化 学 応 用 部 門

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◇研究目的

本部門では，化学的手法を応用する和漢薬の基礎研究として，天然薬物を中心とする生理活性分子の医薬化学的及び生物有機化学的研究を行っている。即ち，天然薬物の成分単離，構造解析，合成等の，和漢薬成分に関する化学的研究を行う。さらに，その過程で構造が明らかとなる天然薬物成分について，その構造・活性相関，構造・機能相関の化学的解明に取り組んでいる。本年度の主な研究課題は下記の通りである。

◇研究概要

Ⅰ．天然薬物成分の単離，構造解析，合成，作用

1）人参，丹参，草豆蔻，藜蘆等の和漢生薬

2）インドネシア，ベトナム，ミャンマー，ネパール等の薬用植物

3）麝香から単離した新規成分ムスクライド類の合成及び誘導体化

4）肝臓病や骨粗鬆症に有効な天然薬物成分の開発研究

Ⅱ．薬物・生体高分子相互作用系の生物有機化学

1）構造・機能相関解析に有用な独自の化学的手法の開発

2）機能性生体高分子の構造生物学

上記の研究課題によって得られた本年度の成果（原著及び学会報告）は下記の通りである。

◇原　著

1) **Xiong Q., Hase K., Tezuka Y., Tani T., Namba T., and Kadota S.: Hepatoprotective Activity of Phenylethanoids from *Cistanche deserticola.* Planta Medica, 64: 120–125, 1998.**

Abstract: Four phenylethanoids isolated from the stems of *Cistanche deserticola, viz.* acteoside (**1**), 2'-acetylacteoside (**2**), isoacteoside (**3**) and tubuloside B (**4**), significantly suppressed NADPH/CCl_4-induced lipid peroxidation in rat liver microsomes. Addition of them to primary cultured rat hepatocytes efficiently prevented cell damage induced by exposure to CCl_4 or D-galactosamine (D-GalN). Acteoside (**1**) further showed pronounced anti-hepatotoxic activity against CCl_4 *in vivo.*

2) **Tezuka Y., Kasimu R., Li J. X., Basnet P., Tanaka K., Namba T., and Kadota S.: Constituents of Roots of *Salvia deserta* Schang. (Xinjiang-Danshen). Chem. Pharm. Bull., 46: 107–112, 1998.**

Summary: *Salvia deserta* Schang. (Lamiaceae) is a plant grown in Xinjiang province in China, and its dried roots are called Xinjiang-Danshen. This plant has not been used as a medicine or a food, but recently it was reported that Xinjiang-Danshen is mixed in Danshen (roots of *S. miltiorhiza* Bunge), a well-known Chinese crude drug, at Xinjiang province when latter was in short supply. We examined the constituents of the roots of S. *deserta* (Xinjiang-Danshen) and identified a new caffeic acid trimer [salvianolic acid K (**1**)], along with two known caffeic acid dimers [salviaflaside (**2**), rosmarinic acid (**3**)], a known caffeic acid tetramer [lithospermic acid B (**4**)], seven known abietane-type diterpenes [6,7-dehydroroyleanone (**5**), royleanone (**6**), taxodione (**7**), ferruginol (**8**), 7-*O*-methylhorminone (**9**), 7-*O*-acetylhorminone (**10**), horminone (**11**)], and a known steroid [daucosterol (**12**)]. Five of the diterpenes (**5**, **6**, **9-11**) were "royleanones" and the main caffeic acid derivative was the trimer **1**. These differed from the constituents of roots of *S. miltiorhiza,* which contains "tanshinones" as diterpenes and magnesium lithospermate B as the main caffeic acid derivative. Thus, the mixing of Xinjiang-Danshen with Danshen is not appropriate and two should be considered different drugs.

3) **Kasimu R., Tanaka K., Tezuka Y., Gong Z.-N., Li J. X., Basnet P., Namba T., and Kadota S.: Comparative Study of Seventeen *Salvia* Plants; Aldose Reductase Inhibitory Activity of Water and MeOH Extracts and Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis of Water Extracts. Chem. Pharm. Bull., 46: 500–504, 1998.**

