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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の役割，② 薬物代謝に関する腸内細菌遺伝子の解明，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析
3．AIDS，C型肝炎の予防 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

本年度の主な研究を列挙すると：

1．ヒト腸内細菌によるゴボウの種子に多量に含まれるarctiinの哺乳動物リグナン（エンテロジオール，エンテロラクトン）への変換を検討し，この化合物が容易に加水分解，脱メチル化，脱水酸基化反応を経て 種々の代謝物に変換されることを明らかにした。また，これらの代謝物のエストロジェン様作用，抗エストロジェン様作用を検討した。さらに ヒト腸内細菌と化学反応を併用して 簡便なエンテロラクトン，エンテロジオールの合成法を開発した。
2．HIV-インテグラーゼ阻害活性を指標にタイ薬用植物を探索し，*Coleus parvifolius, Thevetia peruviana* などに強い酵素阻害活性を見出した。又，これらのエキスから阻害活性成分を単離し，その阻害様式を検討した。
3．中国 および タイで用いられている薬用植物の抗ヘルペスウイルス活性 また，C型肝炎ウイルス由来のRNA ポリメラーゼ阻害活性を検討した。
4．腎疾患増悪因子のラジカルの産生部位を証明するとともに，蛋白・糖修飾をsurrogate マーカーとして利用し，腎不全合併症における病態把握に着手した。また酸化ストレスを抑制する新たな治療手段を探索するために，地楡成分のsanguiin H-6，ソバ成分，温脾湯，桂枝茯苓丸を中心に検討した。

◇著　書　Books

1）Yokozawa T., Dong E.: Radical-Scavenging Activity of Green Tea Polyphenols. "Free Radicals in Foods: Chemistry, Nutrition and Health", edited by M. Morello, F. Shahidi and C.T. Ho, Quaker Oats Co., Barrington, 2002, pp.224-240.

2）横澤隆子：腎障害を伴う高血圧における緑茶ポリフェノール．“茶の機能---生体機能の新たな可能性”，村松敬一郎，小國伊太郎，伊勢村　護，杉山公男，山本万里，学会出版センター，東京，2002，pp.145-151.

3）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 -第6集-”，横澤隆子，湯川　進監修，宗　正敏，青柳一正編，東京医学社，東京，2002，pp.67-73.

4）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（内因性抗酸化物）の慢性腎不全進展抑制作用．“腎とフリーラジカル -第6集-”，横澤隆子，湯川　進監修，宗　正敏，青柳一正編，東京医学社，東京，2002，pp.74-76.

5）大久保　勉，レカ•ラジュ•ジュネジャ，横澤隆子：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 -第6集-”，横澤隆子，湯川　進監修，宗　正敏，青柳一正編，東京医学社，東京，2002，pp.120-123.

6）中川孝子，横澤隆子，寺澤捷年：糖尿病性腎症における漢方方剤の役割．“腎とフリーラジカル -第6集-”，横澤隆子，湯川　進監修，宗　正敏，青柳一正編，東京医学社，東京，2002，pp.138-141.

7）Yokozawa T., Kim H.Y., Cho E.J., Choi J.S.: Antioxidant Activities of Mustard Leaf (*Brassica juncea*). “腎とフリーラジカル -第6集-”，横澤隆子，湯川　進監修，宗　正敏，青柳一正編，東京医学社，東京，2002，pp.142-148.

8）横澤隆子，中川孝子：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 -第6集-”，横澤隆子，湯川　進監修，宗　正敏，青柳一正編，東京医学社，東京，2002，pp.206-210.

◇原　著　Original papers

1）**Abdel-Hafez A. A., Nakamura N., and Hattori M.: Biotransformation of phorbol by human intestinal bacteria. *Chem. Pharm. Bull.*, 50, 160-164 (2002).**

Anaerobic incubation of phorbol (**1**) from *Croton tiglium* with human intestinal bacteria afforded five metabolites : isophorbol (**2**), deoxyphorbol (**3**), 4β, 9α, 20-trihydroxy-13, 15-seco-1, 6, 15-tigliatriene-3, 13-dione (**4**), 4β, 9α, 20-trihydroxy-15, 16, 17-trinor-1, 6-tigliadiene-3, 13-dione (**5**) and 4β, 9α, 20-trihydroxy-14(13→12)-abeo-12αH-1, 6-tigliadiene-3, 13-dione (**6**). All these metabolites (**2-6**) were identified and characterized by spectroscopic means, including two-dimensional (2D)-NMR. Nine defined strains from the human intestine showed an ability to transform **1** to these metabolites.

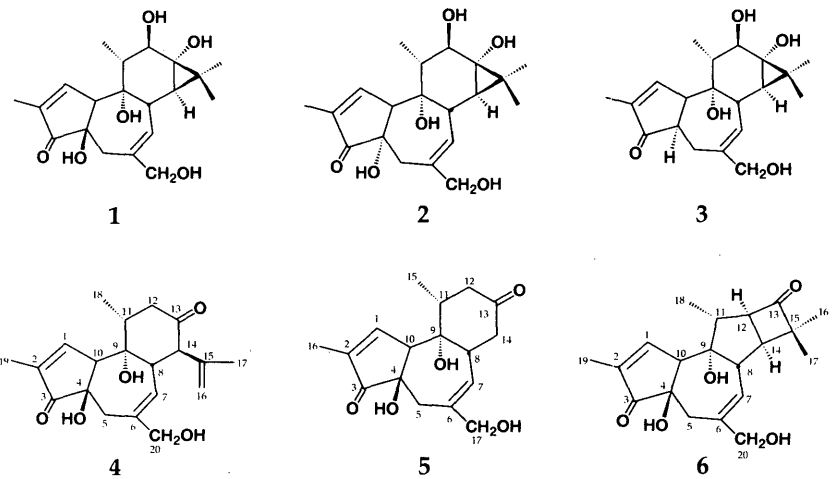
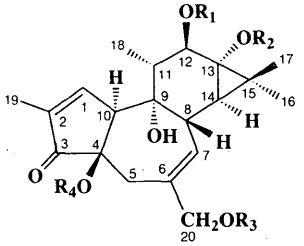


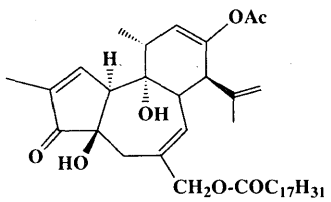
Chart 1.

2) **El-Mekkawy S., Meselhy M. R., Abdel-Hafez A. A., Nakamura N., Hattori M., Kawahata T., and Otake T.: Inhibition of cytopathic effect of human immunodeficiency virus type-1 by various phorbol derivatives.** ***Chem. Pharm. Bull.*****, 50, 523-529 (2002).**

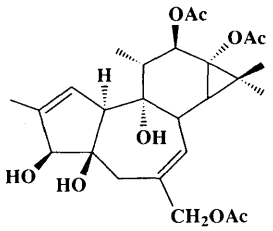
Forty-eight derivatives of phorbol (**9**) and isophorbol (**14**) were evaluated for their inhibition of HIV-1 induced cytopathic effects (CPE) on MT-4 cells, as well as their activation of protein kinase C (PKC), as indices of anti-HIV-1 and tumor promoting activities, respectively. Of these compounds, the most potent inhibition of CPE was observed in 12-*O*-tetradecanoylphorbol 13-acetate (**8**) and 12-*O*-acetylphorbol 13-decanoate (**6**). The former also showed the strongest PKC activation activity, while the latter showed no activity at 10 ng/ml. Both activities were generally observed in those phorbol derivatives with an A/B *trans* configuration, but not in the isophorbol derivatives with an A/B *cis* configuration. Acetylation of 20-OH in the phorbol derivatives significantly reduced the inhibition of CPE, as shown in 12-*O*-, 20-*O*-diacetylphorbol 13-decanoate (**6a**) (IC_{100}=15.6 μg/ml) *vs* compound **6** (IC_{100}=0.0076 μg/ml), and 12-*O*-tetradecanoylphorbol 13, 20-diacetate (**8a**) (IC_{100}=15.6 μg/ml) *vs* 12-*O*-tetradecanoylphorbol 13-acetate (**8**) (IC_{100}=0.00048 μg/ml), except in the case of 12-*O*-decanoylphorbol 13-(2-methylbutyrate) (**4**) and phorbol-12, 13-diacetate (**9c**). The reduction of a carbonyl group at C-3 abruptly reduced the inhibition of CPE, as observed in 3β-hydroxyphorbol 12, 13, 20-triacetate (**9f**) (IC_{100}=500 μg/ml) *vs* phorbol 12, 13, 20-triacetate (**9d**) (IC_{100}=62.5 μg/ml).

