[*Org. Lett.*, **5**, 565-567 (2003)] [Lab. of Pharm. Chemistry]

Development of Novel Diastereoselective Alkenylation of Enolates Using Alkenylselenonium Salts.

Shin-ichi WATANABE, Takahiro IKEDA, Tadashi KATAOKA,*
Genzoh TANABE, and Osamu MURAOKA

A novel alkenylation of enolates using alkenylselenonium salts is described. A reaction of lithium enolates, which were prepared *in situ* by the reaction of LiHMDS and carbonyl compounds, with alkenylselenonium salts gave the ethenylation products of carbonyl compounds in high yield. Diastereoselective alkenylation was also accomplished by the reaction of the enolates derived from *N*-acyl-1,3-oxazolidin-2-ones with the alkenylselenonium salt to afford good results (up to 92% yield and up to 95% de).

[*Chem. Eur. J.*, **9**, 1496-1502 (2003)] [Lab. of Pharm. Chemistry]

Chalcogenide-Lewis Acid Mediated Reactions of Electron-Deficient Alkynes with Aldehydes.

Sayaka KINOSHITA, Hironori KINOSHITA, Tatsunori IWAMURA,
Shin-ichi WATANABE, and Tadashi KATAOKA*

Reactions of but-3-yn-2-one with aldehydes in the presence of $TiCl_4$ or $TiBr_4$ and dimethyl sulfide predominantly gave (*E*)-α-(halomethylene) aldols in high yields, while reactions of methyl propiolate mainly afforded (*Z*)-3-halogeno-2-(hydroxymethyl)-acrylates in low to moderate yields. Although reactions of acetylenic ketones proceeded without dimethyl sulfide, ratios of the geometrical isomers were different from those obtained from the reactions using the sulfide. Reactions of methyl propiolate did not proceed without the sulfide.

[*Angew. Chem. Int. Ed.*, **42**, 2889-2891 (2003)] [Lab. of Pharm. Chemistry]

Asymmetric Induction of Three Consecutive Chiral Centers by Reactions of *N*-Enoylthioamides with Aldehydes.

Tadashi KATAOKA*, Hironori KINOSHITA, Sayaka KINOSHITA, Takashi OSAMURA, Shin-ichi WATANABE,
Tatsunori IWAMURA, Osamu MURAOKA, and Genzoh TANABE

The reaction of *N*-cinnamoyl-4-methyl-5-phenyl-1,3-oxazoline-2-thione with aromatic aldehydes in the presence of $BF_3 \cdot Et_2O$ diastereoselectively gave tricyclic adducts, which contain three consecutive asymmetric centers and a chiral bridgehead bound to four heteroatoms in high yields with a high diastereomer ratio.

[*Eur. J. Org. Chem.*, 4852-4861, (2003)] [Lab. of Pharm. Chemistry]

Chalcogeno Morita–Baylis–Hillman Reaction of 2-(Methylchalcogeno)phenyl Vinyl Ketones with Aldehydes, Ketones, and α-Dicarbonyl Compounds.

Hironori KINOSHITA, Sayaka KINOSHITA, Yukari MUNECHIKA, Tatsunori IWAMURA,
Shin-ichi WATANABE, and Tadashi KATAOKA*

Reactions of 2-(methylchalcogeno)phenyl vinyl ketones **1** and **2** with aldehydes were conducted in the presence of $BF_3 \cdot Et_2O$. The reaction was quenched by addition of Et_3N and gave the Morita–Baylis–Hillman (MBH) adducts in good yields. When the reaction mixture of the sulfur derivative **1** with an aldehyde was worked up with saturated aqueous $NaHCO_3$, the sulfonium salt was obtained together with MBH adducts. Ketones, α-diketones, and α-oxo esters, which hardly react in the traditional MBH reaction, similarly reacted with 2-(methylchalcogeno)phenyl vinyl ketones to give the MBH adducts. Selenochromanones were obtained together with MBH adducts from reactions of seleno derivative **2** with aldehydes, α-dicarbonyl compounds as by-products. The formation mechanism for the sulfonium salt is discussed.

[*J. Org. Chem.*, **68**, 7532-7534 (2003)] [Lab. of Pharm. Chemistry]

Chalcogeno–Morita–Baylis–Hillman Reaction of Enones with Acetals: Simple α-Alkoxyalkylation of Enones.

Hironori KINOSHITA, Takashi OSAMURA, Sayaka KINOSHITA, Tatsunori IWAMURA, Shin-ichi WATANABE, Tadashi KATAOKA*, Genzoh TANABE, and Osamu MURAOKA

1-[2-(Methylsulfanyl)phenyl]prop-2-en-1-one and the seleno congener reacted with acetals in the presence of $BF_3 \cdot Et_2O$ to give α-alkoxyalkyl enones in good yields. When the reaction mixtures were worked up with a saturated $NaHCO_3$ solution instead of Et_3N, onium salts were obtained together with α-alkoxyalkyl enones. Reactions with a cyclic acetal gave α-(β-hydroxyethoxy)enones accompanied by dimeric products. $BF_3 \cdot Et_2O$ worked for the formation of both the enolates and α-alkoxy carbocations.

[*Chem. Commun.*, 654-655 (2003)] [Lab. of Medicinal Chemistry]

A remarkable solvent effect toward the Pd/C-catalyzed cleavage of silyl ethers.

Hironao SAJIKI, Takashi IKAWA, Kazuyuki HATTORI and Kosaku HIROTA*

We have discovered a remarkable solvent effect toward the Pd/C-catalyzed cleavage of TBDMS and TES ethers and it was applied to the development of a chemoselective hydrogenation method for olefin, benzyl ether and acetylene functionalities distinguishing from the TBDMS and TES protective groups of a hydroxy group by the employment of EtOAc or MeCN as a solvent. The ready availability of the catalyst (commercially available), the high yields, simplicity of the procedure, and selective nature of the hydrogenation, render this new and simple methodology advantageous for work involving TBDMS and TES ether manipulation.

[*Tetrahedron Lett.*, 44, 171-174 (2003)] [Lab. of Medicinal Chemistry]

Preparation of silk fibroin-supported Pd(0) catalyst for chemoselective hydrogenation: reduction of palladium(II) acetate by methanol on the protein.

Hironao SAJIKI, Takashi IKAWA, Hiromi YAMADA. Kozo TSUBOUCHI and Kosaku HIROTA*

The present study provides indication that the rapid reduction of the silk-fibroin conjugated $Pd(OAc)_2$ proceeded using MeOH as a reductant at room temperature. The Pd/Fib (palladium/fibroin) catalyst displays good chemoselectivity in the hydrogenation of olefins and azides in the presence of aromatic carbonyls and/or halogens or an *O*-benzyl protective group. This catalyst provides a simple and practical protocol for chemoselective hydrogenation and reinforces the versatility of aromatic carbonyls and halides in organic synthesis.

[*Tetrahedron Lett.*, 44, 2179-2181 (2003)] [Lab. of Medicinal Chemistry]

A novel chemical modification at the 5-position of uridine derivatives.

Hironao SAJIKI, Akira YAMADA, Kanoko YASUNAGA, Takashi TSUNODA, Mumen F. A. AMER and Kosaku HIROTA*

A novel chemical modification was achieved at the 5-position of 2,3-*O*-isopropylideneuridine (6) in a one-pot procedure and a remarkable effect of the base on the progress of the reaction was found. The present reaction seems like an intramolecular base-catalyzed Baylis–Hillman reaction. Although numerous examples of Baylis–Hillman-type reaction for aldehydes with electron-deficient olefins are reported, a general and intramolecular base-catalyzed procedure for nucleic acids has not been established. Therefore, the present coupling demonstrates sufficient usefulness in nucleic acid chemistry. It discloses a new α-hydroxybenzylation at the 5-position of uridine derivatives under mild conditions.

[*Tetrahedron Lett.*, **44**, 7407-7410 (2003)] [Lab. of Medicinal Chemistry]

Significant supplier-dependent disparity in catalyst activity of commercial Pd/C toward the cleavage of triethylsilyl ether.

Hironao SAJIKI, Takashi IKAWA and Kosaku HIROTA*

We have clearly demonstrated the unreliability of the palladium-catalyzed cleavage of TES ethers in the absence of hydrogen conditions. The cleavage should be interpreted as an acid-catalyzed solvolysis. This methodology will be an attractive tool for the organic chemist if the supplier of Pd/C is specified and the reproducibility of the data is indicated using some different lots of the Pd/C. Furthermore, we were also able to demonstrate Pd/C catalysts exhibit remarkable supplier-dependent difference in the property and quality. When a Pd/C catalyst is used in an article, the name of the supplier and the product number of the catalyst must be clarified. Finally, it is noteworthy that hydrogen is an obligatory condition for the real palladium-catalyzed cleavage of silyl ethers in MeOH and that 10% Pd/C of Aldrich (20,569-9) is a quite safe and nearly neutral catalyst within our investigation. According to all of the results indicated in this paper, the 10% Pd/C (Aldrich)-catalyzed TES cleavage mechanism under hydrogen conditions3 can involve a direct hydrogenolysis of the silyl group or a true palladium-catalyzed methanolysis by the 10% Pd/C activated by hydrogen.

[*Tetrahedron Lett.*, **44**, 8437-8439 (2003)] [Lab. of Medicinal Chemistry]

Markedly chemoselective hydrogenation with retention of benzyl ester and *N*-Cbz functions using a heterogeneous Pd-fibroin catalyst.

Hironao SAJIKI, Takashi IKAWA and Kosaku HIROTA*

We have developed a mild and chemoselective hydrogenation method using 2.5% Pd/Fib as a catalyst, which is widely applicable to the selective hydrogenation of a variety of olefin, azido and acetylene functionalities leaving intact the benzyl esters and aromatic *N*-Cbz protective groups. The hydrogenolysis of the extremely reducible benzyl ester and aromatic *N*-Cbz protective groups could be completely suppressed. We believe that the present method should find broad application in organic synthesis.

[*Org.Biomol. Chem.*, **1**, 1354-1365 (2003)] [Lab. of Medicinal Chemistry]

Efficient synthesis of 2,9-disubstituted 8-hydroxyadenine derivatives.

Kosaku Hirota,* Kazunori Kazaoka, Itaru Niimoto, and Hironao Sajiki

An efficient and general method for the synthesis of 2,9-disubstituted 8-hydroxyadenines, which are expected to have various biological activities, was realized. 5-Amino-4-cyano-2-hydroxyimidazoles (**1**) were prepared from aminomalononitrile and isocyanates as key intermediates. The condensation of **1a** with amidines, imidates, guanidine, urea and thioureas afforded 8-hydroxyadenines (**2**–**6**) possessing various substituents at the 2-position. Furthermore, selective alkylation of 2-amino- and 2-hydroxyadenines (**4** and **6**) successively proceeded to give the corresponding 2-alkylamino- and 2-alkoxyadenines (**5** and **7**), respectively. 2-Alkylthioadenines (**15**) were prepared by an analogous reaction of **1a** with benzoylisothiocyanate and subsequent *S*-alkylation. The imidazoles **1** are most useful intermediates for the synthesis of 8-hydroxyadenine derivatives.

[*Bioorg. Med. Chem.*, **11**, 2715-2722 (2003)] [Lab. of Medicinal Chemistry]

Synthesis and biological evaluation of 2,8-disubstituted 9-benzyladenines: discovery of 8-mercaptoadenines as potent interferon-inducers.

Kosaku HIROTA,* Kazunori KAZAOKA and Hironao SAJIKI

Recently, we have identified 9-benzyl-8-hydroxyadenines bearing an appropriate substituent (a butoxy, propylthio or butylamino group) at the 2-position as potent interferon (IFN)-inducers. Herein we report the design, synthesis, and IFN-inducing activity of 8-substituted 9-benzyladenines possessing such an appropriate substituent at the 2-position. Introduction of the appropriate substituent into the 2-position of the adenine nucleus gave rise to expression of the activity even in 9-benzyladenines bearing no hydroxyl group at the 8-position. An amino group at the 6-position and a hydroxyl or thiol group carrying an acidic proton at the 8-position are required to express excellent IFN-inducing activity. 9-Benzyl-2-butoxy-8-mercaptoadenine indicated the most potent activity with MEC of 0.001 μM.

[*Bioorg. Med. Chem.*, 11, 3641-3647 (2003)] [Lab. of Medicinal Chemistry]

Synthesis and structure-activity relationships of 2-substituted 8-hydroxyadenine derivatives as orally available interferon inducers without emetic side effects.

Yoshiaki ISOBE, Masanori TOBE, Haruhisa OGITA, Ayumu KURIMOTO, Tetsuhiro OGINO, Hajime KAWAKAMI, Haruo TAKAKU, Hironao SAJIKI, Kosaku HIROTA,* and Hideya HAYASHI

Recently we have reported 2-substituted 8-hydroxyadenine derivatives as a novel class of interferon (IFN) inducing agents. In the present study, we conducted a detailed structure-activity relationship study of 2-alkyl analogues of 8-hydroxyadenines on IFN inducing activity. We found that 2-butyl-9-benzyl-8-hydroxyadenine (1) exhibited the most potent IFN inducing activity *in vitro* with a minimum effective concentration of 0.01 mM, and 1 also showed strong IFN-inducing activity at dose of more than 0.3 mg/kg by oral administration in mice. This potency was 10-fold stronger than that of Imiquimod. Moreover, 1 did not cause emesis in ferrets even at doses as high as 10 mg/kg, whereas, 80% of animals were emetic when orally administered with the same dose of Imiquimod. These results indicate that compound 1 is superior to Imiquimod with respect to efficacy and safety.

[*Bioorg. Med. Chem.*, 11, 5501-5508 (2003)] [Lab. of Medicinal Chemistry]

Synthesis and structure-activity relationships of 2-amino-8-hydroxyadenines as orally active interferon inducing agents.

