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) **Fan W., Tezuka Y., Komatsu K., Namba T., and Kadota S.: Prolyl Endopeptidase Inhibitors from Underground Part of *Rhodiola sacra* S. H. Fu. Biol. Pharm. Bull., 22 : 157–161, 1999.**

Summary : Prolyl endopeptidase (PEP, EC 3.4.21.26) is an enzyme which plays a role in the metabolism of proline-containing neuropeptides, *e.g.*, vasopressin, substance P and thyrotropin-releasing hormone (TRH), which have been suggested to be involved in learning and memory processes. In our systematic screening for PEP inhibitors from traditional Chinese medicines, we found that MeOH extract from the underground part of *Rhodiola sacra* S. H. Fu shows significant inhibitory activity against PEP from *Flavobacterium meningosepticum*. Examination of the constituents of the extract resulted in the isolation of nineteen known compounds, identified as hydroquinone (**1**), 4-hydroxybenzoic acid (**2**), caffeic acid (**3**), 4-hydroxycinnamic acid (**4**), suberic acid (**5**), protocatechuic acid (**6**), gallic acid (**7**), (−)-epigallocatechin 3-*O*-gallate (**8**), 2-phenylethyl β-D-glucopyranoside (**9**), 3-*O*-galloylepigallocatechin-(4β→8)-epigallocatechin 3-*O*-gallate (**10**), 2-phenylethyl α-L-arabinopyranosyl-(1→6)-β-D-glucopyranoside (**11**), sacranoside A (**12**), β-D-glucopyranosyl 4-hydroxybenzoate (**13**), rhodiocyanoside A (**14**), rhodiooctanoside (**15**), sarmentosin (**16**), heterodendrin (**17**), arbutin (**18**) and 4-*O*-(β-D-glucopyranosyl)-gallic acid (**19**). Among these, **1, 2, 5, 8**—**10, 13, 16, 18** and **19** have been isolated for the first time from *R. sacra*, among which **5, 9, 10, 13, 16, 18** and **19** have been isolated from *Rhodiola* plants for the first time. On the PEP inhibition, seven compounds (**6**—**8, 10, 12, 18, 19**) showed inhibition with an IC_{50} of 27.8, 487, 1.47, 0.437, 348, 391 and 215 μM, respectively. The kinetic study of these inhibitors indicated that they are noncompetitive inhibitors, except for **6** which is a competitive inhibitor.

2) **Tezuka Y., Terazono M., Kusumoto I. T., Kawashima Y., Hatanaka Y., Kadota S., Hattori M., Namba T., Kikuchi T., Tanaka K., and Supriyatna S.: Helisterculins A and B, Two New (7.5',8.2')-Neolignans, and Helisorin, the First (6.4',7.5',8.2')-Neolignan, from Indonesian Medicinal Plant *Helicteres isora*. Helv. Chim. Acta, 82: 408–417, 1999.**

Summary : During a chemical study of Indonesian medicinal plants, we examined the constituents of fruits of *Helicteres isora* L. (Sterculiaceae), one of the famous Jamu medicines. From a water extract of the fruits, we isolated three new neolignans, helisterculins A (**1**) and B (**2**) and helisorin (**3**), and elucidated their structures by spectral analyses. Helisterculins A (**1**) and B (**2**) are (7.5',8.2')-neolignans with a bicyclo[2.2.2]octene C-framework, while helisorin (**3**) is a (6.4',7.5',8.2')-neolignan with a very rare 4,4a,9,9a-tetrahydro-3,9-methano-3*H*-fluorene C-framework. The natural product with the latter C-framework has no literature precedent. The neolignans **1**—**3** showed weak inhibitory activity against reverse transcriptase from avian myelobrastosis virus.