Summary: The dry root and rhizome of *Salvia miltiorhiza* (Lamiaceae) are used as a crude drug Danshen, while those of *S. deserta* (Xinjiang-Danshen) are mixed in Danshen at Xinjiang province when the former is in short supply. The water and MeOH extracts of *S. deserta* showed strong aldose reductase (AR) inhibitory activity, and their active constituents were determined to be polar compounds different from "tanshinones" of *S. miltiorhiza,*

i.e., lithospermic acid B (**1**), salvianolic acid K (**2**), salviaflaside (**3**), and rosmarinic acid (**4**) (IC50, 2.63-3.91 μM). We also examined the AR inhibitory activity of water and MeOH extracts of seventeen *Salvia* plants, including ten species of Danshen resources (*S. bowleyana, S. deserta, S. miltiorhiza, S. miltiorhiza* var. *miltiorhiza* f. *alba, S. paramiltiorhiza, S. paramiltiorhiza* f. *purpureo-rubra, S. przewalskii, S. przewalskii var. mandarinorum, S. sinica* f. *purpurea, S. trijuga*), and their water extracts were also analyzed by liquid chromatography-mass spectrometry (LC-MS). The results indicated that there were four types with regard to the AR inhibitory activity and three types with regard to the amount of **1**. Ten species used as Danshen resources showed good correlation between the AR inhibitory activity and the morphological classification. However, the intensities of their AR inhibitory activity varied, and they contained **1** in varying amounts. These facts suggested that the ten species were not the same, and thus their use as a Danshen resource should be based on their activity and/or active constituents.

4) **Prasain J. K., Li J.-X., Tezuka Y., Tanaka K., Basnet P., Dong H., Namba T., and Kadota S.: Calyxin H, Epicalyxin H, and Blepharocalyxins A and B, Novel Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*: J. Nat. Prod., 61 : 212–216, 1998.**

Summary : Four unprecedented diarylheptanoids, calyxin H (**1**) and epicalyxin H (**2**), pos sessing a diarylheptanoid unit and a chalcone moiety, and blepharocalyxins A (**3**) and B (**4**), possessing two diarylheptanoid units and a chalcone moiety, were isolated from the seeds of *Alpinia blepharocalyx*. The structures of **1-4**, including absolute stereochemistry, were elucidated by spectroscopic means and after a consideration of their biogenesis.

5) **Prasain J. K., Tezuka Y., Hase K., Basnet P., Dong H., Namba T., and Kadota S.: Inhibitory Effect of Diarylheptanoids on Nitric Oxide Production in Activated Murine Macrophages. Biol. Pharm. Bull., 21 : 371–374, 1998.**

Summary : Thirteen novel diarylheptanoids bearing a chalcone or a flavanone moiety (**1-13**), a new curcumin derivative, 1,2-dihydrobis(de-*O*-methyl)curcumin (**14**), and two known flavonoids (**15** and **16**) isolated from the seeds of *Alpinia blepharocalyx* K. Schum. were tested for their inhibitory effects on nitric oxide (NO) production in lipopolysaccaride (LPS)-activated murine macrophages J774.1 *in vitro*. All the tested compounds inhibited NO production in a concentration-dependent manner (IC_{50} = 36-568 μM). Among the compounds examined, blepharocalyxin B (**13**) was the most potent inhibitor of NO production (IC_{50} = 36 μM). Analysis of the structure activity relationship among these novel diarylheptanoids led to the conclusion that the position of attachment of a chalcone or a flavanone to a diarylheptanoid does not affect their inhibitory potency although their presence in association causes a substantial enhancement of the inhibitory activity. Moreover, a conjugated double bond in a chalcone moiety potentiated the inhibitory activity. On the other hand, hexamethoxydeoxycalyxin A (**17**) and pentamethoxycalyxin B (**18**), a methylated product

of calyxin A (**1**) and an epimeric mixture of calyxin B, showed greatly reduced activity suggesting that phenolic hydroxyl groups are involved in the inhibitory activity.

6) **Prasain J. K., Tezuka Y., Li J.-X., Tanaka K., Basnet P., Dong H., Namba T., and Kadota S.: Novel Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*: Revised Structure of Calyxin A. J. Chem. Res., (S) 22–23, (M) 265–279, 1998.**

Summary: Calyxins A (**1**), E (**2**), F (**3**), 6-hydroxycalyxin F (**4**), calyxin G and epicalyxin G (**5** and **6**), a series of stereochemically complex, novel diarylheptanoids having a chalcone or a flavanone moiety, were isolated from the seeds of *Alpinia blepharocalyx* K. Schum. and their structures were elucidated by spectroscopic methods. The structure of calyxin A (**1**) was revised and its stereochemistry was deduced by the NMR spectral analysis of its MTPA esters.