Ayumu KURIMOTO, Tetsuhiro OGINO, Shinji ICHII, Yoshiaki ISOBE, Masanori TOBE, Haruhisa OGITA, Haruo TAKAKU, Hironao SAJIKI, Kosaku HIROTA* and Hajime KAWAKAMI

Recently we have reported the 2-substituted 8-hydroxyadenine derivatives as a novel class of interferon (IFN) inducing agents. In the present study, a series of 8-hydroxyadenines, which possess various amino moieties at the adenine C(2)-position, were synthesized and evaluated for their ability to induce endogenous interferon in comparison to the known active agent, Imiquimod. Among the compounds prepared, 9-benzyl-8-hydroxy-2-(2-methoxyethyl)aminoadenine (1) was found to exhibit potent IFN inducing activity *in vivo*. Compound 1 induced IFN from the dosage of 0.1mg/kg, which was 30-fold potent than that of Imiquimod, and showed a good oral bioavailability (F= 81%)

[*Chem. Pharm. Bull.*, 51, 320-324 (2003)] [Lab. of Medicinal Chemistry]

Pd/C-catalyzed chemoselective hydrogenation in the presence of a phenolic MPM protective group using pyridine as a catalyst poison.

Hironao SAJIKI and Kosaku HIROTA*

Employment of a Pd/C-pyridine combination as a catalyst is a very useful method for the selective removal (hydrogenolysis) of phenolic *O*-benzyl, *N*-Cbz and benzyl ester protective groups and for the selective hydrogenation of nitro and olefin functions of phenol derivatives protected with the MPM group. These discriminatory results are apparently attributable to the effect of pyridine. The MPM group could be extensively applied to chemoselective hydrogenation as a protective group for phenolic hydroxyl functions.

[*Chem. Pharm. Bull.*, 51, 608-611 (2003)] [Lab. of Medicinal Chemistry]

Synthesis of 6-substituted 9-benzyl-8-hydroxypurines with potential interferon-inducing activity.

Kazunori KAZAOKA, Hironao SAJIKI and Kosaku HIROTA*

Various 6-substituted 9-benzyl-8-hydroxypurines were synthesized in order to investigate the structure-activity relationship at the 6-position of 9-benzyl-8-hydroxyadenine (1), which is a lead compound for the screening of interferon (IFN)-inducing activity. 6-Unsubstituted, mercapto-, methylthio- and hydroxy-9-benzyl-8-hydroxypurines were prepared from 5-amino-1-benzyl-4-cyano-2-hydroxyimidazole. Synthesis of a 6-methoxy analog was conducted from 5-amino-4-benzylamino-6-chloropyrimidine. 6-Alkylamino and acylaminopurines were also prepared by alkylation and acylation of 1, respectively. Since these compounds indicated no activity, it was found that a free amino group of 1 is required for the expression of IFN-inducing activity.

[*Chem. Pharm. Bull.*, **51**, 1451-1454 (2003)] [Lab. of Medicinal Chemistry]

Synthesis of pyrimidine derivatives possessing an antioxidative property and their inhibitory effects on picryl chloride-induced contact hypersensitivity reaction.

Yoshiaki ISOBE and Kosaku HIROTA*

We conducted a preliminary structure-activity relationship study of some barbituric acid and uracil derivatives against the picryl chloride-induced contact hypersensitivity reaction (PC-induced CHR). The introduction of an antioxidative moiety to the side chain of the *C*(6)-position of uracil was effective against this model. 6-(3,5- di-*t*-butyl-3-hydroxybenzylidene)hydrazino-3-methyl-1-phenyluracil (**1**) exhibited the most potent activity toward PC-induced CHR. The introduction of dimethoxyphenol or dimethylphenol of **1** instead of di-*t*-butylphenol as an antioxidative moiety gave diminished activities, so, the reactive oxygen would contribute to the inflammation of this model, and an antioxidative activity was required for exhibiting the inhibitory activity. The inhibitory activity was significantly affected by the substituent at the *N*(1)-phenyl moiety.

[*Chem. Lett.*, **32**, 4—5 (2003)] [Lab. of Pharm. Synthetic Chemistry]

Stereospecific Construction of Chiral Quaternary Carbon Compounds from Chiral Secondary Alcohol Derivatives

Yukio MASAKI, * Hideki ARASAKI, and Masashi IWATA

Chiral tertiary dichloromethylcarbinol derivatives, prepared by stereospecific α C-H insertion reaction of dichlorocarbene with protected chiral secondary alcohols, were converted into intermediary α-chloroepoxides which gave stereospecifically chiral quaternary carbon compounds, α-aminoacids via α-azide-aldehydes and α-cyanoacetic acids through cyanation, respectively. The fashion generating the quaternary centers from dichloromethylcarbinols via α–chloroepoxides was proved to be quite different depending on the substrates: inversion of configuration of non-benzylic substrates and apparent retention with benzylic one.

[*Synthesis*, 1511—1516 (2003)] [Lab. of Pharm. Synthetic Chemistry]

Asymmetric Tandem Conjugate Addition–Allylation of Chiral (*p*-Tolylsulfinyl)pyrrolyl Cinnamoyl Amide.

Yoshitsugu ARAI,* Makoto KASAI, Kimio UEDA, and Yukio MASAKI

The alkylation by allyl halides of the intermediate enolates, prepared *in situ* by conjugate addition of di-*p*-tolylcuprate to chiral (*p*-tolylsulfinyl)pyrrolyl cinnamoyl amide, gave the (2*R*,3*R*)-adducts as the major products with 81% to 94% de's. Methanolysis of the products afforded the corresponding methyl esters, together with efficient recovery of the chiral sulfinyl auxiliary without loss of optical purity.

[*Synthesis*, 2289—2291 (2003)] [Lab. of Pharm. Synthetic Chemistry]

Oxidation of the Methyl Group at the Aromatic Nucleus Molecular Oxygen in the Presence of *N*-Bromosuccinimide under Photoirradiation.

Akichika ITOH,* Tomohiro KODAMA, Shouei HASHIMOTO, Yukio MASAKI,

A methyl group at the aromatic nucleus can be oxidized to the corresponding carboxylic acid directly in the presence of *N*-bromosuccinimide (NBS) under photoirradiation. By considering both the promoting effect of oxygen and a catalytic amount of NBS, aerobic oxidation via hydroperoxide, which is thought to be generated by abstraction of a hydrogen with a bromo radical from NBS, proceeded.

[*Surface and Coatings Technology*, 169-170, 587-591 (2003)] [Lab. of Pharm. Physical Chemistry]

Plasma Technique for the Fabrication of a Durable Functional Surface on Organic Polymers.

Masayuki KUZUYA,* Takashi SAWA, Motoaki MOURI, Shin-ichi KONDO and Osamu TAKAI

We report a novel method to fabricate a durable hydrophilic surface on hydrophobic polymer materials modified by plasma treatment. The method involves immobilization of maleic anhydride-containing polymer onto polyethylene (PE) by a plasma-induced cross-link reaction of PE, followed by hydrolysis of the maleic anhydride moiety to generate hydrophilic carboxyl groups on the PE surface. In fact, the PE sheet surface thus treated has shown effective durability of wettability for a long period of time based on the water contact angle measurement.

[*J. Photopolym. Sci. Technol.*, 16, 71-74 (2003)] [Lab. of Pharm. Physical Chemistry]

Plasma-Assisted Immobilization of Bio-Molecules on LDPE Surface.

Shin-ichi KONDO, Takashi SAWA and Masayuki KUZUYA*

In this communication, we report an extended work on the plasma-assisted immobilization of oligo-DNA on low-density polyethylene (LDPE) surface. LDPE-VEMAC sheet was prepared by the immobilization of vinylmethylether-maleic anhydride copolymer (VEMA) into the surface layer of LOPE by plasma-assisted cross-link reaction, followed by hydrolysis of VEMA. The immobilization of 5'-aminolinker-$(dT)_8$ on the surface of LDPE-VEMAC sheet was carried out with DMTMM to obtain the LDPE-VEMAC-DNA sheet. It was shown that the LDPE- VEMAC-DNA sheet could detect the complementary oligo-DNA. We performed the hybridization and dehybridization of $(dA)_8$ on this sheet repeatedly. It was shown that this LDPE-VEMAC-DNA sheet could be reusable at least several times under this experimental condition.

[*J. Chromatgr. Sci.*, 41, 337-342 (2003)] [Lab. of Pharm. Anal. Chemistry]

Evaluation of a New Reagent: Anthraquinone-2-Sulfonyl Chloride for the Determination of Phenol in Water by Liquid Chromatography Using Precolumn Phase-Transfer Catalyzed Derivatization.

Fang FENG, Bunji UNO,* Masashi GOTO, Zhengxi Zhang, Dengkui An

A new reagent, anthraquinone-2-sulfonyl chloride, is used for the derivatization of phenols. Several compounds with different polarities are selected to evaluate the new reagent and derivatives of these phenols that are prepared via a facile pathway. The optimal conditions for analytical derivatization and mechanism of the derivatization reaction are discussed. The derivatization procedure involves an ion-pair extraction of the deprotonated phenols with a tetrabutylammonium counter ion in the organic phase. At the interface of two phases, the derivatization reaction occurs quantitatively at room temperature within 3 min. The derivatives are stable and readily amenable to analysis by normal-phase (NP) and reversed-phase (RP) high-performance liquid chromatography (HPLC). Excellent linearity response was demonstrated over the concentration range of 0.2-200 μmol/L at 320 nm for NP-HPLC and at 256 nm for RP-HPLC.

[*J. Chromatogr.A*, 987, 341-347 (2003)] [Lab. of Pharm. Anal. Chemistry]

Analysis of DNA Adducts of Acetaldehyde by Liquid Chromatography/Mass Spectrometry

Shinsuke INAGAKI, Yukihiro ESAKA,* Yoshihiro DEYASHIKI, Magoichi SAKO, Masashi GOTO

A high-sensitive method using liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) was developed for the analysis of DNA adducts of acetaldehyde (AA). AA, which is the primary oxidative metabolite of ethanol, is considered to possess carcinogenic activity. AA reacts with the exocyclic amino group of guanine in DNA to form N^2-ethylguanine (Et-Gua) and 1,N^2-propanoguanine (Pr-Gua) adduct. In the present method, such adducts were detected as the base forms cut off from DNA chains by depurination in the pretreatment process. In our measurement with LC/ESI-MS, detection limits of Et-Gua and Pr-Gua adducts of the base forms were 3.0×10^{-10} M and 5.0×10^{-9} M, respectively, and the limits are decreased about two orders of magnitude compared with those of the nucleoside forms. This method was also applied to study whether these two adducts were formed in DNA of cultured HL-60 cells during exposure to AA for 24 hours in the medium. Pr-Gua was detected clearly and a trace of Et-Gua also would be detected in the DNA of the cell. The present method will be a useful tool for studies of Et-Gua and Pr-Gua adducts in organisms, especially in human beings in connection with their cancer.

[*Electrophoresis*, **24**, 1635-1640 (2003)] [Lab. of Pharm. Anal. Chemistry]

Electrophoretic analysis of quinone anion radicals in acetonitrile solutions using an on-line radical generator.

Yukihiro ESAKA,* Noriko OKUMURA, Bunji UNO, Masashi GOTO

We have investigated separation analysis of anion radicals of phenanthraquinone (PhQ) and anthraquinone (AQ) using acetonitrile-CE under anaerobic conditions. PhQ and AQ have relatively highly negative reduction potentials meaning that their anion radicals are re-oxidized quite readily by surrounding O_2 to disappear during analysis and we failed to detection of them with our previous system. In this work, we have developed an on-line system combining a unique electrolysis cell for generation of the radicals and a CE unit to keep the analysis system free from external O_2 molecules and to reduce analysis time remarkably. As a result, electrophoretic detection of the anion radicals of PhQ and AQ have been achieved. Furthermore, we have observed hydrogen-bonding interaction between the anion radicals and dimethylurea (DMU) using the present system and have indicated a characetristic interaction of the anion radical of PhQ as an *ortho*-quinone with DMU.

[*J. Control Release*, **86**, 235-242 (2003)] [Lab. of Pharm. Engineering]

Mucoadhesive properties of carbopol or chitosan-coated liposomes and their effectiveness in the oral administration of calcitonin to rats.

Hirofumi TAKEUCHI*, Yuuji MATSUI, Hiromitsu YAMAMOTO, Yoshiaki KAWASHIMA

Mucoadhesive liposomes were prepared by coating multilamellar liposomes with Carbopol (CP) in a similar manner to that used in the preparation of chitosan-coated liposomes (CS-Lip) previously reported. The order of mucoadhesive properties of both the resultant polymer-coated liposomes (CP-Lip and CS-Lip) and the positively or negatively charged noncoated liposomes (Non-Lip) was CS-Lip>/= CP-Lip>positively charged Non-Lip>negatively charged Non-Lip.Administration of CP-Lip and CS-Lip containing calcitonin showed an enhanced and prolonged reduction in blood calcium concentration. The overall pharmacological effect of CP- and CS-Lips evaluated by means of the area under the plasma calcium concentration curve was 2.4 and 2.8 times higher than that of negatively and positively charged Non-Lips, respectively.

[*J. Control Release*, **88**, 23-33 (2003)] [Lab. of Pharm. Engineering]

A new agglomerated KSR-592 beta-form crystal system for dry powder inhalation formulation to improve inhalation performance in vitro and in vivo.