3) **Prasain J. K., Tezuka Y., Li J.-X., Tanaka K., Basnet P., Dong H., Namba T., and Kadota S.: New Diarylheptanoid from the Seeds of *Alpinia blepharocalyx*. Planta Medica, 65 : 196, 1999.**

Abstract : *Alpinia blepharocalyx* K. Schum. (Zingiberaceae) is used in traditional Chinese medicine for the treatment of stomach disorder (**1**). We have recently reported fifteen novel diarylheptanoids together with six known phenolic compounds (**2**—**5**). Further investigation on the ether soluble fraction of the seeds of *A. blepharocalyx* has led to the isolation of a new diarylheptanoid, 5,6-dehydro-4"-de-*O*-methylcentrolobin (**1**).

4) **Li H., Miyahara T., Tezuka Y., Namba T., Suzuki T., Dowaki R., Watanabe M., Nemoto N., Tonami S., Seto H., and Kadota S.: The Effect of Kampo Formulae on Bone Resorption *in Vitro* and *in Vivo*. II. Detailed Study of Berberine. Biol. Pharm. Bull., 22 : 391–396, 1999.**

Summary : We previously isolated berberine from aqueous extracts of tsu-kan-gan, a Kampo formula used for the treatment of osteoporosis. Berberine caused an inhibitory effect on parathyroid hormone (PTH)-stimulated bone resorption in neonatal mouse bone. In this report we describe the inhibitory effect of berberine on the formation of osteoclast-like multinucleated cells (OCLs) in the co-culture of mouse osteoblastic cells and bone marrow cells in the presence of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$], PTH and interleukin-1α (IL-1α). Berberine dose-dependently inhibited the formation of tartrate-resistant acid phosphatase (TRAP)-positive OCLs induced by 1α,25$(OH)_2D_3$, PTH and IL-1α. We prepared OCLs in the co-culture of osteoblastic cells and bone marrow cells. The effect of berberine on pit formation by OCLs was examined using dentin slices. As OCLs are terminally differentiated multinucleated cells, the survival of OCLs affects the bone-resorbing activity of OCLs. This prompted us to count the number of TRAP-positive OCLs on the slices. Berberine dose-dependently inhibited pit formation and caused a decrease in the number of TRAP-positive OCLs. Calcitonin (CT) inhibited pit formation without affecting the number of OCLs. Berberine accelerated the cell death in OCLs cultivated on a culture plate, but CT did not affect the cell death of OCLs. This suggests that the decrease in the number of OCLs on dentin slices may be due to apoptotic cell death in OCLs. In fact, Hoechst 33258 staining revealed that the treatment of OCLs with berberine resulted in condensed nuclei and a decrease in cell size. Oral administration of the berberine (30 and 50 mg/kg/d) to ovariectomized rats prevented a decrease in bone mineral density (BMD) of the lumbar vertebra without affecting the weight of the uterus and plasma concentration of estradiol. These results suggested that berberine prevented a decrease in BMD *in vivo* by inhibiting osteoclastic bone resorption.

5) **Stampoulis P., Tezuka Y., Banskota A. H., Tran K. Q., Saiki I., and Kadota S.: Staminol A, a Novel Diterpene from *Orthosiphon stamineus*. Tetrahedron Lett., 40:**

4239–4242, 1999.

Abstract: From the aerial part of a Vietnamese medicinal plant, *Orthosiphon stamineus* BENTH. (Lamiaceae), staminol A (**1**), a diterpene with a novel carbon framework, was isolated together with four new isopimarane-type diterpenes, orthosiphols F–I (**2**–**5**). Their structures were elucidated by the spectroscopic analyses.

6) **Xiong Q., Hase K., Tezuka Y., Namba T., and Kadota S.: Acteoside Inhibits Apoptosis in D-Galactosamine and Lipopolysaccharide-Induced Liver Injury. Life Sciences, 65 : 421–430, 1999.**