7) **Hasegawa H., Suzuki R., Wakabayashi C., Murata J., Tezuka Y., Saiki I., and Kadota S.: Synthesis of a Biologically Active Fluorescent Derivative of GM1, a Main Ginseng Saponin Metabolite Formed by Intestinal Bacteria. Biol. Pharm. Bull., 21 : 513–516, 1998.**

Summary: A fluorescent derivatives of GM1 [20-O-β-D-glucopyranosyl-20(*S*)-protopanaxadiol], a main Ginseng saponin metabolite formed by intestinal bacteria, was obtained from the condensation of its trisnor-aldehyde derivative with dansyl hydrazine. The dansylated GM1 fluoresced strongly and showed almost the same properties as its parent compound in lipophilicity and biological activities, so this fluorescent compound might provide an insight into the mechanism of pharmacological activities of GM1.

8) **Li J.-X., Li P., Tezuka Y., Namba T., and Kadota S.: Three Phenylethanoid Glycosides and An Iridoid Glycoside from *Picrorhiza scrophulariiflora*. Phytochemistry, 47 : 537–542, 1998.**

Abstract: Three new phenylethanoid glycosides, named scrosides A-C and a new iridoid glycoside, named picroside IV, have been isolated from the underground part of *Picrorhiza scrophulariiflora*, together with 11 known compounds. Their structures were elucidated by the means of 2D NMR spectroscopy and chemical method.

9) **Li H., Li J., Prasain J. K., Tezuka Y., Namba T., Miyahara T., Tonami S., Seto H., Tada T., and Kadota S.: Antiosteoporotic Activity of the Stems of *Sambucus sieboldiana*. Biol. Pharm. Bull., 21 : 594–598, 1998.**

Abstract: We previously found that a methanolic extract of the stems of *Sambucus sieboldiana* inhibited bone resorption in organ culture. In this study, we further fractionated the methanol extract guided by the activity towards bone resorption stimulated by parathyroid hormone (PTH) *in vitro*. The ethyl acetate fraction (EtOAc Fr.) of the methanolic extract inhibited PTH-stimulated bone resorption of neonatal mouse bones, and the inhibitory

activity was more potent than those of other fractions. Oral administration of the EtOAc Fr. (50 and 100 mg/kg/d) to ovariectomized (OVX) rat prevented the decrease in bone mineral density (BMD) of the lumbar (L2-4) vertebra, indicating that the EtOAc Fr. is effective *in vivo*. Furthermore, the EtOAc Fr. (50, 100 and 150 mg/kg/d) decreased the serum calcium level elevated in low calcium dietary rats. The phenolic constituents of the EtOAc fraction were examined for their inhibitory effect on bone resorption stimulated by PTH in neonatal mouse bone. Among them, vanillic acid, vanillin and coniferyl alcohol showed significant inhibitory effects on bone resorption. Of the compounds examined, vanillic acid was found to have a significant inhibitory effect on the decrease of BMD in OVX mice. Therefore, the EtOAc Fr. of *S. sieboldiana* showed a suppressive effect on bone resorption *in vitro* and *in vivo*. In addition, the inhibitory effects of the EtOAc Fr. on bone resorption may be at least partly due to the inhibitory action of vanillic acid.

10) **Matsumoto K., Kohno S., Ojima K., Tezuka Y., Kadota S., and Watanabe H.: Effects of Methylenechloride-soluble Fraction of Japanese Angelica Root Extract, Ligustilide and Butylidenephthalide, on Pentobarbital Sleep in Group-housed and Socially Isolated Mice. Life Sci., 62 : 2073–2082, 1998.**

Summary : We previously showed the extract of Japanese angelica root (JAR-E) reversed the decrease in pentobarbital (PB) sleep induced by isolation stress and yohimbine and methoxamine, stimulants of central noradrenergic systems, in mice. Here, we tested the effects of several fractions from JAR-E and ligustilide and butylidenephthalide, phthalide components of JAR-E, on PB sleep in isolated mice to elucidate the mechanism of the action of JAR-E. Methanol-soluble (Met-S) and -insoluble (Met-IS) fractions were obtained from JAR-E. Methylenechloride-soluble (MC-S) and -insoluble fractions (MC-IS) were prepared from Met-S. MC-S (11.4-76 mg/kg, p.o.) reversed the isolation stress-induced cecrease in PB sleep, but neither Met-IS (0.8-2.4 g/kg, p.o.) nor MC-IS (0.7-2 g/kg, p.o.) had the same effect. The i.p. administration of MC-S exhibited a similar activity to that observed after the p.o. administration of the same fraction. Ligustilide (5-20 mg/kg, i.p.) and butylidenephthalide (10-30 mg/kg, i.p.) reversed PB sleeep decrease in isolated mice. Both compounds (20 mg/kg, i.p.) attenuated the suppressive effects of yohimbine (30 nmol, i.c.v.), methoxamine (200 nmol, i.c.v.) and a benzodiazepine inverse agonist FG7142 (10 mg/kg, i.p.) on PB sleep in group-housed mice. These results suggest the contribution of ligustilide and butylidenephthalide to the effect of JAR-E on PB sleep in isolated mice, and implicate central noradrenergic and/or GABAA systems in the effects of these components.

