薬 物 代 謝 工 学

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薬物代謝工学部門は和漢薬の薬効，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遺伝子の解析，②薬物代謝機能調節遺伝子の解明とその応用，③腎毒性物質産生機構の分子生物学的解明とその制御に関する研究を課題として取りあげ，和漢薬の薬効発現機構，生体へのレスポンスなどの基礎的研究を通じて，和漢薬の科学的評価や臨床応用をはかることを目指している。主な研究題目を以下に示す。

1．天然物のバイオトランスフォーメイション
2．和漢薬の薬効発現に関与する腸内細菌遺伝子の解明
3．AIDS の予防および治療薬の開発
4．腎疾患における病態の解明と腎臓病治療薬の開発

本年度の主な研究成果を列挙すると：

1．ヒト腸内細菌による*C*-配糖体 mangiferin の還元的脱離反応を触媒する菌種の同定および secoisolariciresinol 配糖体からエストロジェン様作用物質 enterodiol, enterolactone へ変換に関与する *Peptostreptococcus* SDG-1, *Eubacterium* SDG-2 の単離同定に成功した。前者は脱メチル化を，後者は脱ヒドロキシル化を効率良く行なう菌種であった。

2．霊芝 *Ganoderma lucidum* 胞子から新規トリテルペンを単離，構造決定し，その代表的成分 ganoderic acid A の酵素免疫測定法を開発した。

3．Sennoside の薬効発現に関与するヒト腸内細菌 *Bifidobacterium sp.* SEN から３種の異なる β-グルコシダーゼ遺伝子の塩基配列を決定し，それぞれの大腸菌による発現を行なった。

4．増悪因子のフリーラジカルの関与について，緑茶，地楡，薬用人参サポニン，丹参成分 magnesium lithospermate B，漢方方剤温脾湯などの腎における役割を解析した。

◇著　書

1）Meselhy R. M., and Hattori M.: Recent studies on peony roots and a bioactive constituent, paeoniflorin. *Current Topics in Phytochemistry*, Vol 4, Research Trends, Trivandrum, India, 2000, pp. 1-19.

2）Yokozawa T.: The antihypertensive properties of Dan-Shen, the root of *Salvia miltiorrhiza*. "Medicinal and Aromatic Plants-Industrial Profiles", ed. by Kintzios S.E., Harwood Academic Publishers, The Netherlands, 2000, pp.193-205.

3）横澤隆子，三潴忠道，二宮裕幸，田中　隆：腎不全における温脾湯の有用性と活性成分の検索．"腎とフリーラジカル －第５集－"，伊藤克巳，玄番宗一監修，青柳一正編，東京医学社，東京，2000，pp. 65–71.

4）横澤隆子，中川孝子，趙　恩珠：培養腎上皮細胞 LLC-PK_1 を用いたラジカル消去能の検討 －緑茶の場合－．"腎とフリーラジカル －第５集－"，伊藤克巳，玄番宗一監修，青柳一正編，東京医学社，東京，2000，pp. 72–77.

5）Yokozawa T.: Preventive effect of Luobuma leaf against oxidation of low-density lipoproteins. "Recent Research Developments in Agricultural & Biological Chemistry, 4", ed. by Pandalai S.G., Research Signpost, India, 2000, pp. 45-58.

6）横澤隆子：障害腎に対する薬用人参の作用．"薬用人参2000"，熊谷　朗監修，共立出版，東京，2000，pp. 145–150.

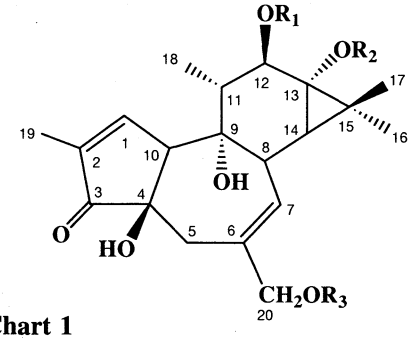
◇原　著

1）**Ahmed A. S., Nakamura N., Meselhy M. R., El-Emary N., Hattori M.: Phenolic constituents from *Grevillea robusta*. *Phytochemistry*, 53: 149-154, 2000.**

Seven new phenolic compound, together with six known ones, were isolated from a MeOH extract of the leaves of *Grevillea robusta*. The structures of these compounds were determined by various spectral methods including 2D NMR.

2）**El-Mekkawy S., Meselhy M. R., Nakamura N., Hattori M., Kawahata T., and Otake T.: Anti-HIV-1 phorbol esters from the seeds of *Croton tiglium*. *Phytochemistry*, 53: 457-464, 2000.**

Five new phorbol diesters (**1-5**), together with three known ones, were isolated from a MeOH extract of the seeds of *Croton tiglium*, and their structures were determined by spectroscopic methods and selective hydrolysis of acyl groups. These compounds were assessed for their abilities to inhibit HIV-induced cytopathic effect on MT-4 cells and to activate protein kinase C (PKC) associated with tumor-promoting action. 12-*O*-Acetylphorbol-13-decanoate (**6**) and 12-*O*-decanoylphorbol-13-(2-methylbutyrate) (**4**) effectively inhibited the cytopathic effect (CPE) of HIV-1 [complete inhibitory concentration (IC_{100} values of 7.6 ng/ml and 7.81 μg/ml, and minimum cytotoxic concentration (CC_0) values of 62.5 and 31.3 μg/ml, respectively]. Compound **6** showed no activation of PKC at concentrations of 10 and 100 ng/ml. 12-*O*-Tetradecanoylphorbol-13-acetate (TPA, **8**) was found to be not only the most potent inhibitor of HIV-1-induced CPE (IC_{100} value of 0.48 ng/ml), but also the most potent activator of PKC (98% activation) (Chart 1 参照).



	R_1=	R_2=	R_3=
1	H	Ac	$C_{18}H_{31}O$
2	H	Tigloyl	$C_{18}H_{31}O$
3	Ac	Tigloyl	H
4	$C_{10}H_{19}O$	2-Me butyryl	H
5	Tigloyl	2-Me butyryl	H
6	Ac	$C_{10}H_{19}O$	H
7	2-Me butyryl	$C_{12}H_{23}O$	H
8	$C_{14}H_{27}O$	Ac	H
9	H	Ac	H

Chart 1

3）**Helal A. M., Nakamura N., El-Askary H., and Hattori M.: Sesquiterpene lactone glucosides from *Sonchus asper*. *Phytochemistry*, 53: 473-477, 2000.**

From the methanolic extract of the roots of *Sonchus asper*, two new sesquiterpene glucosides, together with two known sesquiterpene glucosides and a known aglycone were isolated. Structures were identified as 11 β, 13-dihydrourospermal A, 15-*O*- β -D-glucopyranosyl-11 β, 13-dihydrourospermal A, 15-*O*- β -D-glucopyranosylurospermal A, 15-*O*-[6'-(*p*-hydroxyphenylacetyl)]- β -D-glucopyranosylurospermal A and 14-*O*-methylacetal-15-*O*-[6'-(*p*-hydroxyphenylacetyl)]- β -D-glucopyranosylurospermal A, by spectroscopic means.

4）**Ma C., Nakamura N., Hattori M., Kakuda H., Qiao J., and Yu H.: Inhibitory effects on HIV-1 protease of constituents from the wood of *Xanthoceras sorbifolia*. *J. Nat. Prod.*, 63: 238-242, 2000.**

From a methanol extract of the wood of *Xanthoceras sorbifolia*, two new compounds, 29-hydroxy-3-oxotirucalla-7,24-dien-21-oic acid (**3**, xanthocerasic acid) and epigallocatechin-(4 β →8, 2 β →*O*-7)-epicatechin (**6**), were isolated together with eleven known compounds. Of the isolated compounds, 3-oxotirucalla-7,24-dien-21-oic acid (**2**), oleanolic acid (**4**), and **6** were found to be inhibitory substances against human immunodeficiency virus (HIV-1) protease, with their 50% inhibitory concentrations (IC_{50}) being 20, 10, and 70 μg/ml, respectively. Condensed tannins of high molecular weights with epicatechin and epiafzelechin as the main extender units were found to be the most active principles of this plant (IC_{50} values ca. 6.0 μg/ml).