Summary: We assessed the effect of acteoside, a naturally occurring antioxidative phenylethanoid, on hepatic apoptosis and the subsequent liver failure induced by D-galactosamine (D-GalN) and lipopolysaccharide (LPS). A co-administration of D-GalN (700 mg/kg) and LPS (35 mg/kg) to mice evoked typical hepatic apoptosis characterized by DNA fragmentation and apoptotic body formation, resulting in fulminant hepatitis and lethality of mice. Pre-administration of acteoside at 10 or 50 mg/kg subcutaneously at 12 and 1 h prior to D-GalN/LPS intoxication significantly inhibited hepatic apoptosis, hepatitis and lethality. Tumor necrosis factor-α (TNF-α) secreted from LPS-stimulated macrophages is an important mediator of apoptosis in this model. Acteoside showed no apparent effect on the marked elevation of serum TNF-α, but it partially prevented *in vitro* TNF-α (100 ng/ml)-induced cell death in D-GalN (0.5 μM)-sensitized hepatocytes at the concentrations of 50, 100 and 200 μM. These results indicated that D-GalN/LPS-induced hepatic apoptosis can be blocked by an exogenous antioxidant, suggesting the involvement of reactive oxygen intermediates (ROIs) in TNF-α-dependent hepatic apoptosis.

7) **Fan W., Tezuka Y., Xiong Q., Hattori M., Namba T., and Kadota S.: Apocynins A–D : New Phenylpropanoid-substituted Flavan-3-ols Isolated from Leaves of *Apocynum venetum* (*Luobuma-Ye*). Chem. Pharm. Bull., 47 : 1049–1050, 1999.**

Summary: Four new phenylpropanoid-substituted flavan-3-ols called apocynins A–D (**1**–**4**) have been isolated from the leaves of *Apocynum venetum* (Apocynaceae), together with two known phenylpropanoid-substituted flavan-3-ols, catechin-[8,7-e]-4α-(3,4-dihydroxyphenyl)-dihydro-2(3*H*)-pyranone (**5**) and cinchonain Ia (**6**), and four known flavan-3-ols, (−)-epicatechin, (+)-catechin, (−)-epigallocatechin, and (+)-gallocatechin. Their structures were elucidated on the basis of spectral analysis, including 2D NMR and CD spectra. They showed hepatoprotective activity against D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes.

8) **Tezuka Y., Fan W., Kasimu R., and Kadota S.: Screening of Crude Drug Extracts for Prolyl Endopeptidase Inhibitory Activity. Phytomedicine, 6 : 197–203, 1999.**

Summary: Prolyl endopeptidase (PEP, EC 3.4.21.26) is an enzyme to play a role in

metabolism of proline-containing neuropeptides, such as vasopressin, substance P and thyrotropin-releasing hormone (TRH), which were suggested to be involved with learning and memory processes. Then, specific inhibitor of PEP is expected to have antiamnesic effects, and thus we screened forty-six water- and methanol-extracts from crude drugs selected on the basis of traditional Chinese medicine theory, for *Flavobacterium* prolyl endopeptidase inhibition. Among them, the water-extracts of *Rhodiola sacra* (IC_{50}, 0.77 μg/ml) and the methanol-extracts of *Lycopodium clavatum* (IC_{50}, 1.3 μg/ml), *Paeonia lactiflora* var. *trichocarpa* (IC_{50}, 5.7 μg/ml), *Paeonia veitchii* (IC_{50}, 2.4 μg/ml) and *Rhodiola sacra* (IC_{50}, 0.67 μg/ml) showed strong inhibitory activity. In addition, we also examined the PEP inhibitory activity of eleven compounds from *Salvia deserta*, and found that in addition to a catechol group α-hydroxy-para-quinone group may be related to the PEP inhibition.

9) **Ohsugi M., Fan W., Hase K., Xiong Q., Tezuka Y., Komatsu K., Namba T., Saitoh T., Tazawa K., and Kadota S.: Active-oxygen Scavenging Activity of Traditional Nourishing-tonic Herbal Medicines and Active Constituents of *Rhodiola sacra*. J. Ethnopharmacol., 67 : 111–119, 1999.**

