細胞資源工学部門

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本部門では，和漢薬資源の恒久的維持，育成を図るため，薬用生物に関する細胞工学的研究を行なうと同時に，動植物細胞の持つ遺伝情報を解析して，その薬用資源開発への応用，あるいは微生物および動物細胞を用いて生理活性物質の探索，和漢薬の薬効発現機構の解明を行なうことを目指している。本年度の主な研究テーマと成果は下記の通りである。

Ⅰ．腸内嫌気性菌によるバイオトランスフォーメーション

温脾湯の構成生薬，大黄に含まれるepicatechin 3-*O*-gallateの腸内細菌による変換反応を検討し，13種の生成物を単離，構造決定した。また，この化合物の変換反応がヒトとラットの腸内細菌フローラでは著しく異なることを見いだした。その他，芍薬成分paeoniflorinの腸内細菌代謝物を酵素免疫法により測定し，paeoniflorin投与後のラット血漿中に多量に検出されることを見いだした。

Ⅱ．腸内細菌による薬物の代謝活性化に関与する遺伝子

Paeoniflorinの加水分解に関与する，*Bifidobacterium* sp.由来の酵素遺伝子の塩基配列の決定を試みた。

Ⅲ．抗ウイルス薬の開発

HIV-1プロテアーゼ阻害作用を有する鎖陽から，各種トリテルペン類を単離，同定した。また，これらのジカルボン酸ヘミエステルを合成し，その活性を調べた。その他，インドネシア生薬の抗ヘルペスウイルス作用を探索し，数種のエキスに強い作用を見いだした。

Ⅳ．腎疾患における病態の解明と治療薬の開発

丹参から単離したmagnesium lithospermate Bが・OH付加物のcreatol産生を抑制すること，ESRスペクトルより・OH消去作用を有すること，さらにO_2^-，NOに対しても影響を及ぼしていることを証明し，このようなラジカル消去作用は培養腎上皮細胞を用いたin vitro評価系，cephaloridineによる細胞障害系，急性腎不全モデルを用いた実験でも明らかにした。一方，酸化亢進状態にある透析患者に緑茶タンニンを最長6ヶ月投与し，MG, MG/Cr比の低下，手根管症候群の原因物質のβ_2-MGが投与期間中，低下する所見を得た。

◇ 著　書

1）服部征雄（分担）：猪苓湯，重要構成生薬解説・茯苓，『漢方製剤の知識』日本病院薬剤師会監修，pp. 50-53，薬事新報社，1996.

◇ 原　著

1）Cai, B., Nagasawa, T., Kadota, S., Hattori, M., Namba, T., and Kuraishi, Y.: Processing of Nux Vomica. VII. Antinociceptive Effects of Crude Alkaloids from the Processed and Unprocessed Seeds of *Strychnos nux-vomica* in Mice.
Biol. Pharm. Bull., 19 : 127-131, 1996.

We examined the antinociceptive effects of the

crude alkaloid fractions (CAF) of nux vomica (the dried seeds of *Strychnos nux-vomica* L.) and the influences of various processing methods upon their antinociception in three analgesic tests in mice. In the tail-pressure test, the CAF (0.01-1 μg/kg, i.p.) of nux vomica that was unprocessed or treated with sand-, licorice-, oil- or vinegar and sand-processing showed clear antinocieption. The CAF (1 μg/kg, i.p.) of vinegar-processed nux vomica showed antinociception, without effects at lower doses of 0.01 and 0.1 μg/kg and those treated with urine- or urine and sand-processing were without effects at doses of 0.01-1 μg/kg. Morphine (2 mg/kg, s.c.) showed short-lasting antinoception, without effects at a dose of 1 μg/kg. In the hot-plate test, the CAF (100 μg/kg, i.p.) or nux vomica having undergone sand-processing produced a significant antinociception, without effects at lower doses of 0.01 and 1 μg/kg. The CAF (0.01-100 μg/kg, i.p.) of nux vomica that was unprocessed or treated with oil- or vinegar and sand-processing and morphine (1 and 100 μg/kg, s.c.) were without effects. In the acetic acid-induced writhing test, the CAF (1 μg/kg, i.p.) of nux vomica that was treated with sand-processing significantly inhibited the writhing behavior, while those of nux vomica that was unprocessed or treated with oil- or vinegar and sand-processing and morphine were without effects at a dose of 1 μg/kg. The present results demonstrate the antinociceptive effects of the CAF of nux vomica and suggest that sand-processing is good for the analgesic potency of nux vomica. It is also suggested that the CAF of nux vomica has distinct antinociceptive potency, even after treatment with licorice-, oil-, vinegar- and sand-processing.

2) Akao, T., Che, Q., Kobashi, K., Hattori, M. and Namba, T. : A Purgative Action of Barbaloin Is Induced by *Eubacterium* sp. Strain BAR, a Human Intestinal Anaerobe, Capable of Transforming Barbaloin to Aloe-Emodin Anthrone.
Biol. Pharm. Bull., 19 : 136-138, 1996.

Orally administered barbaloin (100 mg/kg) did not induce any diarrhea in male Wistar rats, in spite of severe diarrhea with sennoside B (40 mg/kg). Also, in gnotobiote rats mono-associated with *Peptostreptococcus intermedius,* a human intestinal anaerobe capable of reducing sennidins to sennidins to rhein anthrone, barbaloin did not induce diarrhea ; the faecal water content (71.9 %) 8 h after the administration of barbaloin was not increased, compared with that (73.9 %) just before the treatment. However, severe diarrhea was induced with barbaloin in gnotobiote rats mono-associated with *Eubacterium* sp. strain BAR, another human intestinal anaerobe capable of transforming barbaloin to aloe-emodinanthrone ; the faecal water content was significantly increased to 85.5 % 8 h after the administration, from 73.2 % before the treatment. At this time, barbaloin was transformed to aloe-emodin anthrone in the feces from the gnotobiote rats mono-associated with the strain BAR, but not in feces from the conventional rats or the gnotobiote rats mono-associated with *P. intermedius.* These facts indicate that barbaloin is inactive as a laxative itself but is activated to aloe-emodin anthrone, a genuine purgative component, by *Eubacterium* sp. strain BAR.

3) Lin, C., Lee, H., Chang, C., Namba, T. and Hattori, M.: Evaluation of the Liver Protective Principles from the Root of *Cudrania cochinchinensis* var. gerontogea.
Phytother. Res., 10 : 13-17, 1996.

Three components in the EtOAc and *n*-BuOH fractions, obtained from the ethanol extract of *Cudrania cochinchinensis* var. *gerontogea* (Moraceae) were evaluated for their hepatoprotective activities in rats on carbon tetrachloride (CCl_4) and D-galactosamine (D-GalN)-induced hepatotoxicity. Three flavonoids (25 mg/kg), wighteone, naringenin and populnin (kaempferol 7-glucoside), exhibited greater hepatoprotective effects on CCl_4-induced liver injury than on D-GalN-induced hepatotoxicity by reversing the altered serum enzymes (SGOT and SGPT) and preventing the development of hepatic lesions, including liver centrilobular inflammation, cell necrosis, fatty change, ballooning degeneration in CCl_4 intoxication and necrosis of the portal area in D-GalN intoxication. Wighteone and naringenin (25 mg/kg) isolated from the EtOAc fraction showed a better hepatoprotective effect against CCl_4-induced liver

injury than that of populnin (25 mg/kg) obtained from the *n*-BuOH fraction. Furthermore, wighteone protected the liver, not only against CCl_4-induced hepatotoxicity, but also against D-GalN-induced liver injury. These results demonstrated that wighteone and naringenin are two active hepatoprotective principles from *Cudrania cochinchinensis var. gerontogea.*

4) Kawahata, T., Otake, T., Mori, H., Morimoto, M., Ueba, N., Kusumoto, I. T., El-Mekkawy, S., Hattori, M. and Namba, T.: Screening of Egyptian Folk Medicinal Plant Extracts for Anti-human Immunodeficiency Virus Type-1 (HIV-1) Activity.
J. Trad. Med., 13 : 59-65, 1996.

As a result of screening the extracts of 41 Egyptian folk medicines, six plants showed inhibitory activity against HIV-1 : methanol extracts of *Abrus precatorius* L., *Artemisia absinthium* L., *Croton tiglium* L. and *Datura stramonium* L., and water extracts of *Bassia muricata* (L.) MURR., *Centaurea scoparia* L., *Croton tiglium* L. and *Datura stramonium* L., Their respective anti-HIV-1 activity (IC_{50}) for MT-4 cells were 2.0, 9.8, 0.025, 4.1, 135, 46.2, 2.0 and 90.0 (μg/ml). *Croton tiglium* L. also suppressed giant cell formation in co-cultures of MOLT-4 cells with MOLT-IIIB cells. Moreover, *Abrus precatorius* L., *Artemisia absinthium* L., *Croton tiglium* L. had direct effects and decreased the infectivity of HIV-1. However, none of them showed any inhibitory effect on the reverse transcriptase and protease activity of HIV-1.

5) Hattori, M., Yang, X., Shu, Y., Heikal, O.A., Miyashiro, H., Kato, H., Kanaoka, M., Akao, T., Kobashi, K. and Namba, T.: Enzyme Immunoassay for Paeonimetabolin I, a Major Metabolite of Paeoniflorin by Intestinal Bacteria.
J. Trad. Med., 13, 73-80, 1996.

For quantitative determination of paeonimetabolin I (PM-I), a mixture of (*7R*)-and (*7S*)-isomers transformed from paeoniflorin (PF) by intestinal bacteria, we established an enzyme immunoassay (EIA) method as follows.

As a hapten, a mixture of (*7R*)-and (*7S*)-8-(2-carboxyethylthio)paeonimetaboplin I (CEPM) was prepared by anaerobic incubation of PF with *Lactobacillus brevis* in the presence of 3-mercaptopropionic acid. Without separation of both isomers, CEPM was coupled with β-galactosidase (β-Gal) and bovine serum albumin (BSA) *via* an *N*-hydroxysuccinimide ester method to give CEPM-β-Gal (enzyme-labeled antigen) and CEPM-BSA (immunogen), respectively. The anti-PM-I antiserum, which had been elicited in rabbits by immunization with CEPM-BSA, was specific to (*7R*)- and (*7S*)-PM-I and their 1 : 1 mixture but not to other related compounds, when the assay was carried out by the double antibody technique. Satisfactory standard curves of (*7R*)-, (*7S*)-PM-I and both isomers were obtained in a range of 0.1-500 ng/tube but appreciably interfered by addition of serum or urine to an assay mixture. By using the newly developed method, quantitative determination of the PM-I [(*7R*)-and (*7S*)-isomers] contents was possible in several fractions taken during the incubation of PF with *L. brevis.*

6) Yang, L., Akao, T., Kobashi, K. and Hattori, M.: A Sennoside-hydrolyzing β-Glucosidase from *Bifidobacterium* Sp. Strain SEN Is Inducible.
Biol. Pharm. Bull., 19 : 701-704, 1996.

Bifidobacterium sp. strain SEN was isolated and characterized by hydrolytic conversion of sennosides to sennidins (Akao *et al., Appl. Environ. Microbiol.,* 60, 1041 (1994)). The sennoside-hydrolyzing capacity of the strain SEN disappeared following the addition of glucose to the media in spite of good bacterial growth and potent activity hydrolyzing *p*-nitrophenyl β-D-glucopyranoside (*p*NPG). In a fructose-containing medium, no such suppressing effect was shown. Following a 10 h incubation in 50 mM potassium phosphate buffer (pH 7.4), the sennoside-hydrolyzing activity of the bacterium increased, dose-dependently, with the addition of sennoside B. Inhibition of the substrate induced increase in sennoside-hydrolyzing activity was observed following the addition of some antibiotics (chloramphenicol, streptomycin, and rifampicin). In particular, chloramphenicol completely inhibited the increase of sennoside-hydrolyzing activity while 38 % *p*NPG-hydrolyzing activity remained. It is

suggested that the strain SEN produces two different β-glucosidases of which the sennoside-hydrolyzing enzyme is inducible. In addition, the glucosides *p*NPG, esculin, salicin, or amygdalin stimulated the induction of the sennoside β-glucosidase, but less markedly than sennoside. Sennidin A or sugars (glucose, fructose, cellobiose, or malyose) did not induce the enzyme.

7) Yang, L., Akao, T., Kobashi, K. and Hattori, M.: Purification and Characterization of a Novel Sennoside-hydrolyzing β-Glucosidase from *Bifidobacterium* Sp. Strain SEN, a Human Intestinal Anaerobe. Biol. Pharm. Bull., 19 : 705-709, 1996.

A novel β-glucosidase, which is inducible and capable of catalyzing the hydrolysis of sennosides, was purified from *Bifidobacterium* sp. strain SEN with Triton X-100 solubilization and DEAE-cellulose column chromatography, by which hydrolytic activities toward sennoside B, 4-methylumbelliferyl β-glucoside (MUG), and *p*-nitrophenyl β-glucoside (pNPG) were obtained together in the same eluted fractions. The activity was stable against detergents such as sodium dodecyl sulfate (SDS) and Triton X-100, but was denatured by SDS and β-mercaptoethanal when heated. The final preparation was shown to be nearly homogeneous on SDS-polyacrylamide gel electrophoresis (PAGE) either after the enzyme was denatured or when it was not denatured. In the non-denaturing SDS-PAGE, a single protein band hydrolyzed MUG on the gel. In the denaturing SDS-PAGE, the subunit mass of the enzyme was estimated to be 110 kDa.

The enzyme was optimally active at pH 6.0 for hydrolysis of sennoside B and MUG. K_m values for sennoside B and MUG are 0.94 and 0.53 mM, respectively. The enzyme also catalyzed the hydrolysis of pNPG, amygdalin, geniposide and salicin. It was less active against methyl β-glucoside and incapable of hydrolyzing cellobiose. The β-glucosidase activity was inhibited by deoxynojirimycin and *p*-chloromercuribenzenesulfonic acid, but was less susceptible to several metals ($FeSO_4$, $ZuCl_2$, and $CuSO_4$), and 5.5'-dithio-bis (2-nitrobenzoic acid).

8) Yang, X., Hattori, M. and Namba, T.: Two New Coumarins from the Roots of *Aegle marmelos*.
J. Chin. Pharm. Sci, 5 : 68-73, 1996.

Two new coumarins, named marminal (**14**) and 7'-*O*-methylmarmin (**15**), along with nineteen known compounds: β-sitosteryl pentadecanoate (**1**), 4-methoxy-1-methyl-2-quinolone (**2**), aurapten (**3**), 4-sitosten-3-one (**4**), lupeol (**5**), imperatorin (**6**), xanthotoxin (**7**), dictamnine (**8**), (+)-epoxyaurapten (**9**), β-sitosterol (**10**), γ-fagarine (**11**), skimmianine (**12**), scoparone (**14**), umbelliferone (**16**), scopoletin (**17**), decursinol (**18**), marmesin (**19**), marmin (**20**), and integriquinolone (**21**) were isolated from the methanolic extract of the roots of *Aegle marmelos* Corr. (family Rutaceae).