Kazuhiko IKEGAMI , Yoshiaki KAWASHIMA*, Hirofumi TAKEUCHI, Hiromitsu YAMAMOTO, Kazuki MIMURA, Den-ichi MOMOSE, Kiyohisa OUCHI

A new agglomerated KSR-592 (steroid) beta-form needle-like crystals with lactose system for dry powder inhalation (DPI) was developed to improve inhalation performance with Jethaler. The drug agglomerates were prepared by the method of spherical agglomeration in liquid so as to control the particle size and the mechanical strength of agglomerates by changing the agitation speed of the agglomeration system. The DPI formulation with these agglomerates exhibited ideal fluidity and provided a larger fine particle fraction than the formulation with agglomerates consisting of alpha-form (plate-like) crystals. Inhaled KSR-592 (beta-form) crystals were found to be uniformly deposited in the lungs of Brown Norway rat sensitized by ovalbumin (OA) and suppressed the increase in eosinophil number in the lungs after OA challenge.

[*J. Control Release*, **90**, 313-322 (2003)] [Lab. of Pharm. Engineering]

Microsphere design for the colonic delivery of 5-fluorouracil.

Alf LAMPRECHT, Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI and Yoshiaki KAWASHIMA*

The treatment of colon cancer has been aimed by approaches of oral drug administration. 5-Fluorouracil is the standard treatment still nowadays and would be a candidate to be delivered orally to the colon. A pH-sensitive polymer Eudragit P-4135F was used to prepare microspheres by a simple oil/water emulsification process. The solvent extraction was preferable over solvent evaporation with a view to the encapsulation rate (extraction: 37%; evaporation: 19%) due to the hydrophilic character of the drug while release pattern were nearly unchanged. Eudragit P-4135F, pure or in mixture, was found to retain drug release at pH 6.8 lower than 35% within 6 h. At pH 7.4, nearly immediate release (within 30 min) was observed for pure P-4135F, while mixtures enabled to prolong the release slightly. Analysis of the morphology led to an inhomogeneous polymer distribution of P-4135F and RS100 throughout the particle core. The formulation proved its applicability in-vitro as a promising device for pH-dependent colon delivery of 5-fluorouracil.

[*J. Control Release*, 93, 39-47 (2003)] [Lab. of Pharm. Engineering]

In vivo evaluation of riboflavin-containing microballoons for floating controlled drug delivery system in healthy human volunteers.

Yasunori SATO, Yoshiaki KAWASHIMA*, Hirofumi TAKEUCHI and Hiromitsu YAMAMOTO

Hollow microspheres (microballoons) floatable in JPXIII no. 1 solution were developed as a floating controlled drug delivery system. Microballoons (MB) were prepared by the emulsion solvent diffusion method utilizing enteric acrylic polymers dissolved in a mixture of dichloromethane and ethanol. Riboflavin powder, riboflavin-containing MB, and riboflavin-containing NF were administrated orally to each of three healthy volunteers. Riboflavin pharmacokinetics was investigated via analysis of urinary excretion of riboflavin. As a result, although urinary excretion of riboflavin following administration of MB was not sustained in the fasted state, urinary excretion of MB was significantly sustained in comparison to riboflavin powder and NF in the fed condition. Additionally, the excretion half-life time ($t_{1/2}$) of MB was prolonged significantly by feeding. Furthermore, MB provided significantly high total urinary excretion (%) of riboflavin compared to NF in the fasted and the fed conditions.

[*Eur. J. Pharm. Biopharm.*, **55**, 147-154 (2003)] [Lab. of Pharm. Engineering]

Improvement of pulmonary absorption of cyclopeptide FK224 in rats by co-formulating with β-cyclodextrin

Toshiomi NAKATA, Hiromitsu YOSHIDA, Atsuo OHIKE, Yuji TOKUNAGA, Rinta IBUKI, Yoshiaki KAWASHIMA*

The objective of this study was to investigate the effect of the pulmonary route on the systemic absorption of FK224 in comparison with other administration routes, and to determine the bioavailability (BA) of FK224 following pulmonary administration in rats using various dosage forms. From absorption studies on the Polyethylene Glycol 400 solution given by various routes, it was shown that pulmonary administration was a potentially attractive route for FK224. In the pulmonary absorption studies, after administration of the aqueous suspension, the BA was reduced to 2.7% compared with 16.8% for the solution. However, β-cyclodextrin (CyD) was found to be an effective additive as far as improving the solubility of FK224 was concerned. The BA of the aqueous suspension containing CyD was increased to 19.2%. Pressurized metered dose inhalers were prepared by formulating CyD with various molar ratios of 1:0, 1:1 and 1:7 (FK224/CyD), and the resulting BAs were 4.3%, 29.0% and 91.2%, respectively.

[*Eur. J. Pharm. Biopharm.*, **55**, 297-304 (2003)] [Lab. of Pharm. Engineering]

Physicochemical properties to determine the buoyancy of hollow microspheres (microballoons) prepared by the emulsion solvent diffusion method

Yasunori SATO, Yoshiaki KAWASHIMA*, Hirofumi TAKEUCHI and Hiromitsu YAMAMOTO

Hollow microspheres (microballoons) floatable on JPXIII No.1 solution were developed as a dosage form capable of floating in the stomach. Hollow microspheres were prepared by the emulsion solvent diffusion method using enteric acrylic polymers with drug in a mixture of dichloromethane and ethanol. It was found that preparation temperature determined the formation of cavity inside the microsphere and the surface smoothness, determining the floatability and the drug release rate of the microballoon. The correlation between the buoyancy of microballoons and their physical properties were elucidated. The drug loading efficiency of microballoons with various types of drug was correlated to the distribution coefficient of drug between dichloromethane and water. The optimum loading amount of riboflavin in the microballoon was found to impart ideal floatable properties to the microballoons. On the other hand, little entrapment was observed for aspirin due to the low distribution coefficient.

[*Adv. Powder Tec.*, 14, 215-229 (2003)] [Lab. of Pharm. Engineering]

A new spherically agglomerated drug composite system with lactose for dry powder inhalation.

Kazuhiko IKEGAMI, Yoshiaki KAWASHIMA*, Hirofumi TAKEUCHI, Hiromitsu YAMAMOTO, Kazuki MIMURA, Den-ichi MOMOSE, Kiyohisa OUCHI

In order to prepare a dry powder inhalation (DPI) formulation for steroids, we designed crystal agglomerates consisting of fine drug crystals suitable for DPI that disintegrated into primary crystals in inhalation device at emission. The drug agglomerates were prepared by the method of spherical agglomeration in liquid. The agglomerates in the DPI formulation disintegrated into respirable primary crystals by collision with coformulated lactose particles in the milling chamber of the device when inhaled. The DPI formulation with these agglomerates exhibited a higher fine particle (= respirable) fraction (FPF: 24.5%) than the formulation with fine drug particles (FPF: 16.1%) as evaluated *in vitro* with a cascade impactor. The inhalation property of the present DPI formulation was determined by the particle size of coformulated lactose particles.

[*Powder Technol.*, **126**, 266-274 (2003)] [Lab. of Pharm. Engineering]

Improved flowability and compactibility of spherically agglomerated crystals of ascorbic acid for direct tabletting designed by spherical crystallization process.

Yoshiaki KAWASHIMA*, Misato IMAI, Hirofumi TAKEUCHI,
Hiromitsu YAMAMOTO, Kazunori KAMIYA, Tomoaki HINO

Spherical agglomerated crystals of ascorbic acid with improved compactibility for direct tableting were successfully engineered by the spherical crystallization technique. In this process, ascorbic acid crystals were precipitated by a solvent change method, followed by their agglomerations with the emulsion solvent diffusion (ESD) or spherical agglomeration (SA) mechanism. The micromeritic properties were preferably improved for direct tableting Under static compression, the acceptable compact (tablet) with a sufficient strength was produced successfully without capping, although the capping occurred with the original unagglomerated crystals. It was also found that the spherically agglomerated crystals were tableted directly using a single punch machine under dynamic compression, although the tensile strength of resultant tablet decreased in tolerable degree with increasing punch velocity.

[*Yakuzaigaku*, **63**, 29-33 (2003)] [Lab. of Pharm. Engineering]

Development of Copoly (DL-Lactic/Glycolic Acid) Nanospheres Coated with Chitosan to Optimize Intra-Articular Delivery System: Binding Enhancement to Synovial Cells from Patients with Rheumatoid Arthritis.

Eijiro HORISAWA, Kenji TOBETTO, Akio KOMURA, Hiroshi TAKEI,
Hiromitsu YAMAMOTO, Hirofumi TAKEUCHI, Yoshiaki KAWASHIMA*

The present study investigated the inflamed synovial cell-adhesive copoly(dl-lactic/glycolic acid) (PLGA) particulate system to optimize intra-articular drug administration. Using fluorescein 5-isothiocyanate bound to PLGA ([FITC]PLGA), [FITC]PLGA nanospheres were prepared by the modified emulsion solvent diffusion method and the surface of the nanospheres (NS) were coated with a bioadhesive polymer, chitosan (MW: 50,000). The surface coating of the [FITC]PLGA NS with chitosan was confirmed by the zeta-potential profile. The effect of coating the [FITC]PLGA NS with chitosan was evaluated by measuring the binding profile to the cultured synovial cells originated from patients with rheumatoid arthritis. The chitosan-coated nanospheres showed high binding ability to the synovial cells as compared non-coated nanospheres. It was assumed that the chitosan-coated PLGA NS was delivered more quickly to inflammatory cells after intra-articular administration.

[*J. Soc. Powder Tec.*, **40**, 157-162 (2003)] [Lab. of Pharm. Engineering]

Design of Solid Dispersion Particles of Drug with Fine Porous Carrier

Hirofumi TAKEUCHI*, Shinsuke NAGIRA, Hiromitsu YAMAMOTO, Yoshiaki KAWASHIMA

Solid dispersion particles were prepared with indomethacin and fine porous carriers having different pore size (Sylysia350, Sylysia740) by use of spray-drying technique. The particles were in the size range of 3-6μm, which is equal to that of the original carrier particles. The indomethacin in solid dispersions was in amorphous state regardless of the type of silica. Specific surface area measurement and DSC analysis of the solid dispersion particles suggested that the difference in pore size of these carrier particles leads to the different drug dispersion of the resultant solid dispersion particles. The drug dissolution properties of solid dispersions were improved compared with crystalline indomethacin of γ-form. Solubility of drug formulated into solid dispersions was higher than that of crystalline indomethacin, indicating that the amorphous indomethacin kept its higher energy state in the porous carrier.

[*Chem Pharm Bull.*, **51**, 1223-1226 (2003)] [Lab. of Pharm. Enginnering]

Optimum heat treatment conditions for masking the bitterness of the clarithromycin wax matrix.

Toshio YAJIMA, Shigeo ITAI, Hirofumi TAKEUCHI, Yoshiaki KAWASHIMA*

The effects of the contents of aminoalkyl methacrylate copolymer E (AMCE) in a wax matrix on the mechanism of polymorphic transformation of glyceryl monostearate (GM) were clarified by evaluating the enthalpy change. The optimum temperature for the transformation of GM was 50 degrees C, at which the enthalpy change was maximum. To prepare the wax matrix preparation of clarithromycin (CAM), we considered 40 degrees C the optimum treatment temperature for the transformation of GM in a CAM wax matrix compounded from CAM, GM and AMCE, since the matrices were mutually welded at above 45 degrees C during the spray congealing process. By applying the tumbling that accelerated the transformation of GM in a CAM wax matrix, almost all of the alpha-form disappeared, and the release of CAM from the wax matrix diminished when the enthalpy change was more than 0.8.

[*Pharm. Res.*, 20, 1309-1316 (2003)] [Lab. of Pharmaceutics]

Sialic Acid 9-*O*-acetylesterase Catalyzes the Hydrolyzing Reaction from Alacepril to Deacetylalacepril.

Shigeyuki USUI, Masafumi KUBATA, Kazuhiro IGUCHI, Tadashi KIHO, Tadashi SUGIYAMA, Yoshihiro KATAGIRI, and Kazuyuki HIRANO*

The alacepril thiolesterase, which catalyzes the hydrolyzing reaction of the thioleste linkage in alacepril and the conversion from alacepril to deacetylalacepril, was purified from rat liver cytosol and characterized. A purification procedure for the thiolesterase consisted of ammonium sulfate fractionation and 4 steps of column chromatographies. The purified thiolesterase is heterodimeric with a molecular mass of 29 and 36 kDa subunits. N-terminal amino acid sequence of these subunits reveals that the thiolesterase is identical to sialic acid 9-*O*-acetylesterase. The thiolesterase hydrolyzes not only the thiolester bond in alacepril, spironolactone, and acetyl coenzyme A but also the carboxylester bond in α-naphtyl acetate.

[*Biol. Pharm. Bull.*, 26, 1455-1460 (2003)] [Lab. of Pharmaceutics]

Metabolite Profiles of Flutamide in Serum from Patients with Flutamide-Induced Hepatic Dysfunction.

Eiji TAKASHIMA, Kazuhiro IGUCHI, Shigeyuki USUI, Hajime YAMAMOTO, and Kazuyuki HIRANO*

We investigated whether hepatic dysfunction could be assessed by the metabolite profiles in serum from patients receiving flutamide. Serum samples were obtained from 15 patients with prostate cancer, 12 patients with no sign of hepatotoxicity and 3 patients with slight hepatic dysfunction during long-term flutamide treatment. We analyzed the metabolite profiles by LC/MS in selected ion monitoring mode and detected a new metabolite (M3) that was an oxidation product of flutamide. However, there were no consistent differences in the serum flutamide metabolites between patients with normal function and those suffering hepatic dysfunction. Therefore, the profile of flutamide metabolites determined in serum may not contribute to the risk prediction of flutamide-related hepatotoxicity.

[*J. Toxicol. Pathol.*, 16, 33-39 (2003)] [Lab. of Pharmaceutics]

Gastric Carcinogenesis and Intestinalization Induced by N-methyl-N-nitrosourea in the Senescence-Accelerated Mouse (SAMP3).