Abstract: Active-oxygen scavenging activity of seventy traditional herbal medicines used in China and Japan as nourishing-tonics were evaluated by electron spin resonance (ESR) technique, in order to evaluate their effectiveness for anti-aging and to search for new active-oxygen scavengers from natural resources. Most of the seventy herbal medicines showed scavenging activity with various intensities. Especially *Areca catechu* (methanol-extract), *Dendrobium plicatile* (methanol-extract), *Juglans regia* (water-extract), *Paeonia lactiflora* (methanol-extract), *Psychotria serpens* (water- and methanol-extracts), *Rhodiola sacra* (water- and methanol-extracts) and *Uncaria rhynchophylla* (water-extract) showed strong scavenging activity against superoxide anion radical ($\cdot O^{2-}$), while *Juglans regia* (water- and methanol-extracts), *Morus alba* (water-extract) and *Schisandra chinensis* (water-extract) revealed strong scavenging activity against hydroxyl radical (HO$\cdot$). In addition, the active-oxygen scavenging activities of nineteen compounds isolated from *R. sacra* was also examined, and hydroquinone (**1**), caffeic acid (**3**), protocatechuic acid (**6**), gallic acid (**7**), (−)-epigallocatechin 3-*O*-gallate (**8**), 3-*O*-galloylepigallocatechin-(4$\beta\rightarrow$8)-epigallocatechin 3-*O*-gallate (**10**), heterodendrin (**17**) and gallic acid 4-*O*-β-D-glucopyranoside (**19**) were found to show mild or strong inhibitory activity against superoxide anion radical ($\cdot O^{2-}$), while 4-hydroxybenzoic acid (**2**), **3**, 4-hydroxycinnamic acid (**4**), **6**—**8** and **19** inhibited hydroxyl radical (OH$\cdot$). These active-oxygen scavengers may contribute, with different extent, to their anti-aging action.

10) **Stampoulis P., Tezuka Y., Banskota A. H., Tran K. Q., Saiki I., and Kadota S.: Staminolactone A, B and Norstaminol A: Three Highly Oxygenated Staminane-type Diterpenes from *Orthosiphon stamineus*. Org. Lett., 1 : 1367–1370, 1999.**

Abstract: Staminolactones A (**1**) and B (**2**) and norstaminol A (**3**), three highly oxygenated staminane-type diterpenes having mild cytotoxic activities against highly liver-metastatic colon 26-L5 carcinoma cells, were isolated from the aerial part of Vietnamese medicinal plant *Orthosiphon stamineus* (Lamiaceae). Their structures were elucidated based on the extensive spectral analyses.

11) **Gewali M. B., Tezuka Y., Banskota A. H., Ali M. S., Saiki I., Dong H., and Kadota S.: Epicalyxin F and Calyxin I; Two Novel Antiproliferative Diarylheptanoids from the Seeds of *Alpinia blepharocalyx*. Org. Lett., 1 : 1733–1736, 1999.**

Abstract: Epicalyxin F (**1**) and calyxin I (**2**), two novel diarylheptanoids were isolated from a residual fraction of an EtOH extract of *Alpinia blepharocalyx*. Calyxin I (**2**) represented a new carbon skeleton and epicalyxin F (**1**) possessed potent antiproliferative activity towards HT-1080 fibrosarcoma and colon 26-L5 carcinoma with ED_{50} values 1.71 and 0.89 μM, respectively.

12) **Tezuka Y., Zhao W., Ishii E., and Kadota S.: Anti-*Helicobacter pylori* Activity of Steroidal Alkaloids Obtained from Three *Veratrum* Plants. J. Trad. Med., 16 : 196–200, 1999.**

Abstract: Anti-*Helicobacter pylori* (HP) activities were examined, by disc method, on three total alkaloid fractions and fourteen steroidal alkaloids obtained from three *Veratrum plants* (*V. maackii, V. nigrum* var. *ussuriense* and *V. patulum*), which are used as a name of "Li-lu (藜蘆)" to treat aphasia arising from apoplexy, wind type dysentery, jaundice, headache, scabies, chronic malaria, etc. Among them, verapatulin (**12**) and veratramine (**13**) revealed anti-HP activities, and the disc-minimum inhibitory concentration (disk-MIC) value (10 μg/ml) of **12** against two standard HP strains, NCTC11637 and NCTC11916, was higher than that of a clinically used antibiotic, erythromycin (< 0.013 μg/ml), but was comparable to those of penicillin G (3.1 μg/ml and 1.6 μg/ml, respectively).