9) Yang, X., Hattori, M. and Namba, T.: Studies on the Anti-lipid Peroxidative Actions of the Methanolic Extract of the Root of *Aegle marmelos* and Its Constituents *In Vitro* and *In Vivo*.
J. Chin. Pharm. Sci, 5 : 132-140, 1996.

The inhibitory effect of the methanolic extract of the root of *Aegle marmelos* (MERA) and its constituents on the lipid peroxidation *in vivo* and *in vitro* were studied. The results suggested that MERA increased the activities of superoxide dismutase (SOD) and GSH-peroxidase in the liver cytosol of mice, but showed no significant effect on the activity of catalase, and one of its major constituents, 4-methoxy-1-methyl-2-quinolone (MMQ) increased the activity of SOD in liver tissue of mice intoxicated with $FeCl_2$-ascorbic acid (AA)-ADP *in vivo*.

Various constituents isolated from the root of title plant inhibited the lipid peroxidation in rat liver homogenate, which was *in vitro* induced by $FeCl_2$-ascorbic acid, CCl_4-NADPH, or ADP-NADPH. Of the test compounds, MMQ and its derivatives integriquinolone were similar to α-tocopherol in inhibiting MDA production in rat liver microsomes induced by Fe^{2+}-ascorbate, CCl_4-NADPH, or ADP-NADPH.

10) Arima, T., Baba, I., Hori, H., Kitamura, Y., Namba, T., Hattori, M., Kadota, K. and Nomura, Y.: Ameliorating Effects of Dan-Shen Methanol Extract on Cognitive Defi-

ciencies in Senescence-Accelerated Mouse. Kor. J. Gerontol., 6 : 14-21, 1996.

Effect of the long-term treatment with a Dan-Shen (*Salviae miltiorrhizae radix*) methanol extract (DME) on memory and learning in scenescence-accelerated mouse was investigated by means of Morris's water maze task. DME treatment significantly decreased the escape latency and floating time in the P8 strain of senescence-accelerated mouse (SAMP8), which strain spontaneously develops learning deficits. These results suggest that DME treatment improved spatial learning and activated emotional function. In neurochemical investigation, although no effect on choline acetyl transferase activity or [^{3}H]QNB (quinuclidinyl benzilate) binding was observed, DME treatment caused increases in [^{3}H]MK-801 ((+)-5-methy-10, 11-dihydro-5H-dibenzo [a,b]-cyclohepten-5, 10-imine maleate) binding to the cortical membrane fraction and [^{3}H]NNA (N^G-nitro-L-arginine) binding to the cytosolic fraction of the brain stem, and decreased [^{3}H]PDBu (phorbol 12,13-dibutyrate) binding to membranes from the hippocampus, striatum, and cereballum. Thus, our data suggest that long-term DME treatment improves spatial learning and emotional functions in SAMP8, As to the mechanism, DME treatment enhances cortical glutamatergic excitatory neurotransmission and nitric oxide synthase-related functions in brain stem, and affects hippocampal and striatal functions mediated by protein kinase C.

11) **Goto, H., Shimada, Y., Akechi, Y., Kohta, K. Hattori, M. and Terasawa, K.: Endothelium-dependent Vasodilator Effect of Extract Prepared from the Roots of *Paeonia lactiflora* on Isolated Rat Aorta. Planta Med., 62, 436-439, 1996.**

Paeoniae Radix (the roots of *Paeonia lactiflora* Pallas) is a crude drug that is used in Asia and Europe to improve blood flow. We studied its vasodilator effect and mechanisms of action *in vitro*. The extract from Paeoniae Radix (PRE) relaxed prostaglandin F2a-precontracted aortic ring preparations of isolated rat aorta that contained endothelium. Relaxation by PRE did not occur in specimens without endothelium. and was inhibited by pretreatment with 10^{-4} M N^G-nitro-1-arginine methyl ester.

Paeoniflorin and paeonol, the main active components of Paeoniae Radix, lacked a vasodilator effect. The effect of the component gallotannin was examined after treating PRE with tannase, but the product lacked a vasodilator effect. Pentagalloylglucose, hexagalloylglucose, heptagalloylglucose, and octagalloylglucose were extracted from PRE ; they relaxed aortic rings with endothelium, but failed to relax aortic rings without endothelium. We conclude that PRE exhibits an endothelium dependent vasodilator effect on isolated rat aorta. The active component is gallotannin.

12) **Takeda, S., Wakui, Y., Mizuhara, Y., Ishihara, K., Amagaya, S., Maruno, M. and Hattori, M.: Gastrointestinal Absorption of Paeoniflorin in Germ-free Rats. J. Trad. Med., 13 : 248-251, 1996.**

The influence of intestinal bacterial metabolism on the gastrointestinal absorption of paeoniflorin was studied using Wistar germ-free and conventional rats.

Plasma concentration profiles of paeoniflorin in germ-free and conventional rats after its oral administration at a dose of 2 mg/kg were compared. In conventional rats, the mean plasma concentration of paeoniflorin rapidly reached Cmax (32.7 ng/ml) at 15 min and decreased ; the mean value of 0.7 ng/ml at 240 min after administration. In germ-free rats, paeoniflorin was rapidly absorbed as well as in conventional rats, but the plasma concentration was kept at the nearly constant level around 25 ng/ml until 240 min after the administration. There were significant differences in AUC and tmax values between germ-free and conventional rats, while the difference of Cmax values was not found. AUC value in germ-free rats was two times larger than that in conventional rats.

These findings suggests that paeoniflorin orally administered is rapidly absorbed from upper part of the gastrointestinal tract, but the more than half of paeoniflorin which must be absorbed from intestine is degraded to some metabolites by the intestinal bacteria.

13) **Park, J., Yu, Y., Lee, J., Hattori, M., Lee, C.,**

Choi, J.: Protective Effect of *Oenanthe javanica* on the Hepatic Lipid Peroxidation in Bromobenzene-Treated Rats and Its Bioactive Component.
Planta Med., 62 : 1-3, 1996.

To investigate the detoxification of bromobenzene-induced hepatic lipid peroxidation by *Oenanthe javanica* DC, the hepatic lipid peroxide level and the activities of enzymes responsible for production and removal of epoxide were studied. The level of lipid peroxide elevated by bromobenzene was significantly reduced by the methanol extract (250 mg/kg) and persicarin (5 mg/kg). The methanol extract and persicarin administered daily over 4 weeks before intoxication with bromobenzene did not affect the activities of aminopyrine *N*-demethylase, aniline hydroxylase, and glutathione *S*-transferase. Epoxide hydrolase activity was decreased significantly by bromobenzene, which was restored to the control level by pretreatment with persicarin. However, the identical pretreatment with isorhamnetin and hyperoside did not change the enzyme activity or lipid peroxide level. The results suggest that the reduction of bromobenzene-induced hepatic lipid peroxidation by *O. javanica* under our experimental conditions is effected through enhancing the activity of epoxide hydrolase, and enzyme removing bromobenzene epoxide. In addition, the bioactive component of this plant responsible for the detoxification of bromobenzene, at least in part, is thought to be persicarin.

14) Yokozawa, T., Tsuji, A., Hattori, M., Oura, H., Kaji, T., Fujiwara, Y., Wakaki, K., Koizumi, F., and Shimizu, M.: Effect of a Chinese Medical Preparation, Hokoei-to, on Lipids in Blood and Involvement of Cells of Vascular Origin.
Phytother. Res., 10 : 224-227, 1996.

A significant increase in high-density lipoprotein-cholesterol and a tendency for total cholesterol and low-density lipoprotein-cholesterol to decrease were found in parous rats given Hokoei-to orally for 25 days, resulting in improvement of the atherogenic index. In experiments using cells of vascular origin, significantly increased proliferation of endothelial cells and suppressed proliferation of smooth muscle cells were obtained in the presence of Hokoei-to. When the action of each component galenical of Hokoei-to was examined, all five components, i.e. Taraxaci Radix, Angelicae Radix, Cyperi Rhizoma, Moutan Cortex and Dioscoreae Rhizoma, promoted the proliferation of endothelial cells. However, Taraxaci Radix and Angelicae Radix also promoted the growth of smooth muscle cells, showing inconsistency with the effect of Hokoei-to as a whole.

15) Yokozawa, T., Houri, T., Hattori, M., Oura, H., Kawamura, K., Yamamura, H., and Nunoura, Y.: Effects on the Proliferation of Smooth Muscle Cells of Oriental Medical Prescriptions Used for the Treatment of Arteriosclerosis.
Nat. Med., 50 : 9-13, 1996.

The effects of seven Oriental medical prescriptions used for the treatment of arteriosclerosis on blood vessel-derived smooth muscle cells were determined in terms of [^{3}H] thymidine uptake by DNA. Saiko-ka-ryukotsu-borei-to, San'o-shashin-to, Bofu-tsusho-san, Tokaku-joki-to, Dai-saiko-to-kyo-daio and Dai-saiko-to inhibited the proliferation of smooth muscle cells except Daio-botampi-to, but the most potent inhibitory effect was shown by Saiko-ka-ryukotsu-borei-to. Similar experiments with 12 crude drugs contained in these seven medical prescriptions revealed that Bupleuri Radix, Ginseng Radix, Zingiberis Rhizoma, Cinnamomi Cortex, Rhei Rhizoma, Scutellariae Radix and Coptidis Rhizoma had proliferation-inhibiting activity.

16) Dong, E., Yokozawa, T., Kashiwagi, H., Hattori, M, Watanabe, H., and Oura, H.: The Role of Total Ginseng Saponin and Its Components in Antiperoxidation in Vitro.
Nat. Med., 50 : 128-134, 1996.

Antisuperoxidation activities of total ginseng saponin and its individual saponins (ginsenoside-Rb_1, -Rb_2, -Rc, -Rd) were assessed using a reaction mixture in which oxygen-containing radicals such as superoxide anion (O_2^-) and hydroxyl radical were generated by addition of H_2O_2, Fe^{2+} or both to normal rat liver homogenate. The level of the final product of this superoxidation reaction—

thiobarbituric acid (TBA)-reactive substances—measured spectrophotometrically was used as a parameter for evaluating the antioxidation ability. All the ginseng samples tested showed distinctive inhibition of the formation of TBA-reactive substances ; under the three different treatment conditions, total ginseng saponin and ginsenoside-Rc showed considerable protection against superoxidation as compared with the other samples tested, although the former was much stronger than the latter. However, we also found that all the ginseng samples except for ginsenoside-Rb_2 were able to selectively suppress O_2^- induced by H_2O_2, On the other hand, spin-trapping and thin-layer chromatography revealed that ginseng saponin was unable to react directly with either oxygen-containing radicals or their inducers such as H_2O_2 and Fe^{2+}, indicating that the antisuperoxidation mechanism of ginseng saponin might take the form of mediation, i.e. activation of the endogenous free radical scavenging system. Furthermore, the present findings prove that saccharides linked at C-20 in protopanaxadiol play an important role in protection against lipid peroxidation.

17) **Yokozawa, T., Chung, H. Y., He, L. Q., and Oura, H. : Effectiveness of Green Tea Tannin on Rats with Chronic Renal Failure.**
Biosci. Biotech. Biochem., 60 : 1000-1005, 1996.

The effects of green tea tannin on nephrectomized rats were examined. There were increases in blood urea nitrogen, serum creatinine, and urinary protein, and a decrease in creatinine clearance in the nephrectomized control rats, whereas better results for these parameters were obtained in rats given green tea tannin after nephrectomy, demonstrating a suppressed progression of the renal failure. When the renal parenchyma was partially resected, the remnant kidney showed a decrease in the activity of radical scavenger enzymes. Green tea tannin, however, was found to lighten the kidney under such oxidative stress. Mesangial proliferation and glomerular sclerotic lesions, which were conspicuous in the rats that were not given green tea tannin after nephrectomy, were also relieved.

18) **Yokozawa, T., Dong, E., Liu, Z. W., Oura, H. and Nishioka, I. : Antiperoxidation Activity of Wen-Pi-Tang *in Vitro*.**
Nat. Med., 50 : 243-246, 1996.

Antiperoxidation tests *in vitro* showed that Wen-Pi-Tang extract and two of its five components — Rhei Rhizoma and Glycyrrhizae Radix extract— protect against superoxidation activity, similarly to another important constituent, Ginseng Radix. However, Aconiti Tuber and Zingiberis Rhizoma extract showed considerably weak activity, indicating that the action of Wen-Pi-Tang extract is determined mainly by the former two extracts. On the other hand, with regard to the chemical ingredients tested, the most active were tannins, especially epicatechin 3-*O*-gallate. Some flavones, such as licochalcone A, and anthraquinones, such as emodin and physcion, also showed favorable activity, and thus their role in this prescription seems very important. However, triterpenoids such as glycyrrhetic acid had very low activity.

19) **Yokozawa, T., Inaba, S., Oura, H., He, L. Q. and Zheng, P. D. : Experimental Evaluation of Traditional Herb Prescriptions Given to Patients with Chronic Renal Failure in China, Using Rats with Subtotal Nephrectomy.**
J. Trad. Med., 13 : 42-49, 1996.

Three traditional herbal prescriptions reported to be effective for treating chronic renal failure in China were studied using nephrectomized rats. Each prescription was given orally to rats for 80 days after excision of five-sixths of their kidney volume. Increased duration of 91 Shen-Shuai-Chong-Ji (91 腎衰衝剤) administration was associated with a progressive decrement of blood urea nitrogen (BUN), compared with the corresponding control values. A significant decrease of BUN was also observed in rats given Da-Huang-Lin-Pi-Chong-Ji (大黄霊脾衝剤) or Bu-Shen-Sheng-Xue-Chong-Ji (補腎生血衝剤), and these effects became more marked as the administration period was extended. Not only were total serum protein and albumin increased in rats given the three prescriptions, but also the serum levels of creatinine, methylguanidine and guanidinosuccinic acid were reduced significantly and urinary protein excretion was suppressed in the Da-Huang-Lin-Pi-Chong-Ji and Bu-Shen-Sheng-Xue-Chong-Ji groups. Histologically,

mesangial proliferation, extracapillary lesions, glomerular sclerosis index and tubulo-interstitial lesions, which were conspicuous in rats not given the prescriptions after nephrectomy, were improved considerably in the above two groups.

20) Yokozawa, T., Yasui, T., and Oura, H.: Molecular Biological Analysis of the Effects of Ginsenoside-Rb_2 on Albumin mRNA in Streptozotocin-induced Diabetic Rats.
J. Pharm. Pharmacol., 48 : 763-767, 1996.

The mechanisms of the mRNA synthesis-promoting action of ginsenoside-Rb_2, were investigated at the gene level.