Masami YAMAMOTO, Tetsuya TSUKAMOTO, Hiroki SAKAI, Akihiko HIRATA, Tokuma YANAI, Toshiaki MASEGI, Kazuyuki HIRANO*, Chie FURIHATA, and Masae TATEMATSU

We investigated induction of both intestinal metaplasia and gastric cancers in the Senescence-accelerated mouse (SAMP3/Aice) treated with N-methyl-N-nitrosourea (MNU). The incidences of adenocarcinomas in the 120, 60, 30 ppm MNU treated and control groups were 60, 31.3, 5.9, and 0% in males, and 28.6, 23.1, 16.7, and 0% in females, respectively. With immuno- and enzyme-histochemistry, intestinal alkaline phosphatase (I-ALP) positive intestinal absorptive cell-like elements were observed in regions of hyperplasia, adenomas, and adenocarcinomas of MNU treated mice, but no phenotypic expression of goblet or Paneth cells was found. The results suggest that Senescence-acceleration may not influence intestinalization in the gastric mucosa or induction of gastric carcinomas.

[*Phytotherapy Research*, 17, 933-937 (2003)] [Lab. of Hygienics]

Inhibitory Effect of Magnolol on Tumor Metastasis in Mice.

Koji IKEDA, Yoshimichi SAKAI and Hisamitsu NAGASE*

The anti-metastatic effects of magnolol were evaluated by an experimental liver and spleen metastasis model using L5178Y-ML25 lymphoma, or an experimental and spontaneous lung metastasis model using B16-BL6 melanoma. Intraperitoneal (i.p.) administrations of 2 or 10 mg/kg of magnolol significantly suppressed liver and spleen metastasis or lung metastasis. As for the spontaneous lung metastasis model using B16-BL6 melanoma, multiple i.p. administrations of 10 mg/kg of magnolol after and before tumor inoculation significantly suppressed lung metastasis and primary tumor growth. In addition, magnolol significantly inhibited B16-BL6 cell invasion of the reconstituted basement membrane (Matrigel, MG) without affecting cell growth. The anti-metastatic action of magnolol is considered to be due to its ability to inhibit tumor cell invasion.

[*J. Liq. Chrom. & Rel. Technol.*, **26**, 819-832 (2003)] [Lab. of Hygienics]

Simultaneous Analysis of Carotenoid Coloring in Foods by Thin-layer Chromatography.

Tomoko HAYASHI, Hisao OKA, Yuko ITO, Tomomi GOTO, Naoko OZEKI, Yuko ITAKURA, Hiroshi MATSUMOTO, Yasuko OTSUJI, Hiromichi AKATUKA, Takahiko MIYAZAWA and Hisamitsu NAGASE*

We established a simultaneous analysis method by TLC for carotenoid colorings (annatto extract, orange color, gardenia yellow, paprika color, tomato color, marigold color, and β-carotene) in foods. Reversed phase C18 TLC using the solvent systems of acetonitrile-acetonc-n-hexane (11: 7 : 2) and acetone-water (9 : 1), and normal phase silica gel TLC, using the solvent systems of n-hexane-diethyl ether-acetic acid (4 : 1 : 1) and benzene-ethyl acetate-methanol (15 : 4 : l) yielded well-delineated spots with good separation. These TLCs were applied to the analysis of a total of 294 commercially available foods, and the Rf value of each color spot was evaluated under the four TLC conditions. The difference in the Rf value was slight between each color extracted from the food samples and the standard color, and the coefficient of variation was small, indicating excellent reproducibility.

[*Carcinogenesis*, **24**, 1123-1132 (2003)] [Lab. of Hygienics]

Metallothionein Deficiency Enhances Skin Carcinogenesis Induced by 7,12-Dimethylbenz[*a*]anthracen and 12-*O*-Tetradecanoylphorbol-13-acetate in Metallothionein-null Mice.

Junko SUZUKI, Noriko NISHIMURA, Baoxu ZHANG, Shizuko KOBAYASHI, Masahiko SATOH*, Chiharu TOHYAMA

We examined the role of metallothionein (MT) in the 7,12-dimethylbenz[*a*]anthracene (DMBA) / 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced two-stage carcinogenesis using MT-null mice. All of the MT-null mice treated with DMBA/TPA developed tumors in the skin, in contrast to only 40 % of wild-type mice. We detected a transversion of A^{182} to T in codon 61 of c-Ha-*ras* gene in the papilloma tissues of MT-null mice and wild-type mice by PCR-RFLP and PCR-SSCP methods. The present study showed that MT has anticarcinogenic potential in the chemical skin carcinogenesis.

[*Int. J. Cancer*, **105**, 707-714, (2003)] [Lab. of Hygienics]

Autocrine Motility Factor Signaling Induces Tumor Apoptotic Resistance by Regulations Apaf-1 and Caspase-9 Apoptosome Expression.

Arayo HAGA*, Tatsuyoshi FUNASAKA, Yasufumi NIINAKA, Avraham RAZ, Hisamitsu NAGASE

We have established that AMF signaling induced anti-apoptotic activity cells that secretes high level of AMF did not express the apoptotic protease activating factor-1 (Apaf-1) and Caspase-9 genes that encode for the proteins forming the apoptosome complex. The disappearance of the Apaf-1 and Caspase-9 gene was recovered by a cellular signaling inhibitor of PKC, PI3K and MAPK of *in vitro* cultured human fibrosarcoma HT-1080 line. Treatment with these inhibitors favored apoptotic cell death induced by anti-cancer drugs of the murine ascites Ehrlich line. Antibodies against AMF induced Ehrlich ascites apoptosis *in vitro,* and effectively aided *in vivo* apoptosis induced by anti-cancer drugs. The results might indicate a novel route by which tumor cells protect themselves with products, such as AMF, and proliferate despite various stresses and chemical insults.

[*J. Urol.*, **170**, 2467-2470 (2003)] [Lab. of Hygienics]

Optimal Administration Schedule of Cisplatin for Bladder Tumor with Minimal Induction of Metallothionein.

Yukihiro KONDO, Kenji YAMAGATA, Masahiko SATOH*, Seiichiro HIMENO, Nobumasa IMURA, Taiji NISHIMURA

We examined the role of metallothionein (MT) in cisplatin resistance using the nude mice inoculated with human bladder tumor (NMB-1). Since MT concentration in the tumor was significantly increased by pretreatment with zinc sulfate (200 μmol/kg x 2 days), the antitumor activity of cisplatin was reduced. The fractioned injections of cisplatin (16 μmol/kg x 4 days) induced the same level of MT in the tumor as that of treatment with zinc sulfate in the NMB-1 bearing nude mice. The present study found that the elevated level of tumor MT caused the cisplatin resistance and the fractioned daily injections of cisplatin induced MT synthesis in the tumor.

[*J. Vet. Med. Sci.*, 65, 511-513 (2003)] [Lab. of Hygienics]

Interleukin-6 Protects Skin Legion Caused by 7,12-Dimethylbenz[*a*]anthracene.

Mie MURATA, Noriko NISHIMURA, Baoxu ZHANG, Masahiko SATOH*, Chiharu TOHYAMA

We examined the effect of interleukin-6 (IL-6) on skin damage of 7,12-dimethylbenz[*a*]anthracene (DMBA) using IL-6 null mice. Severe skin damage such as ulcer was observed by topical treatment with DMBA in the IL-6 null mice. However DMBA-treated wild-type mice induced only epidermal hyperplasia. The occurrence of the skin damage in the DMBA-treated IL-6 null mice was suppressed by treatment with recombinant human IL-6. The present study showed that IL-6 is one of the crucial factors that are responsible for the sensitivity of inflammatory responses.

[*Chem. Biol. Interact.*, 143-144, 353-361 (2003)] [Lab. of Biochemistry]

Cloning, expression and tissue distribution of a tetrameric form of pig carbonyl reductase.

Noriyuki USAMI*, Shuhei ISHIKURA, Hiroko ABE, Makoto NAGANO, Miki UEBUCHI, Akihiko KUNIYASU, Masaki OTAGIRI, Hitoshi NAKAYAMA, Yorishige IMAMURA and Akira HARA

We isolated a cDNA for tetrameric carbonyl reductase (CR) from pig heart. The pig CR showed high amino acid sequence identity (81%) with rabbit $NADP^+$-dependent retinol dehydrogenase (NDRD). The purified recombinant pig CR and NDRD were about 100-kDa homotetramers and exhibited reductase activity towards alkyl phenyl ketones, α-dicarbonyl compounds and all-*trans*-retinal. The identity of NDRD with the tetrameric CR was verified by protein sequencing of the purified rabbit heart CR. Transfection of HeLa cells with vectors expressing pig CR demonstrated that the enzyme is localized in the peroxisomes.

[*Biochem. J.*, 369, 363-368 (2003)] [Lab. of Biochemistry]

Involvement of phospholipase D in insulin-like growth factor-I-induced activation of extracellular signal-regulated kinase, but not phosphoinositide 3-kinase or Akt, in Chinese-hamster ovary cells.

Yoshiko BANNO, Yoh TAKUWA, MomokoYAMADA, NorikoTAKUWA, Kenji OHGUCHI, Akira HARA* and Yoshinori NOZAWA

We previously demonstrated that sphingosine 1-phosphate (S1P)-induced phospholipase D (PLD) activation via the G-protein-coupled receptor endothelial differentiation gene (EDG)3/$S1P_3$ was involved in S1P-induced stimulation of phosphoinositide 3-kinase (PI3K) and Akt. We have herein examined the involvement of two PLD isozymes, PLD1 and PLD2, in insulin-like growth factor (IGF)-I receptor tyrosine kinase-mediated stimulation of PI 3-kinase/Akt and ERKs. IGF-I and to a lesser degree S1P stimulated PI3K activity in Chinese hamster ovary cells overexpressing EDG3/$S1P_3$. IGF-I-induced ERK phosphorylation was suppressed by butan-1-ol, but not butan-2-ol, whereas no effect of butanol was observed in IGF-I-induced Akt activation in $S1P_3$-overexpressing cells. Overexpression of wild-type PLD1 and PLD2 substantially potentiated S1P-, but not IGF-I-, induced activation of PI 3-kinase and Akt, whereas overexpression of the catalytically inactive mutant of PLD1 or PLD2 did not affect the responses to either agonist. On the other hand, overexpression of wild-type PLD1 and PLD2 potentiated IGF-I- and, to much smaller extents, S1P-induced ERK stimulation. ERK activation by IGF-I as well as S1P was dependent on Ras, but Akt activation by IGF-I was not dependent on Ras. These results suggest that PLDs are involved in growth factor regulation of at least two signalling pathways, PI 3-kinase/Akt and ERKs, depending on the class of cell-surface receptors.

[*Chem. Biol. Interact.*, 143-144, 503-513 (2003)] [Lab. of Biochemistry]

Selective and potent inhibitors of human 20α-hydroxysteroid dehydrogenase (AKR1C1) that metabolizes neurosteroids derived from progesterone.

Yu HIGAKI, Noriyuki USAMI, Syunichi SHINTANI, Shuhei ISHIKURA, Ossama El-KABBANI and Akira HARA*

Neuroactive steroids, such as 3α,5α-tetrahydroprogesterone (3α,5α-THP) and 3α,5α-tetrahydrodeoxycorticosterone, have been shown to be synthesized from progesterone in animal brains. Comparison of kinetic constants for the neuroactive steroids and their precursors among four human 3(20)α-hydroxysteroid dehydrogenases (AKR1C1-AKR1C4) suggests that AKR1C1 and AKR1C2 are involved in the catabolism and synthesis, respectively, of the neuroactive steroids in the human brain. Benzbromarone and 3',3",5',5"-tetrabromophenolphthalein were found to be selective and potent inhibitors of AKR1C1 (Ki=0.7 nM). The inhibitors effectively also decreased the reduction of 3α,5α-THP to its 20α-hydroxy metabolite in HepG2 cells treated with ethacrynic acid.

[*Chem. Biol. Interact.*, **143-144**, 543-550 (2003)] [Lab. of Biochemistry]

Identification of amino acid residues involved in substrate recognition of L-xylulose reductase by site-directed mutagenesis.

Shuhei ISHIKURA*, Tomoya ISAJI, Noriyuki USAMI, Junichi NAKAGAWA, Ossama EL-KABBANI and Akira HARA

L-Xylulose reductase (XR) shows high sequence identity with mouse lung carbonyl reductase (MLCR), an enzyme that reduces 3-ketosteroids but not sugars. By the site-directed mutagenesis with rat XR, we have confirmed the roles of Ser136, Tyr149 and Lys153 as the catalytic triad, and suggest the importance of the size and hydrophobicity of the five residues (Gln137, Leu143, His146, Asn190 and Trp191) for substrate recognition by XR and MLCR. Furthermore, the mutant enzymes containing a Q137M mutation were stable against cooling, which provides a structural mechanism of the cold inactivation that is a characteristic of the rodent XR.

[*Amino Acids*, **25**, 41-47 (2003)] [Lab. of Biochemistry]

Protein levels of genes encoded on chromosome 21 in fetal Down syndrome brain: Challenging the gene dosage effect hypothesis (Part IV).

Myeong S. CHEON, Ki S. SHIM, Seong H. KIM, Akira Hara* and Gert. LUBER

Down syndrome (DS) is the most frequent genetic disorder with mental retardation and caused by trisomy 21. The near completion of the sequencing of human chromosome 21 provides unprecedented opportunities to understand the molecular pathology of DS, however, functional information on gene products is limited so far. We therefore evaluated the levels of six proteins whose genes are encoded on chromosome 21 (trefoil factor 1, trefoil factor 2, trefoil factor 3, coxsackie virus and adenovirus receptor, carbonyl reductase 1 and interferon- alpha receptor) in fetal cerebral cortex from DS and controls at the early second trimester using Western blot analysis. None of the investigated proteins showed overexpression in DS compared to controls, suggesting that these proteins are not involved in abnormal development of fetal DS brain and that DS phenotype can not be simply explained by the gene dosage effect hypothesis.