Although **8** was equipotent in the inhibition of CPE, and activation of PKC, both activities were abruptly decreased by the acetylation of 20-OH and methylation of 4-OH [as in **8a** and 4-*O*-methyl-12-*O*-tetradecanoylphorbol 13, 20-diacetate (**8b**), respectively]. On the other hand, its positional isomer (12-*O*-acetylphorbol 13-tetradecanoate (**8c**) showed neither activities. The removal of a long acyl group in **8** led to a substantial loss of both activities, as shown in phorbol 13-acetate (**9b**).





1a



9f

	R_1	R_2	R_3	R_4
1	H	Ac	$C_{18}H_{31}O$	H
1b	Ac	Ac	$C_{18}H_{31}O$	H
2	H	Tig	$C_{18}H_{31}O$	H
3	Ac	Tig	H	H
4	$C_{10}H_{19}O$	2-Me butyryl	H	H
4a	$C_{10}H_{19}O$	2-Me butyryl	Ac	H
5	Tig	2-Me butyryl	H	H
6	Ac	$C_{10}H_{19}O$	H	H
6a	Ac	$C_{10}H_{19}O$	Ac	H
6b	Ac	$C_{10}H_{19}O$	Ac	Me
7	2-Me butyryl	$C_{12}H_{23}O$	H	H
8	$C_{14}H_{27}O$	Ac	H	H
8a	$C_{14}H_{27}O$	Ac	Ac	H
8b	$C_{14}H_{27}O$	Ac	Ac	Me
8c	Ac	$C_{14}H_{27}O$	H	H
8d	H	$C_{14}H_{27}O$	$C_{14}H_{27}O$	H
9	H	H	H	H
9a	Ac	H	H	H
9b	H	Ac	H	H
9c	Ac	Ac	H	H
9d	Ac	Ac	Ac	H
9e	Ac	Ac	Ac	Me
9g	Ac	Ac	Ac	Ac
10	Bz	Bz	Bz	H
11	Ac	$C_6H_{11}O$	H	H
11a	Ac	$C_6H_{11}O$	$C_6H_{11}O$	H
12	Ac	$C_9H_{17}O$	H	H
12a	Ac	$C_9H_{17}O$	$C_9H_{17}O$	H
13	Ac	$C_{12}H_{23}O$	H	H
13a	Ac	$C_{12}H_{23}O$	$C_{12}H_{23}O$	H

Chart 2.

Of the 12-*O*-acetyl-13-*O*-acylphorbol derivatives, the highest inhibition of CPE was observed in **6**, which has a dodecanoyl residue at C-13. Both an increase and decrease in the number of fatty acid carbon chains resulted in significant reduction of the inhibition of CPE.

3) **Wang L., Min B., Li Y., Nakamura N., Qin G., Li C., and Hattori M.: Annonaceous acetogenins from the leaves of *Annona montana*. *Bioorg. Med. Chem.*, 10, 561-565 (2002).**

A novel Annonaceous acetogenin, montanacin F, with a new type of terminal lactone unit, was isolated from the leaves of *Annonna montana*. Its structure was determined on the basis of spectral evidences and chemical methods, and a possible biosynthetic pathway was discussed. In addition, the cytotoxicity of montanacin F was evaluated *in vitro* against Lewis lung carcinoma (LLC) tumor cell lines. Furthermore, the previously isolated cytotoxic acetogenin annonacin against LLC was examined for *in vivo* antitumor activity with LLC tumor cells.

4) **Tewtrakul S., Nakamura N., Hattori M., Fujiwara T., and Supavita T.: Flavanone and flavonol glycosides from the leaves of *Thevetia peruviana* and their HIV-1 reverse transcriptase and HIV-1 integrase inhibitory activities. *Chem. Pharm. Bull.*, 50, 630-635 (2002).**

Two new flavanone glucosides, (*2R*)- and (*2S*)-5-*O*-β-D-glucopyranosyl-7, 4′-dihydroxy-3′, 5′-dimethoxyflavanone [pervianoside I (**3**), peruvianoside II (**4**)] and a new flavonol glycoside, quercetin 3-*O*-{β-D-glucopyranosyl (1→2)-[α-L-rhamnopyranosyl (1→6)]-β-D-galactopyranoside} (peruvianoside III, **13**) were isolated from the leaves of *Thevetia peruviana* Schum., together with nine known flavonol glycosides and two known iridoid glucosides. The structures of all compounds were determined on the basis of chemical and spectroscopic methods. Their inhibitory effects against HIV-1 reverse transcriptase and HIV-1 integrase were also investigated.

5) **Ma C., Nakamura N., Hattori M., and Cai S.: Isolation of malonyl oleanolic acid hemiester as anti-HIV protease substance from the stems of *Cynomorium songaricum*. *Chin. Pharm. J.*, 37, 336-338 (2002).**

OBJECTIVE Further investigation on the bio-active constituents of the stems of *Cynomorium songaricum*. **METHODS** The compounds were isolated with chromatographic technology, including HPLC. Their structures were determined by spectroscopic methods. **RESULTS** Malonyl oleanolic acid hemiester (I) and zingerone 4-*O*-β-D-glucopyranoside (II) were isolated from the plant. **CONCLUSION** Both compounds were isolated from this plant family for the first time and compound I potently inhibited the activity of HIV protease.

6) **Min B., Lee H., Bae K., Gao J., Nakamura N., and Hattori M.: Antitumor activity of cultured mycelia of *Ganoderma lucidum*. *Natural Product Sciences*, 8, 52-54 (2002).**

The cultured mycelia of fungus *Ganoderma lucidum* were investigated for the inhibitory effect on the growth of s.c. transplanted Lewis lung carcinoma (LLC) in BDF-1 mice by intraperitoneal (i.p.) administration. The cultured mycelia showed antitumor activity with T/C values of 89.6 and 50.3 % at doses of 100 and 500 mg/kg, respectively, compared to adriamycin, which was used a positive control, with T/C value of 54.6 % at 2 mg/kg.

7) **Hur J., Park J., Park J., Hyun K., Lee K., Miyashiro H., and Hattori M.: Inhibitory effects of ninety nine Korean plants on human immunodeficiency virus type 1 protease activity. *Nutraceuticals and Food*, 7, 123-127 (2002).**

Ninety nine extracts from Korean plants were screened for their inhibitory activities on human immunodeficiency virus (HIV) type 1 protease by an HPLC method. The protease inhibitory activities were determined by incubating the extracts in reaction mixtures containing protease and substrate (His-Lys-Ala-Arg-Val-Leu-(*p*-NO_2-Phe)-Glu-Ala-

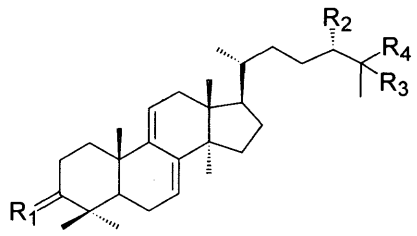
Nle-Ser-NH_2) to perform proteolytic cleavage reactions. Of the extracts tested, the water extracts of *Viburnum awabuki* (stem and leaves) and *Distylium racemosum* (leaves) had the highest protease inhibitory activities at a concentration of 100 μg/mL. Activity-guided fractionation revealed that the *n*-butanol fraction of the *V. awabuki* extract and the ethyl acetate fraction from the *D. racemosum* extract had the greatest inhibitory activity on HIV-1 protease.