5）**Xiong Q., Fan W., Tezuka Y., Adnyana I. K., Stampolis P., Hattori M., Namba T., and Kadota S.: Hepatoprotective effect of *Apocynum venetum* and its active constituents. *Planta Medica*, 66: 127-133, 2000.**

化学応用部門の項を参照

6）**B. Min, N. Nakamura, H. Miyashiro, Y. Kim, and M. Hattori : Inhibition of human immunodeficiency virus type 1 reverse transcriptase and ribonuclease H activities by constituents of *Juglans mandshurica*. *Chem. Pharm. Bull.*, 48: 194-200, 2000.**

From the stem-bark of *Juglans mandshurica*, two new naphthalenyl glucopyranosides, 1,4,8-trihydroxynaphthalene 1-*O*-[α -L-arabinofuranosyl-(1→6)- β -D-glucopyranoside] (**1**) and 1,4,8-trihydroxynaphthalene 1-*O*- β -D-[6'-*O*-(3",5"-dihydroxy-4"-methoxybenzoyl)]glucopyranoside (**4**), and two new α -tetralonyl glucopyranosides, 4 α ,5,8-trihydroxy- α -tetralone 5-*O*- β -D-[6'-*O*-(3",5"-dihydroxy-4"-methoxybenzoyl)]glucopyranoside (**7**) and 4 α ,5,8-trihydroxy- α -tetralone 5-*O*- β -D-[6'-*O*-(3",4",5"-trihydroxybenzoyl)]glucopyranoside (**8**), were isolated together with three known naphthalenyl glucopyranosides (**2, 3** and **5**), one α -tetralonyl glucopyranoside (**6**), four flavonoids (**9-12**), and two galloyl glucopyranosides (**13, 14**). Amongst the isolated compounds, 1,2,6-trigalloylglucopyranose (**13**) and 1,2,3,6-tertagalloylglucopyranose (**14**) exhibited the most potent inhibition of reverse transcriptase (RT) activity with IC_{50} values of 0.067 and 0.040 μM, respectively, while the latter compound also inhibited ribonuclease H (RNase H) activity with an IC_{50} of 39 μM, comparable in potency to illimaquinone used as a positive control. 1,4,8-Trihydroxynaphthalene 1-*O*- β -D-glucopyranoside (**2**), 1,4,8-trihydroxynaphthalene 1-*O*- β -D-[6'-*O*-(4"-hydroxy-3",5"-dimethoxybenzoyl)]glucopyranoside (**3**) and **8** showed moderate inhibition against both enzyme activities, and inhibitory potency of **2** against RNase H activity (IC_{50}=156 μM) was slightly greater than that against the RT activity (IC_{50}=290 μM). The inhibitory potencies of 4 α ,5,8-trihydroxy- α -tetralone 5-*O*- β -D-[6'-*O*-(4"-hydroxy-3",5"-dimethoxybenzoyl)]glucopyranoside (**6**), **7** and **8** against RT activity increased, accompanied by an increase in the number of free hydroxyls on the galloyl residues, as represented by the IC_{50} values of >500, 330 and 5.8 μM, respectively. (Chart 2 参照)

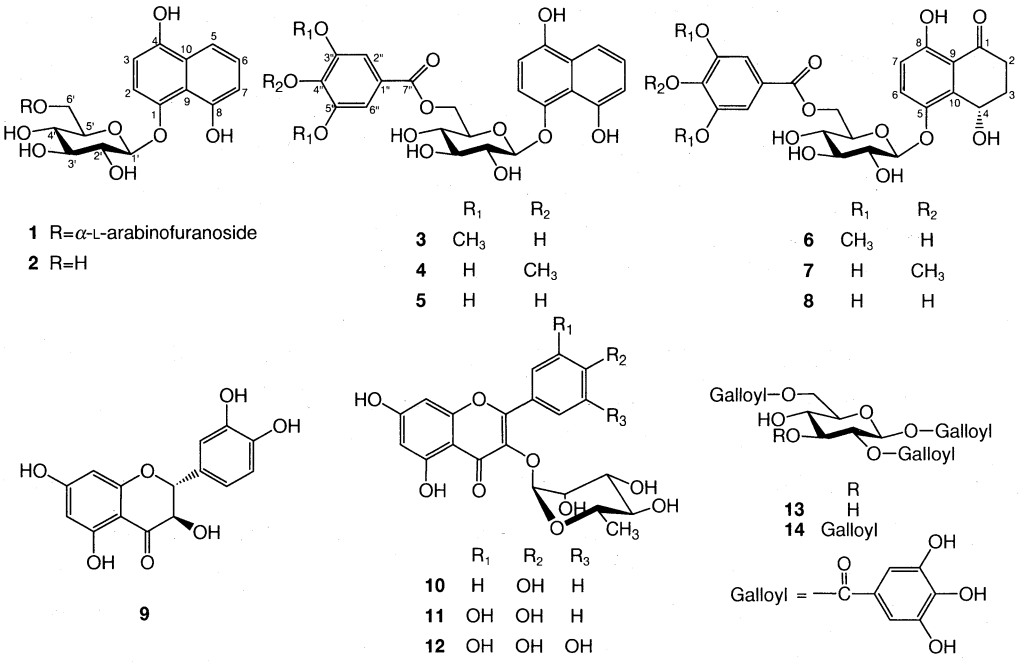


Chart 2

7）**Song S., Nakamura N., Ma C., Hattori M., and Xu S.: Four new saponins from the root bark of *Aralia elata*. *Chem. Pharm. Bull.*, 48: 838-842, 2000.**

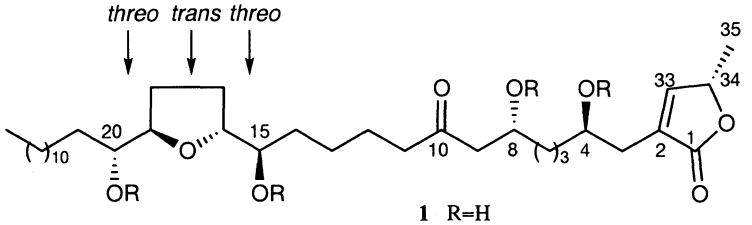
Four new saponins, 3-*O*-[β -D-glucopyranosyl (1→3)- α -L-arabinopyranosyl]-16 α -hydroxyoleanolic acid 28-*O*- β -D-glucopyranosyl ester (called aralia-saponin I), 3-*O*-[β -D-glucopyranosyl(1→3)- α -L-arabinopyranosyl]-16 α -hydroxyhederagenin 28-*O*- β -D-glucopyranosyl ester (aralia-saponin II), 3-*O*-[β -D-glucopyranosyl(1→3)- β -D-glucopyranosyl(1→3)- α -L-arabinopyranosyl]-16 α -hydroxyoleanolic acid 28-*O*- β -D-glucopyranosyl ester (aralia-saponin III), 3-*O*-[β -D-glucopyranosyl(1→3)- β -D-glucopyranosyl(1→3)- β -D-glucopyranosyl]-16α -hydroxyoleanolic acid 28-*O*- β -D-glucopyranosyl ester (aralia-saponin IV), were isolated from the root bark of *Aralia elata* (Miq.) Seem., together with nineteen known compounds including glycosides of (20*S*)-protopanaxadiol and (20*S*)-protopanaxatriol. Their structures were determined on the basis of chemical and spectroscopic methods.