Rot analysis suggested that the previously reported increase in RNA polymerase activity as a result of administration of ginsenoside-Rb_2 might be because of its effect on a specific gene. In this regard, albumin mRNA, which is expressed specifically in the liver, was assayed by northern blot hybridization using albumin cDNA in normal rats, diabetic control rats and diabetic rats given ginsenoside-Rb_2. When the level of albumin mRNA in normal rats was set at 100, the level was reduced markedly to 32 in diabetic control rats. In contrast, in diabetic rats given ginsenoside-Rb_2 the level was 0.54, significantly higher (69 %) than that in diabetic rats given no ginsenoside-Rb_2. In addition, poly $(A)^+$RNA was purified from total RNA and subjected to hybridization, and poly$(A)^+$RNA bands with different charges were measured by densitometry. The results of the measurement revealed changes dependent on the charge, and this was confirmed by autoradiography.

We found no significant difference in the transcription activity of albumin mRNA, however, it showed only a tendency to increase. This suggests that ginsenoside-Rb_2 has some effect on post-transcriptional regulation of the stability of mRNA itself. The results of Rot analysis suggest that ginsenoside-Rb_2 affects a specific gene alone.

21) Yokozawa, T., Oura, H., Shibata, T., Ishida, K., Kaneko, M., Hasegawa, M., Sakanaka, S., and Kim, M.: Effects of Green Tea Tannin in Dialysis Patients.
J. Trad. Med., 13 : 124-131, 1996.

Green tea tannin (daily dose, 400 mg) administered for 6 months to 50 dialysis patients decreased in the blood levels of creatinine (Cr) and methylguanidine (MG) and also the MG / Cr ratio. A decrease in β_2-microglobulin and improvement of arthralgia were also noted in some patients. These findings suggest that green tea tannin ameliorates the state of enhanced oxidation in dialysis patients.

22) Yokozawa, T., He. L. Q., Oura, H., and Nishioka, I.: Evidence Suggesting a Role for Magnesium Lithospermate B, an Active Component Isolated from Dan Shen, in Glycerol-induced Acute Renal Failure Rats.
J. Trad. Med. 13 : 143-150, 1996.

In order to evaluate the effectiveness of magnesium lithospermate B in alleviating acute renal failure, we used a rat model of acute tubular necrosis and designed two experimental methods. In experiment 1, normal rats were administered magnesium lithospermate B (20 mg/kg body weight/day) for 20 days before injection of glycerol. After deprivation of water for 18 h, the rats received an injection of 50 % glycerol into the muscle of the rear limb at 10 ml/kg body weight. The rats were given magnesium lithospermate B continually for 2 days orally, and then sacrificed by decapitation. In experiment 2, we treated rats with acute renal failure induced by the above method by intraperitoneal administration of magnesium lithospermate B (10 or 20 mg/kg body weight/day) once every 8 h for 2 days, after the rats had been deprived of water for 6 or 12 h. The markedly elevated levels of urea nitrogen and creatinine in blood and the reduced creatinine clearance related to progression of renal failure caused by injection of the nephrotoxic drug following changes in the dehydration period were significantly improved by oral and intraperitoneal administration of magnesium lithospermate B. Urine volume and urinary osmolarity, which were markedly lowered with the advance of renal failure in parallel with the various periods of dehydration, were obviously increased, and this compound appeared promising for suppressing the increased excretion of urinary glucose and fractional excretion of sodium due to alteration of tubule function following injection of glycerol.

23) Yokozawa, T., Dong, E., Watanabe, H., Oura,

H., and Kashiwagi, H.: Increase of Active Oxygen in Rats after Nephrectomy is Suppressed by Ginseng Saponin.
Phytother. Res., 10 : 569-572, 1996.

Subtotally nephrectomized rats were found to have decreased activities of superoxide dismutase (SOD), catalase and glutathione peroxidase, and spin-trapping with 5,5-dimethyl-1-pyrroline-N-oxide showed that the amount of hydroxyl radical (·OH) in the residual kidney tissue was greater than that in the normal kidney. In contrast, rats given ginseng saponin (25 mg / kg body weight) orally for 30 days after subtotal nephrectomy showed restoration of catalase activity to almost a normal level. A significant increase in SOD activity was observed. ·OH, which is highly reactive and for which there is no scavenger system in the body, was decreased markedly in kidney homogenates obtained from rats given ginseng saponin. The increased levels of uraemic toxins in the blood were also reduced in rats given ginseng saponin. These findings indicate that ginseng saponin helps to inhibit the progression of renal failure by scavenging radicals.

24) **Hatanaka, Y., Nakamura, N., Wakabayashi, M., Fujioka, T. and Kikuchi, T.: Synthesis and Characterization of Nobel Photoreactive Naltrexone Analogs as Isomeric Carbene-generating Probes for Opioid Receptors.**
Heterocycles, 43 : 519-522, 1996.

A convenient synthesis of *m*-and *p*-CF_3-diazirinylbenzoic acid was developed. A pair of novel photoaffinity probes bearing these diazirines on a naltrexyl framework bind reversibly with high affinity at μ-, δ-, and κ-receptors.

25) **Qui, M., Nie, R., Nakamura, N. and Kikuchi. T.: Paxillarines A and B, New Steroidal Alkaloids from *Pachysandra axillaris*, and Conformation of Their Ring A Moieties.**
Chem. Pharm. Bull., 44 : 2015-2019, 1996.

Two new steroidal alkaloids, paxillarines A (1) and B (2), were isolated from *Pachysandra axillaris*. Their stuctures were determined by means of spectrometric methods as 3α-*N*-methylbenzoylamino-4β-acetoxy-16β-hydroxy-20α-dimethylamino-5α-pregnane and 3α-*N*-methylbenzoylamino-4β-acetoxy-12β-hydroxy-20α-dimethylamino-5α-pregnane, respectively. Some of the expected characteristic ^{1}H-NMR signals were not observed due to hindered rotation when the spectra were measured on a 400 MHz spectrometer, although all of the signals appeared clearly on a 90 MHz machine. The conformation of the ring A moiety of these alkaloids is discussed.

◇ 総　説

1) 服部征雄：漢方薬の薬効発現に及ぼす腸内細菌の役割. Prog. Med. 16 : 201-206, 1996.
2) 横澤隆子：腎不全における温脾湯の有用性. 腎と透析, 41 : 501-508, 1996.

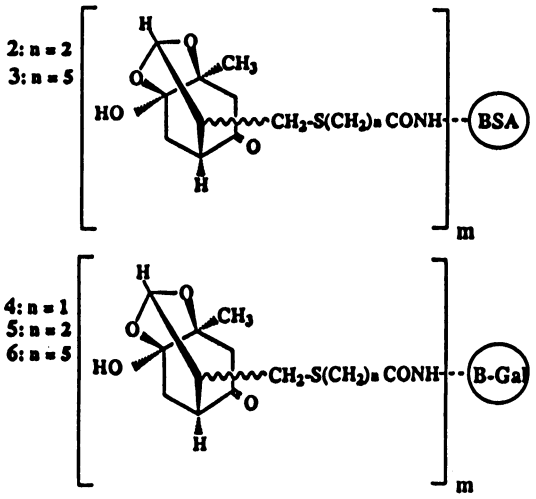
◇ 学会報告

1) **有馬　隆, 松野純子, 堀妃登美, 難波恒雄, 服部征雄, 門田重利, 野村靖幸：老化促進モデルマウス（SAM）の空間認知機能障害に対する Lithospermate B の作用 SAM 研究会, 1996, 2, 大阪.**

Senescence accelerated mouse P8 (SAM P8) substrain exhibits memory disturbance as well as accelerated-aging symtoms. This animal may be useful as a model for the evaluation of drugs for treatment and/or prevention against memory disturbance. Lithospermate B, major ingredient of Danshen, Radix Saliviae Miltioorrhizae, methanol extract was administered orally (60 mg/kg/day) for 3 weeks to 4 month-old SAM P9. Spatial learning ability was assessed with Morris's water maze mothod. A significant decrease in the mean path length was observed in treatment group, Although lithospermate B treatment did not alter choline acetyl transferase activity and [^{3}H]QNB binding in cortex and hippocampus, [^{3}H]PDBu binding was increased significantly in hippocampus. It is suggested that lithospermate B alters the protein kinase C (PKC)-mediated function in hippocampus and improves memory disturbance in SAM P8.

2) **Ola Ahmed Heikal, 宮代博継, 服部征雄, 金岡又雄, 赤尾光昭, 小橋恭一：Paeonimetabolin-I の酵素免疫測定法に付いて II. 日本薬学会第116年会, 1996, 3, 金沢.**

【目的】 芍薬成分 paeoniflorin (1) の代謝物 paeonimetabolin-I の高感度な定量を行う目的で酵素免疫分析法の開発をおこなった。特にハプテンとタンパク



質担体との架橋の長さと定量感度との関係について検討した。

【方法】 HS-$(CH_2)_n$-COOH (n=1, 2, 5) の存在下，**1** を腸内細菌で代謝させ，8-(carboxymethylthio)-, 8-(carboxyethylthio)-, 8-(carboxypenthlthio)-paeonimetabolin を得た。これらはさらに succinimide エステル法で BSA 抱合体 (**2, 3**) および beta-Gal 抱合体 (**4, 5, 6**) とした。家兎に BSA 抱合体 (**2, 3**) を継続的に摂取し，2種類の抗血清を得，長さの異なった架橋からなる beta-Gal 抱合体を用いて paeonimetabolin-I の定量を行った。

【結果】 BSA あるいは beta-Gal とハプテン間を連結する架橋の長さを検討した結果，抗体産生に用いたものより短い架橋をもつ beta-Gal 抱合体を使用した場合に最も高感度であった。

3）中村憲夫，Amr M. Helal，服部征雄：エジプト産生薬 *Centaurea scoparia* の新セスキテルペンラクトンについて．日本薬学会第116年会，1996，3，金沢．

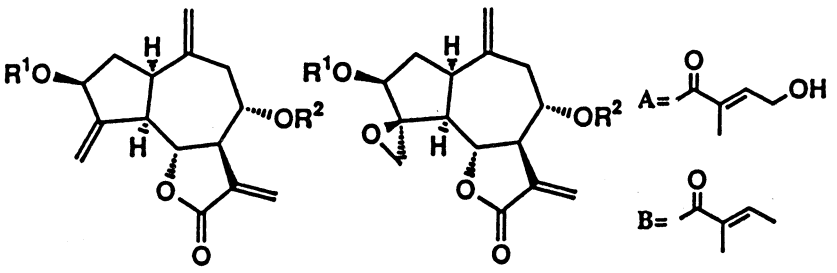
【目的】 天然物由来のエイズ治療薬開発を目的とした研究の一環として，今回，エジプト産生薬 *Centaurea scoparia* の成分研究を行い，5種の新 guaianolide セスキテルペンラクトン scoparin A-E を単離，構造決定したので報告する。

【実験・結果】 *C. scoparia* の地上部 1 kg を水で抽出し，残査をメタノールで抽出した。水抽出エキスはクロロホルムと水で分配後，クロロホルム可溶部を順相及び逆相シリカゲルクロマトを繰り返し3種の新化合物 scoparin A, D, E (**1, 4, 5**) を単離した。メタノールエキスはジクロロメタン，酢酸エチル，水可溶部に分画し，ジクロロメタン可溶部を順相及び逆相シリカゲルクロマトを繰り返し，新化合物 scoparin B, C (**2, 3**) を単離した。Scoparin 類の構造は各種スペクトルデータから下に示すような guaianolide セスキテルペンラクトンであると決定した。

4）横澤隆子，大浦彦吉，西岡五夫：cephaloridine による腎障害を magnesium lithospermate B が軽減した。日本薬学会第116年会，1996，3，金沢．

【目的】 cephaloridine による腎での障害部位は主に近位尿細管であるが，本研究では尿細管障害が primary のアデニン誘発慢性腎不全ラットに対し，その有効性が報告されている magnesium lithospermate B (MLB) が，cephaloridine による腎障害を軽減出来るか否かについて検討した。

【方法】 20日前より MLS (10 mg/kg 体重) を経口投与したラット並びに MLB の代わりに等量の水を投与したラットに cephaloridine (1 g/kg 体重) をそれぞれ静注し，投与後1～2日目の尿を採取，次いで腎を灌流し摘出した。尿浸透圧は氷点降下法を用いたアドバンス浸透圧計 3WII, Na, K は水素イオン電極法を用いた



1: R^1=H R^2=A
2: R^1=R^2=A
3: R^1=H R^2=B
4: R^1=H R^2=A
5: R^1=R^2=H

電解質測定装置（AHS/Japan Corporation），NO_2^-及びNO_3^-の分析はNOX測定装置TCI-NOX 1000（東京化成工業社製）を用い測定した。またグルコースは百瀬法，蛋白はスルフォサリチル酸法，抗酸化酵素活性は比色法で測定した。

【結果及び考察】 cephaloridine投与群の尿中Na，グルコース排泄量はいずれも増加し，また尿量，尿蛋白の増加，尿浸透圧の低下から，近位尿細管における機能不全を呈していることが，本実験においても観察された。しかし前もってMLBを経口投与し，cephaloridineを静注した群のNa，グルコース，蛋白排泄量は有意に低下，特に尿糖は著しく改善していた。また尿量，浸透圧は正常化傾向にあり，MLBが腎障害を軽減する作用を有していることが示された。一方，cephaloridine投与群のNO_2^-/NO_3^-は正常値より低下していたが，MLBを前もって投与し，続いてcephaloridineを投与した群では逆に有意に増加，またO_2^-消去系のSOD，catalase活性もMLB投与群で有意に上昇し，酸素ストレスに対しMLBが防御的に作動していることが示唆された。

5）武藤靖子，横澤隆子，何　立群，服部征雄，大浦彦吉：慢性腎不全ラットにおけるグアニジノ化合物動態．日本薬学会第116年会，1996，3，金沢．

【目的】 腎不全病態におけるグアニジノ化合物の意義が注目されているが，本研究では腎の障害部位が異なる慢性腎不全ラットを用い，グアニジノ化合物動態を検討した。

【実験】 Wistar系雄性ラット（体重約200 g）を用いた。糸球体障害由来の慢性腎不全モデルとして腎摘（1/2～7/8）を施し，市販固型飼料（日本クレア製，CE-2）で飼育した。尿細管障害由来の慢性腎不全モデルは0.75％アデニン含有食を投与し作製した。いずれのモデルも30日間飼育後に血液，尿，腎臓，肝臓，筋肉を採取し，TCAで処理後，step-gradient法によるHPLCで各種グアニジノ化合物を定量した。

【結果】 腎不全の進行程度の指標のblood urea nitrogenとcreatinine clearanceは，腎摘ラットよりアデニン投与ラットにおいて正常値より著しく逸脱していた。一方，腎で産生されるguanidinoacetic acid（GAA）の尿中レベルはいずれのモデルでも著しく低下，逆に血中，尿中のmethylguanidine（MG），血中，腎組織中のguanidinosuccinic acid（GSA）レベルは増加していた。しかし，これらグアニジノ化合物の変化の程度はアデニン投与ラットで顕著であり，腎摘ラットにおいても腎摘の程度のsevereな5/6，7/8腎摘においてMG，GSAレベルが増加する結果が得られた。