11) **Banskota A. H., Tezuka Y., Prasain J. K., Matsushige K., Saiki I., and Kadota S.: Chemical Constituents of Brazilian Propolis and Their Cytotoxic Activities. J. Nat. Prod., 61 : 896–900, 1998.**

Abstract : The ethyl acetate soluble fraction of the methanolic extract of propolis afforded a new prenylated chromane derivative, 3-hydroxy-2, 2-dimethyl-8-prenylchromane-6-

propenoic acid (**1**), along with twenty two known compounds **2-23**. Of the known compounds, **4**, **7**, **12-19** and **22** were isolated for the first time from propolis and the absolute configuration of 23 was established as *2S,3R*. Investigation suggested that *Baccharis spp.* are a significant source of tropical Brazilian propolis, in addition to *Clusia minor, C. major* and *Araucaria heterophylla*. All the compounds were tested for their cytotoxicity towards human HT-1080 fibrosarcoma and murine colon 26-L5 carcinoma cells. Among these compounds, **9** and **19-21** showed potent cytotoxicity, having ED50 values equal to or less than 10 μg/mL.

12) **Zhao W., Guo Y., Tezuka Y., and Kikuchi T.: Isolation and Structure Determination of Aurantiamide Acetate from *Veratrum nigrum* L. var. *ussuriense* Nakai. 中国中薬雑誌, 23 : 41, 1998.**

Abstract: A modified dipeptide has been isolated from *Veratrum nigrum* var. *ussuriense* for the first time and confirmed to be aurantiamide acetate by spectroscopic analysis.

13) **Zhao W., Guo Y., Tezuka Y., and Kikuchi T.: Chemical Research on the Stilbenes from *Veratrum nigrum* L. var. *ussuriense* Nakai. Chinese Journal of Medicinal Chemistry, 8 : 35–37, 1998.**

Abstract: A new stilbene named verussustilbene and two known stilbenes have been iso lated from *Veratrum nigrum* L. var. *ussuriense* Nakai. The structure of verussustilbene (**III**) was determined by the use of two-dimensional nuclear magnetic resonance (2-D NMR) technique, and two other stilbenes were identified as resveratrol (**I**) and 2,3',4,5'-tetrahydroxys tilbene (**II**).

14) **Peungvicha P., Temsiririkkul R., Prasain J. K., Tezuka Y., Kadota S., Thirawarapan S. S., and Watanabe H.: 4-Hydroxybenzoic Acid: a Hypoglycemic Constituent of Aqueous Extract of *Pandanus odorus* Root. J. Ethnopharmacol., 62 : 79–84, 1998.**

Abstract: Hypoglycemic activity-guided fraction led to the isolation of the known compound, 4-hydroxybenzoic acid, from *Pandanus odorus* Ridl. (Thai name: Toei-hom, Pandanaceae). This compound showed a hypoglycemic effect in normal rats after the oral administration of 5 mg/kg. Additionally, the compound increased serum insulin levels and liver glycogen content in normal rats.

15) **Kasimu R., Tezuka Y., Tanaka K., Gong Z.-N., Li J.-X., Basnet P., Namba T., and Kadota S.: Liquid Chromatography-Mass Spectrometry Analysis of Diterpenoid Constituents of Seventeen *Salvia* Plants. J. Trad. Med., 15 : 109–115, 1998.**

Abstract: The MeOH extracts of the seventeen *Salvia* plants, including ten species used as resources of Chinese crude drug, Dan-shen (丹参, Radix Salviae miltiorhizae, Tan-jin in Japanese), were comparatively examined by the liquid chromatography-mass spectrometry (LC-MS) method using thirteen diterpenoids as standards. The principle component analysis

(PCA) on the relative intensity of the protonated molecular ion of standard diterpenoids in LC-MS showed the presence of several groups in genus *salvia* with regard to the diterpenoids, which means that the ten species used as Dan-shen resources were not the same. Their use as a Dan-shen resource, thus, should be based on their activity and/or active constituents.