8）**Min B., Gao J., Nakamura N., and Hattori M.: Triterpenes from the spores of *Ganoderma lucidum* and their cytotoxicity against Meth-A and LLC tumor cells. *Chem. Pharm. Bull.*, 48: 1026-1033, 2000.**

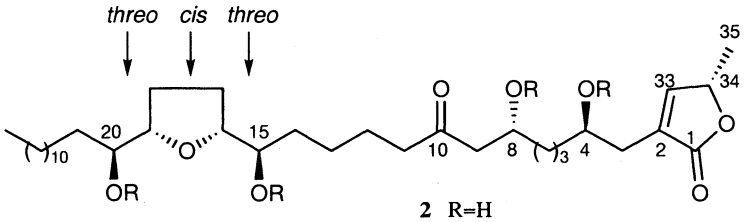
Six new highly oxygenated lanostane-type triterpenes, called ganoderic acid γ (**1**), ganoderic acid δ (**2**), ganoderic acid ε (**3**), ganoderic acid ζ (**4**), ganoderic acid η (**5**) and ganoderic acid θ (**6**), were isolated from the spores of *Ganoderma lucidum*, together with known ganolucidic acid D (**7**) and ganoderic acid C2 (**8**). Their structures of the new triterpenes were determined as (23*S*)-7 β ,15 α ,23-trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic acid (**1**), (23*S*)-7 α ,15 α ,23-trihydroxy-3,11-dioxolanosta-8,24(*E*)-diene-26-oic acid (**2**), (23*S*)-3 β ,7 β ,23-trihydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic acid (**3**), (23*S*)-3 β ,23-dihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic acid (**4**), (23*S*)-3 β ,7 β ,12 β ,23-tetrahydroxy-11,15-dioxolanosta-8,24(*E*)-diene-26-oic acid (**5**) and (23*S*)-3 β ,12 β ,23-trihydroxy-7,11,15-trioxolanosta-8,24(*E*)-diene-26-oic acid (**6**), respectively, by chemical and spectroscopic means, which included the determination of a chiral center in the side chain by a modification of Mosher's method. The cytotoxicity of the compounds isolated from the *Ganoderma* spores was carried out *in vitro* against Meth-A and LLC tumor cell lines.

9) **Wang L., Nakamura N., Meselhy M. R., Hattori M., Zhao W., Cheng K., Yang R., and Qin G.: Four mono-tetrahydrofuran ring acetogenins, montanacins B-E, *from Annona montana.* *Chem. Pharm. Bull.*, 48: 1109-1113, 2000.**

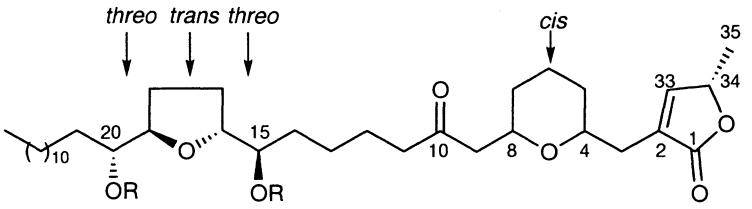
Four novel mono-tetrahydrofuran (THF) acetogenins, montanacins B-E (**1-4**), were isolated from the ethanolic extract of the leaves of *Annona montana*. The structures of **1-4** were established by spectroscopic methods and their absolute stereochemistries were determined by the advanced Mosher ester method. Montancins D (**3**) and E (**4**) bear a non-adjacent tetrahydropyran (THP) ring along with a THF ring and are the most unusual type of acetogenins discovered so far (Chart 3 参照).



1 R=H
1s R=(*S*)-MTPA
1r R=(*R*)-MTPA



2 R=H
2s R=(*S*)-MTPA
2r R=(*R*)-MTPA



3 R=H
3s R=(*S*)-MTPA
3r R=(*R*)-MTPA

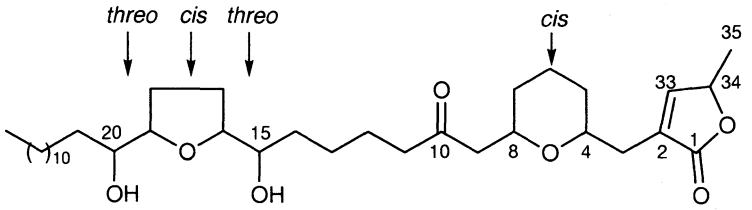


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Chart 3

10) **Li Y., Meselhy M. R., Wang L., Ma C., Nakamura N., and Hattori M.: Biotransformation of a *C*-glycosylflavone, abrusin 2"-*O*-β-D-apioside, by human intestinal bacteria. *Chem. Pharm. Bull.*, 48: 1239-1241, 2000.**

After anaerobic incubation of abrusin 2"-*O*-β-D-apioside (**1**) with a human fecal suspension, five metabolites were isolated and identified as abrusin (**2**), 1-(2', 6'-dihydroxy-3', 4'-dimethoxyphenyl)-3-(4"-hydroxyphenyl)propan-1-one (**5**), 5, 6-dimethoxybenzene-1, 3-diol (**6**), 3-(4"-hydroxyphenyl)propionic acid (**7**) and 3-phenylpropionic acid (**8**). However, methyl ether derivatives of abrusin (4'-*O*-methylabrusin and 4'-*O*-, 5-*O*-dimethylabrusin) resisted degradation under the same conditions.

11) Kim D., Yokozawa T., Hattori M., Kadota S., and Namba T.: Effects of aqueous extracts of *Apocynum venetum* leaves on spontaneously hypertensive, renal hypertensive and NaCl-fed-hypertensive rats. *J. Ethnopharmacology*, 72: 53-59, 2000.

Effects of aqueous extracts of *Apocynum venetum* leaves (Luobuma extracts) on the blood pressure were evaluated in hypertensive animal models, such as spontaneously hypertensive rats (SHR), renal hypertensive rats and NaCl-induced hypertensive rats. In SHR, administration of Luobuma (heat-processed and unprocessed leaves) extracts at a dose of 70 mg/rat/day significantly decreased the systolic blood pressure value, but their decreasing effects were weaker than that of captopril. The urine volume, and the urinary Na^+, K^+ and protein excretions were not significantly different between Luobuma-treated and untreated groups. In 3/4 nephrectomized rats, the Luobuma extracts significantly decreased the systolic blood pressure value, accompanied by significant increases of the urine volume and the urinary Na^+ and K^+ excretions. Furthermore, they decreased the blood urea nitrogen (BUN) level. In NaCl-induced hypertensive rats, the Luobuma extract decreased the systolic blood pressure value. However, it did not change the urinary excretions of Na^+, K^+ and protein. The BUN level was lower than that of control rats, but the serum total cholesterol level was not changed. From these findings, the Luobuma extracts have an anti-hypertensive effect, possibly due to amelioratin of the kidney functions in the three experimental animal models.

12) Yang X., Zou C., and Hattori M.: Harpagometabolins I and II, two new metabolites from harpagoside by human intestinal bacteria. *Chinese Chemical Letters*, 9: 779-782, 2000.

Harpagoside, which is one main iridoid constituent of the dried roots of *Scrophularia ningpoensis* Hemsl., was biotransformated by bacteria isolated from human fecal flora and three metabolites were obtained. The structures of the metabolites, including two new alkaloids, named harpagometabolins I (**1**) and II (**2**), and a known alkaloid aucbinine B (**3**), were identified by chemical methods and the spectroscopic evidence.

13) Min B., Meselhy R. M., Hattori M., Kim H. M., Kim Y. H.: Cytotoxicity of shikonin metabolites with biotransformation of human intestinal bacteria. *J. Microbiol. Biotecnol.*, 10: 514-517, 2000.

