpent-3-enoyl)oxy]pregnan-20-one; **1**) and pachysanone (=(11α,12β)-12-acetoxy-11-[(3,4-demethylpent-3-enoyl)oxy]pregnan-3,20-dion; **2**) have been isolated from *Pachysandra axillaris*. Their structures were determined by spectroscopic methods, and, in the case of **2**, by single-crystal X-ray crystallography (*Figure*). Compound **2** showed significant antitumor activity against Lewis lung carcinoma (LCC) tumor cells, with an IC_{50} value of 0.020±0.006μg/ml, which is equal or even lower than those of the wellknown natual antitumor agents harringtonine (0.02),

homoharringtonine (0.15), and adriamycin (0.06μg/ml; positive control).

5) **Sanugul K., Akao T., Li Y., Kakiuchi N., Nakamura N., and Hattori M.: Isolation of a human intestinal bacterium that transforms mangiferin to norathyriol and inducibility of the enzyme that cleaves a *C*-glucosyl bond. *Biol. Pharm. Bull.*, 28: 1672-1678, 2005.**

Abstract: The *C*-glucosyl bond of *C*-glucosides generally tolerates acid and enzymatic hydrolysis. Many *C*-glucosides are cleaved by human intestinal bacteria. We isolated the specific bacterium involved in the metabolism of mangiferin (2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone), *C*-glucosyl xanthone, from a mixture of human fecal bacteria. The anaerobic *Bacterodies* species named MANG, transformed mangiferin to the aglycone, norathyriol, suggesting cleavage of a *C*-glucosyl bond. However, *B.* sp. MANG cleaved *C*-glucosyl in a dose- and time-dependent manner only when cultivated in the presence of mangiferin. Cleavage was abolished by inhibitors of RNA and protein syntheses, such as rifampicin and chloramphenicol, respectively, indicating that the enzyme that cleaves *C*-glucosyl is induced by mangiferin. In contrast, mangiferin did not affect bacterial α- and β-glucosidase activities under any conditions. The *C*-glucosyl-cleavage in cell-free extracts was not altered by potent glucosidase inhibitors such as 1-deoxynojirimycin and gluconolactone. Therefore, the *C*-glucosyl-cleaving enzyme substantially differs from known glucosidases that cleave *O*-glucosides. This is the first description of a specific intestinal bacterium that is involved in the metabolism of mangiferin and which produces a novel and inducible *C*-glucosyl-cleaving enzyme.

6) **Sanugul K., Akao T., Nakamura N., and Hattori M.: Two proteins, Mn^{2+}, and low molecular cofactor are required for *C*-glucosyl-cleavage of mangiferin. *Biol. Pharm. Bull.*, 28: 2035-2039, 2005.**

Abstract: *C*-Glucosides, in which sugars are attached to the aglycone by carbon-carbon bonds, are generally resistant to acid and enzyme hydrolysis. The *C*-glucosyl bond of mangiferin, a xanthone *C*-glucoside, was cleaved by anaerobic incubation with a human intestinal bacterium, *Bacteroides* sp. MANG, to give norathyriol. A cell-free extract obtained by sonication of *B.* sp. MANG demonstrated cleaving activity for mangiferin to norathyriol by adding NADH, diaphorase, and dithiothreitol. Both high molecular weight (>10k) and low molecular weight (<10k) fractions obtained from the cell-free extract were required for the activity. $MnCl_2$ was necessary for the activity, but other metal ions were not. By purification of the high molecular weight fraction using DEAE-cellulose and Phenyl Sepharose column chromatography, two fractions, designated as proteins A and B, were separated and required for the activity. Neither protein A nor protein B alone showed any activity. This is the first report describing a *C*-glucosyl-cleaving enzyme from human intestinal bacterium that seems to involve a novel enzyme mechanism.