【考察】 最も強力なuremic toxinとされているMGは，障害部位の異なる2種類のモデルのうち尿細管障害由来のアデニン投与ラットにおいて，生体内により多く蓄積する結果が得られたが，類似した結果はGSAレベルでも認められ，グアニジノ化合物動態に尿細管が重要な役割を担っていることが示唆された。

6）金　東郁，横澤隆子，服部征雄，門田重利，難波恒雄：羅布麻葉（*Apocynum venetum* L.）に関する研究－高cholesterol食投与ラットを用いての検討－．日本薬学会第116年会，1996，3，金沢．

【目的】 羅布麻（*Apocynum venetum* L.）は中国では高血圧，心不全，気管支炎，水腫等に有効であることが報告されており，またお茶として中国の西北地方で飲用されている。本研究では循環器系に及ぼす効果に着目し，4種類の羅布麻葉水エキスの高cholesterol投与ラットに及ぼす効果を検討した。

【方法】 Wistar系雄性ラット（体重約170 g）を用いた。日本クレア製粉末飼料（CE-2）に1.0％ cholesterol，0.5％ cholic acidを加え，高cholesterol食を作製し，pair feeding scheduleで飼育した。羅布麻水エキスは産地が異なる中国産羅布麻葉2種類（I，III），中国産茶（II），日本産茶（IV）を実験開始20日までは35 mg/ラット，その後実験終了40日までは70 mg/ラットをそれぞれ連日投与した。各種cholesterolは比色法で定量した。なお動脈硬化指数（atherogenic index；A.I.）はT. Chol.とHDL-Chol.から算出した。

【結果】 高cholesterol食を投与したラットでは血清T. Chol.，LDL-Chol.の上昇，HDL-Chol.の低下をきたし，高cholesterol血症を呈していた。これに対し中国産茶（II）と日本産茶（IV）はこれらcholesterol成分をいずれも改善し，A.I.も低下した。しかし羅布麻葉（I）はLDL-Chol.の低下，羅布麻葉（III）ではLDL-Chol.の低下とHDL-Chol.の上昇とA.I.の改善効果を示した。肝組織中のT. Chol.は4種類の羅布麻水エキスいずれにおいても低下作用を示した。

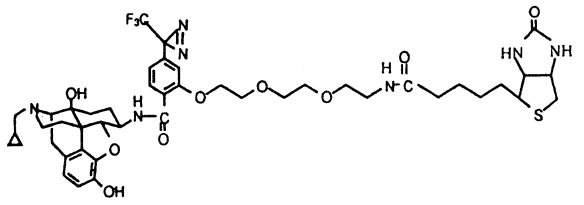
【結論】 羅布麻の葉より培煎した茶において高cholesterol血症低下作用が顕著であった。

7）我妻卓司，中村憲夫，手塚康弘，畑中保丸：光アフィニティーピオチン化（IV）NG108-15細胞を用いたδ-オピオイドレセプターの光アフィニティーラベル．日本薬学会第116年会，1996，3，金沢．

【目的】 光アフィニティーラベルにより，レセプターの薬物結合部位にビオチン基を導入する方法（光アフィニティーピオチン化）は，ラベル生成物の検出，単離

にアビジン－ビオチン系を利用できる点で，通常の光アフィニティーラベル法よりも有利である。我々は，この方法をオピオイドレセプターの薬物結合部位解析に応用する目的で，光反応性ビオチン化ナルトレキソン誘導体を合成した[1]。今回，この試薬を用いてNG108-15細胞に対する実験を行った。

【実験結果】 光アフィニティービオチン化試薬は，μ, δ, κいずれのレセプターに対しても良い親和性を有する[1]。これらのうち，δ-レセプターのみが存在し，大量のレセプター標品を得るのに適したNG108-15細胞を用いて実際の光ラベル実験を検討する方針とし，まず試薬の親和性を測定した。[^{3}H]p-Cl-Phe-DPDPEに対する阻害定数(Ki値)は，ラット脳δ-レセプターで得られた値と同じ10^{-9} Mオーダーとなり，この培養細胞に対しても高い親和性を有することが確認された。さらに，この試薬を利用したアフィニティーカラム，光ラベルも検討したので合わせて報告する。



【結論・考察】 ビオチン化ナルトレキソン誘導体は，NG108-15細胞中の，δ-レセプターに対して強い親和性を有するため，このレセプター薬物結合部位の解析に有用な光ラベル試薬と考えられる。

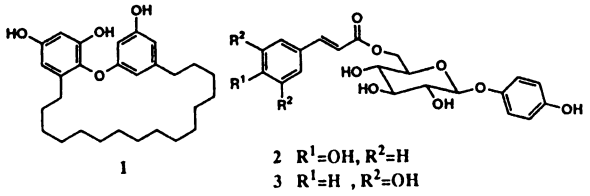
【文献】

1) 畑中保丸　他，日本薬学会第115年会講演要旨集，No.2, p.275

8) Sayed Ahmed Amany, 中村憲夫, 服部征雄, El-Emary Nasr：エジプト産生薬 *Grevillea robusta* 葉の成分研究. 日本薬学会第116年会, 1996, 3, 金沢.

【目的】 天然物由来のエイズ治療薬開発を目的とした研究の一環として，今回，エジプト産生薬 *Grevillea robusta* の乾燥葉のメタノール抽出エキスについての成分研究を行い3種の新フェノール性化合物を単離，構造決定したので報告する。

【実験・結果】 *G. robusta* の乾燥葉2.75 kgをメタノールで抽出し，得られたエキス(388 g)をヘキサン，クロロホルム，酢酸エチル，ブタノール可溶部についてシリカゲルクロマトおよび逆相分取HPLCを繰り返し，新化合物1を含む12種の化合物を単離した。ブタノール可溶部はセファデックスLH-20，シリカゲル，逆相クロマトを行い新化合物2種(**2, 3**)を含む4種の化合物を単離した。これら新化合物の構造は各種スペクトルデータから下のように決定した。



1

2 R^1=OH, R^2=H
3 R^1=H , R^2=OH

9) 横澤隆子，大浦彦吉，鄭　海泳，阪中専二，金　武祚：慢性腎不全ラットにおける緑茶タンニンの有用性. 第50回日本栄養・食糧学会大会, 1996, 4, 京都.

【目的】 慢性腎不全はいったんその病態が確立されるとそれは不可逆性で，程度の差はあれ進行性の経過をとることが知られ，最終的には腎機能の廃絶に至る。この進行性の経過をとる機序については未だに完全には解明されていないが，そのほとんどが糸球体障害によることが指摘されている。先に糸球体障害で見られるメサンギウム細胞の増殖を緑茶タンニンが抑制する知見を示したので，本研究では非炎症性の腎不全モデルとして糸球体障害の発症，進展の機序を解明するうえで重要なモデルとされている腎摘ラットを用い検討した。

【方法】 Wistar系雄性ラット(体重200 g前後)を用いた。左腎の1/2を部分摘除し，その2週間後に右腎を全摘した。約10日後にBUN値を測定し，3群に分けた。1群には水を与え，他の2群には緑茶タンニンをそれぞれ10, 20 mg/kg体重/日を80日間経口投与し，血中，尿中成分，腎組織中のラジカル消去酵素活性を測定した。

【結果】 腎摘ラットではBUN, Crの増加, Ccrの低下，尿蛋白の増加をきたしていたが，緑茶タンニン投与群ではこれらパラメーターがいずれも改善し，腎不全の進行を抑制する知見が得られた。また腎実質を部分切除した場合，残存腎ではSOD, catalase, GSH-Pxの活性低下をきたしていたが，緑茶タンニンはこれら酸化的ストレス状態にある腎を是正する作用を有していることが示された。

【結論】 糸球体障害性の腎不全に緑茶タンニンが有用であることを，実験的に明らかにした。

10) 家永和治，中村　耕，三上博輝，横澤隆子，大浦彦吉：NZ-419の慢性腎不全ラットに及ぼす効果：5/6腎摘並びにアデニン負荷ラットを用いた

検討．第39回日本腎臓学会学術総会，1996，5，倉敷．

【目的】 発症機序の異なる動物モデルを用いてNZ-419が慢性腎不全の進行を抑制するかどうか検討した。

【方法】 (1) 5/6腎摘ラットにNZ-419 (0, 80, 160 mg/kg/日)を投与し，経時的にBUN, 尿蛋白を，最終日90日目に血清，尿中成分，腎血流量を測定し，腎組織の形態学的所見を観察した。(2) アデニン負荷と同時にNZ-419 (0, 250, 500, 1000 mg/kg/日) を24日間投与し，血清，尿成分の測定とCcrの算出を行った。

【結果】 (1) 5/6腎摘ラット：NZ-419投与によりBUN, 尿蛋白の増加が有意に抑制され，腎血流量の低下も有意に抑制された。又，糸球体，尿細管病変の緩和が観測された。(2)アデニン負荷ラット：NZ-419投与によりCcrの低下が有意に抑制され，血中，尿中成分の改善も認められた。特に，尿毒症毒素で活性酸素のマーカーのMG, GSAの増加を著しく抑制した。

【結論】 NZ-419が慢性腎不全の進行を抑制する可能性が示唆された。又，アデニン負荷モデルではMGやGSAの増加を顕著に抑制していることから，進行抑制の機序の1つに活性酸素の消去が関与しているものと考えられた。

11) 横澤隆子，大浦彦吉，河合悦子，玄番宗一：培養腎上皮細胞LLC-PK1における漢方方剤温脾湯の関与．第39回日本腎臓学会学術総会，1996，5，倉敷．

【目的】 大黄を主剤とする温脾湯は中国唐代の“備急千金要方”に記載され，中医学では中程度の慢性腎不全患者の治療に経験的に用いられている方剤である。その作用機序について，筆者らはすでに腎不全ラットを用い究明しているが，本研究では腎細胞に及ぼす影響を検討した。

【方法】 LLC-PK_1細胞を培養，コンフルエンスに達してから温脾湯エキス並びにその構成生薬エキスを添加した(最終濃度2.5～125 μg/ml)。48時間培養後，培養液中のLDHの遊離量と過酸化脂質量 (TBARS) を測定した。

【結果】 細胞から培養液中へのLDH遊離の抑制が温脾湯エキス添加群で用量依存的に認められた。構成生薬では大黄において最も強い作用を発揮し，次いで甘草，薬用人参，乾姜，附子の順に認められたが，TBARSもLDHの変化と類似していた。

【結論】 培養腎上皮細胞株LLC-PK_1における保護作用が温脾湯に認められ，これら作用は構成生薬の複合によるものと考えられた。

12) Park, Jong C., Yu, Young B., Lee, Jong H., Hattori, M., Lee, Chung K., Dhoi, Jong W.: Protective Effect of *Oenanthe javanica* on the Hepatic Lipid Peroxidation Induced by Bromobenzene. The 37th Annual Meeting of the American Society of Pharmacognosy, 1996, 7, Santa Cruz, USA.

The aerial parts of *Oenanthe jauanica* Dc. have been used for treatment of jaundice and hypertension in Korean folklore medicine. To inveatigate detoxification of bromobenzene-induced hepatic lipid peroxidation by title plant, hepatic lipid peroxide level and the activities of enzymes responsible for production and removal of epoxide were studied. The level of lipid peroxide elevated by bromobenzene significantly reduced by methanol extract and persicarin isolated from this plant. The extract and persicarin did not affect the activities of aminopyrine *N*-demethylase, aniline hydroxylase, glutathione S-transferase. Epoxide hydrolase activity was decreased significantly by bromobenzene, which was restored to the control level by pretreatment of persicarin. However, the pretreatment of another components, isorhamnetin and hyperoside did not change the enzyme activity as well as lipid peroxide level. The results suggest that the reduction of bromobenzene-induced hepatic lipid peroxidation by this plant is thought to be due to enhancing the activity of epoxide hydrolase, an enzyme removing bromobenzene epoxide.

13) Park, Jong C., Miyashiro, H., Hattori, M., Yoshida, T.: Screening of Korean Plant Extracts for Inhibitory Effects on Human Immunodeficiency Virus Type 1 Protease. The 37th Anual Meeting of the American Society of Pharmacognosy, 1996, 7, Santa Cruz, USA.

For the purpose of developing new anti-HIV agents from natural sources, various Korean plant extracts were screened for inhibiory effects on HIV-1 protease. The screening assay was measured using His-Lys-Ala-Arg-Val-Leu-(pNO_2-Phe)-Glu-Ala-NIe-Ser-NH_2 as a substrate. Of medicinal plants tested, methanolic extracts of *Camellia japonica*, *Alisma plantago-aquatica* var. *orientale* and *Cedrela sinensis* were shown to have most potent inhibitory

慢性腎不全患者の予後は，透析療法の確立と医療技術の進歩により飛躍的に改善された。しかし維持透析を続けるための患者の精神的，肉体的負担は大きく，また透析患者の増加とともに経済的側面など，社会的な問題も噴出してきている。このような状況のもと，腎不全の進行を抑制し，人工透析への移行を遅らせるための治療薬の開発が要望されている。

中医学では，「扶正降濁」法が慢性腎不全を治療する良い方法であることが経験的に知られている。扶正降濁作用を有する代表的な漢方方剤として温脾湯が挙げられ，腎不全患者の治療に比較的良い改善効果が示され，現在，中国では中程度の慢性腎不全患者の治療に汎用されている方剤の一つである。

筆者らは，臨床的にその有効性が認められている温脾湯の科学的根拠を実験的に明らかにするため，慢性腎不全モデル，培養細胞等を用いた *in vitro* 評価系を駆使して検討し，温脾湯による腎不全改善作用は大黄を基盤として薬用人参，甘草，乾姜，附子による総合作用であることを証明したので，これらについて報告する。

19）横澤隆子，大浦彦吉，柴田　透，金子　稔，長谷川眞常：透析患者における緑茶タンニン投与の影響．第13回和漢医薬学会大会，1996，8，富山．

【目的】 わが国の維持透析患者数は年々増加し，この患者数増加の要因としては透析学の進歩により維持透析患者の生命予後が向上したことが挙げられているが，一方，新たに透析療法を導入せざるをえない末期腎不全患者の発生が後を絶たないことも見逃せない。筆者らは慢性腎不全の薬物療法の有用性を和漢薬に求め，その効果，作用機序，有効成分等について研究を進めてきたが，腎不全では酸化的ストレス状態にあることから，Cr 酸化物の creatol の発見を契機として，Cr 酸化機構の解明に着手し，加えてタンニン含有生薬に抗酸化活性を有することを明らかにした。このようなタンニンは茶葉にも多量に含有することから，緑茶タンニンの腎に対する作用を明らかにした。[1,2] このような基礎研究に立脚し，本研究では酸化亢進状態にある透析患者に投与し，その有用性について検討した。

【対象と方法】 長谷川病院通院の慢性血液透析患者 50 例（男性 37 例，女性 13 例）で，平均年齢は 50.4 歳（27～76 歳），平均血液透析期間は 71.6 ケ月（5～183ケ月）であった。慢性腎不全の原疾患は慢性糸球体腎炎が最も多く 31 例で，次いで嚢胞腎 6 例，悪性高血圧 3 例，その他巣状糸球体硬化症，糖尿病性腎症，ネフローゼ症候群，痛風腎などで，週 2 ないし 3 回の血液透析を受けていた。なお手根管症候群やアミロイド関節症を合併した場合には高性能膜透析を行ったが，普通は非高性能膜透析を行った。緑茶タンニンはゼリーに混合したものを 1 回 200 mg，1 日 2 回投与したが，患者の嗜好の変化を考慮し，カプセルに詰めて投与したケースもあった。採血は 1 ケ月毎に，透析直前に行った。

【結果】 緑茶タンニン（1 日 400 mg）を 6 ケ月間用いたところ，血中 Cr, MG レベルの低下とともに，MG/Cr 比の低下を認め，また β_2-MG の低下と一部の患者で関節痛の改善効果を示した。

【結論】 以上の成績は緑茶タンニンが透析患者の酸化亢進状態を改善する可能性を示唆するものである。

1）Yokozawa T., *et al.*: Biosci. Biotech. Biochem., 58, 855-858 (1994).
2）Yokozawa T., *et al.*: Biosci. Biotech. Biochem., 60, 1000-1005 (1996).