Six shikonin metabolites were obtained from human intestinal bacteria, *Bacteriodes fragilis* subsp. *thetaotus*, follwing biotransformation, The transformation of shikonin (**1**) was performed anaerobically for 3 day at 37°C in the bacterial suspension of *B. fragilis*, which was cultured overnight in GAM broth. The incubation mixture was extracted with EtOAc to give a dark-brown residue. The residue was applied to a silica gel column, which was eluted successively with hexane (Fr. A), $CHCl_3$ (Fr. B), and $CHCl_3$:MeOH (9:1) (Fr. C). Six metabolites, Fr. A (**2** and **3**), Fr. B (**6** and **7**), and Fr. C (**4** and **5**) were isolated by repeated silica gel column chromatography, preparative TLC, followed by Sephadex LH-20. *In vitro* cytotoxicities were tested against human tumor cell lines; PC-3 (prostate), ACHN (renal), A549 (lung), SW620 (colon), K562 (leukemia), and Du145 (prostate). The shikonin metabolites **2**, **4**, **5**, and **6** showed weaker cytotoxicity than the parent shikonin (**1**), whereas shikonin monomeric metabolite **3** (ED_{50} 0.44-1.22 μg/ml) and dimeric metabolite **7** (ED_{50} 0.48-2.35 μg/ml) exhibited stronger activities compared with adriamycin, which was used as the positive control.

14) Hussein G., Miyashiro H., Nakamura N., Hattori M., Kakiuchi N., and Shimotohno K.: Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. *Phytother. Res.*, 14: 510-516, 2000.

One hundred fifty-two methanol and water extracts of different parts of 71 plants commonly used in Sudanese traditional medicine, were screened for their inhibitory effects on hepatitis C virus (HCV) protease (PR) by using *in vitro* assay methods. Thirty-four extracts showed significant inhibitory activity (≥60% inhibition at 100 μg/ml). Of these, 8 extracts, methanol extracts of *Acacia nilotica, Boswellia carterii, Embelia schimperi, Quercus infectoria,*

Trachyspermum ammi, and water extracts of *Piper cubeba, Q. infectoria and Syzygium aromaticum*, were the most active (≥90% inhibition at 100 μg/ ml).

From the *Embelia schimperi* extract, two benzoquinones, embelin (I) and 5-*O*-methyl embelin (II), were isolated and found as potent HCV-PR inhibitors with IC_{50} values of 21 and 46 μM, respectively.

15) Wang L., Meselhy M. R., Li Y., Qin G., and Hattori M.: Human intestinal bacteria capable of transforming secoisolariciresinol diglucoside to mammalian lignans, enterodiol and enterolactone. *Chem. Pharm. Bull.*, 48: 1606-1610, 2000.

Seven metabolites were isolated after anaerobic incubation of secoisolariciresinol diglucoside (**1**) with a human fecal suspension. They were identified as (-)-secoisolariciresinol (**2**), 3-demethyl- (-)-secoisolariciresinol (**3**), 2-(3-hydroxybenzyl)-3-(4-hydroxy-3-methoxybenzyl)butane-1,4-diol (**4**), didemethylsecoisolariciresinol (**5**), 2-(3-hydroxybenzyl)-3-(3,4-dihydroxybenzyl)butane-1, 4-diol (**6**), enterodiol (**7**) and enterolactone (**8**).

Furthermore, two bacterial strains, *Peptostreptococcus* sp. SDG-1 and *Eubacterium* sp. SDG-2, responsible for the transformation of **1** to a mammalian lignan **7**, were isolated from a human fecal suspension. The former transformed **2** to **3** and **5**, as well as **4** to **6**, and the latter transformed **5** to **6** and **7** (Chart 4 参照).

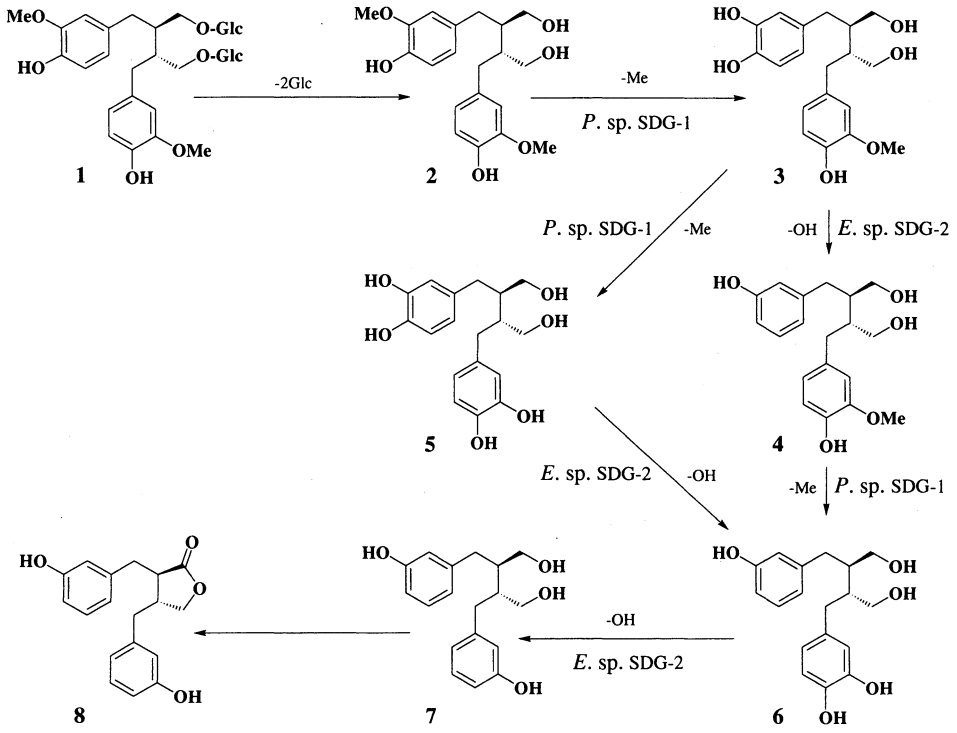

Chart 4

16) Ma C., Nakamura N., and Hattori M.: Chemical modification of oleanene type triterpenes and their inhibitory activity against HIV-protease dimerization. *Chem. Pharm. Bull.*, 48: 1681-1688, 2000.

Oleanolic acid derivatives with different lengths of 3-*O*-acidic acyl chains were synthesized and evaluated for their inhibitory activity against HIV-1 protease. The lengths of the acidic chains were optimized to 6 and 8 carbons. Changing a 3-ester bond to an amide bond or dimerization of the triterpenes retained their inhibitory activity against HIV-1 protease. Introduction of an additional acidic chain to C-28 of oleanolic acid increased the inhibitory activity appreciably, though a derivative with only one acidic chain linked at C-28 also showed potent activity against HIV-1 protease.

The inhibitory mechanism was proved directly by size exclusion chromatography to be inhibition of dimerization of the enzyme polypeptides. The ester bonds of the triterpene derivatives were found to be stable to lipase under mild alkaline conditions.

17) Ma C., Nakamura N., Hattori M., Zhu S., Komatsu K.: Guaiane dimers and germacranolide from *Artemisia caruifolia*. *J. Nat. Prod.*, 63: 1626-1629, 2000.

One new germacranolide (named caruifolin A) and three new guaiane dimers (caruifolins B-D), together with six known compounds, were isolated from the aerial parts of *Artemisia caruifolia* Buch.-Ham. ex Roxb. The structures were determined by chemical and spectroscopic methods.

18) Yokozawa T., Sekiya M., Rhyu D., Hattori M., and Chung H.: Radical- scavenging activity of Wen-Pi-Tang and its component crude drugs : with special reference to the effects on nitric oxide, superoxide and peroxynitrite. *J. Trad. Med.*, 17: 41-47, 2000.

In renal diseases, active oxygen and free radicals play various roles in the development and progression of the pathological condition. Our previous studies have provided evidence that the Oriental medical prescription Wen-Pi-Tang normalizes the kidney under conditions of increased oxidative stress. In the present study, we examined the antioxidant capacity of Wen-Pi-Tang and its component crude drugs in a nitric oxide, superoxide and peroxynitrite generation system. It was found that the radical-scavenging effect of Wen-Pi-Tang is dose-dependent, and that three of its component crude drugs, i.e., Rhei Rhizoma, Zingiberis Rhizoma and Glycyrrhizae Radix, play important roles in the antioxidant action.