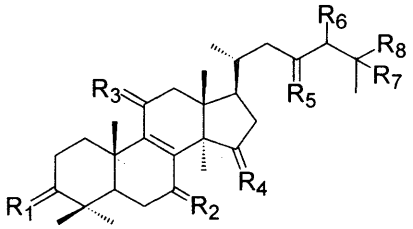
8) **Ma C., Nakamura N., Hattori M., Kawahata T., and Otake T.: Inhibitory effects of triterpene-azidothymidine conjugates on proliferation of human immunodeficiency virus type 1 and its protease. *Chem. Pharm. Bull.*, 50, 877-880 (2002).**

The conjugates of some dicarboxylic acid hemiesters of triterpenes which show potent inhibition against human immunodeficiency virus type 1 protease (HIV-1 PR) with a reverse transcriptase inhibitor azidothymidine (AZT) or anti-HIV alkaloid FK 3000 were prepared, and their inhibitory activities were investigated against HIV-induced cytopathic effects (CPE) and HIV-1 PR. Most of the triterpene-AZT conjugates showed potent anti-HIV activity as well as moderate to potent PR inhibitory activity, though AZT itself showed no PR inhibitory activity at all. However, the triterpene-FK 3000 conjugates showed neither PR inhibitory activity nor anti-HIV activity.

9) **Gao J., Min B., Ahn E., Nakamura N., Lee H., and Hattori M.: New triterpene aldehyde, lucialdehydes A-C, from *Ganoderma lucidum* and their cytotoxicity against murine and human tumor cells. *Chem. Pharm. Bull.*, 50, 837-840 (2002).**

Three new lanostante-type triterpene aldehydes, named lucialdehydes A-C (**1**-**3**), were isolated from the fruiting bodies of *Ganoderma lucidum*, together with ganodermanonol (**4**), ganodermadiol (**5**), ganodermanondiol (**6**), ganodermanontriol (**7**), ganoderic acid A (**8**), ganoderic acid B8 (**9**), and ganoderic acid C1 (**10**). The structures of the new triterpenes were determined as (24*E*)-3β-hydroxy-5α-lanosta- 7,9(11),24-trien-26-al (**1**), (24*E*)-3,7-dioxo-5α-lanosta-8,24-dien-26-al (**2**), and (24*E*)-3β-hydroxy-7-oxo-5α-lanosta-8,24-dien-26-al (**3**), respectively, by spectroscopic means. The cytotoxicity of the compounds isolated from the ganoderma mushroom was tested *in vitro* against LLC, T-47D, Sarcoma 180, and Meth-A tumor cell lines. Lucialdehydes B-C (**2**-**3**), ganodermanonol (**4**) and ganodermanondiol (**6**) showed cytotoxic effect on tested tumor cells. Of the compounds, lucialdehyde C (**3**) exhibited the most potent cytotoxicity against LLC, T-47D, Sarcoma 180, and Meth-A tumor cell lines with ED_{50} values of 10.7, 4.7, 7.1, and 3.8 μg/ml, respectively.





	R_1	R_2	R_3	R_4
1	$\overset{OH}{\underset{H}{}}$	$\Delta^{24(25)}$		CHO
4	O	$\Delta^{24(25)}$		CH_2OH
5	$\overset{OH}{\underset{H}{}}$	$\Delta^{24(25)}$		CH_2OH
6	O	OH	OH	CH_3
7	O	OH	OH	CH_2OH

	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8
2	O	O	H_2	H_2	H_2	$\Delta^{24(25)}$		CHO
3	$\overset{OH}{\underset{H}{}}$	O	H_2	H_2	H_2	$\Delta^{24(25)}$		CHO
8	O	$\overset{OH}{\underset{H}{}}$	O	$\overset{OH}{\underset{H}{}}$	O	H_2	H	COOH
9	O	$\overset{OH}{\underset{H}{}}$	O	$\overset{OH}{\underset{H}{}}$	O	H_2	H	COOH
10	O	$\overset{OH}{\underset{H}{}}$	O	O	O	H_2	H	COOH

Chart 3.

10) Zhao J., Nakamura N., Hattori M., Kuboyama T., Tohda C., and Komatsu K.: Withanolide derivatives from the roots of *Withania somnifera* and their neurite outgrowth activities. *Chem. Pharm. Bull.*, 50, 760-765 (2002).

Five new withanolide derivatives (**1** and **9-12**) were isolated from the roots of *Withania somnifera* together with fourteen known compounds (**2-8**, and **13-19**). On the basis of spectroscopic and physiochemical evidence, compounds **1** and **9-12** were determined to be (20*S*, 22*R*)-3α, 6α-epoxy-4β, 5β, 27-trihydroxy-1-oxowitha-24-enolide (**1**), 27-*O*-β-D-glucopyranosylpubesenolide 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside (withanoside VIII, **9**), 27-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranosylpubesenolide 3-*O*-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside (withanoside IX, **10**), 27-*O*-β-D-glucopyranosylpubesenolide 3-*O*-β-D-glucopyranoside (withanoside X, **11**), and (20*R*, 22*R*)-1α, 3β, 20, 27-tetrahydroxywitha-5, 24-dienolide 3-*O*-β-D-glucopyranoside (withanoside XI, **12**). Of the isolated compounds, **1**, withanolide A (**2**), (20*S*, 22*R*)-4β, 5β, 6α, 27-tetrahydroxy-1-oxowitha-2, 24-dienolide (**6**), withanoside IV (**14**), withanoside VI (**15**) and coagulin Q (**16**) showed significant neurite outgrowth activity at a concentration of 1 μM on a human neuroblastoma SH-SY5Y cell line.

11) Min B., Miyashiro H., and Hattori M.: Inhibitory effects of quinones on RNase H activity associated with HIV-1 reverse transcriptase. *Phytother. Res.*, 16, S57-62 (2002).

In an effort to develop new drugs preventing the growth of human immunodeficiency virus (HIV), we developed an *in vitro* assay method of ribonuclease H (RHase H) activity associated with reverse transcriptase (RT) from HIV-1. Some naphthoquinones, such as 1, 4-naphthoquinone (**1**), vitamin K_3 (**2**), juglone (**3**) and plumbagin (**6**), moderately inhibited RNase H activity, and others, including naphthazarin (**5**) and shikonins (**8-9**, **18-23**), showed weak inhibition. Diterpenoid quinones, tanshinones (**24-28**), had also moderate inhibition against RNase H activity. Of these quinones, compound **1** showed the most potent inhibition on RNase H activity with a 50% inhibitory concentration (IC_{50}) of 9.5 μM, together with moderate inhibition against RNA-dependent and DNA-dependent DNA polymerase (RDDP and DDDP) activities with IC_{50} values of 69 and 36 μM, respectively. Compounds **3** and **5** showed significant inhibition against RDDP (IC_{50} = 8 and 10 μM, respectively) and DDDP (IC_{50} = 5 and 7 μM, respectively) activities. The structure-activity relationship of the naphthoquinones suggested that non-hydroxylated naphthoquinones (**1** and **2**) showed significant inhibition of RNase H activity, whereas 5-hydroxylated naphthoquinones (**3** and **5**) showed potent inhibition against RDDP and DDDP activities.

12) Ma C., Nakamura N., Miyashiro H., Hattori M., Komatsu K., Kawahata T., and Otake T.: Screening of Chinese and Mongolian herbal drugs for anti-human immunodeficiency virus type 1 (HIV-1) activity. *Phytother. Res.*, 16, 186-189 (2002).

Water and methanol extracts of 30 Chinese and Mongolian medicinal plants were tested for their human immunodeficiency virus type-1 (HIV-1) inhibitory activity. Of the 60 extracts, 23 showed anti-HIV activity. Bioassay-guided fractionation of one of the most active extract, the methanol extract of the root tuber of *Stephania cepharantha*, has led to the isolation of two alkaloids, aromoline and FK-3000 as potent inhibitory substances. They completely inhibited the cytopathic effects of HIV-1 on MT-4 cells at 31.3 and 7.8 μg/mL, respectively.

13) Ohsaki M., Kurokawa M., Nawawi A., Nakamura N., Hattori M., and Shiraki K.: Characterization of anti-herpes simplex virus type 1 activity of an alkaloid FK 3000 from *Stephania cepharantha*. *J. Trad. Med.*, 19, 129-136 (2002).