[*J. Molecular Catalysis B: Enzymatic*, **23**, 29-35 (2003)] [Lab. of Biochemistry]

Stereoselective reduction of 4-benzoylpyridine by recombinant pig heart carbonyl reductase.

Hideaki SHIMADA, Sumie FUJIKI, Michiko OGINUMA, Makio ASAKAWA, Tadashi OKAWARA, Keisuke KATO, Shigeo YAMAMURA, Hiroyuki AKITA, Akira HARA* and Yorishige IMAMURA

Optically active (−) and (+)-α-phenyl-4-pyridylmethanol (PPOL) were synthesized from 4-benzoylpyridine and established to possess (*S*)- and (*R*)-configurations, respectively, by X-ray structural analysis. When the reduced product of 4-benzoylpyridine by recombinant carbonyl reductase of pig heart was analyzed on high-performance liquid chromatography, (*S*)-(−)-PPOL was identified as the predominat product. Thus, the enzyme reduces stereoselectively 4-benzoylpyridine to (*S*)-(−)-PPOL with high optical purity.

[*Arch. Biochem. Biophys.*, **416**, 180-187 (2003)] [Lab. of Biochemistry]

Tetrahydrobiopterin is synthesized from 6-pyruvoyl-tetrahydropterin by the human aldo-keto reductase AKR1 family members.

Teruo IINO, Mayuko TABATA, Shinichiro TAKIKAWA, Hiroshi SAWADA, Haruo SHINTAKU, Shuhei ISHIKURA and Akira HARA*

Tetrahydrobiopterin (BH_4) is a cofactor for aromatic amino acid hydroxylases and nitric oxide synthase. The biosynthesis includes two reduction steps catalyzed by sepiapterin reductase. An intermediate, 6-pyruvoyltetrahydropterin (PPH_4) is reduced to 1'-oxo-2'-hydroxypropyl-tetrahydropterin (1'-$OXPH_4$) or 1'-hydroxy-2'-oxopropyl-tetrahydropterin (2'-$OXPH_4$), which is further converted to BH_4. In this study, the reductase activities for the BH_4 intermediates were examined using several human recombinant enzymes belonging to the aldo-keto reductase (AKR) family and short-chain dehydrogenase/reductase (SDR) family. In the reduction of PPH_4 by AKR family enzymes, 2'-$OXPH_4$ was formed by 3α-hydroxysteroid dehydrogenase (HSD) type 2, whereas 1'-$OXPH_4$ was produced by aldose reductase, aldehyde reductase and 20α-HSD, and both 1'-$OXPH_4$ and 2'-$OXPH_4$ were detected as the major and minor products by 3α-HSDs (types 1 and 3). The activities of aldose reductase and 3α-HSD type 2 were higher than those of the other enzymes. 2'-$OXPH_4$ was reduced only by aldose reductase. These results suggest a novel alternative pathway from PPH_4 to BH_4, in which 3α-HSD type 2 and aldose reductase work in concert.

[*Biochem. Biophys. Res. Comunn.*, 308, 68-72 (2003)] [Lab. of Biochemistry]

Structural determinant for cold inactivation of rodent L-xylulose reductase.

Shuhei ISHIKURA, Noriyuki USAMI, Ossama El-KABBANI and Akira HARA*

L-Xylulose reductase (XR) is a homotetramer belonging to the short-chain dehydrogenase/reductase family. Human XR is stable at low temperature, whereas the rodent enzymes are rapidly dissociated into their inactive dimeric forms. We have here performed site-directed mutagenesis of Asp238, Leu242, and Thr244 of mouse XR to the corresponding residues (Glu, Trp, and Cys) of the human enzyme. Effects of the single mutations, double mutations (L242W/T244C, D238E/L242W) on the inactivation and subunit dissocation at low temperature indicates that the determinants for cold inactivation of rodent XRs are Asp238 and Leu242 with small side chains, which weaken the salt bridges between Arg203 and the C-terminal carboxylate group, and lead to cold inactivation.

[*Bioorg. Med. Chem. Lett.*, 13, 1469-1474 (2003)] [Lab. of Biochemistry]

Structure-based design of inhibitors of human L-xylulose reductase modelled into the active site of the enzyme.

Vincenzo CARBONE, Connie DARMANIN, Shuhei ISHIKURA, Akira HARA* and Ossama EL-KABBANI

The program GRID was used to design potential inhibitors of human L-xylulose reductase based on a model of the holoenzyme in complex with n-butyric acid. The inclusion of phosphate or carboxylate functional groups in the ligand suggested an increase in the net binding energy of the complex up to 2.8- and 4.0-fold, respectively. This study may be useful in the development of potent and specific inhibitors of the enzyme.

[*Metabolism*, 52, 42-49 (2003)] [Lab. of Biochemistry]

Glycated high-density lipoprotein regulates reactive oxygen species and reactive nitrogen species in endothelial cells.

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Incubation for 48 h with 100 μg/mL of glycated oxidized HDL (gly-ox-HDL) induced significant release of H_2O_2 from cultured human aortic endothelial cells (HAECs), and gly-ox-HDL-induced H_2O_2 formation was inhibited in the presence of diphenyleneiodonium, an inhibitor of NADPH oxidase. Stimulation of HAECs with gly-ox-HDL elicited a marked downregulation of catalase and Cu^{2+}, Zn^{2+}-superoxide dismutase. Treatment of HAECs with gly-ox-HDL attenuated the expression of endothelial nitric oxide synthase, but not inducible nitric oxide synthase, and this was followed by decreased production of nitric oxide by the cells. Taking all of the above findings together, gly-ox-HDL may lead to the deterioration of vascular function through altered production of reactive oxygen species and reactive nitrogen species in endothelial cells.

[*Pharmacology*, 67, 21-31 (2003)] [Lab. of Pharmacology]

Effect of Synthetic Retinoid, TAC-101, on Experimental Autoimmune Disease.

Naoki MIYAGAWA, Takeyasu HOMMA, Hiroyuki KAGECHIKA, Koichi SHUDO and Hiroichi NAGAI*

The effect of 4-[3,5-bis(trimethylsilyl)benzamido]benzoic acid (TAC-101) on collagen-induced arthritis (CIA) in mice and experimental autoimmune encephalomyelitis (EAE) in rats was studied. TAC-101 inhibited the development of CIA in terms of the swelling of fore- and hind-limbs and bone destruction in knee joints. TAC-101also suppressed the production of anti-type II collagen (CII) IgG antibody and delayed-type hypersensitivity (DTH) against CII. In addition, TAC-101 delayed the onset and development of EAE but did not affect the maximum symptom of EAE in rats. The elevation of serum anti-myelin basic protein (MBP) antibody and DTH to MBP on day 13 clearly suppressed by TAC-101 in EAE rats. Moreover, TAC-101 inhibited the IL-1β-induced PGE_2 production by MG-63 cells through the suppression of cyclooxygenase II mRNA expression. These findings suggest that TAC-101 inhibits CIA in mice and EAE in rats due to the suppression of immune response to auto-antigen and the production of PGE_2.

[*Br. J. Pharmacol.*, **138**, 912-920 (2003)] [Lab. of Pharmacology]

Role of Th2 Responses in the Development of Allergen-Induced Airway Remodelling in a Murine Model of Allergic Asthma.

Masato KOMAI, Hiroyuki TANAKA, Taisei MASUDA, Koichi NAGAO, Masayuki ISHIZAKI, Masatsugu SAWADA and Hiroichi NAGAI*

To clarify the involvement of Th2 responses in the development of airway remodelling, we investigated the effect of anti-CD4 monoclonal antibody (mAb) and anti-CD8 mAb, and the responses of IL-4 gene-knockout (KO) mice. Anti-CD4 mAb inhibited allergen-induced increases in airway responsiveness to acetylcholine, the number of eosinophils in bronchoalveolar lavage fluid, serum OA-specific IgE levels, IL-13 and transforming growth factor-β1 levels in bronchoalveolar lavage fluid, and amount of hydroxyproline in the lung. Furthermore, the antibody also attenuated allergen-induced goblet cell hyperplasia and subepithelial fibrosis. Furthermore, all these parameters were attenuated in IL-4 KO mice.

[*Allergol. Int.*, **52**, 31-36 (2003)] [Lab. of Pharmacology]

Role of T cells in IgE-Dependent Triphasic Cutaneous Reaction Caused by Dinitrofluorobenzene in the Mouse Ear: Participation of $CD8^+$ T Cells.

Nobuaki NAKAMURA, Takashi OCHI, Masatsugu SAWADA, Hiroyuki TANAKA, Naoki INAGAKI, Ikuo SAIKI and Hiroichi NAGAI*

The present study was conducted to elucidate the role of T cells in a triphasic cutaneous reaction. Whereas the magnitudes of immediate phase reaction (IPR) and late PR (LPR) in BALB/c nu/nu T cell-deficient mice were similar to those in BALB/c +/+ mice, very LPR (vLPR) was not observed in nu/nu mice. In addition, FK 506 and cyclosporin A clearly suppressed the onset of vLPR without affecting IPR and LPR. When mice were treated with anti-CD4 or anti-CD8 mAb, the magnitude of the vLPR was augmented by anti-CD4 mAb and suppressed by anti-CD8 mAb, without affecting IPR and LPR. Disruption of the IL-4 gene slightly suppressed IPR, LPR and vLPR, but the lack of the IL-5Rα chain gene did not affect these triphasic responses.

[*Skin Pharmacol. Appl. Skin Physiol.*, **16**, 165-175 (2003)] [Lab. of Pharmacology]

Evaluation and Characterization of Mouse Scratching Behavior by a New Apparatus, MicroAct.

Naoki INAGAKI, Katsuhiro IGETA, Noriko SHIRAISHI, John Fan KIM, Masafumi NAGAO, Nobuaki NAKAMURA and Hiroichi NAGAI*

We evaluated and characterized the mouse scratching behavior using a new apparatus, MicroAct. Scratching behavior was evoked in ICR and BALB/c mice by compound 48/80, passive cutaneous anaphylaxis or repeated hapten application. Under the present experimental condition, MicroAct detected consecutive scratching behavior (events) consisting of 3 or more beats. Although the detecting standard of MicroAct was not identical to that of an observer, the number of events detected by MicroAct and by an observer were almost comparable with each other. The present results demonstrate that MicroAct is a useful tool for evaluating mouse scratching behavior. Mouse scratching behavior seems to have a relatively fixed pattern and the causing stimulus increases mainly in the frequency of event without affecting the scratching speed.

[*Biol. Pharm. Bull.*, **26**, 618-621 (2003)] [Lab. of Pharmacology]

Effects of Prednisolone on the Cutaneous Reaction and Skin Barrier Function in Mice Treated with a Hapten.

Yoshifumi UEDA, Toshiro SONE, Naoki INAGAKI and Hiroichi NAGAI*

Glucocorticoids are effective drugs for the treatment of allergic skin diseases. In the present study, we observed the effects of prednisolone on the cutaneous reaction and skin barrier function in mice treated with a hapten, 2,4-dinitrofluorobenzene. Repeated hapten application onto the mouse ear resulted in a potent ear swelling with an elevation of specific serum IgE. Topical treatment with prednisolone apparently suppressed the swelling, whereas it failed to affect the serum specific IgE level. The hapten apparently caused an increase in transepidermal water loss, which was potently inhibited by prednisolone, although the water content was not affected. Amounts of triglyceride and cholesterol in the ear skin increased after repeated hapten applications, whereas the relative amount of free fatty acid and ceramide diminished. Prednisolone exhibited an inhibitory effect on the changes in lipid content.

[*Clin. Exp. Allergy*, 33, 705-713 (2003)] [Lab. of Pharmacology]

Mast Cells Play a Partial Role in Allergen-Induced Subepithelial Fibrosis in a Murine Model of Allergic Asthma.

Taisei MASUDA, Hiroyuki TANAKA, Masato KOMAI, Koichi NAGAO, Masayuki ISHIZAKI, Daisuke KAJIWARA and Hiroichi NAGAI*

To clarify the role of mast cells in the development of allergen-induced airway remodelling, we compared their responses of mast cell-deficient mice with those of congenic mice. In both sensitized mast cell-deficient mice, the degree of bronchial hyper-responsiveness to Ach, the number of inflammatory cells and the level of transforming growth factor-β1 in bronchoalveolar lavage fluid and goblet cell hyperplasia in the epithelium after repeated allergen provocation were not significantly different from those of congenic mice. In contrast, subepithelial fibrosis observed in congenic mice was partially attenuated in both mast cell-deficient mice. These findings suggest that mast cells play a partial role in allergen-induced subepithelial fibrosis at least in this model.

[*Nature Immunol.*, 4, 694-701 (2003)] [Lab. of Pharmacology]

Thromboxane A_2 Modulates Interaction of Dendritic Cells and T Cells and Regulates Acquired Immunity.

Kenji KABASHIMA, Takahiko MURATA, Hiroyuki TANAKA, Toshiyuki MATSUOKA, Daiji SAKATA, Nobuaki YOSHIDA, Koko KATAGIRI, Tatsuo KINASHI, Toshiyuki TANAKA, Masayuki MIYASAKA, Hiroichi NAGAI*, Fumitaka USHIKUBI and Shuh NARUMIYA

Here we show that dendritic cells (DCs) produce thromboxane A_2 (TXA_2), whereas naive T cells express the thromboxane receptor (TP). *In vitro*, a TP agonist enhances chemokinesis of naive but not memory T cell, impairs DC-T cell adhesion, and inhibits DC-dependent proliferation of T cells. *In vivo*, immune responses to foreign antigens are enhanced in TP-deficient mice, which also develop marked lymphadenopathy with age. Similar responses were seen in wild-type mice treated with a TP antagonist during the sensitization period. Thus, TXA_2-TP signaling modulates acquired immunity by negatively regulating DC-T cell interactions.