19) Yokozawa T., Wang T. S., Chen C. P., and Hattori M.: Inhibition of nitric oxide release by an aqueous extract of *Tinospora tuberculata*. *Phytother. Res.*, 14: 51-53, 2000.

An aqueous extract of *Tinospora tuberculata* stems was found to scavenge nitric oxide (NO) *in vitro* in both cell and cell-free systems. When the aqueous extract was added to lipopolysaccharide-stimulated murine macrophages, it inhibited NO release dose-dependently, and similar activity was found in a cell-free system using sodium nitroprusside as a NO donor. These findings may help to explain, in part, certain pharmacological activities of *Tinospora tuberculata.*

20) Yokozawa T., and Chen C. P.: Role of Salviae Miltiorrhizae Radix extract and its compounds in enhancing nitric oxide expression. *Phytomedicine*, 7: 55-61, 2000.

Excessive production of nitric oxide (NO) and its peroxidant product, peroxynitrite, has been implicated in the pathology of acute and chronic renal failure, and inhibitors of NO production have been shown to exert protective and ameliorative effects against renal epithelial cell damage mediated by enhanced generation of NO. Salviae Miltiorrhizae Radix has a beneficial effect in the improvement of renal failure. For clarifying the mechanism responsible, we investigated whether Salviae Miltiorrhizae Radix extract and several of its related compounds, including caffeic acid and its polymers which were isolated by our research group, can regulate the generation and release of NO. The results demonstrated that Salviae Miltiorrhizae Radix extract and these compounds suppressed NO effectively in the systems employing activated macrophages and the arginine-hydrogen peroxide, and that furthermore the activity shown by the compounds was higher than that shown by the extract. In addition, direct scavenging of NO was also observed. The present findings suggest that Salviae Miltiorrhizae Radix extract and its compounds are potent NO inhibitors, and that their inhibitory effect on the generation and release of NO may contribute to the pharmacological effect of Salviae Miltiorrhizae Radix in improving renal function reported previously.

21) Yokozawa T., Chen C. P., and Kitani K.: Chiyu extract stimulates antioxidant defense ability in senescence-accelerated mice. *J. Trad. Med.*, 17: 73-79, 2000.

The effect of chiyu extract on antioxidant defense alteration in senescence-accelerated mice (SAM) was examined. Comparison with AKR/N Slc mice, a strain consistent with SAM but exhibiting normal aging, showed a lower glutathione (GSH) level and glutathione/glutathione disulfide (GSH/GSSG) ratio in the kidney and liver of SAM, whereas malondialdehyde (MDA), a lipid peroxidation product, was increased significantly. Administration of chiyu extract increased the GSH level and GSH/GSSG ratio, and markedly suppressed MDA production. On the other hand, detection of renal enzymes related to the glutathione redox cycle showed that catalase and glutathione peroxidase activities were largely decreased in SAM, whereas chiyu extract reversed this tendency. The reduced activities of hepatic catalase and glutathione reductase were increased significantly by the extract. These findings suggest that a decline of the antioxidant defense system occurs in SAM, and that chiyu extract may have a beneficial effect in ameliorating oxidative stress or damage.

22) Yokozawa T., and Liu Z. W.: The role of ginsenoside-Rd in cisplatin-induced acute renal failure. *Renal Failure*, 22: 115-127, 2000.

DNA of LLC-PK$_1$ cells cultured with cisplatin was fragmented to produce low-molecular-weight fragments. Agarose gel electrophoresis of the DNA revealed a ladder pattern characteristic of apoptosis, indicating the induction of apoptosis by cisplatin. However, the degree of apoptosis was lower in cells cultured with cisplatin in the presence of ginsenoside-Rd, and this was accompanied by suppressed leakage of lactic dehydrogenase into the culture medium. The ladder pattern was detected on electrophoresis of DNA in renal tissue samples obtained from rats given an intravenous injection of cisplatin. Such DNA fragmentation was less conspicuous in rats given ginsenoside-Rd orally for 30 days prior to cisplatin administration. Significant suppression of the DNA fragmentation was also demonstrated by densitometry, and measurement of urea nitrogen and creatinine in blood also showed a marked decrease in their respective levels in rats administered ginsenoside-Rd. The present findings suggest that ginsenoside-Rd ameliorates cisplatin-induced renal injury, a process in which apoptosis is plays a central role, and thereby causes restoration of renal function.

23) Yokozawa T., Chen C. P., Tanaka T., and Kitani K.: A study on the nitric oxide production-suppressing activity of Sanguisorbae Radix components. *Biol. Pharm. Bull.*, 23: 717-722, 2000.

The active components of an aqueous extract of Sanguisorbae Radix, which possesses nitric oxide (NO) production-suppressing activity, were determined using macrophages that were activated by the addition of lipopolysaccharide. Significant inhibitory activity against the formation of both NO and inducible NO synthase, and NADPH-diaphorase activity, which is involved in NO generation, was shown by Sanguisorbae Radix fractions T-B and T-C. On further fractionation, the subfractions of T-B and T-C all showed high anti-NO activity. Sanguiin H-6, sanguiin H-11, 1,2,3,4,6-penta-*O*-galloyl-β-D-glucose, eugeniin and polymeric proanthocyanidin were isolated from TB-3 and TC-4, and all were identified as exhibiting strong anti-NO activity. We have confirmed that sanguiin H-6 is the most active component of Sanguisorbae Radix with respect to the suppression of NO production. It is suggested that tannin makes a prominent contribution to the biological activity of Sanguisorbae Radix.

24) Yokozawa T., Dong E., and Chen C. P.: Protection of the kidney by Wen-Pi-Tang against ischemia-reperfusion injury. *Phytomedicine*, 7: 185-189, 2000.

Electrophoretic and densitometric data revealed induction of apoptosis in the kidney due to experimentally induced ischemia-reperfusion injury. Such apoptosis in the kidney was reduced in rats given 62.5 or 125 mg/kg body weight/day Wen-Pi-Tang orally for 30 days prior to ischemia-reperfusion. An increase in the dose of Wen-Pi-Tang was associated with suppressed fragmentation of DNA, a ladder pattern of low-molecular-weight molecules, resulting in a beneficial effect on renal function.

25) Chung H. Y., Yokozawa T., Kim M. S., Lee K. H., Kim K. W., Yang R., and Choi J. H.: The mechanism of nitric oxide and/or superoxide cytotoxicity in endothelial cells. *Exp. Toxic. Pathol.*, 52: 227-233, 2000.

We examined the mechanism of nitric oxide (NO) and/or superoxide (O_2^-)-induced cytotoxicity and the importance of thiols in endothelial cells by treating the cells with superoxide dismutase (SOD), catalase (CAT) and hemoglobin (Hb). Pyrogallol, a O_2^- generator and precursor of hydrogen peroxide (H_2O_2), had potent cytotoxic effects on the endothelial cells, but this effect was completely abolished by SOD/CAT. Hb, a NO scavenger, protected the endothelial cells from sodium nitroprusside-induced cytotoxicity. The cytotoxic effect of 3-morpholinosydnonimine (SIN-1), which is thought to form peroxynitrite ($ONOO^-$) as a simultaneous O_2^- and NO generator, was completely blocked by SOD/CAT or Hb. On the other hand, pretreatment of endothelial cells with diethylmaleate, a glutathione depleter, aggravated the cytotoxicity induced by SIN-1, which was prevented by addition of exogenous glutathione and/or SOD/CAT. These data suggest that the cytotoxicity induced by NO, O_2^- and $ONOO^-$ can be blocked by glutathione, and that this is an important cellular protective mechanism against these reactive oxygen species.