A morphinane alkaloid FK 3000 (6, 7-di-*O*-acetylsinococuline) from the root tubers of *Stephania cepharantha* showed antiviral activity against acyclovir- and phosphonoacetic acid (PAA)-resistant herpes simplex virus type 1 (HSV-1), influenza virus, measles virus, and poliovirus. The anti-HSV action of FK 3000 was assessed in comparison with that of PAA that inhibits the activity of HSV DNA polymerase and HSV DNA synthesis. FK 3000 inhibited

the growth of thymidine kinase-deficient and ACV and PAA-resistant HSV-1 strains, as well as wild type HSV strains in Vero cells. This compound, as well as PAA, interfered with the synthesis of late viral proteins but not early viral proteins. The analysis of HSV DNA synthesis by slot blot hybridization showed that FK 3000 inhibited the viral DNA synthesis in a dose-dependent manner. However, the viral RNA was partially synthesized in the presence of FK 3000 (even at a dose that HSV DNA synthesis was inhibited) and PAA, indicating that FK 3000, as well as PAA, allowed early viral RNA synthesis but not viral DNA synthesis. Since partially purified HSV DNA polymerase activity was not inhibited by FK 3000, this compound was suggested to inhibit HSV DNA synthesis by a mechanism different from that of PAA.

14) Matsuse I. T., Nakamura N., Basnet P., Hattori M., Kamimura K., and Funada H.: Amino acids and phosphates stimulate hatching of *Ochlerotatus koreicoides* (Diptera: Culicidae) eggs. *Med. Entomol.* Zool., 53, S47-54 (2002).

A tree-hole mosquito species, *Ochlerotatus (Finlaya) koreicoides* has been reared in laboratory by stimulating their hatch using dried yeast powder. This method shows to be more effective than lowering the dissolved oxygen concentration by mechanical, chemical or biological means. From a hatch test guided fractionation of the water extract of dried yeast, glutamic acid was isolated as the main compound of the hatch stimulating fraction. This amino acid alone did not show hatch stimulating effect but it was effective in combination with the phosphates that are also constituents of dried yeast. Among 20 amino acids, only histidine and proline showed similar effects as glutamic acid. A concentration of 0.1 mg/ml of each glutamic acid and trisodium phosphate gave high percentage hatch in 18 h.

15) Zhang C., Nakamura N., Tewtrakul S., Hattori M., Sun Q., Wang Z., and Fujiwara T.: Sesquiterpenes and alkaloids from *Lindera chunii* and their inhibitory activities against HIV-1 integrase. *Chem. Pharm. Bull.*, 50, 1195-1200 (2002).

Three new eudesmane type sesquiterpenoid lindenanolides E (**1**), F (**2**) and G (**3**), and two new aporphine alkaloid lindechunines A (**18**) and B (**20**) were isolated from roots of *Lindera chunii* MERR., together with seven known

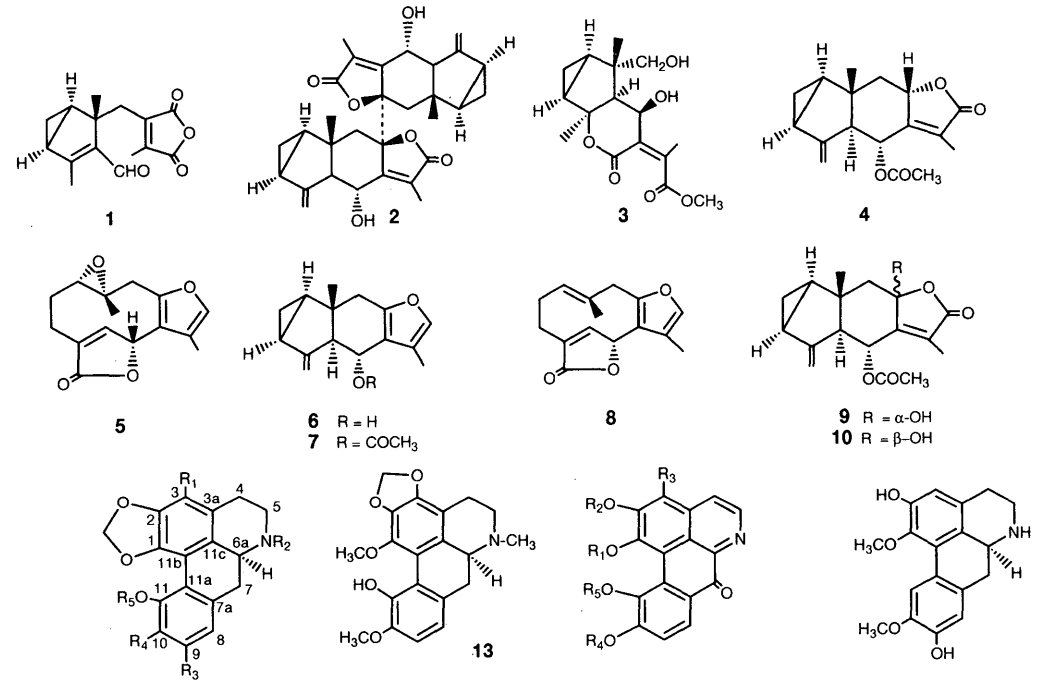


Chart 4.

sesquiterpenes including a new naturally-occurring lindenanolide H (**4**) and eigth known aporphine alkaloids. The structures of these compounds were determined by spectroscopic means. Of the isolated compounds, hernandonine (**14**), laurolistine (**16**), 7-oxohernangerine (**17**) and linedechunine A (**18**) showed significant anti-human immunodeficiency virus type 1 (HIV-1) integrase activity with IC_{50} values of 16.3, 7.7, 18.2 and 21.1 μM, respectively. The major alkaloids presented in the roots of *L. chunii* were quantitatively analyzed by an HPLC method.

16) **Akao T., Yoshino T., Kobashi K., and Hattori M.: Evaluation of salicin as an antipyretic prodrug that does not cause gastric injury. *Planta Med.*, 68, 714-718 (2002).**

Pharmacokinetic and pharmacological studies were performed to compare the antipyretic effects of salicin (SL), saligenin (SG, an aglycone of SL) and salicylic acid (SA, an active metabolite of SL) in rats. When SL was administered orally to rats, SA appeared slowly in the plasma and levels increased gradually, in contrast to the rapid appearance observed after oral administration of sodium salicylate (SANa) or SG. Orally administered SL did not affect the rectal temperatures of afebrile rats at a dose of 5 mmol/kg; at this dose, SANa and SG lowered body temperature significantly. However, it significantly reduced yeast-induced fever, producing a normal body temperature, and completely prevented fever when administered simultaneously with yeast. SL did not induce gastric lesions even at a dose of 5 mmol/kg; conversely, SANa and SG induced severe gastric lesions in a dose-dependent manner at 1, 2.5 and 5 mmol/kg. Poor absorption of SL and rapid absorption of SA and SG were confirmed in an *in vivo* system, as well as in an *in vitro* system using everted rat jejunal sacs. Only small amounts of SA and SG were detected in the intestinal tracts of rats 1 h after oral administration, whereas more than 50% of an SL dose was recovered an SL and SG from the intestinal tracts 1 h after treatment and 15.8% of the dose was still present as SG 4 h after administration. When given to germ-free rats, 19.8% of the SL dose was recovered intact, mainly from the cecum, and no SG was detected even at 4 h after treatment. These results indicate that SL is a prodrug which is gradually transported to the lower part of the intestine, hydrolyzed to SG by intestinal bacteria, and converted to SA after absorption. It thus produces an antipyretic action without causing gastric injury.

17) **Park J., Hur J., Park J., Hatano T., Yoshida T., Miyashiro H., Min B., and Hattori M.: Inhibitory effects of Korean medicinal plants and camelliatannin H from *Camellia japonica* on human immunodeficiency virus type 1 protease. *Phytother. Res.*, 16, 422-426 (2002).**

To identify substances with anti-human immunodeficiency virus (HIV) activity in traditional medicines, 101 extracts of Korean medicinal plants were screened for their inhibitory effects on HIV type 1 protease (PR). The enzyme activity was determined by HPLC. Of the extracts tested, strong inhibitory effects were observed in the acetone extracts of the pericarp and leaves of *Camellia japonica*, the water extract of the leaves of *Sageretia theezans* and the methanol extract of the aerial part of *Sophora flavescens*. Camelliatannin H from the pericarp of *C. japonica*, showed a potent inhibitory activity on HIV-1 PR with an IC_{50} of 0.9 μM.