[*Am. J. Respir. Cell Mol. Biol.*, 29, 314-320 (2003)] [Lab. of Pharmacology]

Role of Prostaglandin I_2 in Airway Remodeling Induced by Repeated Allergen Challenge in Mice.

Koichi NAGAO, Hiroyuki TANAKA, Masato KOMAI, Taisei MASUDA, Shuh NARUMIYA and Hiroichi NAGAI*

We examined the role of prostaglandin (PG) I_2 in allergen-induced airway remodeling using IP gene-deficient mice. In wild-type mice, prolonged allergen exposure in sensitized animals induced the increases in the numbers of inflammatory leukocytes (including eosinophils and lymphocytes), levels of T helper type 2 (Th2) cytokines, levels of OVA-specific immunoglobulin (Ig)E and IgG1 in serum, and amount of hydroxyproline in the right lungs associated with transforming growth factor-β1 levels in bronchoalveolar lavage fluid. Moreover, goblet cell hyperplasia and subepithelial fibrosis were also appreciated after repeated allergen challenge. In contrast, the disruption of IP gene significantly augmented all these parameters. These findings suggest that PGI_2 has a regulatory role in allergen-induced airway remodeling as well as airway eosinophilic inflammation, Th2 cytokine production and IgE production, and that a PGI_2 agonist is a therapeutic approach for the treatment of airway remodeling in allergic asthma.

[*Pharmacology*, 69, 51-58 (2003)] [Lab. of Pharmacology]

Effects of RS-601, a Novel Leukotriene D_4/Thromboxane A_2 Dual Receptor Antagonist, on Asthmatic Responses in Guinea Pigs.

Takatoshi YAMADA, Yoshimasa TAKAHASHI, Masayuki ISHIZAKI, Keiichi MUSOH, Tetsuo Ohashi, Hiroyuki TANAKA, Naoki INAGAKI and Hiroichi NAGAI*

The effects of 4-[4-[5,5,6,6,6-pentafluoro-1-(4-fluoroben-zene-sulfonamido)hexyl]phenyl]butyric acid (RS-601), a novel leukotriene D_4 (LTD_4)/thromboxane A_2 (TxA_2) dual receptor antagonist, on bronchial asthmatic responses in guinea pigs were examined. RS-601 inhibited the increase in airway resistance caused by LTD_4 and TxA_2 mimetic compound, U-46619, but not by histamine. RS-601 and pranlukast but not S-1452 inhibited an antigen-inhibited late asthmatic response. In addition, RS-601 inhibited an antigen-induced airway hyperresponsiveness (AHR), whereas pranlukast and S-1452 had no effect on the AHR. These findings indicate that RS-601 has a potent antiasthmatic efficacy, especially on AHR.

[*J. Pharmacol. Exp. Ther.*, **306**, 1174-1181 (2003)] [Lab. of Pharmacology]

The Orally Available Spleen Tyrosine Kinase Inhibitor 2-[7-(3,4-dimethoxyphenyl)-imidazo [1,2-c]pyrimidin-5-ylamino] nicotinamide dihydrochloride (BAY 61-3606) Blocks Antigen-Induced Airway Inflammation in Rodents.

Noriyuki YAMAMOTO, Keisuke TAKESHITA, Michitaka SHICHIJO, Toshio KOKUBO, Masako SATO, Kosuke NAKASHIMA, Mina ISHIMORI, Hiroichi NAGAI*, LI Ying-Fu, Takeshi YURA and Kevin B BACON

We identified 2-[7-(3,4-dimethoxyphenyl)-imidazo[1,2-c]pyrimidin-5-ylamino]-nicotinamide dihydrochloride (BAY 61-3606), a potent (Ki = 7.5 nM) and selective inhibitor of Syk kinase. Oral administration of BAY 61-3606 to rats significantly suppressed antigen-induced passive cutaneous anaphylactic reaction, bronchoconstriction, and bronchial edema at 3 mg/kg. Furthermore, BAY 61-3606 attenuated antigen-induced airway inflammation in rats. Based on these anti-inflammatory effects of BAY 61-3606 both in vitro and in vivo, it was demonstrated that Syk may play a very critical role in the pathogenesis of allergic reactions.

[*Biol. Pharm. Bull.*, **26**, 1685-1690 (2003)] [Lab. of Pharmacology]

Inhibition of Syk Activity and Degranulation of Human Mast Cells by Flavonoids.

Michitaka SHICHIJO, Noriyuki YAMAMOTO, Hideki TSUJISHITA, Masahiro KIMATA, Hiroichi NAGAI* and Toshio KOKUBO

To investigate the effect of flavonoids on the activation of $p72^{syk}$ (Syk) protein tyrosine kinase which plays a pivotal role in the high affinity IgE receptor-mediated degranulation of mast cells, we picked out 10 flavonoids, classified them into 4 series, and examined their effects on the activation of Syk and on the degranulation of human mast cells. Flavones and flavonols showed clear inhibition, whereas flavanones and isoflavones had either weak or no effect on Syk enzymatic activity induced by amino acid peptide corresponding to the activation loop domain and on IgE-dependent degranulation of human cultured mast cells (HCMC). These results suggested that the impairment of mast cell degranulation by several flavonoids classified into flavones and flavonols might be mediated *via* inhibition of the intracellular activation of Syk.

[*Cytokine*, **24**, 293-303 (2003)] [Lab. of Pharmacology]

The Negative-Feedback Regulation of the IL-13 Signal by the IL-13 Receptor α2 Chain in Bronchial Epithelial Cells.

Shin-ichiro YASUNAGA, Noriko YUYAMA, Kazuhiko ARIMA, Hiroyuki TANAKA, Shuji TODA, Miyako MAEDA, Keiko MATSUI, Chiho GODA, Qing YANG, Yuji SUGITA, Hiroichi NAGAI* and Kenji IZUHARA

In this article, we analyzed the expression of the IL-4 receptor α chain, IL-13Rα1 and IL-13Rα2 in bronchial epithelial cells (BEC). Either IL-4 or IL-13 induced intracellular expression of IL-13Rα2 in BECs, which was STAT6-dependent and required de novo protein synthesis. IL-13Rα2 expressed on the cell surface as a monomer inhibited the STAT6-dependent IL-13 signal. Furthermore, expression of IL-13Rα2 was induced in lung tissues of ovalbumin-induced asthma model mice. Taken together, our results suggested the possibility that IL-13Rα2 induced by its ligand is transferred to the cell surface by an unknown mechanism, and it down-regulates the IL-13 signal in BECs, which functions as a unique negative-feedback system for the cytokine signal.

[*Planta*, **216**(3), 432-436 (2003)] [Lab. of Pharmacognosy]

Formation of benzoquinol moiety in cornoside by salidroside mono-oxygenase, a cytochrome P450, from *Abeliophyllum distichum* cell suspension cultures.

Hirobumi YAMAMOTO, Mitsuko HORI, Hiroshi KUWAJIMA and Kenichiro INOUE*

A microsomal fraction prepared from *Abeliophyllum distichum* Nakai (Oleaceae) cell suspension cultures oxidized salidroside, a glucoside of 4-hydroxyphenylethyl alcohol, to cornoside possessing a unique benzoquinol ring. The enzyme named salidroside mono-oxygenase required NADPH as the only cofactor, and molecular oxygen. The reaction was strongly inhibited by CO as well as several cytochrome P450 inhibitors, such as cytochrome c and miconazole, indicating the involvement of a cytocrome P450 enzyme. Salidroside mono-oxygenase accepted salidroside as the only substrate, but did not oxidize 4-hydroxyphenylethyl alcohol, the salidroside aglucone, and 4-hydroxybenzoic acid. The optimum pH of the reaction was 7.5, and apparent *Km* values for salidroside and NADPH were 44 μm and 33 μm, respectively. The benzoquinol ring formation mechanism is discussed in comparison to the mechanism for ipso substitution of 4-hydroxybenzoate by active oxygen species followed by elimination leading to hydroquinone.

[*Phytochemistry*, 62(7), 1093-1099 (2003)] [Lab. of Pharmacognosy]

Efficient production and capture of 8-prenylnaringenin and leachianone G - biosynthetic intermediates of sophoraflavanone G - by the addition of cork tissue to cell suspension cultures of *Sophora flavescens*.

Ping ZHAO, Chie HAMADA, Kenichiro INOUE*, Hirobumi YAMAMOTO

It has previously been demonstated that the addition of cork tissue to cell suspension cultures of *Sophora flavescens* stimulates the production of sophoraflavanone G, most of which has been recovered from the added cork tissue. In the present study, it was found that two precursors of sophoraflavanone G, 8-prenylnaringenin (sophoraflavanone B) and leachianone G, both of which have never been detected either in cultured cells or in the original plants, also accumulated in the added cork tissue. Thirteen minor flavonoids including three prenylated flavonoids, in addition to 8-prenylnaringenin and leachianone G, were isolated from the cork tissue co-incubated with *S. flavescens* cells. The new compounds flavescenones A, B and C, were determined to be (3R)-5,7,2'-trihydroxy-6-γ,γ-dimethylallyl-4',5'-methylenedioxyisoflavanone;5,7,2'-trihydroxy-6-γ,γ-dimethylallyl-4',5'-methylened ioxyisoflavone and 2-[2',4'-dihydroxy-3'-(γ-hydroxymethyl-γ-methylally)phenyl]-5,6- methylenedioxybenzofuran, respectively, by means of spectroscopic analysis that included 2D-NMR techniques.

[*Plant Physiology*, 133 (11), 1306-1313 (2003)] [Lab. of Pharmacognosy]

Characterization of leachianone G 2''-dimethylallyltransferase, a novel prenyl side-chain elongation enzyme for the formation of the lavandulyl group of sophoraflavanone G in *Sophora flavescens* Ait. cell suspension cultures.

Ping ZHAO, Kenichiro INOUE*, Isao KOUNO and Hirobumi YAMAMOTO

Leachianone G (LG) 2''-dimethylallyltransferase, a novel prenyl side-chain elongation enzyme, was identified in *Sophora flavescens* Ait. cultured cells. The enzyme transfers, a dimethylallyl group to the 2' position of another dimethylallyl group attached at position 8 of LG to form sophoraflavanone G a branched monoterpenoid-conjugated flavanone characteristic to this plant. This membrane-bound dimethylallyltransferase required Mg^{2+} (optimum concentration was 10 mM) for the reaction and had an optimum pH of 8.8. It utilized dimethylallyl diphosphate as the sole prenyl donor, and the 2'-hydroxy function in LG was indispensable to the activity. The apparent Km values for dimethylallyl diphosphate and LG were 59 and 2.3 μm, respectively.

[*Plant Cell Physiol.*, 44, 404-411 (2003)] [Lab. of Pharmacognosy]

Up-regulation of Soyasaponin Biosynthesis by Methyl Jasmonate in Cultured Cells of *Glycyrrhiza glabra*.

Hiroaki HAYASHI*, Pengyu HUANG and Kenichiro INOUE

Exogenous applied methyl jasmonate (MeJA) stimulated soyasaponin biosynthesis in the cultured cells of *Glycyrrhiza glabra*. mRNA level and enzyme activity of beta-amyrin synthase (bAS), an oxidosqualene cyclase (OSC) situated at the branching point for oleanane-type triterpene saponin biosynthesis, were up-regulated by MeJA, whereas those of cycloartenol synthase, an OSC involved in sterol biosynthesis, were relatively constant. Two mRNAs of squalene synthase (SQS), a common enzyme to both triterpene and sterol biosyntheses, were also up-regulated by MeJA. In addition, enzyme activity of UDP-glucuronic acid: soyasapogenol B glucuronosyltransferase, an enzyme situated at later step of soyasaponin biosynthesis, was also up-regulated by MeJA. Accumulations of bAS and two SQS mRNAs were not transient but lasted for 7 days after exposure to MeJA, resulting in the high-level accumulation (more than 2% of dry weight cells) of soyasaponins in cultured licorice cells. In contrast, bAS and SQS mRNAs were coordinately down-regulated by yeast extract.

[*Biol.Pharm.Bull.*, 26, 867-871 (2003)] [Lab. of Pharmacognosy]

Field Survey of *Glycyrrhiza* Plants in Central Asia (1). Characterization of *G. uralensis*, *G. glabra* and the Putative Intermediate Collected in Kazakhstan.

Hiroaki HAYASHI*, Sayaka HATTORI, Kenichiro INOUE, Kanat SARSENBAEV, Michiho ITO and Gisho HONDA

The characteristics of *Glycyrrhiza* plants in Kazakhstan were investigated. At 4 sites near Shu, and 1 site near Almaty, *G. glabra* and *G. uralensis* grew together forming a mixed population, and intermediate-type plants between them were also observed at 3 sites. Although two nucleotide substitutions of the chloroplast *rbcL* gene were observed between *G. uralensis* and *G. glabra*, *rbcL* sequences of the intermediate-types were divided into *G. uralensis*-type and *G. glabra*-type. HPLC analysis of the roots indicated that species-specific flavonoids, glabridin and glycycoumarin, were detected in the roots of *G. glabra* and *G. uralensis*, respectively, but neither flavonoid was detected in underground parts of the intermediate-types. HPLC analysis of their leaves indicated a significant difference among *G. uralensis*, *G. glabra* and the intermediate-type plants. Both *G. glabra*-specific and *G. uralensis*-specific compounds were detected in the leaves of the intermediate-type, thus suggesting that the intermediate plants are hybrids of *G. glabra* and *G. uralensis*.