26) Song Q. H., Toriizuka K., Iijima K., Yabe T., Yokozawa T., and Cyong J. C.: Effects of Hokoei-to (Pu-gong-ying-tang), Kampo formula, on estradiol and progesterone contents in brain regions and serum in ovariectomized mice. *J. Trad. Med.*, 17: 180-185, 2000.

We investigated the effect of the Hokoei-to on the estradiol and progesterone contents of ovariectomized mice. The Hokoei-to was decocted from crude drugs, and freeze-dry extracts were prepared. The experimental mice were given the Hokoei-to extract (240mg/kg body weight/day) for 20 days after ovariectomy at 7 weeks of age. Contents of estradiol and progesterone in brain tissues and serum were determined by enzyme immunoassay. Hokoei-to increased the levels of estradiol and progesterone in the brain tissues, and tended to increased the levels of estradiol but not progesterone in the serum of ovariectomized mice. At the climacteric, mental and physical disorders develop with the diminished release of sexual hormones, and the Hokoei-to may have a regulatory effect to these hormones.

27) Kim D. W., Yokozawa T., Hattori M., Kadota S., and Namba T.: Inhibitory effects of an aqueous extract of *Apocynum venetum* leaves and its constituents on Cu^{2+}-induced oxidative modification of low density lipoprotein. *Phytother. Res.*, 14: 501-504, 2000.

An aqueous extract of *Apocynum venetum* leaves and its constituents inhibited thiobarbituric acid reactive substances (TBARS) and conjugated-diene formations in the Cu^{2+}-induced oxidation of low density lipoprotein (LDL) *in vitro*. The TBARS formation was most strongly inhibited by chlorogenic acid with an IC_{50} value of 1.9 μM, but other constituents were in a range of 2.3～23.3 μM.

On the other hand, the lag time in the conjugated-diene formation was dose-dependently prolonged by addition of the aqueous extract. Catechin prolonged the lag time more than 300 min and other constituents such as chlorogenic acid, epicatechin, epigallocatechin, hyperoside and isoquercitrin led to no conjugated-diene formation within 700 min under the experimental conditions.

28) Cho E. J., Yokozawa T., Rhyu D. Y., Mitsuma T., Terasawa K., and Park J. C.: Protective activity from hydrophilic and lipophilic free radical generators of Wen-Pi-Tang and its crude drug extracts in LLC-PK_1 cells. *J. Trad. Med.*, 17: 245-252, 2000.

We investigated Wen-Pi-Tang and its crude drug extracts to determine their protective effect from oxidative stress caused by the hydrophilic and lipophilic free radical generators, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN) in LLC-PK_1 renal tubular epithelial cells. In response to AAPH and AMVN treatment, cell viability decreased significantly and significantly enhanced thiobarbituric acid-reactive substances (TBARS) formation was observed. However, Wen-Pi-Tang and its crude drug extracts showed

scavenging of peroxyl radicals, which were generated by AAPH and AMVN, resulting in greater cell viability and lower TBARS formation than controls treated only with free radical generators. In particular, Wen-Pi-Tang, Rhei Rhizoma and Ginseng Radix demonstrated high protective activity, whereas Aconiti Tuber, Zingiberis Rhizoma and Glycyrrhizae Radix showed relatively low activity. This result suggests that the antioxidant activity of Wen-Pi-Tang was attributable to the crude extracts, and that both act as hydrophilic and lipophilic antioxidants.

29) Yokozawa T., Cho E. J., Nakagawa T., Terasawa K., and Takeuchi S.: Inhibitory effect of green tea tannin on free radical-induced injury to the renal epithelial cell line, LLC-PK$_1$. *Pharm. Pharmacol. Commun.*, 6: 521-526, 2000.

We examined the effect of green tea tannin on the viability of renal epithelial LLC-PK$_1$ cells treated with 3-morpholinosydnonimine (SIN-1), sodium nitroprusside (SNP) or pyrogallol. SIN-1 treatment significantly decreased cell viability, while a mixture of tannin and SIN-1 led to a recovery of viability from the cellular damage induced by free radicals generated by SIN-1. Moreover, (-)-epigallocatechin 3-*O*-gallate (EGCg) and (-)-epigallocatechin (EGC), the main components of tannin, produced higher activity than tannin alone. On the other hand, caffeine and theanine, other components of green tea, did not show activity. However, tannin did not protect the cell against nitric oxide (NO) or superoxide anion (O_2^-) (produced by SNP and pyrogallol, respectively). This result suggests that green tea tannin protects LLC-PK$_1$ cells from oxidative stress caused by free radicals generated by SIN-1, but not from stress induced by either NO or O_2^-. Moreover, the radical scavenging activity of green tea is mainly attributable to tannin and its components, EGCg and EGC.

30) Yokozawa T., Cho E. J., Hara Y., and Kitani K.: Antioxidative activity of green tea treated with radical initiator 2,2'-azobis(2-amidinopropane) dihydrochloride. *J. Agric. Food Chem.*, 48: 5068-5073, 2000.

This study investigated the antioxidative activity of green tea extract, and a green tea tannin mixture and its components under conditions of radical generation using the hydrophilic azo compound, 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) to generate peroxyl radicals at a constant and measurable rate in the cultured renal epithelial cell line, LLC-PK$_1$, which is susceptible to oxidative damage. Treatment with AAPH decreased cell viability and increased the formation of thiobarbituric acid-reactive substances. However, green tea extract, and the tannin mixture and its components, comprising (-)-epigallocatechin 3-*O*-gallate (EGCg), (-)-gallocatechin 3-*O*-gallate (GCg), (-)-epicatechin 3-*O*-gallate, (-)-epigallocatechin (EGC), (+)-gallocatechin (GC), (-)-epicatechin and (+)-catechin, showed protective activity against AAPH-induced cellular damage. The tannin mixture and its components exhibited higher antioxidative activity than the green tea extract. Furthermore, EGCg and GCg had higher activity than EGC and GC, respectively. In particular, EGCg exerted the most significant cellular protective activity against AAPH. These results indicate that green tea tannin may inhibit cellular loss and lipid peroxidation resulting from the peroxyl radical generated by AAPH, and that the chemical structure of tannin is also involved in the activity, suggesting that the *O*-dihydroxy structure in the B ring and the galloyl groups are important determinants for radical scavenging and antioxidative potential.

31) Choi J. S., Chung H. Y., Jung H. A., Park H. J., and Yokozawa T.: Comparative evaluation of antioxidant potential of alaternin (=2-hydroxyemodin) and emodin. *J. Agric. Food Chem.*, 48: 6347-6351, 2000.

The antioxidant activities of alaternin (2-hydroxyemodin) and emodin were compared for their respective potentials to inhibit lipid peroxidation in the linoleic acid system by the thiocyanate method, to inhibit total reactive oxygen species generation in kidney homogenates using 2',7'-dichlorodihydrofluorescein diacetate, to inhibit peroxynitrite formation by the 3-morpholinosydnonimine system, which generates superoxide radical and nitrogen monooxide,

and to scavenge authentic peroxynitrites. Both alaternin and emodin were found to inhibit the peroxidation of linoleic acid by the thiocyanate method in a dose-dependent manner. Whereas the former shows inhibitory activities in reactive oxygen- and nitrogen-mediated reactions, the latter does not. These results indicate that alaternin is a potentially effective and versatile antioxidant and can be used to protect biological systems and functions against various oxidative stresses.

◇総　説

1）服部征雄：アロエエキスは医者いらずか？—瀉下効果発現のプロセスについて—. *Biotherapy*, **14**: 983–989, 2000.

2）横澤隆子：腎における丹参成分 lithospermic acid B Mg 塩の役割. MINOPHAGEN MEDICAL REVIEW, **45**: 323–331, 2000.

◇学会報告

1）Kim J. W., An H. J., Park K. J., Cho E. J., Yokozawa T. and Chung H. Y.: Identification of herbs for oxidant scavenging activities. The 8th Congress of International Association of BioMedical Gerontology, 2000, 2, Pusan, Korea.