18) **Min B., Lee H., Lee S., Kim Y., Bae K., Otake T., Nakamura N., and Hattori M.: Anti-human immunodeficiency virus -type 1 activity of constituents from *Juglans mandshurica*. *Arch. Pharm. Res.*, 25, 441-445 (2002).**

Three naphthalene glycosides (**1-3**), four flavonoids (**4-7**), and two galloyl glycosides (**8-9**) were isolated from the stem-bark of *Juglans mandshurica* (Juglandaceae). Their structures were determined by chemical and spectral means, including with 2D-NMR (COSY, HMQC, and HMBC) experiments. Amongst the isolated compounds, taxifolin (**4**) showed the most potent HIV-induced cytopathic activity against MT-4 cells with a complete inhibitory concentration (IC_{100}) value of 25 μg/ml and a maximum cytotoxic concentration (CC_{100}) value of above 100 μg/ml. However, naphthalene glycosides (**1-3**), flavonoids (**5-7**), and galloyl tannins (**8-9**) were inactive against anti-HIV-1 activity.

19) Akanitapichat P., Kurokawa M., Tewtrakul S., Pramyothin P., Sripanidkulchal B., Shiraki K., and Hattori M.: Inhibitory activities of Thai medicinal plants against herpes simplex type 1, poliovirus type 1, and measles virus. *J. Trad. Med.*, 19, 174-180 (2002).

Forty-eight ethanol- and 43 water-extracts of 49 traditional Thai medicines were evaluated for antiviral activities by a plaque reduction assay. For preliminary characterization of the mode of their antiviral action, poliovirus type 1, measles virus and herpes simplex virus type 1 (HSV-1) that are different in nucleic acid component and enveloped structure were used in this study. Fifty-two, 28 and 29 extracts exhibited inhibitory activities against poliovirus, measles virus and HSV-1, respectively. Of 29 extracts with anti-HSV-1 activities, the inhibitory activities of *Rhinacanthus nasutus* (leaf), *Terminalia citrina* (fruit) and *Thevetia peruviana* (leaf) were observed in both ethanol and water extracts. The ethanol extracts of *Derris scandens* (leaf) and *Plumbago indica* (leaf) and the water extract of *Capsicum frutescens* (fruit) were active against only HSV-1, suggesting the mechanism of their antiviral action likely unique to HSV-1 but neither poliovirus nor measles virus. Contrarily, 26 extracts displayed inhibitory activities against poliovirus and/or measles virus. These findings suggest that the 29 extracts from traditional Thai medicines are potential candidates for anti-HSV agents.

20) Yokozawa T., Chen C.P., Tanaka T., Kitani, K.: Effects of sanguiin H-6, a component of Sanguisorbae Radix, on lipopolysaccharide-stimulated nitric oxide production. *Biochem. Pharmacol.*, 63, 853-858, 2002.

The present study was conducted to evaluate the effect of sanguiin H-6, a component of Sanguisorbae Radix, on the production of nitric oxide (NO), using macrophages activated by lipopolysaccharide (LPS). Sanguiin H-6 inhibited nitrite production, taken as an index for NO, in a concentration-dependent fashion. This compound decreased inducible NO synthase (iNOS) activity, with the inhibitory effect at a concentration of 25 μM being equal to that of the known iNOS inhibitor aminoguanidine at 50 μM. However, unlike aminoguanidine, sanguiin H-6 was associated with improved cell viability. Reverse transcription-polymerase chain reaction analysis revealed that the expression of iNOS mRNA in activated macrophages was suppressed by sanguiin H-6 in a concentration-dependent manner. In addition, sanguiin H-6 even at a low concentration showed a clear scavenging effect on the NO generated from sodium nitroprusside (an NO donor). These findings indicate that not only does sanguiin H-6 act directly as an NO scavenger, but it also inhibits NO production in LPS-activated macrophages by the concomitant inhibition of iNOS mRNA induction and enzyme activity.

21) Nakagawa T., Yokozawa T., Terasawa K., Shu S., Juneja L.R., Kitani K.: Protective activity of green tea against free radical- and glucose-mediated protein damage. *J. Agric. Food Chem.*, 50, 2418-2422, 2002.

Protein oxidation and glycation are post-translational modifications that are implicated in the pathological development of many age-related disease processes. This study investigated the effects of green tea extract, and a green tea tannin mixture and its components, on protein damage induced by 2,2′-azobis(2-amidinopropane) dihydrochloride, which is a free radical generator, and glucose in vitro assay systems. We found that green tea extract can effectively protect against protein damage, and showed that its action is mainly due to tannin. In addition, it was shown that the chemical structures of tannin components are also involved in this activity, suggesting that the presence of the gallate group at the 3 position plays the most important role in the protective activity against protein oxidation and glycation, and that there is also a contribution by the hydroxyl group at the 5′ position in the B ring and the sterical structure. These findings demonstrate the mechanisms of the usefulness of green tea in protein oxidation- and glycation-associated diseases.

22) Kim J.W., No J.K., Ikeno Y., Yu B.P., Choi J.S., Yokozawa T., Chung H.Y.: Age-related changes in redox status of rat serum. *Arch. Gerontol. Geriatr.*, 34, 9-18, 2002.

Aging and age-related diseases are known to be associated with increased oxidative stress. To protect against the deleterious effects of oxidative stress, a well-co-ordinated network of enzymatic and nonenzymatic anti-oxidant defense systems is essential. In the present study, we investigated the age-related redox status of serum by analyzing hydrogen peroxide, hydroxyl radical, superoxide-scavenging abilities, and other redox markers. Results showed the anti-oxidative capacity to be significantly decreased in serum of aged rats, which was accompanied by a marked increase in peroxide levels. Our analyses also revealed that levels of nitrated proteins, induced by peroxynitrite treatment, were higher in old rats than in young rats. Our results clearly indicated that the serum redox balance shifted toward oxidation during aging. To further confirm this age-related redox shift, we quantified the changes in thiol content. The total thiol level was found to be significantly decreased in the aged group. We also noticed an age-related reduction in serum albumin, which may be partially responsible for the decreased serum thiol levels. A similar pattern can be explained by low levels of serum GSH in old rats compared to young rats. The significance of the present study is the data showing increased oxidative stress in serum during aging, attributed to a decrease in major antioxidant components in serum.

23) Yokozawa T., Kim H.Y., Nonaka G., Kosuna K.: Buckwheat inhibits progression of renal failure. *J. Agric. Food Chem.*, 50, 3341-3345, 2002.

Rats subjected to partial resection of the parenchyma showed reduced radical-scavenging activity in the remaining kidney and increased severity of renal tissue lesions. However, in similarly nephrectomized rats given buckwheat extract, the state of oxidative stress improved by restoring the decreased activities of reactive oxygen species-scavenging enzymes such as superoxide dismutase and catalase. The degree of mesangial proliferation, severity of extratubular lesions such as crescents and adhesions, glomerulosclerosis index, and severity of tubular interstitial lesions also improved. In addition, nephrectomized rats given buckwheat extract showed improvement in renal function, as indicated by decreased serum level of creatinine, with a significant decrease in level of methylguanidine, a uremic toxin produced from creatinine in the presence of hydroxyl radical.

24) Yokozawa T., Kashiwada Y., Hattori M., Chung H.Y.: Study on the components of Luobuma with peroxynitrite-scavenging activity. *Biol. Pharm. Bull.*, 25, 748-752, 2002.

The origin of the antioxidant activity of Luobuma aqueous extract was examined by measuring the peroxynitrite ($ONOO^-$)-eliminating activities of fractions of this extract obtained by Sephadex LH-20 column chromatography. Three of the four fractions obtained, *i.e.*, those excluding the H_2O-eluted fraction, were found to possess $ONOO^-$-eliminating activity. These three fractions were combined and fractionated again by Sephadex LH-20 column chromatography, which yielded five fractions. Seven different compounds were isolated from two of these five fractions with high activity. Epigallocatechin-(4β-8)-epicatechin showed the highest $ONOO^-$-eliminating activity.

25) Yokozawa T., Nakagawa T., Kitani K.: Antioxidative activity of green tea polyphenol in cholesterol-fed rats. *J. Agric. Food Chem.*, 50, 3549-3552, 2002.

We investigated the effects of green tea polyphenol on the serum antioxidative activity and cholesterol levels of cholesterol-fed rats and compared them with those of probucol, an antioxidant hypocholesterolemic agent. To evaluate the antioxidative activity, we measured the susceptibility to oxidative modification of low-density lipoprotein (LDL) isolated from the serum of cholesterol-fed rats and the serum antioxidative activity using the spontaneous autoxidation system of brain homogenate. Administration of green tea polyphenol effectively inhibited LDL oxidation and elevated serum antioxidative activity to the same degree as probucol. However, higher amounts of polyphenol than probucol needed to be administered to reduce the total, free and LDL-cholesterol levels.