[*Chem.Pharm.Bull.*, **51**, 1147-1152 (2003)] [Lab. of Pharmacognosy]

Field Survey of *Glycyrrhiza* Plants in Central Asia (2). Characterization of *G. uralensis*, *G. glabra* and the Putative Intermediate Collected in Kazakhstan.

Hiroaki HAYASHI*, Shui-Li ZHANG, Tomoko NAKAIZUMI, Kumiko SHIMURA, Misako YAMAGUCHI, Kenichiro INOUE, Kanat SARSENBAEV, Michiho ITO and Gisho HONDA

A new prenylated flavanone, licoleafol, and a new prenylated dihydrostilbene, uralstilbene, together with four known compounds were isolated from the leaves of *Glycyrrhiza uralensis* collected in Kazakhstan. HPLC analysis of the leaves of *Glycyrrhiza* plants collected in Kazakhstan showed that both *G. uralensis*-specific and *Glycyrrhiza glabra*-specific compounds were detected in the leaves of the morphologically intermediate-type plants, suggesting that the intermediate-type plant is a hybrid of *G. glabra* and *G. uralensis*. In addition, HPLC profiles of leaf extracts from offspring of intermediate-type plants were divided into the three types: the *G. uralensis* type, *G. glabra* type, and the intermediate type. From these results, it appears likely that the intermediate-type plant back-crosses with *G. glabra* and *G. uralensis* to generate a *G. glabra*-type plant and a *G. uralensis*-type plant, respectively.

[*Chem.Pharm.Bull.*, **51**, 1338-1340 (2003)] [Lab. of Pharmacognosy]

Field Survey of *Glycyrrhiza* Plants in Central Asia (3). Chemical Characterization of *G. glabra* Collected in Uzbekistan.

Hiroaki HAYASHI*, Sayaka HATTORI, Kenichiro INOUE, Olimjon KHODZHIMATOV, Ozodbek ASHURMETOV, Michiho ITO and Gisho HONDA

The chemical characteristics of *Glycyrrhiza glabra* L. were investigated at a habitat in Uzbekistan. HPLC analysis of the underground parts indicated that glycyrrhizin contents varied from 3.3 to 6.1% of dry weight, and that glabridin, a species-specific flavonoid for *G. glabra*, was detected in all underground samples (0.08-0.35% of dry weight). HPLC analysis of the leaves indicated that *G. glabra* plants collected in the present study could be divided into two types, **RT**-type and **IQ**-type, according to their major flavonol glycosides, rutin or isoquercitrin, respectively.

[*J Neurosci Res..*, **71**, 648-658 (2003)] [Lab. of Molecular Biology]

Neurotrophins facilitate neuronal differentiation of cultured neural stem cells via induction of mRNA expression of basic helix-loop-helix transcription factors Mash1 and Math1.

Hisanori ITO, Aki NAKAJIMA, Hiroshi NOMOTO, Shoei FURUKAWA*

Neurogenesis is promoted bHLH transcription factors Mash1, Math1, or NeuroD but suppressed by another set, Hes1 and Hes5. Each neurotrophin increased Mash1 and Math1 mRNAs of the stem cells growing in the presence of FGF-2, but did not alter Hes1, Hes5, or NeuroD mRNA levels. Simultaneously, most of the cells expressed nestin but not MAP2, and remained undifferentiated. FGF-2 removal from the medium reduced the levels of Hes1 and Hes5 mRNAs and increased those of Mash1, Math1, and NeuroD mRNAs, resulting in substantial neuronal differentiation. When the cells were pretreated with BDNF, FGF-2 removal enhanced earlier NeuroD expression and generated many more MAP2-positive cells. The high level of Mash1 and Math1 that had been elevated at FGF-2 withdrawal accelerated NeuroD expression in cooperation with the reduced Hes1 and Hes5 expression. Our present results suggest that neurotrophins stimulate neuronal differentiation by altering the balance of expression of various bHLH transcription factors.

[*Neurosci Lett*, **339**, 231-234 (2003)] [Lab. of Molecular Biology]

Neurotrophins facilitate synthesis of choline acetyltransferase and tyrosine hydroxylase in cultured mouse neural stem cells independently of their neuronal differentiation.

Hisanori ITO, Hiroshi NOMOTO, Yoshiko FURUKAWA, Shoei FURUKAWA*.

Effects of three neurotrophins, i.e., NGF, BDNF, and NT-3, on the expression of four neurotransmitter-synthesizing enzymes, i.e. ChAT, TH, DBH, and glutamate decarboxylase 65 were investigated in cultured mouse neural stem cells. All three neurotrophins enhanced the mRNA expression of ChAT, TH, or DBH of the cells caused to differentiate by the removal of FGF-2 from the culture medium, and increased the protein and mRNA levels of ChAT and TH of even the undifferentiated proliferating neural stem cells due to the presence of FGF-2. These results demonstrate that neurotrophins stimulate the synthesis of ChAT and TH of the neural stem cells prior to neuronal differentiation, and suggest that neurotrophins may play roles in the commitment to neuronal cells and choice of specific neurotransmitter phenotypes in early stages of neurogenesis.

[*J Neurosci Res.*, 72, 211-217 (2003)] [Lab. of Molecular Biology]

Growth arrest of PC12 cells by nerve growth factor is dependent on the phosphatidylinositol 3-kinase/Akt pathway via p75 neurotrophin receptor.

Hisanori ITO, Hiroshi NOMOTO, Shoei FURUKAWA*

We recently isolated mutant PC12 cell clones (PC84 cells) by transfection of PC12 cells with NGF cDNA. These cells secreted active NGF and extended short processes, but proliferated faster than the parental PC12 cells. The expression level of p75 was significantly low, and it was shown that NGF signaling via p75 was necessary for the growth arrest of the PC12 cells. In PC84 cells, MAP kinase was phosphorylated but the phosphorylation level of Akt was very low under the serum-free condition. We found PC12 cells treated with Wortmannin did not cease proliferation in the presence of NGF and anti-p75 neutralizing antibody reduced NGF-induced phosphorylation of Akt in PC12 cells under the serum-free condition. These results suggest that NGF activates Akt via p75, which is necessary for the NGF-induced growth arrest of PC12 cells.

[*Neuroscience.*,118, 409-415 (2003)] [Lab. of Molecular Biology]

Materno-fetal coordination of stress-induced Fos expression in the hypothalamic paraventricular nucleus during pregnancy.

Takashi FUJIOKA, Hisashi ENDOH, Yoshiyuki SAKATA ,Shoei FURUKAWA *, Shoji NAKAMURA

This study investigates whether maternal stress during pregnancy induces maternal and fetal hypothalamic PVN neuronal activation and the effects of maternal stress on fetal hypothalamic and PVN BDNF expression. Pregnant rats were exposed to three types of maternal stress with varying severity (restraint, forced walking and immobilization) for 30 min on gestational day 21. Forced walking and immobilized stress, but not restraint stress, significantly increased BDNF expression in the fetal hypothalamus. These findings suggest that the fetal (hypothalamic-pituitary-adrenal) HPA response following maternal stress mirrors maternal HPA activation. In addition, BDNF may play a role in protecting fetal brain neurons from damage caused by severe stress.

[*Acta Neuropathol (Berl).*,106, 29-86 (2003)] [Lab. of Molecular Biology]

Increased expression of neurotrophins and their receptors in the mechanically compressed spinal cord of the spinal hyperostotic mouse (twy/twy).

Kenzo UCHIDA, Hisatoshi BABA, Yasushisa MAEGAWA, Shoei FURUKAWA*, Makoto OHMIYA, Yasuo KONDOH, Chikara KUBOTA , Hideaki NAKAJIMA .

To identify any compensatory changes at the site of chronic compression of the spinal cord and neighboring segments, serial immunohistochemical and immunoblot analyses were performed in 24 tip-toe walking Yoshimura mice (twy/twy) aged 12-24 weeks. Immunoreactivities for BDNF, NT-3, trkB and trkC were preferentially localized in the gray matter, particularly in the anterior horn cells. In 24-week-old twy mice with severe compression, expression levels of these neurotrophins at the site of maximal compression were significantly lower than at the less- or non-compressed sites. In contrast, the expression levels of BDNF, NT-3, trkB and trkC were significantly higher at the rostral and caudal sites immediately adjacent to the maximal compression site. Our results suggest that overexpression of BDNF, NT-3, trkB and trkC in motoneuron areas neighboring the site of mechanical compression may represent compensatory changes in response to the compromised neuronal function at the level of compression, and that these proteins possibly contribute to neuronal survival and plasticity.

[*J. Pharmacol. Sci.*, 91, 219-228 (2003)] [Lab. of Microbiology]

A murine model of enterohemorrhagic *Escherichia coli* O157:H7 infection to assess immunopotentiating activity of drugs on mucosal immunity.

Keiji NAGANO, Takeshi SUGISAKI, Kazuki TAGUCHI, Takumi HARA, Mitsuru NAIKI and Hiroshi MORI*

An enterohemorrhagic *Escherichia coli* (EHEC) O157 oral infection murine model was established to examine the potentiating activity of drugs on mucosal immune responses. Groups of ICR mice inoculated intragastrically with EHEC O157 showed chronic intestinal infection and resulted in the high levels of antigen specific fecal IgA antibody synthesis. Intraperitoneal administration of Neurotropin, an immunopotentiator, augmented the antigen specific mucosal immune responses to EHEC O157. When mice were immunized intranasally with a mixture of ovalbumin and cholera toxin, co-administration of Neurotropin significantly potentiated the synthesis of fecal IgA and serum IgG antibodies. These results suggest that Neurotropin has potential as a mucosal adjuvant to promote secretory IgA antibody production.

[*Infect. Immun.*, **71**, 2598-2606 (2003)] [Lab. of Microbiology]

Increased adherence to Caco-2 cells caused by disruption of the *yhiE* and *yhiF* genes in enterohemorrhagic *Escherichia coli* O157:H7.

Ichiro TATSUNO, Keiji NAGANO, Kazuki TAGUCHI, Li RONG, Hiroshi MORI* and Chihiro SASAKAWA

To identify genes modulating the adherent capacity enterohemorrhagic *Escherichia coli* (EHEC) to intestinal epithelium, mini-Tn*5*Km2-mutagenized EHEC O157:H7 strains were screened and eight mutants with increased adherence to Caco-2 cells were isolated. DNA sequence analyses indicated that one possessed the insertion within an O157 antigen gene cluster, another possessed within the *yhiF* gene, and the others had in the *yhiE* gene. The *yhiE* or *yhiF* mutants showed enhanced type III secretion and increased expression of the LEE genes. When one of the *yhiE* mutants was orally inoculated into ICR mice, the number of bacteria shed into feces by day 14 was greater than that for the wild type. These results suggest that *yhiE* and *yhiF* are involved in the adherence to epithelial cells as negative regulators for the gene expression of the type III secretion system.

[*Microbiol. Immunol.*, **47**, 125-132 (2003)] [Lab. of Microbiology]

Adhesion and colonization of enterohemorrhagic *Escherichia coli* O157:H7 in cecum of mice.

Keiji NAGANO, Kazuki TAGUCHI, Takumi HARA, Shin-ichiro YOKOYAMA, Kenji KAWADA and Hiroshi MORI*

Infectious diseases due to enterohemorrhagic *Escherichia coli* (EHEC) are characterized by diarrhea, hemorrhagic colitis and hemolytic uremic syndrome. The adherence of EHEC on intestinal epithelial cells is a first step for developing these diseases. In the present study, we examined whether EHEC O157:H7 adhere to intestinal epithelial cells of mice and cause F-actin accumulation in the epithelial cells following the intragastric inoculation of the pathogen. Fecal shedding of the EHEC O157:H7 strain was observed in ICR mice up to 3 weeks. Fecal shedding periods of the type III secretion system-related gene (*espA* and *sepL*) deletion mutants were clearly shorter than that of the wild-type EHEC O157:H7 strain. The EHEC O157:H7 colonies were found on the epithelial surfaces of the ceca in association with F-actin accumulation beneath the attached bacteria.

[*Natural Medicines*, **57**, 1-6 (2003)] [Lab. of Herbal Garden]

Morphological, Chemical and Molecular Biological Comparison among *Artemisia capillaries, A. japonica* and their Natural Hybrids.

Motoyasu MINAMI, * Keizo HOSOKAWA, Md. Wahiduzzaman Mia, Eiji. SAKAI, Motoyoshi SATAKE, Seizo KONDO, Kenji OKA, Yasunori KOGA-BAN, Toshiaki KAYANO, Hiroshi TANAKA and Toshiro SHIBATA

The heads of *Artemisia capillaries* have been used for medicinal purposes as their choleretic effect of capillarisin (CAP) and 6,7-dimethylesculetin (DME) are known. However, hybrid plants between *A. capillaries* and *A. japonica* are often found in the crude drugs sold in the Japanese market, therefore observation of flowering dates, the ten morphological characteristics of flower heads, and CAP and DME contents, as well as RAPD (Random Amplified Polymorphic DNA) analysis were carried out for A. capillaries, *A. japonica* and their natural hybrid plants grown in the flood plain of the Sho-river, Takaoka, Toyama, Japan. Further, the pharmaceutical status of flower heads derived from the hybrid plants was discussed. The result showed that hybrid plants were similar to *A. japonica*, and when flower heads from the hybrid plants were mixed in crude drugs, the quality of the crude drug could decline because of traces of CAP and DME. Flower heads of hybrid plants could be detected with certainly by the presence of secretory sacs on corolla lobes of disk flowers and lower content of CAP and DME and removed.