16) **Tezuka Y., Kikuchi T., Zhao W., Chen J., and Guo Y.: Two New Steroidal Alkaloids, 20-Isoveratramine and Verapatuline, from the Roots and Rhizomes of *Veratrum patulum*. J. Nat. Prod., 61 : 1078–1081, 1998.**

Summary : Roots and rhizomes of *Veratrum patulum* L. (Liliaceae), used as a source of the Chinese crude drug "Li-lu", have yielded two new steroidal alkaloids, 20-isoveratramine (**1**) and verapatuline (**2**), along with three known alkaloids, veratramine (**3**), veratrosine (**4**), and jervine (**5**). Structures of the new alkaloids, **1** and **2**, were determined to be a C-20 epimer of **3** and *N*-(methoxycarbonyl)jervine, respectively, by the use of spectral data including 2D NMR.

17) **Tezuka Y., Kudoh M., Hatanaka Y., Kadota S., and Kikuchi T.: Synthesis of Musclide-A1 Diastereomers; Confirmation of Absolute Stereochemistry. J. Trad. Med., 15 : 168–175, 1998.**

Abstract : (2*R*,5*R*)-Musclide-A1 and (2*S*,5*R*)-musclide-A1 were synthesized from L-valine and their diastereomers from D-valine in order to determine the structure of natural musclide-A1 isolated from musk. Comparisons of spectral data of synthetic, natural, and previously synthesized musclide-A1 confirmed the structure suggested previously; *i.e.*, natural musclide-A1 is a mixture of (2*R*)-hydroxy-6-methyl-(5*R*)-heptyl hydrogen sulfate and (2*R*)-hydroxy-6-methyl-(5*S*)-heptyl hydrogen sulfate.

18) **Li J.-X., Shi Q., Xiong Q.-B., Prasain J. K., Tezuka Y., Hareyama T., Wang Z.-T., Tanaka K., Namba T., and Kadota S.: Tribulusamide A and B, New Hepatoprotective Lignanamides from the Fruits of *Tribulus terrestris*: Indications of Cytoprotective Activity in Murine Hepatocyte Culture. Planta Medica, 64 : 628–631, 1998.**

Abstract : Tribulusamides A (**1**) and B (**2**), new lignanamides embracing two cinnamic amide parts joined in a *cis* configuration, were isolated from the fruits of *Tribulus terrestris*, together with four known compounds, *N-trans*-feruloyltyramine (**3**), terrestriamide (**4**), *N-trans*-coumaroyltyramine (**5**), and β-sitosterol. The structures were elucidated by 2D-NMR spectroscopy. Addition of compounds **1-5**, especially **1** and **2**, to primary cultured mouse hepatocytes significantly prevented cell death induced by D-galactosamine (D-GalN)/tumor necrosis factor α (TNF-α).

19) **Tezuka Y., Kikuchi T., Zhao W., Chen J., and Guo Y.: (+)-Verussurine, a New Steroidal Alkaloid from the Roots and Rhizomes of *Veratrum nigrum* var. *ussuriense* and Structure Revision of (+)-Verabenzoamine. J. Nat. Prod., 61: 1397–1399, 1998.**

Summary: Two minor steroidal alkaloids, 1 and 2, have been isolated from the roots and rhizomes of *Veratrum nigrum* var. *ussuriense*. Their structures have been determined by the use of spectral data as 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**1**) and 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**2**). By spectral data comparison with verabenzoamine, the structure of the latter compound has been revised from the previously reported 7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**1**) to 15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine (**2**). Accordingly, alkaloid **1** [7-*O*-acetyl-15-*O*-(2-methylbutyroyl)-3-*O*-veratroylgermine] must be new, and it was given the trivial name verussurine.

20) **Banskota A. H., Tezuka Y., Phung L. K., Tran K. Q., Saiki I., Miwa Y., Taga T., and Kadota S.: Cytotoxic Cycloartane-type Triterpenes from *Combretum quadrangulare*. Bioorg. Med. Chem. Lett., 8: 3519–3524, 1998.**

Abstract: Seven novel cycloartane-type triterpenes were isolated from *Combretum quadrangulare*, and their structures were elucidated on the basis of spectral analysis. All these compounds were tested for their cytotoxicity against murine colon 26-L5 carcinoma cells. Methyl quadrangularate B (**2**) and methyl quadrangularate D (**4**) exhibited potent cytotoxicity having ED50 values 9.54 and 5.42 μM, respectively.