Furthermore, green tea polyphenol increased the levels of high-density lipoprotein (HDL)-cholesterol, leading to dose-dependent improvement of the atherogenic index, an effect that was not seen with probucol. Thus, green tea polyphenol may exert an antiatherosclerotic action by virtue of its antioxidant properties and by increasing HDL-cholesterol levels.

26) Yokozawa T., Chen C.P., Rhyu D.Y., Tanaka T., Park J.C., Kitani K.: Potential of sanguiin H-6 against oxidative damage in renal mitochondria and apoptosis mediated by peroxynitrite *in vivo*. *Nephron*, 92, 133-141, 2002.

Potential of sanguiin H-6, a component of Sanguisorbae Radix, to protect against oxidative damage in renal mitochondria and apoptosis mediated by peroxynitrite ($ONOO^-$) was examined using a model in which rats were injected with lipopolysaccharide (LPS) and then subjected to renal ischemia followed reperfusion (LPS plus ischemia-reperfusion). Ischemia-reperfusion was achieved by occluding bilateral renal artery for 60 min and then releasing for 350 min. At 50 min after ischemia started, LPS was injected intravenously. LPS plus ischemia-reperfusion induced a large amount of 3-nitrotyrosine, an oxidative product of protein that is produced via $ONOO^-$ nitration, which was not detectable in normal group. Oxidative damage of mitochondria was indicated by an accumulated thiobarbituric acid (TBA)-reactive substance, glutathione (GSH) depletion and glutathione peroxidase (GSH-Px) inactivation in the mitochondria. Treatment of rats with sanguiin H-6 (10 mg/kg body weight/day) for 30 days prior to LPS plus ischemia-reperfusion attenuated the oxidative damage in the mitochondria. The amount of TBA-reactive substance was decreased and the GSH level significantly increased as compared with that in control group. However, its effect on GSH-Px activity was much weaker. Apoptosis induced by LPS plus ischemia-reperfusion was detected by fluorescence staining, TdT-mediated dUTP-biotin nick end labeling and electrophoretic analysis. Sanguiin H-6 appeared to inhibit apoptosis, and this was associated with the suppression of caspase-3 activity. These beneficial effects of sanguiin H-6 against oxidative damage in mitochondria and apoptosis contributed to the improvement in renal function by reversing the elevated evels of blood urea nitrogen and creatinine caused by $ONOO^-$.

27) Yokozawa T., Muto Y., Wakaki K., Kashiwagi H.: Site of methylguanidine production and factors that influence production levels. *Nephron*, 92, 356-362, 2002.

The site of methylguanidine (MG) production in the kidney was investigated using animal models of renal disease and cultured renal epithelial cells. In rats with proximal tubular injury induced by adenine, the blood and urinary levels of MG increased as the severity of injury increased. In contrast, in cases of glomerular injury, there were no such changes in MG levels. Thus, it was apparent that proximal tubular injury served to promote MG production. In addition, a marked increase was observed in the intensities of bands attributable to 5,5-dimethyl-1-pyrroline-N-oxide (DMPO)-OH in the electron spin resonance spectrum of the kidney in the rats given adenine. In these rats, the activity of the radical-scavenging enzymes superoxide dismutase, catalase, and glutathione peroxidase was decreased. This suggests that the formation of excessive radicals and deterioration of defense mechanisms that contribute to the development of oxidative stress underlie the enhanced MG production. The experiments using cultured cells revealed that an oxide of adenine, 2,8-dihydroxyadenine (DHOA), directly induced renal tubular injury. These findings indicate that the accumulation of creatinine due to DHOA, combined with oxidative stress, resulted in increased MG production.

28) Yokozawa T., Kim H.Y., Cho E.J.: Erythritol attenuates the diabetic oxidative stress through modulating glucose metabolism and lipid peroxidation in streptozotocin-induced diabetic rats. *J. Agric. Food Chem.*, 50, 5485-5489, 2002.

We investigated the effects of erythritol on rats with streptozotocin (STZ)-induced diabetes mellitus. Oral administration of erythritol (100, 200 or 400 mg/kg body weight/day for 10 days) to rats with STZ-induced diabetes

resulted in significant decreases in the glucose levels of serum, liver and kidney. Erythritol also reduced the elevated serum 5-hydroxymethylfurfural level that is glycosylated with protein as an indicator of oxidative stress. In addition, thiobarbituric acid-reactive substance levels of serum, and liver and kidney mitochondria were dose-dependently lower in the erythritol-treated groups than the control diabetic group. Furthermore, the serum creatinine level was reduced by oral administration of erythritol in a dose-dependent manner. These results suggest that erythritol affects glucose metabolism and reduces lipid peroxidation, thereby improving the damage caused by oxidative stress involved in the pathogenesis of diabetes.

29) **Yokozawa T., Kim H.Y., Cho E.J., Choi J.S., Chung H.Y.: Antioxidant effects of isorhamnetin 3,7-Di-*O*-β-D-glucopyranoside isolated from mustard leaf (*Brassica juncea*) in rats with streptozotocin-induced diabetes. *J. Agric. Food Chem.*, 50, 5490-5495, 2002.**

To investigate the effects of isorhamnetin 3,7-di-*O*-β-D-glucopyranoside (isorhamnetin diglucoside), a major flavonoid compound of mustard leaf, on oxidative stress due to diabetes mellitus, in vivo and in vitro studies were carried out. Oral administration of isorhamnetin diglucoside (10 or 20 mg/kg body weight/day for 10 days) to rats with streptozotocin-induced diabetes significantly reduced serum levels of glucose and 5-hydroxymethylfurfural (5-HMF), which is glycosylated with hemoglobin and is an indicator of oxidative stress. After intraperitoneal administration, isorhamnetin diglucoside did not show these activities. In addition, after oral administration, the thiobarbituric acid-reactive substance levels of serum, and liver and kidney mitochondria declined significantly compared with the control group in a dose-dependent manner, while after intraperitoneal administration these levels only fell slightly. Based on the oral and intraperitoneal results, we hypothesized that isorhamnetin diglucoside was converted to its metabolite in vivo and we confirmed the conversion to its aglycone, isorhamnetin, by β-glucosidase and isorhamnetin acted as an antioxidant. Moreover, we observed that isorhamnetin diglucoside had no effect on the 1,1-diphenyl-2-picrylhydrazyl radical, whereas isorhamnetin showed a potent antioxidant effect in vitro. In addition, intraperitoneal administration of isorhamnetin reduced serum glucose and 5-HMF levels. Furthermore, lipid peroxidation in blood, liver and kidney associated with diabetes mellitus declined after the administration of isorhamnetin. These results suggest that isorhamnetin diglucoside is metabolized in vivo by intestinal bacteria to isorhamnetin and then isorhamnetin plays an important role as an antioxidant.

30) **Nakagawa T., Yokozawa T.: Direct scavenging of nitric oxide and superoxide by green tea. *Food Chem.* Toxicol., 40, 1745-1750, 2002.**

In this study, we investigated the free radical scavenging effects of green tea extract and green tea tannin mixture and its components using a nitric oxide (NO) and superoxide (O_2^-) generating-system in vitro. Green tea extract showed direct scavenging activity against NO and O_2^- and green tea tannin mixture, at the same concentration, showed high scavenging activity. Comparison of the activities of seven pure compounds isolated from green tea tannin mixture showed that (-)-epigallocatechin 3-*O*-gallate (EGCg), (-)-gallocatechin 3-*O*-gallate (GCg) and (-)-epicatechin 3-*O*-gallate (ECg) had higher scavenging activities than (-)-epigallocatechin (EGC), (+)-gallocatechin (GC), (-)-epicatechin (EC) and (+)-catechin (C), showing the importance of the structure of flavan-3-ol linked to gallic acid for this activity. Among the gallate-free tannins, EGC and GC were more effective O_2^--scavengers than EC and C, indicating the *O*-trihydroxy structure in the B ring is an important determinant of such activity. However, this structure did not affect the NO-scavenging activity. These findings confirm that green tea tannin has excellent antioxidant properties, which may be involved in the beneficial effect of this compound.