13) **Li H., Miyahara T., Tezuka Y., Watanabe M., Nemoto N., Seto H., and Kadota S.: The effect of low molecular weight Chitosan on bone resorption *in vitro* and *in vivo*. Phytomedicine, 6 : 305–310, 1999.**

Summary: We studied the effect of low molecular weight chitosan (LMWC) on the formation of osteoclast-like multinucleated cells (OCLs) in the co-culture of mouse osteoblastic cells and bone marrow cells in the presence of 1α,25-dihydroxyvitamin D_3 [1α,25$(OH)_2D_3$]. LMWC at 440 μg/ml inhibited the formation of tartrate-resistant acid phosphatase (TRAP)-positive OCLs induced by 1α,25$(OH)_2D_3$. We prepared OCLs in the co-culture of osteoblastic cells and bone marrow cells. The effect of LMWC on pit formation by OCLs was examined using dentin slices, and LMWC inhibited pit formation at 440 μg/ml. Oral administration of the LMWC to ovariectomized rats prevented a decrease in bone mineral density (BMD) of the lumbar vertebra without affecting the body and uterus weights. These

results suggested that LMWC prevented a decrease in BMD *in vivo* by inhibiting osteoclastic bone resorption.

14) **Hase K., Xiong Q., Basnet P., Namba T., and Kadota S.: Inhibitory Effect of Tetrahydroswertianolin on Tumor Necrosis Factor-α-Dependent Hepatic Apoptosis in Mice. Biochem. Pharmacol., 57 : 1431–1437, 1999.**

Abstract : We investigated the effect of tetrahydroswertianolin (THS), a hepatoprotective agent from *Swertia japonica*, on tumor necrosis factor-α (TNF-α)-dependent hepatic apoptosis induced by D-galactosamine (D-GalN) (700 mg/kg, i.p.) and lipopolysaccharide (LPS) (10 μg/kg, i.p.) in mice. Apoptotic symptoms were observed at the initial stage of liver damage. By 5 hr after intoxication, hepatic DNA fragmentation had risen to 2123%, with the value in untreated mice set at 100%, without a significant elevation of serum alanine transaminase (ALT) activity. There was a parallel increase in hepatocytes undergoing chromatin condensation and apoptotic body formation. By 8 hr after intoxication, serum ALT activity had risen to 3707 U/L. Pretreatment with THS (50 mg/kg, p.o.) at 18 and 2 hr before intoxication significantly reduced DNA fragmentation to 821% of that in untreated mice and prevented the emergence of chromatin condensation and apoptotic body formation. A significant and dose-dependent reduction in serum ALT activity at 8 hr also was observed with THS pretreatment. These effects of THS were different from those observed from pretreatment with glycyrrhizin (GCR), which is a clinically used hepatoprotective agent with membrane-stabilizing activity. GCR pretreatment (100 mg/kg, p.o.) did not inhibit hepatic DNA fragmentation (1588% of untreated mice), although this compound significantly protected against serum ALT elevation (1463 U/L). These data suggest that an inhibitory effect on the progression of hepetic apoptosis prior to liver injury may be involved in the hepatoprotective mechanisms of THS, whereas it appears that GCR affects the processes after apoptosis. In a separate experiment, we found that the concentration of serum TNF-α rose to 2016 pg/mL at 1 hr after intoxication of mice with D-GalN and LPS, but this increase was suppressed by THS pretreatment (10, 50, or 200 mg/kg, p.o.) to 716, 454, or 406 pg/mL, respectively. Further study with a reverse transcriptase-polymerase chain reaction method showed that THS blocked TNF-α production at the transcriptional level. Because TNF-α is a critical mediator to elicit apoptosis in this model, the property of suppressing TNF-α production may be of prime importance for THS inhibition of hepatic apoptosis.