21) **El-Mekkawy S., Meselhy M. R., Nakamura N., Tezuka Y., Hattori M., Kakiuchi N., Shimotohno K., Kawahata T., and Otaka T.: Anti-HIV-1 and Anti-HIV-1-protease Substances from *Ganoderma lucidum*. Phytochemistry, 49: 1651–1657, 1998.**

Abstract: A new highly oxygenated triterpene named ganoderic acid α has been isolated from a methanol extract of the fruiting bodies of *Ganoderma lucidum* together with twelve known compounds. The structures of the isolated compounds were determined by spectroscopic means including 2D-NMR. Ganoderiol F and ganodermanontriol were found to be active as anti-HIV-1 agents with an inhibitory concentration of 7.8 μg ml-1 for both, and ganoderic acid B, ganoderiol B, ganoderic acid C1, 3β,5α-dihydroxy-6β-methoxyergosta-7, 22-diene, ganoderic acid A, ganoderic acid H and ganoderiol A were moderately active inhibitors against HIV-1 PR with a 50% inhibitory concentration of 0.17-0.23 mM.

22) **Shang M., Cai S., Han J., Li J., Zhao Y., Zheng J., Namba T., Kadota S., Tezuka Y., and Fan W.: Studies on Flavonoids from Fenugreek (*Trigonella foenumgraecum* L.). 中国中薬雑誌, 23: 614–616, 1998.**

Abstract: Objective: To study the flavonoids in fenugreek (*Trigonella foenumgraecum*) produced in China. **Method**: The flavonoids were isolated with silica gel, polyamide and Sephadex LH-20 chromatography, and their structures were identified by physical, chemical

properties and spectral analysis. **Result** : Five flavonoid compounds were isolated from fenugreek seeds and identified as vitexin, tricin, naringenin, quercetin and tricin-7-*O*-β-D-glucopyranoside. **Conclusion** : Three flavonoids, tricin, natingenin and tricin-7-*O*-β-D-glucopyranoside, were isolated from fenugreek as well as from the plants of *Trigonella* for the first time.

23) **Shang M., Cai S., Li J., Kadota S., Tezuka Y., Fan W., and Namba T.: Studies on Triterpenoids from Common Fenugreek (*Trigonella foenum-graecum*). 中草薬, 29 : 655–657, 1998.**

Abstract : Seven triterpenoids were isolated for the first time from the ethanol extract of seed of *Trigonella foenum-graecum* L., six of them were identified as lupeol, 31-norcycloartanol, betullin, betulinic acid, soyasaponin I, and soyasaponin I methyl ester by physico-chemical properties and spectral analysis.

24) **Li H., Miyahara T., Tezuka Y., Namba T., Nemoto N., Tonami S., Seto H., Tada T., and Kadota S.: The Effect of Kampo Formulae on Bone Resorption *in Vitro* and *in Vivo*. I. Active Constituents of Tsu-kan-gan. Biol. Pharm. Bull., 21 : 1322–1326, 1998.**

Abstract : Four water extracts of Kampo formulae (Yi-kkan-sen, Dai-ho-in-gan, Ni-chi-gan, Tsu-kan-gan) were screened for their inhibitory activities on bone resorption induced by parathyroid hormone (PTH) in organ culture using neonatal mouse parietal bones. Among the Kampo formulae, Tsu-kan-gan (TKG) showed the most potent inhibitory activity. We further fractionated the TKG water extract by monitoring the inhibitory activity on bone resorption stimulated by PTH *in vitro*. The MeOH fraction of the water extract inhibited PTH-stimulated bone resorption, and its inhibitory activity was more potent than those of other fractions. The MeOH fraction was then subjected to Sephadex LH-20 column chromatography to give fractions I, II and III, which were examined for bone resorption activity. Fraction I inhibited PTH-stimulated bone resorption, and its inhibitory activity was more potent than those of the other fractions. Upon oral administration of the three fractions (100 mg/kg/d) to ovariectomized (OVX) mice, fractions I and III prevented the decrease of bone mineral density (BMD) of the lumbar vertebra. Eleven compounds isolated from the MeOH fraction were examined for their inhibitory effect on PTH-stimulated bone resorption. Among them, berberine (**1**), syringin (**3**), limonin (**4**) and mangiferin (**10**) showed a significant inhibitory effect on bone resorption. In the formation assay of osteoclast-like cells, these compounds decreased the number of tartrate-resistant acid phosphatase (TRAP)-positive multinucleated cells (MNCs). The inhibitory effect of TKG on bone resorption may be at least partly due to the inhibitory action of these compounds.