7) **Park H.J., Kurokawa M., Shiraki K., Nakamura N., Choi J.S., and Hattori M.: Antiviral activity of the marine alga *Symphyocladia latiuscula* against herpes simplex virus (HSV-1) infection in mice. *Biol. Pharm. Bull.*, 28: 2258-2262, 2005.**

Abstract: The antiviral activities of extracts from 5 species of marine algae collected at Haeundae (Pusan, Korea), were examined using plaque reduction assays. Although the activity of a methanol (MeOH) extract of *Sargassum ringoldianum* (Sargassaceae) was the most potent against several types of viruses, it was also cytotoxic. A MeOH extract of *Symphyocladia latiuscula* (Rhodomelaceae) and its fractions exhibited antiviral activities against acyclovir (ACV) and phosphonoacetic acid (PAA)-resistant (AP^r) herpes simplex type 1 (HSV-1), thymidine kinase (TK^-) deficient HSV-1 and wild type HSV-1 *in vitro* without cytotoxicity. The major component, 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether (TDB) of a CH_2Cl_2-soluble fraction was active against wild type HSV-1, as well as AP^r HSV-1 and TK^- HSV-1 (IC_{50} values of 5.48, 4.81 and 23.3μg/ml, respectively). The therapeutic effectiveness of the MeOH extract and TDB from *S. latiuscula* was further examined in BALB/c mice that were cutaneously

infected with HSV-1 strain 7401H. Three daily oral administrations of the MeOH extract and TDB significantly delayed the appearance of score 2 skin lesions (local vesicles) and limited the development of further score 6 (mild zosteriform) lesions in infected mice without toxicity compared with controls. In addition, TDB suppressed virus yields in the brain and skin. Therefore TDB should be a promising anti HSV agent.

8) Jo M., Nakamura N., Kurokawa M., Komatsu K., Shiraki K., and Hattori M.: Anti-herpes simplex virus activities of traditional Chinese medicines, used in Yunnan and Tibetan provinces of China. *J. Trad. Med.*, 22: 321-328, 2005.

Abstract: One hundred and sixty-eight traditional Chinese medicines collected in the Yunnan and Tibetan provinces were screened for their anti-herpes simplex type 1 (HSV-1) activity by using a plaque reduction assay using Vero cells. Of these, 24 extracts exhibited appreciable inhibitory activities against HSV-1. They were further examined for their therapeutic efficacies in mice infected with HSV-1; mice were infected cutaneously with HSV-1 and the extracts were orally administrated three times daily. Among them, nine extracts of *Terminalia chebula* (T42), *Tripterygium hypoglaucum* (Y42M), and *Moghania philippinensis* (Y86M), and a water extract of *Tripterygium hypoglaucum* (Y44H) delayed the deveropment and progression of skin lesions. Methanol extracts of *Cassia fistula* (T59), and *Choerospondias axillaries* (T73), and water extracts of *Begonia evansiana* (Y27H), *Maytenus fookerii* (Y60H) and *Potentilla griffithii* (Y63H) showed therapeutic effects. These extacts may be candidates for the development of anti-HSV-1 compounds.

◇総　説

1) Meselhy M. R., El-Mekkawy S., Ma C., Nakamura N., Tewtrakul S., and Hattori M.: Developing of anti-HIV agents from natural resources. *J. Trad. Med.*, 22 (suppl. 1) : 116-128, 2005.

2) Gao J. and Hattori M.: Metabolic activation of lignans to estrogenic and antiestrogenic substances by human intestinal bacteria. *J. Trad. Med.*, 22 : 213-221, 2005.

◇学会報告　(*: 特別講演、シンポジウム、ワークショップ等)

1) Park J. C., Kim S. C., Hur J. M., Choi M. R., Miyashiro H., Hattori M.: Screening of Rosa family plants for anti-HIV protease activity and inhibitor, rosamultin from *Rosa rugosa*. 日本農芸化学会, 2005, 3, 28-30, 札幌.

2) 左 風, 中村憲夫, 赤尾光昭, 服部征雄 : Pharmacokinetics of berberine and its main metabolites in conventional and pseudo germ-free rats. 日本薬学会第 125 年会, 2005, 3, 29-31, 東京.