31) **Rhyu D.Y., Yokozawa T., Cho E.J., Park, J.C.: Prevention of peroxynitrite-induced renal injury through modulation of peroxynitrite production by the Chinese prescription Wen-Pi-Tang. *Free Radic. Res.*, 36, 1261-1269, 2002.**

The effect of Wen-Pi-Tang extract on renal injury induced by peroxynitrite ($ONOO^-$) production was investigated using rats subjected to intravenous lipopolysaccharide (LPS) injection and then renal ischemia followed by reperfusion. The plasma level of 3-nitrotyrosine, a marker of cytotoxic $ONOO^-$ formation in vivo, was enhanced markedly in control rats subjected to LPS plus ischemia-reperfusion, but was significantly reduced by the oral administration of Wen-Pi-Tang extract, at doses of 62.5 and 125 mg/kg body weight/day, for 30 days prior to LPS plus ischemia-reperfusion. The activities of inducible nitric oxide synthase (iNOS) and xanthine oxidase (XOD) in renal tissue of control and Wen-Pi-Tang extract-treated rats did not change significantly, while those of the antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase, were significantly increased by the administration of Wen-Pi-Tang extract, indicating that Wen-Pi-Tang improved the defense system by scavenging free radicals, not by directly inhibiting nitric oxide and superoxide production by iNOS and XOD. In addition, the levels of the hydroxylated products, *m*- and *p*-tyrosine, declined, whereas that of phenylalanine increased, after oral administration of Wen-Pi-Tang extract. Furthermore, the elevated plasma urea nitrogen and creatinine levels resulting from LPS plus ischemia-reperfusion process were significantly reduced by Wen-Pi-Tang extract, implying amelioration of renal impairment. The present study indicates that Wen-Pi-Tang extract contributes to the regulation of $ONOO^-$ formation and plays a beneficial role against $ONOO^-$-induced oxidative injury and renal dysfunction in vivo.

32) Nakagawa T., Yokozawa T., Oya T., Sasahara M., Terasawa K.: Evaluation of Keishi-bukuryo-gan in a diabetic nephropathy model by comparison with aminoguanidine, butylated hydroxytoluene and captopril. *J. Trad. Med.*, 19, 200-208, 2002.

A study was done to investigate whether Keishi-bukuryo-gan can delay the progression of diabetic nephropathy in an experimentally induced diabetic nephropathy model. The efficacy of Keishi-bukuryo-gan against renal functional and structural changes and its influence on accumulation of advanced glycation end-products (AGEs) and oxidative stress were also examined by comparison with aminoguanidine (an AGEs inhibitor), butylated hydroxytoluene (BHT; an antioxidant) and captopril (an angiotensin converting enzyme inhibitor). Treatment with Keishi-bukuryo-gan for 10 weeks preserved renal function, as assessed in terms of proteinuria and serum creatinine, and prevented the morphological changes peculiar to diabetic nephropathy. However, its renoprotective activity was inferior to that of captopril and comparable to that of aminoguanidine. BHT lacked any of these effects. On the other hand, renal AGEs accumulation and oxidative stress were significantly enhanced in rats with untreated diabetic nephropathy compared with normal rats. Keishi-bukuryo-gan, captopril and BHT showed significant reduction of AGEs levels, but not to the extent shown by aminoguanidine. Renal lipid peroxidation levels were significantly lowered in the groups given Keishi-bukuryo-gan and captopril, but not to the extent shown in the rats given BHT. The reduction of serum lipid peroxidation levels by captopril was stronger than that by BHT. The effects of Keishi-bukuryo-gan and aminoguanidine on serum lipid peroxidation levels were similar to those of BHT. These results suggest that the pharmaceutical characteristics of Keishi-bukuryo-gan may differ from those of the other three medicines examined.

33) Yokozawa T., Kim H.Y., Cho E.J., Choi J.S.: Protective effects of the BuOH fraction from mustard leaf in a renal ischemia-reperfusion model. *J. Nutr. Sci. Vitaminol.*, 48, 384-389, 2002.

The effects of the BuOH fraction from mustard leaf in rats subjected to renal ischemia-reperfusion were examined. The elevated serum superoxide anion (O_2^-) level and renal xanthine oxidase (XOD) activity in rats subjected to 6 h reperfusion following 1 h ischemia significantly and dose-dependently declined after oral administration of the BuOH fraction at doses of 50 and 200 mg/kg body weight/day for 10 days prior to ischemia-reperfusion. These findings indicate that this fraction might scavenge O_2^- or inhibit the generation of O_2^- by XOD activated by the ischemia-reperfusion process. In addition, the thiobarbituric acid-reactive substance level of the renal mitochondrial fraction of rats given the BuOH fraction orally was significantly lower than that of control rats given physiological saline (vehicle), implying that this fraction exerted protective action against lipid peroxidation caused by ischemia-reperfusion.

Furthermore, oral administration of the BuOH fraction reduced the serum urea nitrogen and creatinine levels, indicators of renal function. These results suggest that the BuOH fraction has protective effects against ischemia-reperfusion injury, acting as an antioxidant by scavenging O_2^-, inhibiting O_2^- generation by XOD, protecting against lipid peroxidation and ameliorating renal functional impairment.

◇総　説　Review paper

1）Yokozawa T.: Examination of the nitric oxide production-suppressing activity in Sanguisorbae Radix. *Oriental Pharmacy and Experimental Medicine*, **2**, 69-79, 2002.

2）横澤隆子, 野中源一郎, 藤井　創, 小砂憲一：ソバポリフェノールの利用. New Food Industry, **44**, 49-56, 2002.

◇学会報告　Scientific presentation

1）Supinya Tewtrakul, 中村憲夫, 藤原民雄, 服部征雄：Flavanone and flavonol glycosides from the leaves of *Thevetia peruviana* and their HIV-1 reverese transcriptase (RT) and integrase (IN) inhibitory activities. 日本薬学会第122年会, 2002, 3, 千葉.

2）石田あい, 横澤隆子, 中川孝子, 柳　東泳, 服部征雄：フリーラジカル惹起モデルを用いた黄連の検討. 日本薬学会第122年会, 2002, 3, 千葉.

3）金　賢栄, 横澤隆子, 崔　在洙, 趙　恩珠, 崔　弘植, 鄭　海泳：芥子菜は体内で抗酸化物を作る. 日本薬学会第122年会, 2002, 3, 千葉.

4）柳　東泳, 横澤隆子, 服部征雄, 朴　鐘哲　：温脾湯のパーオキシナイトライト消去作用. 日本薬学会第122年会, 2002, 3, 千葉.

5）中川孝子, 横澤隆子：糖尿病性腎症における桂枝茯苓丸の役割. 第45回日本腎臓学会学術総会, 2002, 5, 大阪.

6）柳　東泳, 横澤隆子：エピカテキンガレートのパーオキシナイトライト消去作用. 第45回日本腎臓学会学術総会, 2002, 5, 大阪.

7）Ahn, Eun-Mi, Norio Nakamura, Teruaki Akao, Masao Hattori and Tsutomu Nishihara : Searching for estrogenic activity from natural medicines. KSP-JSP-CCTCNM Joint Seminar 2002, 2002, 8, Daejeon, Korea.

8）Akiko Hirakawa, Min Byung-Sun, Gao Jiang-Jing, Norio Nakamura, and Masao Hattori : Cytotoxicity and antitumor activity of triterpenes from *Ganoderma lucidum* KARST. KSP-JSP-CCTCNM Joint Seminar 2002, 2002, 8, Daejeon, Korea.

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10）山辺典子, 横澤隆子, 中川孝子, 服部征雄, 大和田　滋：糖尿病性腎症における八味地黄丸の影響. 第19回和漢医薬学会大会, 2002, 8, 千葉.

11）中川孝子, 横澤隆子, 後藤博三, 大和田　滋, 服部征雄, 柴原直利, 寺澤捷年：桂枝茯苓丸による糖尿病性腎症の進展抑制作用. 第19回和漢医薬学会大会, 2002, 8, 千葉.

12）高橋京子, 小松かつ子, 渡辺麻里子, 欧陽新叹, 呂　紅然, 高橋幸一, 服部征雄, 東　純一：心疾患治療薬としての丹参の有効性と薬物間相互作用発現の可能性：心臓由来培養細胞ならびにヒト肝CYP代謝での検討. 第19回和漢医薬学会大会, 2002, 8, 千葉.