2）Yokozawa T. and Dong E.: Radical-scavenging activity of green tea polyphenols. "Free Radicals in Food: Chemistry, Nutrition, and Health" Symposium at the 219th American Chemical Society, 2000, 3, San Francisco, USA.

3）馬　超美，中村憲夫，宮代博継，服部征雄，大竹　徹，川畑拓也：オレアナン型トリテルペン誘導体の HIV-1 プロテアーゼ阻害活性について. 日本薬学会第120年会，2000，3，岐阜.

4）Gao J., Min B., Nakamura N., and Hattori M.: Seven new triterpenoids from the spores of *Ganoderma lucidum*. 日本薬学会第120年会，2000，3，岐阜.

5）松田久司，來住明宣，村上敏之，下田博司，吉川雅之，服部征雄：羅布麻（*Apocynum venetum* L.）焙煎葉の生物活性成分. 日本薬学会第120年会，2000，3，岐阜.

6）Song S., Nakamura N., Ma C., Hattori M., Xu S.: Saponins from the root bark of *Aralia elata*. 日本薬学会第120年会，2000，3，岐阜.

7）Li Y., Meselhy M. R., Akao T., and Hattori M.: Cleavage of a *C*-glucosyl bond of mangiferin by human intestinal bacteria. 日本薬学会第120年会，2000，3，岐阜.

8）佐野光代，横澤隆子，竹内茂彌，中川孝子，服部征雄，寺澤捷年：腎における緑茶タンニンの役割. 日本薬学会第120年会，2000，3，岐阜.

9）Wang L., Meselhy M. R., Li Y., Qin G., and Hattori M.: Metabolism of secoisolariciresinol diglucoside by huamn intestinal bacteria. 日本薬学会第120年会，2000，3，岐阜.

10）横澤隆子，王　天山，金　東郁，深沢千春，服部征雄，中西和夫：イボツヅラフジの肝・胆管系に及ぼす効果. 日本薬学会第120年会，2000，3，岐阜.

11）Tewtrakul S., Miyashiro H., and Hattori M.: Screening of Thai medicinal plant extracts on HIV-1 integrase inhibitory activity. 日本薬学会第120年会，2000，3，岐阜.

12）宮代博継，新酒めぐみ，林　隆幸，服部征雄，赤尾光昭：Sennoside 代謝活性化に関与するヒト腸内細菌 *Bifidobacterium* sp. SEN 遺伝子の解析. 日本薬学会第120年会，2000，3，岐阜.

13）欧陽新収，高橋京子，小松かつ子，山内葉子，馬場明道，中村憲夫，服部征雄，東　純一：Angiotensin II 誘導による心肥大に対する「丹参」の防御作用. 第73回日本薬理学会年会，2000，3，23–25，横浜.

14）Park JC., Park JG., Hur JM., Park SJ., Park KY., Shin DY., Kim MS., Miyashiro H.,Yokozawa T., and Hattori M.: Anti-HIV-1 protease activity and phytochemical study on the aerial parts of *Orostachys japonicus*. 韓国薬学会，2000，4，釜山，韓国.

15）Yokozawa T.: Effectiveness of green tea tannins in renal failure. International Conference of Traditional

Chinese Medicine (2000 Beijing), 2000, 4, Nanjing, China.
16) 横澤隆子，藤塚直樹：Methylguanidine 上昇因子について．第43回日本腎臓学会学術総会，2000，5，名古屋.
17) 中川孝子，横澤隆子：緑茶タンニンの NO に及ぼす影響．第43回日本腎臓学会学術総会，2000，5，名古屋.
18) 横澤隆子：食品栄養学研究の現状．第40回日本栄養・食糧学会中部支部大会シンポジウム，2000，5，名古屋.
19) 欧陽新収，高橋京子，小松かつ子，四方絵里子，中村憲夫，服部征雄，東　純一：心臓由来培養細胞を用いた Angiotensin II 誘導心肥大モデルに対する丹参の効果：丹参基原植物による比較．第97回日本薬理学会近畿部会，2000，6，16，大阪.
20) Nakamura N., Ma C., and Hattori M.: Survey of anti-HIV agents among traditional medicines. The Third International Symposium on Natural Medicine and Microflora, 2000, 7, 31- 8, 3, Hohhot, China.
21) Yokozawa T.: Antioxidant activity of traditional crude drugs. Vascular Aging Research Society (special lecture), 2000, 8, Pusan, Korea.
22) 佐藤亜希子，中村憲夫，閔　炳善，服部征雄，山田智弘，済木育夫，谿　忠人：アレルギー性疾患モデルを用いた桃核承気湯の活性成分の探索研究．第17回和漢医薬学会大会，2000，9，2-3，名古屋.
23) 高　江静，閔　炳善，中村憲夫，赤尾光昭，Meselhy R. Meselhy，服部征雄：酵素免疫測定法（EIA）による ganoderic acid A の微量定量法の開発．第17回和漢医薬学会大会，2000，9，2-3，名古屋.
24) 中川孝子，横澤隆子，関谷倫子，服部征雄，ジュネジャ・レカ・ラジュ，寺澤捷年：動脈硬化の進展抑制における緑茶タンニンの役割．第17回和漢医薬学会大会，2000，9，2-3，名古屋.
25) 横澤隆子，陳　翠萍，関谷倫子，服部征雄：パーオキシナイトライト由来腎障害モデルを用いた地楡の検討．第17回和漢医薬学会大会，2000，9，2-3，名古屋.
26) 横澤隆子，陳　翠萍，田中　隆，木谷健一：地楡からの NO 産生抑制成分の検索とフリーラジカル由来腎障害モデルを用いた評価．第17回和漢医薬学会大会，2000，9，名古屋.
27) 坂東みゆ紀，柴原直利，赤尾光昭，服部征雄，関矢信康，萬谷直樹，寺澤捷年：ヒトにおける芍薬・甘草・芍薬甘草湯煎液の反復経口投与時のペオニフロリン・ペオニメタボリン-1・グリチルレチン酸の血中動態に関する研究．第17回和漢医薬学会大会，2000，9，2-3，名古屋.
28) Wang Z., Chou G., Zhang C., Xu L., Xu G., and Hattori M.: The utilization, chemical constituents and quality evaluation of roots and leaves of *Lindera aggregata* (Wuyaku). 日本生薬学会第47回年会，2000，9，7-8，東京.
29) 汪　礼権，李　燕，中村憲夫，服部征雄，秦　国偉，李　燦軍：*Annona montana* の葉に含まれる新 acetogenin 類について．日本生薬学会第47回年会，2000，9，7-8，東京.
30) 三潴忠道，横澤隆子，二宮裕幸：大黄ならびに大黄含有漢方方剤・温脾湯の慢性腎不全に対する効果．腎とフリーラジカル研究会シンポジウム，2000，9，富山.
31) 家永和治，三上博輝，西端良治，内木　充，中村　耕，横澤隆子，大浦彦吉，青柳一正，遠藤　仁：NZ-419（内因性抗酸化剤）の慢性腎不全進展抑制作用．腎とフリーラジカル研究会シンポジウム，2000，9，富山.
32) 中川孝子，横澤隆子，寺澤捷年：シスプラチン誘発急性腎不全における緑茶タンニンの関与．腎とフリーラジカル研究会，2000，9，富山.
33) 大久保　勉，阪中専二，朱　政治，レカ・ラジュ・ジュネジャ，横澤隆子，柴田　透，長谷川眞常，仲川清隆，宮澤陽夫：緑茶ポリフェノールの生体内抗酸化と透析患者への試み．腎とフリーラジカル研究会，2000，9，富山.
34) Hattori M.: Searching for anti-HIV agents among traditional medicines. The First International Conference for Pharmaceutical Industry in Egypt and the Middle East, 2000, 9, 27-29, Cairo.
35) Hattori M.: Recent studies on bitter principles from the fruiting bodies and spores of *Ganoderma lucidum.*

2000 International Symposium on Ganoderma Research, 2000, 10, 12-14, Beijing.

36) 久保山友晴，東田千尋，趙　静，服部征雄，小松かつ子：Ashwagandha 成分 withanolide A と withanoside VI による神経突起伸展作用. 第43回日本神経化学会大会, 10, 18–20, 金沢.