20）横澤隆子，劉　中武，董　而博，織田　聡，服部征雄：腎虚血－再灌流モデルを用いた大黄牡丹皮湯の効果．第13回和漢医薬学会大会，1996，8，富山．

【目的】 虚血に陥った組織への血流再開により，虚血時に加え，さらに新たなる障害が負荷され，その障害のメディエーターとして，再灌流時に供給される酸素分子に由来するフリーラジカルが注目され，その消去系あるいは産生系からの解明が進められている。本研究では代表的な駆瘀血作用を有する構成生薬からなる大黄牡丹皮湯の効果を検討した。

【方法】 1．動物実験：Wistar 系雄性ラット（6 週齢，体重 150 g 前後）に各エキス剤（大黄牡丹皮湯，大黄，牡丹皮，桃仁）を 200 mg/kg B.W./day 経口投与した。20 日後に左腎動･静脈，尿管を 45 分間遮断，その後 2 時間再灌流し，腹部下大動脈から採血した。次いで氷冷した生理食塩水で灌流後，腎臓を採取し，各種血清成分，腎組織中のラジカル消去酵素活性を測定した。
2．細胞培養実験：LLC-PK_1 細胞を D-MEM/F-12 に 5 % FCS を添加した培地で培養し，コンフルエンスに達してから細胞を 1 穴あたり 10^4 個ずつ seed した。2 時間後にサンプルを添加し，41 時間培養した。次いで低酸素下（酸素濃度 2 % 未満）で 6 時間培養後，通常の条件下（95 % air, 5 % CO_2）で 1 時間培養することで再酸素化とした。また通常の条件下のみで 48 時間培養した群も設けた。細胞障害の指標として培地中へ漏出した LDH 活性を測定した。

【結果】 大黄牡丹皮湯群では BUN, Cr が低下し，血清，腎組織中の MDA も低下した。抗酸化酵素では GSH-Px が有意に上昇し，構成生薬の中では大黄において類似した作用が認められた。LLC-PK_1 細胞を用

いた実験でも大黄牡丹皮湯と大黄に細胞障害を抑制する知見が認められた。

【結論】 大黄牡丹皮湯に酸素ストレスに対する抑制作用が認められた。

21) 武藤靖子，何　立群，横澤隆子，鄭　平東，服部征雄：温脾湯と大黄の腎における役割．第13回和漢医薬学会大会，1996，8，富山．

【目的】 大黄を主剤とする温脾湯は中国唐代の“備急千金要方”に記載され，中医学では中程度の慢性腎不全患者の治療に経験的に用いられている。筆者らはこれまで実験的にその作用機序を解明するために，アデニン誘発慢性腎不全ラットを用い温脾湯並びにその構成生薬について検討してきた。[1-3] 本研究では非炎症性の腎不全モデルとして糸球体障害の発症，進展の機序を解明するうえで重要なモデルの腎摘ラットを用い，温脾湯とその構成生薬の大黄について検討するとともに，糸球体構成細胞のメサンギウム細胞（MC）に及ぼす効果について検討した。

【方法】 Wistar 系雄性ラット（体重 200 g 前後）の左腎の 2/3 を部分摘除し，その 2 週間後に右腎を全摘した。約 10 日後に BUN 値を測定し，5 群に分けた。1 群には水を与え，他の群には温脾湯，大黄（62.5，125 mg/kg 体重/日）を 80 日間経口投与した。BUN 値は 20 日ごとに，他の血中成分，尿中成分は 80 日目に測定した。MC はマウス腎から Iwano らの方法に従って採取し，各種濃度のサンプルを添加し，^{3}H-thymidine uptake による増殖活性を測定した。

【結果】 腎摘ラットでは BUN, Cr, P の増加，Ca, 総蛋白，アルブミンの低下，尿蛋白の増加をきたしていたが，温脾湯エキス投与群ではこれらパラメーターがいずれも改善し，腎不全の進行を抑制する知見が得られた。このような作用は大黄エキスでも認められたが，温脾湯よりはやや弱い傾向にあり，他の構成生薬の関与が示唆された。また，MC の増殖抑制活性は温脾湯では 25 μg/ml から，大黄では 6.25 μg/ml から認められた。

【結論】 糸球体障害性の腎不全に温脾湯並びに大黄が有用であることを，腎摘ラットを用いて明らかにし，これら作用に MC の関与が示唆された。

【文献】

1）大浦彦吉ら：和漢医薬学会誌，**1**, 209-217 (1984).

2）大浦彦吉ら：和漢医薬学会誌，**2**, 351-356 (1985).

3）Yokozawa T. *et al.*: J. Med. Pharm. Soc. WAKAN-YAKU, **6**, 64-69 (1989).

22) 金　東郁，横澤隆子，服部征雄，門田重利，難波恒雄：酸化 LDL に対する羅布麻（*Apocynum venetum* L.）の効果．第13回和漢医薬学会大会，1996，8，富山．

【目的】 粥状動脈硬化において血管壁に沈着するコレステロールは主に LDL であるが，LDL が何らかの修飾または変性を受け，スカベンジャー受容体に認識されるようになってはじめて泡沫化をきたすことから，近年，酸化的変性を受けた LDL（酸化 LDL）が注目されている。本研究では高コレステロール血症低下作用を有する羅布麻葉[1] の酸化 LDL に及ぼす効果を検討した。

【方法】 羅布麻（*Apocynum venetum* L.）は中国の山東省で採取した。2 種類の未焙煎葉（羅布麻 I，II）と羅布麻 II を 1 回焙煎した羅布麻 III，羅布麻 III をさらに焙煎した羅布麻 IV の 4 種類の水エキスを用いた。LDL はラット（Wistar 系雄性，10 週齢）並びに健康な男性（30 歳代）の血清より超遠心法にて分離し，次いで 0.15 M NaCl 溶液（pH 7.4，4°C）で 48 時間透析した。酸化 LDL は Kuzuya らの方法に従い，透析した LDL に 10 μM $CuSO_4$ と羅布麻エキス（0.25，1 mg/ml）を添加して，37°C で 4 時間インキュベーション後，生成したチオバルビツール酸反応物質（TBARS）を Naito らの方法で測定した。

【結果】 ラットから分離した LDL を $CuSO_4$ とインキュベーションすると，TBARS 量が約 7.8 倍増加した。ところが羅布麻水エキスを 0.25 mg/ml 添加した場合，TBARS 量は著しく減少した。特に羅布麻 I において顕著であった。添加量を 1 mg にした場合，羅布麻 III においてさらに TBARS 量が減少した。ヒト LDL を用いた実験においてもラット LDL と類似した作用を認めたが，羅布麻 III でやや作用が強かった。

【結論】 粥状硬化の初期病変の形成に重要な役割を果たしている酸化 LDL の生成を羅布麻葉水エキスが抑制した。これら作用は焙煎を施した羅布麻において強い傾向を示した。

【文献】

1）金　東郁ら：日本薬学会第 116 年会，講演要旨集 2, p.192 1996.

23) Baek, B.S., Paik, K.J., Shim, K.H., Chung, H. Y., Yokozawa, T., Oura, H.: The active sites of antioxidative action of magnesium lithospermate B from Salviae Miltiorrhizae Radix. 第13回和漢医薬学会大会，1996，8，富山．

【Purpose】 Natural compounds, phenolic derivatives and flavonoids have antioxidant action, and can function as free radical terminators and often, as metal chelators. In this study, we attempt to

examine an antioxidant isolated from naturally occurring herb medicine. A phenolic compound, magnesium lithospermate B (MLS), was isolated and identified as a major biologically active component of an herbal material called Saviae Miltiorrhizae Radix (SMR). The present study was carried out to elucidate the active sites of antioxidative action of MLS.

【Method】 MDA was determined by TBA method.

【Results and Conclusion】 Antioxidative effects of the caffeic acid isolated from Salviae Miltiorrhizae Radix, its dimer (trans-rosmarinic acid), trimer (lithospermate A), and tetramer (lithospemate B) were studied in mouse liver homogenate. Caffeic acid inhibited *in vitro* lipid peroxidation induced by H_2O_2 and $FeSO_4$ to 70 % of the control values. To elucidate the structural requirement, essential functional groups of MLS were investigated by modifying the structure. The derivatives, which changed structure at dihydroxyphenyl moiety of caffeic acid, ferulic acid, 3,4-dimethoxycinnamic acid, and cinnamic acid, showed a decreased antioxidative effect compared to that of caffeic acid. In addition, caffeic acid derivatives which have changed structure at side chain part, p-hydroxyphenylpropionic acid, p-hydroxyphenylacetic acid, p-hydroxybenzoic acid, and hydrocinnamic acid also have less antioxidative effects than that of caffeic acid. These results suggest that dihydroxyl group caffeic acid and the double bond of its side chain an essential role in its antioxidative action.

24) Shim, K. H., Song, S. H., Huh, J. I., Chung, H. Y., Yokozawa, T., Oura, H.: Regulation of PGI2/TXA2 by phenolic acids from Salviae Miltiorrhizae Radix. 第13回和漢医薬学会大会, 1996, 8, 富山.

【Purpose】 Magnesium lithospermate B from an aqueous extract of Salviae Miltiorrhizae Radix has been reported to enhance renal function through prostaglandin system. However, effects of their derivatives on protaglandin system is unknown. The present study has been designed to examine the effects of magnesium lithospermate B (MLS), lithospermate B (LSB), lithospermate A (LSA) and trans-rosmaric acid (RMA) on PGI_2 and TXA_2 synthesis and their action mechanism of increased PGI_2 synthesis.

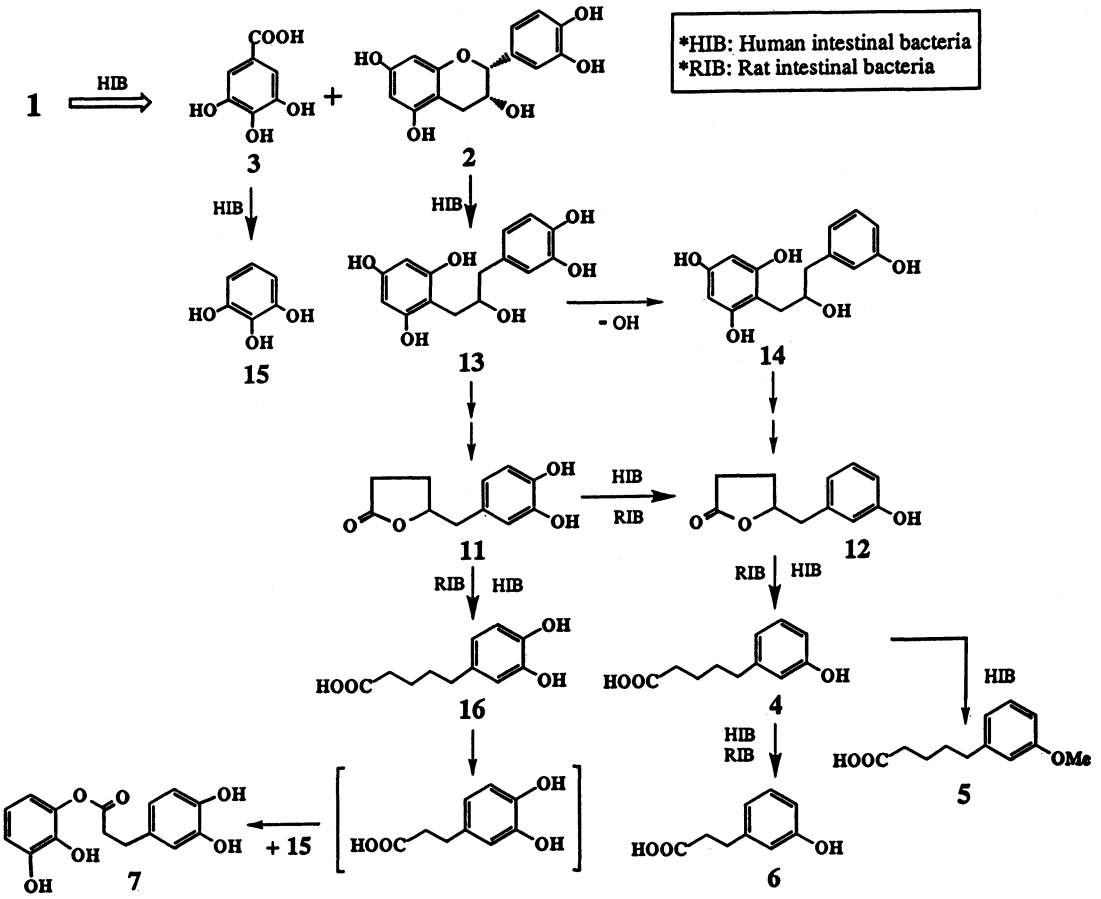
【Method】 Phenolic acids dissolved in saline was injected intraperitoneally into ICR mice at a dose of 5 or 15 μl/kg, while control mice were treated with an equal volume of saline. 6-Keto-PGF1α and TXB_2 were measured by EIA.

【Results and Conclusion】 MLS, LSB, LSA and RMA significantly increased serum 6-keto-PGF1α level, while they didn't change TXB_2 level. Especially, their action mechanism of LSA and RMA were investigated because LSA and RMA were most effective for PGI_2 synthesis. LSA and RMA slightly increased constitutive cyclooxygenase I, whereas they slightly decreased cyclooxygenase II activity. They didn't change PG endoperoxidase activity by estimating cooxygenation of diphenylisobenzofuran, whereas they increased GSH and GSH S-transferase activity which might be alternative pathway of PGH_2 synthesis. These results suggest that LSA and RMA stimulate PGI_2 synthesis through the stimulation of GSH S-transferase and constitutive cyclooxygenase I without change of PG endoperoxidase activity.

25) Meselhy R. Meselhy, 中村憲夫, 服部征雄：ヒト腸内細菌による (-)-epicatechin 3-O-gallate の代謝. 日本生薬学会第43回年会, 1996, 9, 東京.