25) **Ma J.-Y., Wang Z.-T., Xu L.-S., Xu G.-J., Kadota S., and Namba T.: Sesquiterpene Lactones from *Ixeris sonchifolia*. Phytochemistry, 48 : 201–203, 1998.**

Abstract : The whole plant of *Ixeris sonchifola* afforded two new guaianolide sesquiterpene

lactones named 8-dsoxyartelin [3-hydroxy-1(10),3-guaiadiene-12,6-olide-2-one] and ixerin Z [1(10),3,11(13)-guaiatriene-12,6-olide-2-one-3-*O*-glucopyranoside], along with the known compound 9α-hydroxyzaluzalin C, whose structure and stereochemistry wee determined by spectroscopic methods.

26) Hayakawa Y., Fujii H., Hase K., Ohnishi Y., Sakukawa R., Kadota S., Namba T., and Saiki I.: Anti-metastatic and Immunomodulating Properties of the Water Extract from *Celosia argentes* Seeds. Biol. Pharm. Bull., 21 : 1154–1159, 1998.

Abstract : We have investigated the anti-metastatic effect of *Celosia argentea* seed extracts (CAE), which have traditionally been used as a therapeutic drug for eye and hepatic diseases in China and Japan. Intraperitoneal (i.p.) administration of CAE for 7 d before tumor inoculation significantly inhibited liver metastasis caused by intraportal injection of colon 26-L5 carcinoma cells in a dose-dependent manner. CAE also showed concentration dependent mitogenic activity on BALB/c whole splenocytes, whereas incubation of the non-adherent fraction of splenocytes with CAE did not induce this activity. CAE has the ability to induce interleukin (IL)-12 production from macrophages *in vitro*. Following i.p. administration of CAE the maximal levels of IL-12 and interferon (IFN)-γ production in serum were achieved at 2-3 and 6 h, respectively. Experiments using macrophage- or NK cell-deficient mice revealed that CAE-induced IL-12 in serum was not mediated by macrophages and that IFN-γ production was mainly dependent on natural killer (NK) cells. Since CAE was inactive when the contributions of macrophages were removed in our system, its inhibitory mechanism is likely to be mainly associated with the activation of macrophages to an anti-metastatic state rather than NK cells. CAE administration resulted in increased production of IL-2, IFN-γ and decreased production of a Th2 cytokine (IL-4) from splenocytes stimulated by PMA and A23187. Thus, the anti-metastatic effect by CAE is based on its immunomodulating properties including induction of cytokines such as IL-12, IL-2 and IFN-γ leading to a Th1 dominant immune state and activating macrophages to the tumoricidal state. This may provide a basis for the inhibition of cancer metastasis.

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Abstract : Shuang Huang Lian (双黄連, SHL) is one of the Chinese traditional medicines composed of the extracts of Lonicerae Flos, Scutellariae Radix, and Forsythiae Fructus and has been used for the treatment of various chronic and acute diseases including viral hepatitis. It also has shown efficacy in infections caused by antibiotic-resistant bacteria. SHL and the extracts of each constituent plant medicine were tested for suppression of hepatitis B virus surface antigen (HBsAg) secretion in an experimental model for chronic HBV infection using the PLC/PRF/5 cell line. As a result, SHL as well as the extracts of Lonicerae Flos and Scutellariae Radix suppressed the secretion of HBsAg without cytotoxicity.

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Abstract : The technique of photoaffinity labeling has become increasingly appreciated as a powerful chemical methodology for the detailed structural analysis of ligand binding domains. This review describes our recent approach to the development of new phenyldiazirine family, the design of biotinyl photoprobes, and their application in the field of glycobiology.

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Abstract : A rapid and efficient strategy has been developed for identification of photoaffinity labeled peptides. The strategy involves a radioisotope-free approach in which the photoaffinity label is biotinylated. The method then utilizes a novel derivatization of PVDF membrane for identification and analysis of peptide fragments derived from the photoaffinity labeled binding site in a simple dot blot assay. In the present study, the method has been applied to identification of the binding site region of β1,4-galactosyltransferase (GalT). Sequence analysis has revealed that the biotinylated photoprobe is localized in a tryptic GalT fragment (Y197-R208). These data are consistent with previous suggestions concerning the GalT acceptor site and clearly demonstrate the effectiveness of our approach for rapid identification of photolabeled peptides.