37) Hattori M.: Perspectve of regulation system for Japanese herbal drugs. International Symposium The Evolution of Regulations in Oriental Medicine among China, Japan, Korea. 2000, 10, 20, Seoul.

38) Hattori M.: Development of anti-HIV agents from natural sources. The Fifth NRCT-JSPS Joint Seminor on Pharmaceutical Sciences. 2000, 11, 15-17, Bangkok.

39) Hattori M.: Anti-HIV Agents from traditional medicines. Asian Symposium on Plants, Spices and Other Natural Products (ASOMPS X), 2000, 11, 18-23, Dhaka, Bangladesh.

40) Kitani K., Minami C., Isobe K., Maehara K., Kanai S., Carrillo M. C., Ivy G. O. and Yokozawa T.: Why (-)deprenyl prolongs survivals of experimental animals: Increase of antioxidant enzymes in brain and other body tissues as well as mobilization of various humoral factors may lead to systemic anti-aging. The Fifth International NILS Workshop on Longevity Sciences, 2000, 11, Obu.

41) 服部征雄：小柴胡湯の成分とその代謝. 日本生薬学会・和漢医薬学会 合同シンポジウム, 2000, 12, 2–3, 富山.

◇講演会

1) Hattori M.: Metabolism of natural products by human intestinal bacteria and development of anti-HIV agents from natural products. 重慶市中葯研究院, 2000, 5, 4, 重慶, 中国.

2) Hattori M.: New pharmacological effects of herbal ter and coffee. 2000, 9, 26, National Research Center, Cairo.

3) Hattori M.: Some biologically active natural products discovered by our department. 2000, 9, 30, Cairo Univ., Cairo.

4) Hattori M.: Metabolic activation of crude drug components by human intestinal bacteria. 中医研究院中葯研究所, 2000, 10, 11, 北京.

5) 横澤隆子：緑茶タンニンの腎における役割. 第12回飛鳥プロジェクトフォーラム, 2000, 12, 富山.

◇その他

1) 服部征雄：腸内細菌による代謝活性化を利用した新しい薬物の開発. 科学研究費補助金研究成果報告書, 2000, 3.

2) 服部征雄：HIV由来DNA・RNAハイブリッド分解酵素をターゲットとした天然薬物の探索. 科学研究費補助金研究成果報告書, 2000, 3.

3) 野村靖幸：新規高次神経変性疾患モデル動物・細胞の開発，神経変性機序の解析と薬効評価法の確立. 科学研究費補助金研究成果報告書, 2000, 12, 3.

4) Hattori M.: Some phorbol esters potently inhibited the cytopathic effect of HIV-1 without activation of protein kinase C. Proceedings of the 3rd International Symposium on Recent Advances in Natural Products Research and 1999 Korea-Japan Joint Symposium, pp. 30-36. Natural Products Research Institute, Seoul National University. 1999, 11, 19.

5) Chou G., Nakamura N., Ma C., Wang Z., Hattori M., Xu L., Xu G.: Seven new sesquiterpene lactones from *Lindera aggregata*. *J. China Pharm. Univ.* **31**: 339, 2000.

6) 高橋京子，欧陽新収，小松かつ子，山内葉子，馬場明道，中村憲夫，服部征雄，難波 恒雄，東　純一：Angiotensin II 誘導による心肥大に対する「丹参」の作用. 心筋の構造と代謝 **22**: 145–152, 2000.

7) 横澤隆子：フリーラジカル由来腎障害における地楡の評価. 平成11年度厚生科学研究費補助金長寿科学総合研究事業研究報告書, pp. 43–47, 2000.

8) 横澤隆子：シスプラチンによるアポトーシスに及ぼす薬用人参サポニン Rd の影響. The GINSENG

REVIEW, No. 28, 7-11, 2000.

9）横澤隆子：パーオキシナイトライト消去活性を有する緑茶成分．上原記念生命科学財団研究報告集，Vol.14, 65-67, 2000.

◇共同研究

1）小松かつ子：薬効解析センター，「痴呆脳に対するコーヒーの作用」
2）木谷健一：国立療養所中部病院長寿医療研究センター，「抗老化薬に関する研究」
3）鄭　海泳：釜山大学校薬学大学，「抗酸化物に関する研究」
4）家永和治：日本臓器製薬株式会社，「5-Hydroxy-1-methylhydantoin の創薬研究」
5）田中　隆：長崎大学薬学部，「和漢薬の活性成分に関する研究」
6）三潴忠道：飯塚病院漢方診療科，「腎不全治療薬に関する基礎的，臨床的研究」

◇非常勤講師

1）服部征雄：京都大学非常勤講師，2000，4月～9月．
2）横澤隆子：富山大学非常勤講師，2000，4月～9月．

◇研究費取得状況

1）財団法人ヒューマンサイエンス振興財団「HIV インテグラーゼおよび複製を制御する蛋白質を標的とする抗ウイルス剤の開発」（継続，服部分担）350万円．
2）全日本コーヒー協会（継続，服部代表）「痴呆脳に対するコーヒーの作用」150万円．
3）財団法人富山第一銀行奨学財団「天然物由来の抗エイズウイルス薬の開発研究」（新規，服部代表）50万円．
4）文部省科学研究費基盤研究(A)(1)「高次脳機能障害モデルの作出，新規薬効評価の確立と創薬」（新規，服部分担）50万円．
5）財団法人平和中島財団「中国雲南省少数民族薬物を基原とするエイズ治療薬の探索研究」（新規，服部代表）195万円．
6）科学研究費補助金（特別研究員奨励費）「微生物を利用した新しい薬物の開発」（新規，服部代表）90万円．
7）平成12年度教育研究学内特別経費 および 研究所長裁量経費「アトピー性皮膚炎モデルに有効な漢方方剤の作用と作用機序および活性成分の研究」120万円．
8）文部省科学研究費基盤研究(C)（継続，横澤代表）「抗酸化物としての羅布麻の探索」50万円．
9）厚生省長寿科学総合研究費（継続，横澤分担）「老年・老年病に対する栄養学的・薬理学的・分子遺伝学的手法による干渉に関する総合的研究」300万円．
10）薬用人参研究会（新規，横澤代表）「薬用人参の加齢病態の制御と抗酸化作用」100万円．
11）つくし奨学・研究基金（新規，横澤代表）「NOを中心としたラジカルの相互作用と和漢薬の関与」120万円．

◇学位（修士・博士）

修士：富山みゆき「HIV-1 RNase H 阻害活性を有する伝統薬物の探索」
関谷倫子「温脾湯の抗酸化能の評価－*in vivo* ESR，インフルエンザウイルス感染モデルを用いた場合－」
高　江静「霊芝成分の酵素免疫測定法の開発」
Betty Lika Sada「Nitric Oxide Production-Suppressing Activity of Components from *Tinospora tuberculata*」
佐藤亜希子「アレルギー疾患モデルにおける桃核承気湯および治頭瘡一方の活性成分」，

下田　勝「ヒト腸内細菌由来 Sennoside 水解 β−Glucosidase 遺伝子の同定および大腸菌での発現」
牧野圭吾「ヒト腸内細菌による Anthrone および Oxyanthrone C−配糖体の開裂」

博士：陳　翠萍「伝統生薬の NO 消去活性と NO 並びに NO 酸化物による腎障害に対する地楡の役割と機序」
馬　超美「抗 HIV-1 プロテアーゼ活性を有する数種の天然薬物の研究」

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