【Introduction】 (-)-Epicatechin 3-O-gallate (**1**) is a main component of rhubarb tannins. Oral administration of an aqueous rhubarb extract proved effective to decrease the uremic toxins which are responsible for renal failure. Through extensive studies on rhubarb and its tannins, **1** was found to contribute mainly to the antiuremic action of the rhubarb extract. Metabolic studies of related phenolic compounds, such as (-)-epicatechin (**2**) and (+)-catechin, have been carried out in rats and guinea pigs. However, the metabolic fate of **1** in the human intestine has not yet been reported. The present report deals with the biotransformation of **1** using a human fecal suspension.

【Method】 Anaerobic incubation of **1** and related compounds with a human fecal suspension or a rat fecal suspension was carried out in 0.5 M K-phosphate (pH 7.25) in an anaerobic incubator for 24 and 48 h. The products were purified by filtration through Diaion HP-20, followed by elution with



MeOH. Repeated CC of the MeOH eluates afforded 15 metabolites.

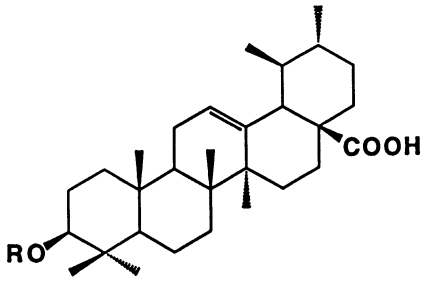
【Results】 Using various spectroscopic means, 13 metabolites were identified, and other metabolites are still under investigation. Possible metabolic pathway of **1** could primarily speculated as shown in the above chart.

26）宮代博継，林　隆幸，馬　超美，垣内信子，服部征雄，下遠野邦忠：C型肝炎ウイルスプロテアーゼ阻害剤の探索 II. 和漢薬エキスおよびその成分について．日本生薬学会第43回年会，1996，9，東京．

【目的】 日本における肝炎のほとんどはウイルス性であると言われているが，その中でもC型肝炎は慢性化率および肝硬変，肝癌への移行率が非常に高い。しかし，現在行なわれているインターフェロン療法では，約半数の患者しか治癒できない現状である。よって，C型肝炎治療において，抗ウイルス薬の開発は最重要課題である。すでに我々は，パナマ産生薬エキスおよびある種のジテルペノイドについてC型肝炎ウイルス（HCV）プロテアーゼの阻害効果について検討してきた。[1] 同様の方法により阻害剤の探索を進め，C型肝炎治療薬開発にむけてのリード化合物を見いだすことを目的とした。

【実験】 21種類の和漢薬から水エキスを熱時抽出により調製した。さらに鎖陽（*Cynomorium songaricum* Rupr.）からは，シリカゲルカラムクロマトグラフィーにより，ursolic acid, 3-*O*-acetyl ursolic acid, β-sitosterol, β-sitosteryl glucoside, β-sitosteryl glucoside 5'-palmitateの5種の既知化合物および1種の新規化合物，ursolic acid propanedioic acid monoester（UPM）を単離し，NMRおよびマススペクトルにより化学構造を決定した。HCVプロテアーゼは，遺伝子工学的に調製したリコンビナント蛋白質を，HCVプロテアーゼの基質は，N末端にダンシル基をもった17量体の合成ペプチドを使用した。阻害率の計算は，反応混合物のHPLCによる定量分析から行った。

【結果・考察】 和漢薬エキスの中では，桂枝エキスと鎖陽エキスに比較的強い阻害活性が認められた。鎖陽成



R=H : Ursolic acid
R=$COCH_3$: 3-*O*-Acetyl ursolic acid
R=$COCH_2COOH$: Ursolic acid propanedioic acid monoester (UPM)

分の中では，UPMに最も強い阻害活性が観察された。UPMはエイズウイルス（HIV）プロテアーゼ（アスパラギン酸プロテアーゼ）に対してはそれほど強い阻害活性は示さないことから，HCVプロテアーゼ（セリンプロテアーゼ）にやや特異的な阻害剤であると考えられる。さらに，反応速度論的解析から，HCVプロテアーゼに対するUPMの阻害様式を検討した。

【文献】

1）日本生薬学会第42回年会（福山）講演要旨集，p.200（1995）

27）横澤隆子，董　而博，大浦彦吉，河合悦子，玄番宗一：Magnesium lithospermate Bはcisplatinによる腎細胞障害を軽減する．第8回腎とフリーラジカル研究会，1996，9，東京．

【目的】抗悪性腫瘍薬cisplatinによる腎障害に，ラットのみならずインビトロ実験においても，フリーラジカルの関与が示唆されている。本研究では，尿細管障害モデルのアデニン誘発腎不全ラットの障害を緩和するmagnesium lithospermate B（MLB）が，[1,2] cisplatinによる腎細胞障害をも軽減出来るか否かについて，培養腎上皮細胞を用い検討した。

【方法】LLC-PK_1細胞をDMEM/F-12に5％FCSを添加した培地で培養し，コンフルエンスに達してから1穴あたり10^4個ずつ細胞をseedした。2時間後にcisplatin, MLBの順に添加し，48時間培養した。細胞障害の指標として培養液中へ漏出したLDH活性を測定した。

【結果】培養液中にcisplatinを添加すると，細胞から培養液中へのLDH遊離の増大（275 mIU/ml前後）がみられた。このcisplatinによる障害は，MLB 2.5 μM添加群では影響されなかったが，12.5 μM添加群では231.5 mIU/mlと有意に抑制され，この作用は添加量の増加とともに著しく，125 μM添加群では128.5 mIU/mlと，cisplatinを添加しない場合のLDH遊離（116.6 mIU/ml）に匹敵する値にまで低下させた。

【結論】MLBは，濃度に依存してcisplatinによる細胞障害に対する軽減作用を示した。

【文献】

1）横澤隆子等：和漢医薬学雑誌，11，277-281（1994）.
2）横澤隆子等：臨牀透析，11，95-99（1995）.

28）Jong-Cheol Park, Jong-Ho Lee, Masao Hattori, Jong-Won Cho : Effects of Methanol Extract and Persicarin of *Oenanthe javanica* on the Hepathotoxicity Induced by Bromobenzene in Rat. IUFoST '96 Regional Symposium on Nonnutritive Health Factors for Future Foods, 1996, 10, Seoul.

The effects of the methanol extract from *Oenanthe javanica* Dc., and the components isolated from this plant, isorhamnetin, hyperoside and persicarin on the hepatic lipid peroxidation induced by bromobenzene were studied. Methanol extract and persicarin significantly reduced the level of lipid peroxide induced by bromobenzene. Pretreatment of the extract or constituents did not influence the activies of aminopyrine N-demethylase, aniline hydroxylase, glutathione S-transferase. Epoxide hydrolase activity was decreased significantly by bromobenzene, which was restored to the control level by pretreatment of persicarin. However, the identical pretreatment of isorhamnetin and hyperoside did not change the enzyme activity. The results suggest that the reduction of bromobenzene-induced hepatic lipid peroxidation by *O. javanica* unde our experimental conditions is thought to be due to enhancing the activity of epoxide hydrolase, an enzyme removing bromobenzene epoxide.

29）Yokozawa, T.: Increase of active oxygen in rats after nephrectomy is suppressed by ginseng saponin. '96 Korea-Japan Ginseng Symposium, 1996, 10, Seoul.

30）Masao Hattori : Traditional Medicine as a Source of Anti-HIV Agents. Symposium of the 2nd Meeting of the Chinese Doctor's Forum of New Medicine, 1996, 10, Beijing.

Since the first patient having an acquired immunodeficiency syndrome (AIDS) was reported in 1981 in the United States, the numbers of AIDS

patients and human immunodeficiency virus (HIV) infected persons increased all over the world. WHO reported that 21,000,000 persons were estimated to be infected by HIV in 1995. For treatment of this disease, many attempts have been carried out but no specially effective drugs are available at present, except for some nucleoside derivatives and peptide analogues.

For the purpose of developing new anti - HIV agents from traditional medicines, various extracts of crude drugs were screened for inhibitory effects on HIV - reverse transcriptase, HIV - protease, growth of HIV and giant cell formation. Some medicinal plant extracts were found to inhibit the viral specific enzymes, the growth of HIV and giant cell formation. The present results show a possiblity of some traditional medicines as anti-HIV drugs in the prevention and therapy of AIDS.

31) Che Qing Ming, Huang Xin Li, Zhao Yu Ying, Masao Hattori, Tsuneo Namba : A Novel *C*-Glucosyl Quinochalcone from *Carthamus tinctorius* petals. Symposium of the 2nd Meeting of the Chinese Doctor's Forum on New Medicine, 1996, 10, Beijing.

The flower petals of *Carthamus tinctorius* L. (Compositae) provide one of the most important drugs in traditional Chinese medicine, used for the treatments of gynecological diseases, heart diseases, and inflammation.[1] We reported several new flavonoids and phenolic compounds[2] from the flower petals of *C. tinctorius,* some of which inhibit Na^+-K^+ ATPase activity.[3] A continuation of these studies has led to the isolation of the novel *C*-glucosyl quinochalcone. carthamoside from a BuOH - soluble fraction of the petals of *C. tinctorius.* Carthamoside was obtained as an amorphous yellow powder, its HRFABMS indicated that it had the molecular formula $C_{27}H_{29}NO_{13}$: and its structure was determined by chemical and spectrographic methods including 2D-NMR. We here describe the isolation and structure elucidation of this compound. Literature Cited.

1) Jiang Su New Medical College, "Dictionary of Chines Materia Medica", Publishing House of Science and Technology of Shanghai, Shanghai, 1995 : 992.

2) Hattori, M., Huang XL, Che QM, Kawata, Y, Tezuka, Y, Kikuchi, T, and Namba, T. *Phytochem.* 1992, 31 : 4001.

3) Huang XL, Hattori, M. and Namba, T. *Shoyakugaku Zasshi* 1992, 46, 901 : 210.

32) Che Qing Ming, Huang Xin Li, Zhao Yu Ying, Teruaki Akao, Kyoichi Kobashi, Masao Hattori, Tsuneo Namba : Inhibitory Effects of Components of *Carthamus tinctorius* on 3α-Hydroxysteroid Dehydrogenase of Rat Liver Cytosol. Symposium of the 2nd Meeting of the Chinese Doctor's Forum on New Medicine, 1996, 10, Beijing.

The flower petals of *Carthamus tinctorius* have been commonly used for treatments of women's diseases in traditional Chinese medicine. In resent years, the drug is also used as a remedy of diseases for cardiovascular system and as an anti-inflammatory agent.

Penning and Talalay have reported that a good correlation between the concentration of anti - inflammatory agents required to inhibit 3α - Hydroxysteroid Dehydrogenase (3α - HSD) and human anti-inflammatory doses.

The flower petals of *C. tinctorius* were examined for their inhibitory effects on 3α-HSD of rat liver cytosol, and we found that a methanolic extract of the flower petals of *C. tinctorius* has an inhibitory effect on 3α-HSD of rat liver cytosol. The extract then was purified by various chromatographic techniques for looking for the anti-inflammatory components. In the course of our study, except well-known pigments, carthamin, safflor yellow, reported from the flower petals of *C. tinctorius.* we isolated and identified 20 of compounds including six new flavonoids, some of which showed potent inhibition against 3α-HSD ($IC_{50} \times 10^{-6}M \sim 10^{-5}M$). The flavonoid glucosides and some phenolic compounds isolated from *C. tinctorius* did not show appreciable inhibitory actions on 3α-HSD comparing with their aglycones. We will describe the inhibitory actions of these compounds obtained from the flower petals of *C. tinctorius* on 3α-HSD of rat liver cytosol.

33) Cai Baochang, Kurokawa Masahiko, Kadota Shigetoshi, Hattori Masao, Namba Tsuneo, Shiraki Kimiyasu : Antiviral Strychnos Alka-

loids from the Processed and Unprocessed Seeds of *Strychnos nux-vomica* against Herpes Simplex Virus (HSV-1), Poliovirus and Measles Virus *in vitro* and Their Therapeutic Efficacies for HSV-1 Infection in Mice. Symposium of the 2nd Meeting of the Chinese Doctor's Forum on New Medicine, 1996, 10, Beijing.

Eight crude strychnos alkaloid fractions from seeds of *Strychnos nux-vomica* processed or unprocessed with various traditional processing methods and fourteen pure strychnos alkaloids from the fractions were examined for antiviral activity against herpes simplex type 1 (HSV-1), measles virus and poliovirus type 1 by using plaque reduction assay. For eight crude strychnos alkaloid fractions, the effective concentrations for 50 % plaque reduction (EC_{50}) for HSV-1 and measles virus were 60.5-88.3 and 64.7-74.9 μg/ml, respectively The EC_{50} values for poliovirus was 13.2-35.4 μg/ml. These EC_{50} values were 1.3 to 11.0 folds higher than the concentrations reducing cell viability by 50 % (CC_{50}). Although antiviral activity of the unprocessing (A) was similar to that of the processing (B) *in vitro,* the cytotoxicity of the former was 1.55 and 2.12 folds stronger than that of the latter. Strychnine and brucine are two main components in *S. nux-vomica,* in which the IC_{50} values inhibiting the plaque formation of three viruses were differently 244.8, 138.2, 63.5 μM by strychnine, and 212.0, 150.0, 40.1 μM by brucine, but selectivity index of brucine was 1.5-2.1 folds of that of strychnine.

Two crude strychnos alkaloid fractions from the processed seeds with a sand bath and unprocessed seeds were further examined for their therapeutic efficacies of HSV-1 infection in mice. In the first experiment, the dose of 1.5 mg/kg was found to be significantly effective in decreasing mortality of HSV-1 virus infected mice ($p < 0.05$ *vs.* control group). In repeat experiment, 1.5 mg/kg of the crude strychnos alkaloid fractions from the processed seeds were found to be significantly effective in prolonging the development of skin lesions and/or in prolonging the mean survival times of HSV-1 infected mice ($p < 0.05$ *vs.* control group). In all groups tested, viral quantity entering skin and brain of the mice infected by HSV-1 was significantly decreased ($p < 0.05$ *vs.* control group) except 3.0 mg/kg of the unprocessed seeds. Toxicity testing in normal mice with 1.5 mg/kg or 3.0 mg/kg of the crude strychnos alkaloid fractions from the processed seeds with a sand bath and the unprocessed seeds were not shown.

34) Ma Chaomei : Inhibitory Effects of Chinese and Mogolian Herbal Drugs on HIV-1 Protease. Symposium of the 2nd Meeting of the Chinese Doctor's Forum on New Medicine, 1996, 10, Beijing.