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Abstract : New cell impermeant bis-mannose photolabels have been developed which have biotinyl groups attached to 4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis(D-mannos-4-yloxy)-2-propylamine (ATB-BMPA) by either a polyethoxy spacer (Bio-ATB-BMPA) or an additional hexanoic acid spacer (Bio-LC-ATB-BMPA). The half-maximal inhibition constants, Ki values, for inhibition of glucose transport activity in insulin-stimulated rat adipocytes are 359 $\pm$ 10 μM and 273 $\pm$ 28 μM for Bio-ATB-BMPA and Bio-LC-ATB-BMPA, respectively. These values are similar to those previously reported for the non-biotinylated compound ATB-BMPA. Following uv-irradiation induced crosslinking of the biotinylated photolabels to rat adipocytes, the biotinylated GLUT4 can be detected by non-radioactive and radioactive methods that utilise the interaction with streptavidin. Biotinylated GLUT4 from 1-2 μg of adipose cell membranes, precipitated onto magnetic streptavidin beads, can be sensitively and quantitatively detected using an electrochemiluminescent assay method. This utilises a ruthenium-tagged anti-GLUT4 antibody that on excitation at an electrode generates a electrochemiluminescent signal in an Origen analyser. Alternatively, surface-biotinylated GLUT4 can be easily, but less sensitively, detected in streptavidin agarose

precipitates which are analysed by conventional GLUT4 Western blotting. Data obtained using the non-radioactive methods compare favourably with those using tritiated versions of the biotinylated probes. Insulin treatment of adipocytes increases the levels of signals from surface-biotinylated GLUT4 by ≈10-fold or ≈20-fold respectively when the electrochemiluminescent or the Western blot detection methods are used and these signals are blocked by cytochalasin B.

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Abstract : Synthesis of a 3-phenyl-3-trifluoromethyldiazirine derivative having a cleavable 1,2-diol moiety for photocross-linking technique is described. An ester of cinnamic acid derivative was prepared by the Wittig reaction of 2-methoxy-4-[3-trifluoromethyl-3*H*-diazirin-3-yl]benzaldehyde which can be readily prepared by the Friedel-Crafts reaction of corresponding phenyldiazirine. The osmium oxidation of the olefinic bond gave the desired diol derivative, ethyl 2,3-dihydroxy-3-[2-methoxy-4-(3-trifluoromethyl-3H-diazirin-3-yl)phenyl]propionate, in a high yield. The cleavability of the diol group was confirmed by the conventional periodate oxidation to give the starting aldehyde.

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Abstract : We have developed a simple method for the preparation of photoreactive membrane carrying carbohydrate ligands on its surface. Firstly, a polyvinylidene difluoride (PVDF) membrane was photochemically modified with two carbene-generating diazirine derivatives for the construction of two chemically different amino fuctional groups on the membrane surface, aliphatic and aromatic amines, respectively. A photoreactive benzophenone carboxylic acid was then selectively coupled to the aliphatic amine site. The remaining aromatic amine was diazotized and subjected to the diazo coupling reaction with the pyridylamino derivative of *N*-acetylneuramine lactose. To evaluate the ability of this photoreactive affinity membrane, photocrosslinking of a sialic acid binding lectine from *Maackia amurensis* was examined.

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◇研究費取得状況

1）平成10年度富山県受託研究：和漢薬・バイオテクノロジー研究（第3年度），（分担：門田重利）「癌転移を抑制する伝承薬物に関する研究」，37万5千

2）文部省科学研究費，国際学術研究（平成9～10年度　増額分），（代表：門田重利）「ベトナム，タイ，ミャンマーにおける伝統医学，並びに天然薬物資源の調査研究」，200万

3）文部省科学研究費，国際学術研究（平成10年度），（代表：門田重利）「ベトナム，タイ，ミャンマーにおける伝統医学，並びに天然薬物資源の調査研究」，300万

4）文部省科学研究費，基盤研究A1（分担：門田重利）「新規高次神経変性疾患モデル動物，細胞の開発，神経変性機序の解析と薬物評価法の確立」，50万

5）（財）東京生化学研究会国際共同研究助成（代表：門田重利）「マクロファージからのNO

産生を抑制する伝統薬物の研究」，150万

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8）（財）公益信託医用薬物研究奨励富岳基金（代表：畑中保丸）「高い認識能を持つ光反応性糖鎖分子の設計と応用」，100万

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◇学位（修士・博士）取得者

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大杉瑞恵「天然薬物のTNF-a誘発肝細胞死に対する作用」

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