15) **Kurokawa M., Basnet P., Ohsugi M., Hozumi T., Kadota S., Namba T., Kawana T., and Shiraki K.: Anti-Herpes Simplex Virus Activity of Moronic Acid Purified from *Rhus javanica In Vitro* and *In Vivo*. J. Pharmacol. Exp. Ther., 289 : 72–78, 1999.**

Abstract : *Rhus javanica*, a medicinal herb, has been shown to exhibit oral therpeutic anti-herpes simplex virus (HSV) activity in mice. We purified two major anti-HSV compounds, moronic acid and betulonic acid, from the herbal extract by extraction with ethyl acetate at pH 10 followed by chromatographic separations and examined their anti-HSV activity *in*

vitro and *in vivo*. Moronic acid was quantitatively a major anti-HSV compound in the ethyl acetate-soluble fraction. The effective concentrations for 50% plaque reduction of moronic acid and betulonic acid for wild-type HSV type 1 (HSV-1) were 3.9 and 2.6 μg/ml, respectively. The therapeutic index of moronic acid (10.3−16.3) was larger than that of betulonic acid (6.2). Susceptibility of acyclovir-phosphonoacetic acid-resistant HSV-1, thymidine kinase-deficient HSV-1, and wild-type HSV type 2 to moronic acid was similar to that of the wild-type HSV-1. When this compound was administered orally to mice infected cutaneously with HSV-1 three times daily, it significantly retarded the development of skin lesions and/or prolonged the mean survival times of infected mice without toxicity compared with the control. Moronic acid suppressed virus yields in the brain more efficiently than those in the skin. This was consistent with the prolongation of mean survival times. Thus, moronic acid was purified as a major anti-HSV compound from the herbal extract of *Rhus javanica*. Mode of the anti-HSV activity was different from that of ACV. Moronic acid showed oral therapeutic efficacy in HSV-infected mice and possessed novel anti-HSV activity that was consistent with that of the extract.

16) **Watanabe C., Satoh T., Tahara E., Murakami K., Hayashi K., Hase K., Andoh T., Kuraishi Y., Kadota S., Nagai H., and Saiki I.: Inhibitory Mechanisms of Glycoprotein Fraction Derived from *Miscanthus sinensis* for the Immediate Phase Response of an IgE-Mediated Cutaneous Reaction. Biol. Pharm. Bull., 22 : 26–30, 1999.**

Summary: We investigated the inhibitory effect of the glycoprotein fraction (fraction 2) extracted from *Miscanthus sinensis* ANDERSSON (*M. sinensis*) on biphasic cutaneous reactions in mice passively sensitized with IgE. Biphasic skin reactions with peak responses at 1 (IPR, immediate phase reaction) and 24 h (LPR, late phase reaction) were caused by passive sensitization with an anti-dinitrophenol IgE monoclonal antibody (anti-DNP IgE mAb) followed by an epicutaneous challenge of 0.1% dinitrofluorobenzene (DNFB) in 100% ethanol. Intraperitoneal injection of fraction 2 before the DNFB challenge significantly inhibited the biphasic ear swelling response in passively sensitized mice in a dose-dependent manner (1−30 mg/kg). We also found that fraction 2 was effective at inhibiting the vascular permeability in mouse ear induced by an injection of compound 48/80, histamine or serotonin. In addition, fraction 2 inhibited scratching behavior as well as ear edema observed within 2 h after DNFB challenge. Marked inhibition was observed in both passively sensitized and non-sensitized mice. The locomotor activity of mice was also reduced by the administration of fraction 2 as well as by diphenhydramine. These results suggest that the inhibitory effect of glycoprotein fraction 2 of *M. sinensis* on an IgE-mediated allergic inflammatory reaction is due to the protection of mediator-induced vascular permeability and that in addition to the inhibition of an inflammatory reaction, a sedative action is responsible for the inhibition of allergy-induced scratching responses.