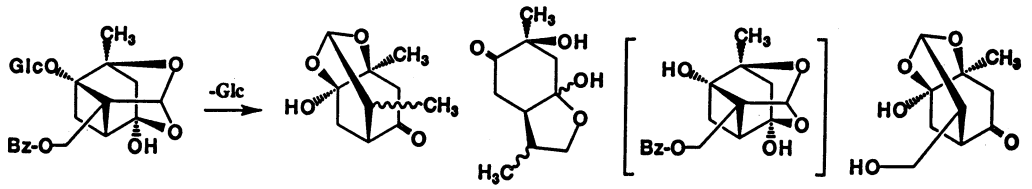
In order to find out active components against human immunodeficiency virus (HIV) from herbal drugs, HIV-1 protease, an essential enzyme for the replication of this virus, was used for evaluating the activity fo plant extracts and compounds. In the present experiment, we investigated the *in vitro* inhibitory effects of some Chinese and Mongolian herbal drugs.

By screening the extracts of 30 crude drugs at a concentration of 0.2 mg/ml, significant inhibitory effects (> 80 %) were observed in the aqueous extracts of the stem of *Cynomorium songaricum* and the rhizoma of *Dryopteric crassirhizoma.* Moderate inhibitory activities were also observed in some other extracts.

From *C. songaricum,* ursolic acid derivatives, β-sitosterol derivatives and some phenolic compounds were isolated and identified. Of these compounds, ursolic acid derivatives showed relatively potent inhibitory activity. Other related compounds of ursolic acid were synthesized and tested for their activity. It was found that the triterpenes having a hydroxy or a carboxy alkyl side chain at the 3-position and a carboxyl group at the 17-position, showed potent inhibitory activities.

35) Masao Hattori : Metabolism of Paeonia Component, Paeoniflorin, by Human Intestinal Bacteria. The First International Symposium on Natural Medicine and Microecology, 1996, 10, Dalian.

Paeoniflorin (PF) is one of the characteristic constituents of peony roots. PF was shown to have hypotensive, vasodilative, anti-oxytocic actions, as well as relaxative and inhibitory actions on the



Paeoniflorin I II III IV

movement and tonus of smooth muscle. Recently, PF was found to attenuate aging - induced and nucleus basalis magnocellularis lesion - induced learning deficits and a spatial working memory deficit produced by scopolamine. On the other hand, we reported that PF was converted to paeonimetabolins I-IV by incubation with a bacterial mixture of human feces and various defined strains of human intestinal bacteria. In the present paper, we report development of an enzyme immunoassay method for paeonimetabolin I (PM-I), a major metabolite by intestinal bacteria. Three PM-I derivatives possessing spacers of different length at C-8 were employed as haptens for preparation of antisera and enzyme-labeled antigens. Two types of antisera were prepared by immunization for the BSA conjugates in rabbits. The sensitivity and cross reactivity were compared in the homologous and heterologous systems with respect to antigens and immunogens used. The sensitivity was higher in the heterologous system using the labeled antigens linked with a shorter spacer than that used for antibody production. The cross reactivity was, however, lower in the heterologous system. By using these enzyme immunoassay systems, the PM-I level in the plasma was measured at time intervals after oral administration of PF (20 mg/kg) in rats. The plasma concentration of PM-I reached a C_{max} (400 ng/ml) at 140 min, then decreased to 0.5 ng/ml at 480 min after administration.

These findings suggest that the metabolite produced by human intestinal bacteria was absorbed from the intestinal tract into the body fluid. Further studies are now under investigation in vew of the metabolite of PF.

36) Ling Yang, Teruaki Akao, Kyoichi Kobashi, Masao Hattori : Sennoside β-Glucosidase. The First International Symposium on Natural Medicine and Microecology, 1996, 10, Dalian.

Sennoside A or B, rhein dianthrone diglucoside, is one of the main constituents of senna and rhubarb, and also considered to be inactive as a laxative itself, but to be transformed to rhein anthrone, a genuine purgative component in the intestine. We have isolated an anaerobic bacterium (*Bifidobacterium* sp. SEN), which hydrolyzes sennoside to sennidin, and demonstrated that the bacterial hydrolysis of sennoside in human intestine plays a key role in activation of sennoside. The present study will describe purification and characterization of sennoside-hydrolyzing β-glucosidase from the anaerobe.

Sennoside β-glucosidase was solubilized with Triton X-100 from the bacterial culture and purified by DEAE-cellulose column chromatography. The activity was stable against detergents such as SDS and Triton X-100, but was denatured by SDS and β - mercaptoethanal when heated. The final preparation was showed to be nearly homogeneous on SDS-PAGE either after the enzyme was denatured or not denatured. In the denaturaing SDS-PAGE, the subunit mass of the enzyme was estimated to be 110 kDa. The enzyme was optimally active at pH 6.0 for hydrolysis of sennoside B and 4-methylumbelliferyl β-glucoside (MUG). Km values for sennoside B and MUG are 0.94 mM and 0.53 mM, respectively. The enzyme also catalyzed the hydrolysis of *p* - nitrophenyl β - glucoside, amygdalin, geniposide and salicin. It was less active against methyl β-glucoside and incapable of hydrolyzing cellobiose. The β-glucosidase activity was inhibited by deoxynojirimycin and pCMS, but was less susceptible to several metals ($FeSO_4$, $ZnCl_2$, and $CuSO_4$), and DTNB.

37) Tetsuro Yoshino, Teruaki Akao, Matao Kanaoka, Norio Nakamura, Masao Hattori, Kyoichi Kobashi : Salicin Is a Good Prodrug with Sustained Release. The First International Symposium on Natural Medicine and Microecology, 1996, 10, Dalian.

We have clarified that some plant glycosides are naturally-occurring prodrugs activated by the intestinal flora. Salicin (SL) (*Salix sp.*) has been a well-known antipyretic agent, from which salicylates and aspirin have been developed for clinical application. In the present study, we investigated whether SL and salicylic acid 2-O-β-glucopyranoside (SAG) are useful prodrugs without stomach ulcers.

SL, its aglycone saligenin (SG), sodium salicylate (NaSA) and SAG were orally administered to Wistar male rats. When SG and NaSA were administered to normal rats, the plasma SA concentrations rapidly increased and the rectal temperatures were reduced maximally 3°C. Furthermore, acute stomach ulcers occurred as side effects. Whereas, when SL and SAG were administered, the plasma SA appeared and increased slowly 1 hr after and no stomach ulcers were observed. On this occasion, the normal rectal temperatures were hardly affected but the fever with yeast was reduced to normal levels. SL and SAG were rapidly hydrolyzed to SG and SA, respectively, by rats feces *in vitro*. These results indicate that SL and SAG administered orally are hydrolzed to aglycones and the aglycones are gradually absorded at the lower part of the intestine to show pharmacological effects. Thus, they seem to be useful prodrugs as mild antipyretic agents with sustained release without side effects.

38) Hiroaki Kida, Teruaki Akao, Masao Hattori : Metabolism of Saikosaponins by Human Intestinal Bacteria. The First International Symposium on Natural Medicine and Microecology, 1996, 10, Dalian.

Introduction Saikosaponins, the main constituents in Bupleui Radix, have been extensively investigated for their pharmacological actions. However, metabolic studies of saikosaponins are limited. We report here on the biotransformation of saikosaponins by human intestinal bacteria, and isolation of two bacterial strains capable of hydrolyzing saikosaponins from a human fecal suspension.

Results The TLC-densitometric analysis of the metabolites obtained by incubation of saikosaponins with a human fecal suspension revealed that saikosaponins a, b_1, b_2 and d were converted to the respective prosaikogenins and saikogenins in order. In the case of saikosaponin c, only saikogenin E was detected. Of 31 defined bacterial strains, *Eubacterium sp.* A-44 could metabolize saikosaponins a, b_1, b_2 and s to the respective prosaikogenins, and further metabolize produced-prosaikogenins F and A to the respective saikogenins. However, saikosaponin c was not transformed by this strain. Two bacrterial strains isolated from fuman feces in this time showed ability to metabolize saikosaponins a, b_1, b_2 and d to the respective prosaikogenins. However, both strains could not hydrolyze the produced-prosaikogenins or saikosaponin c. The strains were identified as *Bifidobacterium* spp., named *Bifidobacterium* sp. Saiko-1 and *B.* sp. Saiko-2, close to *B. breve* ss *breve* and *B. adolescentis,* respectively.

39) Baochang Cai, Norio Nakamura, Masao Hattori, Tsuneo Namba, Masahiko Kurokawa, Seiji Kageyama, Kimiyasu Shiraki : Effects of Alkaloids from the Processed Seeds of *Strychnos nux-vomica* on Herpes Simplex Virus, Polio Virus and Measles Virus. The First International Symposium on Natural Medicine and Microecology, 1996, 10, Dalian.

Eight crude alkaloid fractions from the processed and unprocessed seeds of *S. nux-vomica* and pure strychnos alkaloids were tested for their antiviral activities by using a plaque reduction assay method. The most of the crude alkaloid fractions inhibited the plaque formation with EC_{50} of 60.5-88.3, 64.7-74.9 and 13.2-35.4 μg/ml for HSV-1, measles virus and poliovirus, in this order. IC_{50} values of strychnine and brucine were 244.8, 212.0 μM for HSV-1, 138.2, 150.0 μM for measles virus and 63.5, 40.1 μM for poliovirus, respectively.

On the other hand, the crude alkaloid fractions (1.5 mg/kg) from the processed (parching in a sand bath) and unprocessed seeds significantly prolonged the development of skin lesions and the mean survival time in HSV-1 infected mice.

40) 武藤靖子，横澤隆子，董　而博，服部征雄，大浦

彦吉：腎不全における methylguanidine の動態．第18回グアニジノ化合物研究会，1996，10，岡山．

【目的】Methylguanidine（MG）の動態について，障害部位の異なる腎不全ラットを用い検討した。

【方法】Wistar 系雄性ラット（体重約 200 g）を用い，以下の 4 群に分け，10 日間飼育した。なお，0.75 %アデニン含有 18 % カゼイン食（アデニン食）投与以外の群は，18 % カゼイン食を与えた。飼育期間終了前日から 24 時間尿を採取し，次いで断頭により屠殺して血液を採取した。

I 群：無処置群
II 群：5/6 腎摘群
III 群：アデニン食投与群
IV 群：5/6 腎摘後，アデニン食投与群

【結果】BUN 値は，II，III，IV 群いずれも I 群より有意に上昇していたが，特に IV 群で顕著であった。Ccr は逆にいずれの群においても I 群より有意に低下していたが，FE_{Na} は III，IV 群において著しく上昇していた。

MG レベルは血中，尿中いずれにおいても III 群が II 群より高い値を示し，IV 群ではさらに増加していた。また，MG/Cr 比は IV 群において最も高い値を示していた。

【考察】3 種類の腎不全モデルいずれにおいても，糸球体機能の低下が認められたが，アデニン投与ラットでは，顕著な尿細管機能の障害が見られた。腎摘を施した後にアデニンを投与したラットの尿細管機能障害の程度は，アデニン投与のみよりさらに拡大し，これら尿細管機能障害の程度が，血液と尿の MG レベルに影響を及ぼしていることが示された。また MG レベルの上昇とともに，MG/Cr 比が著しく上昇していたが，すでに報告しているごとく，Cr からの creatol 産生に ·OH が関与していることから（大浦，横澤ら，"老化と脳"，共立出版，東京，1992，p.66-90），MG 動態にフリーラジカルの関わりが推測された。

【結論】尿細管障害が MG レベルの上昇をもたらす可能性が示唆された。

41）我妻卓司，中村憲夫，手塚康弘，門田重利，畑中保丸：光反応性ナルトレキサミン：オピオイドレセプター用新規プローブの性質．第 16 回メディシナルケミストリーシンポジウム，1996，10，富山．

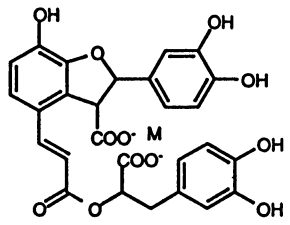
The three major types of opioid receptors, termed μ, δ, and κ, have been cloned from different species and sequenced. Recent success in the construction of chimeric opioid receptors demonstrated the presence of several extracellular regions which could be responsible for the distinct ligand-binding profiles of the each type of opioid receptors. However, it is generally impossible to exclude potential conformational changes which may result in alterations of the ligand binding. The method of photoaffinity labeling is an alternative approach to verify the genetically determined ligand binding sites.[1] We have already developed a pair of carbene generating photoprobes with a naltrexyl framework as specific ligand.[2] Here, we wish to report a novel photoreactive naltrexamine analog as the first example of biotinylated carbene-generating photoprobe for opioid receptors. The probe have a photoreactive phenyldiazirine which is connected to a biotin moiety through a hydrophilic ether spacer for providing a nonradioisotopic and highly sensitive detection of labeled products as well as for trapping photolabeled products on an avidin containing matrix. The probe seems to retain a sufficient affinity to μ-, δ-, and κ-receptors for the photoaffinity labeling study of these receptors.

42）小島ルスマリア，リンジャスミナ，中村憲夫，服部征雄：*Cordia spinescens* L. 成分の HIV-1 逆転写酵素活性について．薬学会北陸支部例会，1996，11，金沢．

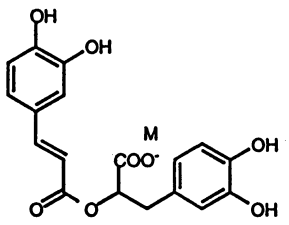
【目的】天然薬物由来のエイズ治療薬開発を目的とした研究の一環として，強い逆転写酵素（RT）阻害活性の見られたパナマ産生薬 *Cordia spinescens* L. について活性成分の検討を行った。

【実験・結果】RT 阻害作用の検定は $(rA)_n \cdot (dT)_{12-18}$ をテンプレート・プライマーとして用いた反応系で行った。*C. spinescens*（葉）をメタノール及び水で抽出した。得られたエキスの IC_{50} はそれぞれ 36.6 μg/ml であった。メタノールエキスをヘキサン，ジクロロメタン，酢酸エチル，ブタノール，水可溶部に分画すると，水画分にのみ RT 阻害活性が見られた。水画分について Diaion HP-20, Sephadex LH-20, ODS カラムクロマトを組み合わせて分離を行い lithospermic acid (**1**) を活性成分として得た。またジクロロメタン可溶部についても成分検索を行いダマランタイプの新トリテルペンを 2 種（**3**，**4**）単離した。水エキスはイオン交換樹脂で分離し，lithospermic acid（**1**）及び rosmarinic acid（**2**）を活性成分として得た。

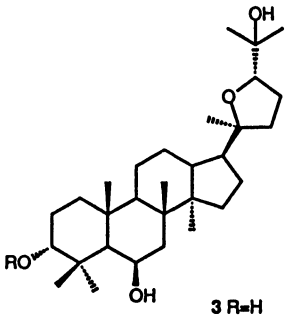
今回単離した lithospermic acid 及び rosmarinic acid は energy-dispersive 型 X-ray 分析から Mg 及