3) 条 美智子, 楳原琢哉, 垣内信子, 中村憲夫, 小松かつ子, 西川 諭, 服部征雄, 下遠野久美子, 下遠野邦忠 : チベット生薬の C 型肝炎ウイルスプロテアーゼ阻害活性の検討. 日本薬学会第 125 年会, 2005, 3, 29-31, 東京.

4) 大川美和, 条 美智子, 中村憲夫, 小松かつ子, 下遠野久美子, 下遠野邦忠, 服部征雄 : タイ民族薬物の C 型肝炎ウイルスポリメラーゼ阻害活性を指標とした探索. 日本薬学会第 125 年会, 2005, 3, 29-31, 東京.

5) 渥美聡孝, 垣内信子, 中村憲夫, 服部征雄, 御影雅幸 : 茯苓の性状と成分との相関. 日本薬学会第 125 年会, 2005, 3, 29-31, 東京.

6) * Hattori M.: Pharmacological effects of *Apocynum venetum* tea for life-style related diseases. International Conference on Chinese Medicine, 2005, 4, 1-3, Macao.

7) * Hattori M.: Naturally occurring estrogenic precursors. 2005 International Symposium in Beijing on Pharmacognosy, 2005, 5, 13-14, Beijing.

8) 左 風, 中村憲夫, 服部征雄 : Absorption, metabolism and excretion of orally administreted

berberine in rats, monitored by LC/MS/MS. 第 59 回北陸質量分析談話会, 2005, 5, 14, 富山.

9) 服部征雄：ヒト腸内細菌による新規な代謝反応−*C*-配糖体の開裂反応について−. 第 26 回和漢薬研究所特別セミナー「和漢薬と消化管−消化管常在菌の役割および消化管疾患をめぐる最新の話題−」, 2005, 7, 13-14, 富山.

10) 陳琮湜, 趙 宇峰, 中村憲夫, 赤尾光昭, 垣内信子, 服部征雄：Arctiin の脱メチル化反応に関与するヒト腸内細菌の単離と同定. 日本薬学会北陸支部第 112 回例会, 2005, 7, 23, 富山.

11) 韓 号峰, 中村憲夫, 左 風, 横澤隆子, 服部征雄：Structural analysis and hepato-protective activity of a neutral polysaccharide from the mycelium of *Antrodia camphorata*. 第 22 回和漢医薬学会大会, 2005, 8, 20-21, 東京.

12) 高 江静, 中村憲夫, 張 群, 服部征雄：ヒトにおける Arctiin の代謝及びその個人差の LC/MS/MS による解析. 第 22 回和漢医薬学会大会, 2005, 8, 20-21, 東京.

13) 左 風, 李 建栄, 張 磊, 中村憲夫, 服部征雄：Quantitative determination of alkaloids and acid components in the precipitation of Xiexin-Tang decoction by HPLC. 第 22 回和漢医薬学会大会, 2005, 8, 20-21, 東京.

14) 近藤直子, 中村憲夫, 吉村千秋, 増山明弘, 高野俊明, 服部征雄：数種の乳酸菌による植物リグナンからエストロゲン様物質への代謝反応について. 第 22 回和漢医薬学会大会, 2005, 8, 20-21, 東京.

15) 条 美智子, 中村憲夫, 小松かつ子, 服部征雄, 垣内信子, 下遠野久美子, 下遠野邦忠, 邱 明華：紫金皮(*Tripterygium hypograucum*)の C 型肝炎ウイルスポリメラーゼ阻害活性成分の検討. 日本生薬学会第 52 回年会, 2005, 9, 16-17, 金沢.

16) 鄭 美和, 中村憲夫, 東田道久, 服部征雄：女性ホルモン調節作用を持つ和漢薬−当帰芍薬散、桂枝茯苓丸−の脳下垂体に与える影響. 日本生薬学会第 52 回年会, 2005, 9, 16-17, 金沢.