17) **Hashimoto M. and Hatanaka Y.: Identification of Photolabeled Peptides for the Acceptor Substrate Binding Domain of β1,4-Galactosyltransferase. Chem. Pharm. Bull., 47 : 667–671, 1999.**

Abstract : We successfully applied a carbene-generating *N*-acetylglucosamine derivative carrying a biotinyl group to the radioisotope-free identification of peptides within bovine UDP-galactose:*N*-acetylglucosamine β1,4-galactosyltransferase (GalT, EC 2.4.1.38) catalytic domain. Owing to the low yield of cross-linking, conventional photoaffinity labeling experiments usually encounter a thorny problem in attempting to isolate labeled components from very complex mixtures. A biotin tag introduced with our photoaffinity probe enabled us to separate the photolabeled protein from a large amount of coexisting unlabeled GalT. The introduction of biotin was also useful for the radioisotope-free detection of a labeled protein based on a highly sensitive chemiluminescent technique. We developed a novel polyvinylidene difluoride membrane for the identification of labeled peptides in a simple dot blot assay. Using this membrane, we successfully identified biotinyl peptides among a number of HPLC separated fragments derived from the protease digestion of photolabeled GalT proteins. The sequence analysis revealed that the biotin tag was incorporated within a tryptic GalT fragment of Y197-R208. Our approach yields, for the first time, information on the acceptor substrate binding-site fragment in this enzyme, that has been difficult to obtain using other approaches. 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.

18) **Kempin U. and Hatanaka Y.: A Novel Reagent for Preparation of Photoaffinity Probes from Unprotected Carbohydrates. Glycoconjugate J., 16 : S54, 1999.**

Abstract : We have developed an efficient method for the preparation of photoaffinity carbohydrate probes. Photoreactive carbohydrates are powerful chemical tools for the structural analysis of proteins that specifically interact with glycoconjugates. Conventional method of probe synthesis, however, usually requires the multiple sequence of reactions involving protection, activation and deprotection steps. Here we report a novel reagent for the one-step introduction of a carbene-generating photoreactive group to the reducing terminus of unprotected carbohydrates. The synthesis of probes takes advantage of the oxime formation reaction between an aminooxy group of the reagent and an aldehyde at the reducing end. Thus, the photoreactive derivative of *N*-acetyllactosamine, 3'-sialyllactose, 3'-sialyl-*N*-acetyllactosamine, Lewis x trisaccharide, or Lewis x tetrasaccharide was easily prepared. Combination of the present method with recently developed non-radioactive approach will provide a powerful strategy for photoaffinity labeling.

19) **Kempin U. and Hatanaka Y.: Photocrosslinking of Carbohydrate-Lectin Interacting Systems. Photomedicine and Photobiology, 21: in press, 1999.**

Abstract : We have developed an efficient method for the preparation of photoaffinity

carbohydrate probes. Photoreactive carbohydrates are powerful chemical tools for the structural analysis of proteins that specifically interact with glycoconjugates. Conventional method of probe synthesis, however, usually requires the multiple sequence of reactions involving protection, activation and deprotection steps. Here we report a novel reagent for the one-step introduction of a carbene-generating photoreactive group to the reducing terminus of unprotected carbohydrates. The synthesis of probes takes advantage of the oxime formation reaction between an aminooxy group of the reagent and an aldehyde at the reducing end. Thus, the photoreactive derivatives of *N*-acetyllactosamine, Lewis x trisaccharide, or Lewis x tetrasaccharide were easily prepared. Combination of the present method with recently developed non-radioactive approach will provide a powerful strategy for photoaffinity labeling.

◇学会報告

1）Arjun H. Banskota, Yasuhiro Tezuka, Le Kim Phung, Kim Qui Tran, Ikuo Saiki, Yoshihisa Miwa, Tooru Taga, and Shigetoshi Kadota: Seven Novel Cycloartane-type Triterpenes from *Combretum quadrangulare* and Their Cytotoxic Activities. 日本薬学会第119年会，1999，3/29–31，徳島.

2）李　慧英，手塚康弘，難波恒雄，門田重利，宮原龍郎，渡辺　誠，堂脇理沙，根本信雄：漢方方剤による抗骨粗鬆活性成分の研究（II）－ベルベリンの骨吸収抑制機構について－. 日本薬学会第119年会，1999，3/29–31，徳島.

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