13）謝　麗華, 安　恩美, 赤尾光昭, 服部征雄：Transformation of arctiin to estrogenic substances by human intestinal bacteria. 第19回和漢医薬学会大会, 2002, 9, 千葉.

14）趙　静, 中村憲夫, 高　江静, 赤尾光昭, 服部征雄, 楊　秀偉：Development of an enzyme immunoassay for quantitative analysis of 12-*O*-acetylphorbol 13-decanoate. 第19回和漢医薬学会大会,

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15) 趙　静, 中村憲夫, 服部征雄, 小松かつ子, 楊　秀偉, 邱　明華 : Isolation and structure elucidation of new triterpenoid saponins from the roots of *Sinocrassula asclepiadea*. 日本生薬学会第49回年会, 2002, 9, 福岡.

16) 中川孝子, 田中　隆, 横澤隆子 : 温脾湯構成生薬並びに大黄・甘草成分の advanced glycation endproducts (AGEs) 形成抑制作用. 第14回腎とフリーラジカル研究会, 2002, 9, 東京.

17) 中川孝子, 横澤隆子, 後藤博三, 寺澤捷年, 大和田 滋 : 糖尿病性腎症に対する桂枝茯苓丸の有用性. 第14回腎とフリーラジカル研究会, 2002, 9, 東京.

＊18) Hattori M.: Utilization of intestinal anaerobes for development of new drugs and searching useful enzymes, Nanjin International Biological & Pharmaceutical Technology Market & Forum. 2002, 10, 南京.

19) 下遠野久美子, 高橋礼子, 平沢絵美, 山門正和, 長崎由希子, 藤本善徳, 中村憲夫, 服部征雄 : C 型肝炎ウイルスの RNA ポリメラーゼ阻害物質の探索. 第46回日本薬学会関東支部大会, 2002, 10, 東京.

20) 中川孝子, 横澤隆子, 佐野光代, 竹内茂彌, 陳　建斌, 源　伸介, 金　武祚 : Cr からの MG 産生に及ぼす(-)-Epigallocatechin 3-*O*-gallate の影響. 第23回グアニジノ化合物研究会, 2002, 9, 富山.

21) 土屋真澄, 中村憲夫, Meselhy R. Meselhy, 趙　宇峰, 安　恩美, 服部征雄 : ヒト腸内細菌を利用した Enterolactone, Enterodiol の簡易合成方法の開発. 日本薬学会北陸支部例会, 2002, 11, 福井.

22) Park Hye-Jin, 条　美智子, 中村憲夫, 服部征雄, 黒川昌彦, 白木公康, Choi Jae-Sue : *In vivo* and *in vitro* antiviral activity of red alga *Symphyocladia latiuscula* against herpes simplex viruses. 日本薬学会北陸支部例会, 2002, 11, 福井.

＊ は 招待講演

◇講演会　Lecture

1) Hattori M.: Metabolic activation of crude drug components by human intestinal bacteria, 北京大学医学部設立60周年記念講演会. 2002, 10, 北京.

2) Hattori M.: Metabolic activation of crude drug components by human intestinal bacteria, 2002, 11, 慶州, 韓国.

3) Yokozawa T.: Protective action of Sanguisorbae Radix against oxidative damage in kidney. 国立順天大学校韓医薬研究所セミナー, 2002, 6, 韓国.

4) 横澤隆子 : 糖尿病性腎症における漢方方剤の役割. 第17回茨城県東洋医学研究会, 2002, 7, つくば.

◇その他　Others

1) 服部征雄 : WFWP 女子留学生日本語弁論大会審査員, 2002年10月 5 日, 福井.

2) 横澤隆子 : 一日富山県教育委員会委員, 2002年 7 月17日, 高岡.

3) 横澤隆子 : 第23回グアニジノ化合物研究会世話人, 2002年 9 月28日, 富山.

4) 横澤隆子 (分担) : 老化・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. 厚生科学研究費補助金長寿科学総合研究事業研究成果報告書, 2002, 3.

5) 横澤隆子, 何　立群 : 2001年度日中医学協会助成金　調査・共同研究助成報告　糖尿病性腎症に有効な伝統薬物の探索. 日中医学, Vol.17 No.2, pp.29, 2002.

◇共同研究　Co-operative research

1) 下遠野邦忠 (京都大学ウイルス研究所), 下遠野久美子 (共立薬科大学), 垣内信子 (金沢大学薬学部) : 「C 型肝炎 RNA ポリメラーゼ阻害活性を指標とした抗 HCV 剤の開発研究」

2) 白木公康, 小松かつ子 (富山医科薬科大学医学部および和漢薬研究所) : 「抗ヘルペスウイルス作用を指標とした中国少数民族薬物の探索」

3）大竹　徹（大阪府立公衆衛生研究所）:「天然からの抗エイズウイルス薬の開発」
4）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」
5）鄭　海泳（釜山大学校薬学大学）:「抗酸化物に関する研究」
6）田中　隆，柏田良樹（長崎大学薬学部，新潟薬科大学）:「活性成分に関する研究」
7）小砂憲一（アミノアップ化学）:「機能性食品の開発研究」

◇非常勤講師　Part-time teacher

1）横澤隆子：富山大学大学院「栄養学特論」，4月～9月
2）横澤隆子：富山大学「栄養学」，4月～9月

◇研究費取得状況　Acquisition of research funds

1）日本学術振興会特別研究員奨励費「微生物を利用した新しい薬物の開発」（継続，服部代表）100万円.
2）つくし奨学・研究基金「加齢過程におけるフリーラジカルの役割と紅花の効果」（新規，横澤代表）120万円.
3）平成14年度教育研究学内特別経費「生薬の細胞保護作用に関する研究」（新規，横澤分担）75万円.
4）興和生命科学振興財団「抗 HIV 活性を有するホルボールエステル類の探索研究」（中村代表）100万円.
5）漢方医薬研究振興財団「C 型肝炎ウイルスの抗ウイルス剤の開発研究－HCVのポリメラーゼに対して阻害活性を示す中国少数民族薬物の探索－」（中村代表）150万円.
6）三島海雲記念財団「ヒト腸内細菌により植物性エストロゲン様作用物質に代謝活性化される食品成分に関する研究」（中村代表）100万円.
7）日本科学協会 平成14年度笹川科学研究助成「ヒト腸内細菌によりエストロジェン活性を発現する天然薬物に関する研究」（安恩美代表）55万円.
8）富山県受託研究「和漢薬・バイオテクノロージ研究」（新規，服部分担）50万円.

◇学位および論文名　Academic degrees and theses

課程博士（2002年3月）

Supinya Tewtrakul : Inhibitory effects of Thai medicinal plants, their constituents and related compounds on HIV-1 integrase

修　士（2002年3月）

木村貴子 : 抗逆転写酵素，抗 HCV ポリメラーゼ阻害活性を指標とした中国少数民族薬物の探索

修　士（2002年9月）

Kanjana Sangul : ヒト腸内細菌によるマンギフェリン *C*-配糖体の開裂

学　士（2002年3月）

平川暁子 : 霊芝苦味成分の細胞毒性及び抗腫瘍効果の検討

学　士（2002年3月）

山辺典子 : 糖尿病性腎症における八味地黄丸の影響

◇研究室在籍者　Research member

3年次学 : 近藤直子，和田江美子
4年次学生 : 西畑友尋
大学院前期1年 : 平川暁子，山辺典子
大学院前期2年 : 石田あい，土屋真澄，条 美智子
大学院後期1年 : 佐藤亜希子，Kanjana Sangul
大学院後期2年 : 高　江静，安　恩美
大学院後期3年 : 謝　麗華，柳　東泳

外国人研究生：湯　俊
外国人客員研究員：趙　静（博士），趙　宇峰，Atef A. Abdel-Hafez（博士），金　賢榮，趙 恩珠（博士），Hye-jin Park（博士），于　超，孫　暁飛，Gafur Md. Abdul（博士），左　風（博士），Suchitra Thongpraditchote（博士），Lamek Marpaung，朴　鐘喆（博士）
受託研究員：八田　明（博士）
事務補佐員：新井恵子，黒岩純子