17) * Hattori M.: Maleic and succinic acid derivatives (Hepasim), and polysaccharides from the Mycelium of *Antrodia camphorata*. 第一届中日樟芝研討会, 2005, 9, 23, 台北.

18) * Hattori M.: Cytotoxic effect of maleic and succinic acid derivatives (Hepasim; antrodins A-E) from the mycelium of *Antrodia camphorata*, and hepatoprotective effect of a neutral polysaccharide (camphoratan A) on mice treated with *P. acnes*-LPS. 第一届中日樟芝研討会, 2005, 9, 23, 台北.

19) * Hattori M. and Gao J. J.: The transformation of phytoestrogen precursors to enterodiol and enterolactone by human intestinal bacterial flora and its individual variation. The 4th International Congress for Chinese Medicine, 2005, 10, 30-31, Kawasaki.

◇その他

1) 服部征雄：乳癌リスクと植物エストロゲン. 第 10 回和漢薬研究所夏期セミナー, 2005, 8, 24-26, 富山.

2) 服部征雄：和漢薬研究所から和漢医薬学総合研究所へ−過去、現在、そして未来へ−. 新富山大学和漢医薬学総合研究所開所記念講演会, 2005, 10, 1, 富山.

3) 服部征雄：「天然薬物」拠点大学交流発表会, 2005, 10, 4-6, 東京.

4) 服部征雄：WFWP2005 留学生日本語弁論大会審査委員長, 2005, 11, 13, 富山.

◇共同研究

国内

1) 抗 HCV 薬の開発研究　京都大学ウイルス研究所　下遠野邦忠
共立薬科大学　下遠野久美子
金沢大学自然科学研究科　垣内信子

和漢医薬学総合研究所　小松かつ子

2)　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

3)　抗 HSV 薬の開発研究　　富山大学医学部　白木公康

◇研究費取得状況

1)　「漢方薬の効果を遺伝子発現レベルで評価する系の開発」経済産業省 地域新生コンソーシアム研究開発事業（服部征雄 代表継続）2000 万円.

2)　「C 型肝炎ウイルスレプリカーゼ及びプロテアーゼをターゲットとした抗 HCV 剤の開発」漢方医薬研究振興財団（服部　分担）50 万円.

◇研究室在籍者

学部 3 年生：佐藤 直人、當房 貴文
学部 4 年生：中村 賢一
大学院前期 1 年：和田 明穂、鈴木 佐和子、Kitalong Christopher、張 群（10 月入学）
大学院前期 2 年：近藤 直子、大川 美和
大学院後期 1 年：王 志剛、李 柱相、Riham Salah El Din、陳琮湜（10 月入学）
大学院後期 2 年：鄭 美和、Ali Mohamoud（10 月入学）
大学院後期 3 年：条 美智子、韓 号峰
COE 研究員：高 江静（博士）
機関研究員：左 風（博士）
外国人客員研究員：Sukonpan Chanokporn (シラパコーン大学薬学部、2005.10.2 ～ 2005.11.29)
Palida Abulizi（新疆医科大学葯学院、2005.10.13～）
侯 集瑞（吉林農業大学中葯材学院、2005.10.26～）
肖怀（大理医科大学、2005.11.21～）
外国人留学生：楊 莉（中国薬科大学博士課程学生、2005.5.23～）
事務補佐員：黒岩 純子

◇学位（修士、博士）取得者

修士論文：

西畑友尋 : Puerarin 代謝に関するヒト腸内細菌の単離及びその性質（3 月）

陳琮湜: Arctiin の脱メチル化反応に関与するヒト腸内細菌の単離と同定（9 月）

博士論文：

佐藤亜希子：加齢による酸化ストレスに対する冠元顆粒の作用とその機序について（3 月）

Kanjana Sanugul : Cleavage of a *C*-Glucosyl Bond in Mangiferin by Enzyme from a Human Intestinal Bacterium, *Bacteroides* sp. Strain MGNG.（9 月）

◇人事異動

横澤隆子助教授 民族薬物研究センター・薬効解析部に配置換え。
中村憲夫助手、助教授に昇進。