1 M=Ca^{2+} or Mg^{2+}



2 M= 1/2 Ca^{2+} or 1/2 Mg^{2+}



3 R=H
4 R=$COCH_3$

びCa塩であることが判った。

43）馬　超美，中村憲夫，宮代博継，服部征雄：内蒙古産生薬鎖陽のHIV-1プロテアーゼ阻害活性について．薬学会北陸支部例会，1996，11，金沢．

【目的】 HIV-1プロテアーゼ（PR）阻害活性を指標とした漢薬および内蒙古産生薬のスクリーニングにおいて強い阻害活性の見られた鎖陽（*Cynomorium songaricum* Rupr.）について活性成分の検索を行った。

【実験・結果】 鎖陽をジクロロメタン，メタノールで順次抽出し，メタノールエキスは更に酢酸エチル，ブタノール可溶部に分けた。各画分を各種カラムクロマトで分離し，ジクロロメタンエキスからursolic acid誘導体（3種）及びβ-sitosterol誘導体（4種）を得た。また，酢酸エチル及びブタノール画分からはフェノール性化合物（9種）を単離した。この中でHIV-1 PRに対する阻害活性はmalonyl ursolic acid hemiesterが最も強く（IC_{50}＝6 μM），次いでursoli acidであった（IC_{50}＝8 μM）。

次に，トリテルペン類の構造と活性の相関を調べるためにursolic acid, oleanolic acid, betulinic acidについて数種の誘導体を合成した。その結果，α-amyrin, β-amyrinや3-アセチル，17-メチルエステル体の活性は弱く，3位をdicarboxylic acid hemiesterに変えた化合物はもとのトリテルペンより強い活性が見られ，glutaric acid hemiesterでIC_{50}は4 μMであった。これらの結果よりトリテルペンの3位の高極性基と17位のカルボキシン基が活性発現に重要であると思われる。

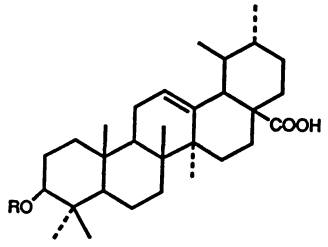
44）服部征雄：芍薬の薬理，代謝，第12回生薬に関する懇談会，1996，12，東京．

はじめに

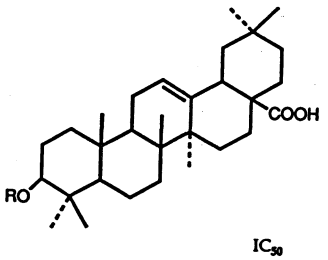
芍薬は当帰芍薬散，芍薬甘草湯をはじめとする種々の漢方処方に含まれる重要生薬である。したがって，これまで非常に多くの化学的および薬理学的研究がなされているが漢方で期待される収斂，緩和，鎮痙，鎮痛作用を現代科学的にまだ充分証明できていない。演者らは芍薬の代表的成分であるpaeoniflorin, albiflorinの腸内細菌による代謝の研究を行ってきたが，最近，代謝物の酵素免疫測定法（EIA）を開発し，paeoniflorin経口投与後のラット血漿を調べた結果，多量の代謝物が検出されたことから，芍薬の薬効を理解する場合にはこの代謝物の薬理作用を検討する必要があることがわかった。また，渡邊らはpaeoniflorinがラットの空間認知機能障害に有効であるとの新しい作用を見いだしている。以下，これまでの研究の歩みを概説する。

Paeoniflorinの腸内細菌による代謝

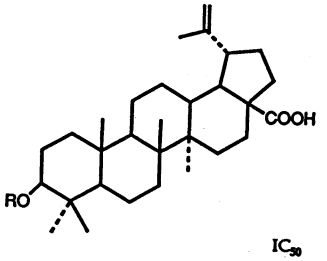
ヒト腸内細菌とpaeoniflorinを嫌気的条件下でインキュベートすると3種の代謝物に変換される（paeonimetabolin I, II, III）。Paeonimetabolin Iは*7R-, 7S-*体の混合物である。Paeonimetabolin IIIは真性アグリコンと考えられるが単離には成功していな



	IC_{50}
ursolic acid (R=H)	8μM
R=-O-(CH2)2-COOH	4μM



	IC_{50}
oleanolic acid (R=H)	8μM
R=-O-(CH2)2-COOH	4μM



	IC_{50}
betulinic acid (R=H)	9μM
R=-O-(CH2)2-COOH	4μM

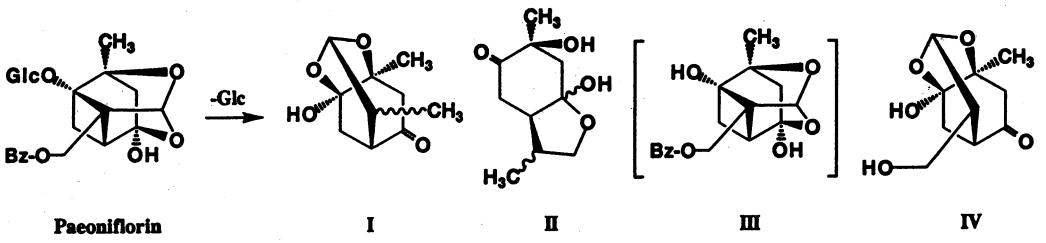


Fig. 1　Paeoniflorin の代謝産物の構造

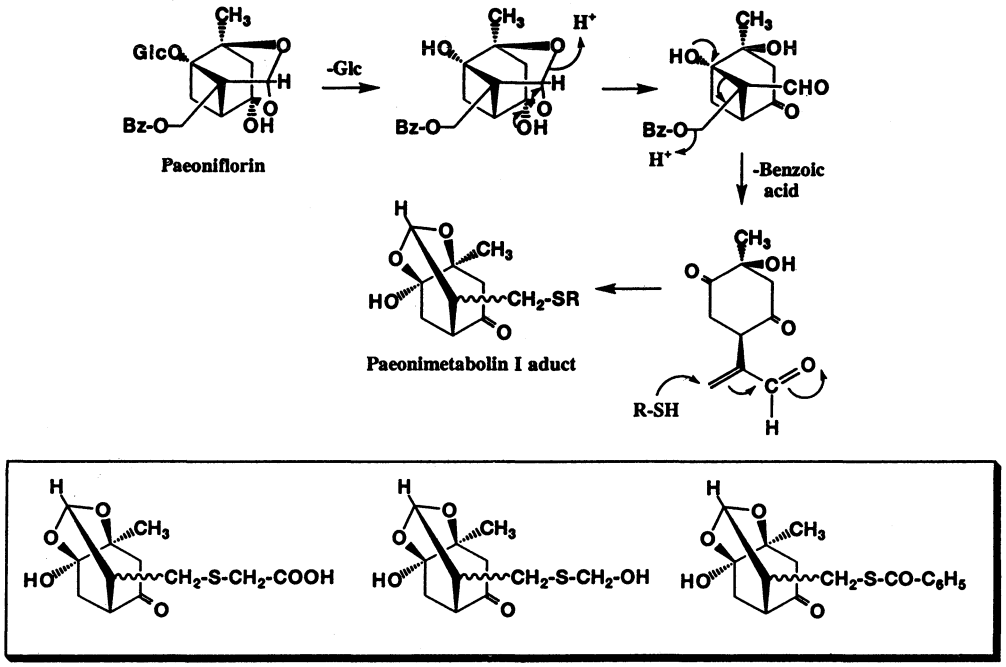


Fig. 2　SH- 化合物との付加体の生成

い。最近，paeonimetabolin IV の構造を持つ代謝物も生成することが見いだされた。ほとんどのヒト腸内嫌気性菌は paeoniflorin を代謝する能力を有するが，この初期段階での反応に関与するβ-glucosidase は細菌の膜と結合した酵素で，可溶化が困難で精製には至っていない。SH 基を持つ化合物の存在下，paeoniflorin を代謝させると，この SH 化合物が付加した生成物が得られる。Mercaptoacetic acid, mercaptoethanol, thiobenzoic acid を添加すると Fig. 2 に示す付加体が生成する。この反応は paeoniflorin の糖部分が水解され，四員環の開裂にともない生成する共役アルデヒド体に SH を持つ化合物が Michael 付加した後，ヘミケタール・アセタールを形成するものと思われる。SH 基以外では付加体の生成はみられなかった。

Paeonimetabolin I の酵素免疫測定法の開発

上記反応を利用するとハプテンと蛋白を結合する種々の長さの架橋を有する免疫原，標識抗原を作製することができる。すなわち，Fig. 3 に示すように n=1-5 の mercaptocarbonic acids の存在下，paeoniflorin を代謝させそれぞれの付加体を合成し，次いで *N*-hydroxysuccinimide ester とした後，β-galactosidase, bovine serum albumin (BSA) と反応させ，標識抗原，免疫原を作製した。免疫原をウサギに継続的に投与し，抗血清を得た。

Paeoniflorin の体内動態

Paeoniflorin をラットに経口投与し，経時的に腸内容物を分析すると，消化管下部で paeonimetabolin I の生成が確認される。ラットに 20 mg/kg 経口投与し，血漿中の paeoniflorin, paeonimetabolin I の濃度を酵素免疫測定法で調べると，血漿中の paeoniflorin 濃度

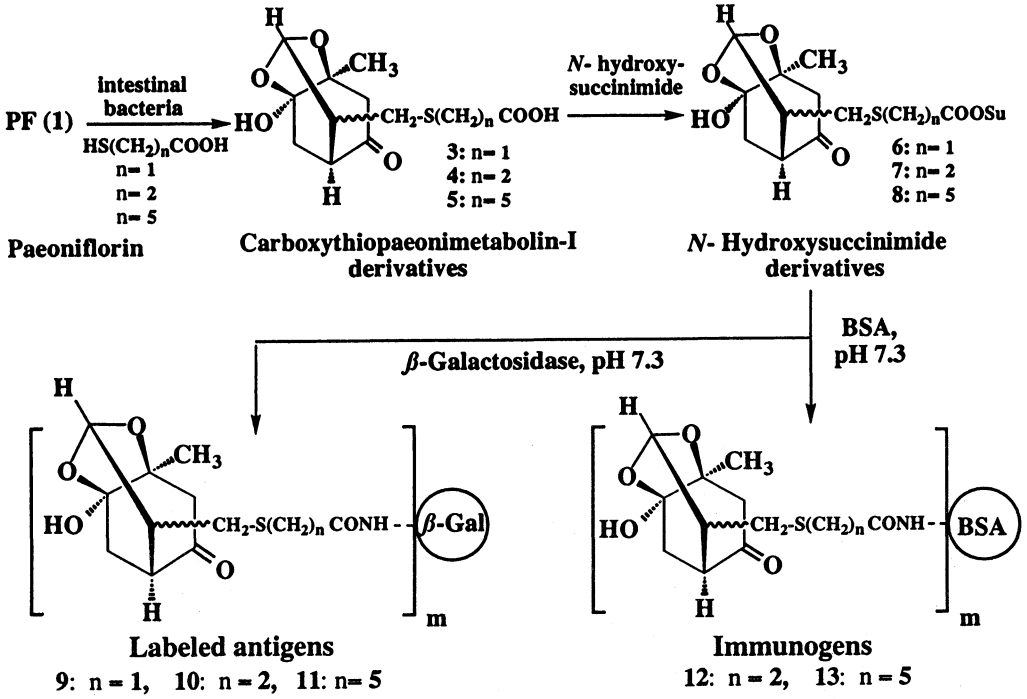


Fig. 3　標識抗原，免疫原の合成

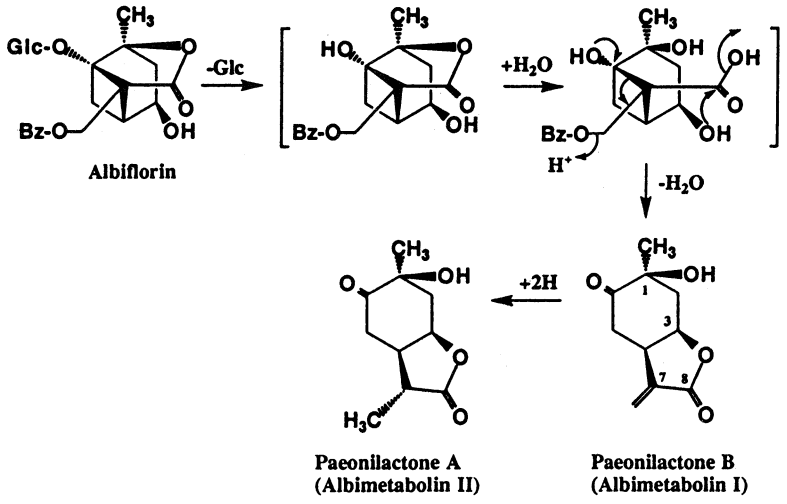


Fig. 4　Albiflorin の代謝経路

は 30 分で急激に最高濃度(C_{max} 85 ng/ml)に達し，240 min で 0.2 ng/ml に減少した。一方，paeonimetabolin I の血漿中の濃度は 140±24.7 min で最高濃度 (400 ng/ml) に達し，480 min 後に 0.5 ng/ml まで減少した。Paeoniflorin および paeonimetabolin I の AUC はそれぞれ 7700±900，524000±17500 ng·min·ml^{-1} であり，後者の代謝物が前者より長時間，高い濃度で血漿中に存在することを示した。このことは，腸内細菌により生成する代謝物が消化管から吸収され，しかも paeoniflorin より血中濃度が高いことから paeoniflorin の薬効発現にはこの代謝物が関与している可能性が高い。

Albiflorin の腸内細菌による代謝

Albiflorin を腸内嫌気性菌とインキュベーションす

ると2つの代謝物が生成する。Paeoniflorinの場合と同様に，glucoseの脱離とともに環開裂が起こりラクトン環の再構成がおこる。これら代謝物は芍薬から単離されているpaeonilactone A, Bと同一の構造をとることがわかった。

芍薬の薬理

最近渡邊らはスコポラミンで起こした迷路行動障害に四物湯が有効であり，構成生薬では地黄，川芎にその改善作用は認められず，当帰，芍薬に改善作用があることを報告している。また芍薬中に含まれるpaeoniflorinおよび関連化合物は極く微量の経口投与においてラットのスコポラミン誘発記憶障害に有効であるという。

Paeoniflorin代謝物のpaeonimetabolin Iをラットの脳室内に投与するとpentylene tetrazole誘発痙攣を阻害した。El癲癇マウスに経口投与，十二指腸投与いずれにおいても痙攣を阻害した。

おわりに

和漢薬の薬効発現に至る過程で，腸内細菌が関与する例が次第に明らかにされつつある。天然薬物は配糖体の形で存在するものが多く，これらの配糖体はそのままの形では消化管から吸収されにくいため，消化管下部まで輸送され腸内細菌による代謝を受けやすい。これら代謝物が生体にとって疾病の予防，治療に結びつくならばプロドラッグの活性化であり，逆に生体に発癌や種々の悪影響を及ぼすならば毒性の発現となる。腸内細菌は善玉でもあり，悪玉でもある。腸内細菌の特性を良く理解し，上手に利用するならば腸内細菌は我々の健康維持に欠かせない良き共生者である。

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◇ **そ の 他**

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◇ **社会的活動**

1）服部征雄：「和漢薬と腸内細菌」1996年9月5日リカレント教育学習コース。