[*Natural Medicines*, **57**, 105-109 (2003)] [Lab. of Herbal Garden]

Contents Variation of Aristolochia Acid in the Plants of Aristolochiaceae; About the Relatied Plants of Chinese Herb Xixin

Tomoko KAWAMURA,* Yuuki OSADA, Kazuyo OKUDA, Youichi HISATA, Eiji SAKAI, Toshihiro TANAKA and Isamu TATEMATSU

With concerns about the Chinese herb Xixin (Saishin, Asiasarum root), the amount of aristlochia acid (AA) in the plants of *Asarum* 48 species, *Aristolochia* 3 species and *Saruma* species were assayed by HPLC. Quantitative analysis of AA-I and AA-II was carried out on each part. The petiole samples of the *Asarum* plants contained the greatest amount of AA-I, and some of their underground parts contained a small amount of AA-I. Variable AA contents were shown in the allied plants of *Asarum*, even in the same genus. High values of AA-I and AA-II were detected in whole plants of *Aristrochia*. The younger aerial parts of *Aristolochia debilis* contained the highest value of AA, and those underground parts collected at different times were found to scarcely vary in AA contents.

[*J. Toxicol. Pathol.*, **16**, 231-236 (2003)] [Lab. of Radiochemistry]

Promoting Effect of Sodium L-Ascorbate on *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine-induced Renal Pelvic Carcinogenesis in SD/cShi Rats of Both Sexes.

Takashi MURAI, Akihiro KOIDE, Hideyuki MIYAUCHI, Satoshi INOUE, Toshiyuki MARUYAMA, Susumu MAKINO, Satoru MORI, Hideki WANIBUCHI, Yukio MORI* and Shoji FUKUSHIMA

Susceptibility to the promoting effects of sodium L-ascorbate(Na-AsA) on the development of pelvis and urinary bladder tumors in male and female SD/cShi rats, featuring spontaneous hydronephrosis, were investigated. Rats received 0.05% *N*-Butyl-*N*-(4-hydroxybutyl)nitrosamine(BBN) in their drinking water for 4 weeks and subsequently given basal diet with or without a 5% Na-AsA supplement for 32 weeks. Histopathological examination revealed the promoting effect of Na-AsA on not only the development of urinary bladder tumors but also renal pelvic tumors in the animals of both sexes in this two-stage carcinogenesis experiment, the effect being more prominent in males. *N*-Butyl-*N*-(3-carboxypropyl)nitrosamine, which is a metabolite of BBN and proximate carcinogen, was found more in the urine of urinary bladder than that of renal pelvis. These results indicate that the urothelium of the renal pelvis and urinary bladder in SD/cShi rats is susceptible to promoting effects of Na-AsA in the present two stage model urinary tract carcinogenesis, with the urinary bladder of male rats as the most sensitive organ.

[*Mutagenesis*, **18**, 87-93 (2003)] [Lab. of Radiochemistry]

Effects of Cigarette Smoke and a Heterocyclic Amine, MeIQx on Cytochrome P-450, Mutagenic Activation of Various Carcinogens and Glucuronidation in Rat Liver.

Yukio MORI, * Akihiro KOIDE, Yoshinori KOBAYASHI, Fumio FURUKAWA, Masao HIROSE and Akiyoshi NISHIKAWA

Immunoblot analyses for microsomal CYP proteins revealed induction of CYP1A1 and constitutive CYP1A2 (2.3－2.7-fold), but not CYP2B1/2, 2E1 or 3A2, by cigarette smoke (CS) exposure for 16 weeks. CS exposure also elevated the mutagenic activities of MeIQx and other five heterocyclic amines (HCAs) 1.4~3.7-fold in strain TA98. In contrast, feeding of 300 ppm MeIQx in diet produced no significant alterations in the levels of these CYP species and mutagenic activities. On the other hand, feeding of MeIQx for 16 weeks enhanced UDPGT activities towards 4-nitrophenol and testosterone, while CS exposure induced that towards 4-nitrophenol; the combined treatment of CS with MeIQx showed a summation effect on induction of the UDPGT1A6 activity. In conjunction with findings of *N*-hydroxy-MeIQx being much poor substrate for rat liver UDPGT, our results clearly indicate that enhancement by CS of MeIQx-induced hepatocarcinogenesis in F344 rats can be attributed to an increase in metabolic activation of MeIQx by hepatic CYP1A2 during the initiation phase.

[*Phytochemistry*, **62**, 601-606 (2003)] [Lab. of Instrumental Center]

Stilbene Derivatives from *Gnetum gnemon* Linn.

Ibrahim ILIYA, Zulfiqar ALI, Toshiyuki TANAKA, Munekazu IINUMA,* Miyuki FURUSAWA, Ken-ichi NAKAYA, Jin MURATA, Dedy DARNAEDI, Nobuyasu MATSUURA and Makoto UBUKATA

Four new stilbene derivatives, gnemonols K and L (both resveratrol trimers), M (isorhapontigenin dimer), and gnemonoside K (glucoside of resveratrol trimer) together with eleven known stilbenoids and a lignan were isolated from acetone, methanol and 70% methanol soluble parts of the root of *Gnetum gnemon* (Gnetaceae). The structures of isolates were determined by spectral analysis. Gnemonol K is formed by the oxidative coupling of resveratrol with ε-viniferin, and it is a geometric isomer of gnemonol L (*Z*). The antioxidant activity of the stilbenoids on lipid peroxide inhibition and the superoxide scavenging activity were also investigated.

[*J. Nat. Prod.*, **66**, 558-560 (2003)] [Lab. of Instrumental Center]

Phenolic Constituents of *Gnetum klossii*.

Zulfiqar ALI, Toshiyuki TANAKA, Ibrahim ILIYA, Munekazu IINUMA,* Miyuki FURUSAWA, Tetsuro ITO, Ken-ichi NAKAYA, Jin MURATA and Dedy DARNAEDI

The genus *Gnetum* (Gnetaceae) consists of about 40 species distributed in South America (Amazon region), South Africa, and the tropical and subtropical zones of Asia. Various species have been used in folk medicine for the treatment of arthritis, bronchitis, and asthma. The genus *Gnetum* is well known for its abundant polyphenolic constituents. In a continuation of our phytochemical studies on *Gnetum* species, we report herein the isolation and structure elucidation of four new phenolic derivatives, gnetofurans A-C and dihydropinosylvindiol, along with nine known compounds (gnetifolin F, isorhapontigenin, gnetol, viniferin, gnetulin, gnetins C and E, latifolol and *trans*-resveratrol).

[*Tetrahedron*, **59**, 1255-1264 (2003)] [Lab. of Instrumental Center]

Two New Oligostilbenes with Dihydrobenzofuran from the Stem Bark of *Vateria indica*.

Tetsuro ITO, Toshiyuki TANAKA, Munekazu IINUMA,* Ken-ichi NAKAYA, Yoshikazu TAKAHASHI, Ryuichi SAWA, Hiroshi NAGANAWA and Veliah CHELLADURAI

Two new stilbenoids, vateriaphenols A and B, were isolated from the stem bark of *Vateria indica* along with known ten stilbenoids and bergenin. The structure of vateriaphenol A is composed of two resveratrol tetramer units (tetramers 1 and 2). The tructure of the tetramer 1 is as same as that of hopeaphenol. Another tetramer 2 has two dihydrobenzofuran rings and a benzocyclopentane ring. The relative structure was determined as same as those of vaticanols B and C. Thus vateriaphenol A, as the first instance of resveratrol octamer in nature, is biologically synthesized by coupling of two different tetramers. Although the planer structure of vateriaphenol B is identical to those of hopeaphenol or isohopeaphenol, vateriaphenol B is composed of two diastereometric dimers of hemsleyanol A and ampelopsin A.

[*Biol. Pharm. Bull.*, **26**, 569-571 (2003)] [Lab. of Instrumental Center]

Cytotoxic Benzophenone Derivatives from *Garcinia* Species Display a Strong Apoptosis-Inducing Effect against Human Leukemia Cell Lines.

Kenji MATSUMOTO, Yukihoro AKAO, Emi KOBAYASHI, Tetsuro ITO, Kenji OHGUCHI, Toshiyuki TANAKA, Munekazu IINUMA* and Yoshinori NOZAWA

We examined the *in vitro* effects of the benzophenone derivatives contained in *Garcinia* species, garcinol, isogarcinol and xanthochymol, on cell growth in four human leuckemia cell lines. All the compounds exhibited significant growth suppression due to apoptosis mediated by the activation of caspase-3. A loss of mitochondorial membrane potential was found in garcinol-isogarcinol-induced apoptosis, but not in xanthochymol-induced apoptosis. These findings suggest that garcinol and isogarcinol may target the molecule(s) on mitochondria in the early phase of the apoptotic process, because no activation of caspase-8 was found. These compounds appeared to exert a negligible toxic effect on normal human lymphocytes at the concentrations lower than 5 μM, although they exhibited slight growth inhibition at 10-15 μM.

[*Tetrahedron*, **59**, 5347-5363 (2003)] [Lab. of Instrumental Center]

New Resveratrol Oligomers in the Stem Bark of *Vatica pauciflora*.

Tetsuro, ITO, Toshiyuki TANAKA, Munekazu IINUMA,* Ibrahim ILIYA, Ken-ichi NAKAYA, Zulfiqar ALI, Yoshikazu TAKAHASHI, Ryuichi SAWA, Yoshiaki SHIRATAKI, Jin MURATA and Dedy DARNAEDI

Five new resveratrol oligomers; pauciflorols A-C, isovaticanols B and C, and three new oligostilbene glucosides; pauciflorosides A-C were isolated from the stem bark of *Vatica pauciflora* (Dipterocarpaceae) together with seventeen known resveratrol oligomers. The planer structure of pauciflorol A is as same as that of vaticanols A and E or suffruticosol B. Although pauciflorol A or vaticanol A has similar ^{1}H-^{1}H coupling constants, the orientation is quite different. The planar structure of pauciflorol B is also as same as pauciflorol A. The strong anisotropic effect of the benzene ring in pauciflorol B was caused the upper field shift of a hydroxyl group. Such anisotropic effect is sometimes applicable to the confirmation of stereo structure in oligostilbenoid chemistry.

[*J. Nat. Prod.*, **66**, 1124-1127 (2003)] [Lab. of Instrumental Center]

Induction of Apoptosis by Xanthones from Mangosteen in Human Leukemia Cell Lines.

Kenji MATSUMOTO, Yukihiro AKAO, Emi KOBAYASHI, Kenji OHGUCHI, Tetsuro ITO, Toshiyuki TANAKA, Munekazu IINUMA* and Yoshinori NOZAWA

We examined *in vitro* cytotoxic effects of six xathones from the pericarps of mangosteen, *Garcinia mangostana*, in HL-60 cells at 72 h after the start of treatment of concentration from 5 to 40 μM. Although all the xanthones exhibited significant growth inhibition, α-, β- and γ-mangostin were particularly effective even at the low dose of 10 μM. α-Mangostine which is a major component in the pericarps showed the strongest activity (completely inhibition at 10 μM). Then we further examined the cell growth inhibitory activity of α-mangostin against other leukemia cell lines, K562, NB4 and U937. α-Mangostin showed no significant effect on the cell growth at the concentrations lower than 2 μM, but was inhibitory to all cell lines at 5 μM. A 10 μM α-mangostin concentration markedly inhibited growth of all cell lines tested, especially HL60, NB4 and U937, with complete suppression of growth at 72 h after treatment.

[*Heterocycles*, **60**, 2077-2083 (2003)] [Lab. of Instrumental Center]

New Luteolin 3'-*O*-Acetylated Rhamnosides from Leaves of *Bursera graveolens*.

Tsutomu NAKANISHI, Yuka INATOMI, Satomi ARAI, Takeshi YAMADA, Hideyuki FUKATSU, Hiroko MURATA, Akira INADA, Nobuyasu MATSUURA, Makoto UBUKATA, Jin MURATA, Munekazu IINUMA,* Miguel A. FARREA and Toshiyuki TANAKA

Three new luteolin 3'-*O*-rhamnopyranosides were isolated from leaves of *Bursera graveolens* (Burseraceae) along with five known flavonol glycosides, β-sitosterol 3-*O*-glucopyranoside and α-amyrin. The structures isolated as new compounds were determined to be luteolin 3'-*O*-α-L-(3"-acetyl-2"-*E*-*p*-coumaroyl)rhamnopyranoside, and luteolin 3'-*O*-α-L-(-2"-*E*-*p*-coumaroyl)-rhamnopyranoside and luteolin 3'-*O*-α-L-(3"-*E*-*p*-coumaroyl)rhamnopyranoside, respectively by spectroscopic analysis. The inhibitory activity for the Maillard reaction of the luteolin rhamnosides was investigated.

[*Heterocycles*, **60**, 2557-2563 (2003)] [Lab. of Instrumental Center]

Flavonol Glycosides in Two *Diospyros* Plants and Their Radical Scavenging Activity

Miyuki FURUSAWA, Tetsuro ITO, Ken-ichi NAKAYA, Toshiyuki TANAKA, Ibrahim ILIYA, Munekazu IINUMA,* Hiroko MURATA, Yuka INATOMI and Tsutomu NAKANISHI

Methanol extracts of leaves of *Diospyros glaucifolia* and *D. kaki* were showed the scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Chromatographic separation and purification of the methanol extracts resulted in the isolation of three new flavonol glycosides [2"-(*E*)-*p*-coumaroylmyricitrin, 3"-(*E*)-*p*-coumaroylmyricitrin and mearsetin 3-*O*-β-glucopyranoside] along with known phenolic compounds. These structures were determined by means of spectroscopic analysis. The scavenging activities of DPPH radical of the isolated compounds were examined.

[*Biochem. Biophys. Res. Comm.*, **307**, 861-863 (2003)] [Lab. of Instrumental Center]

Effects of Hydroxystilbene Derivatives on Tyrosinase Activity

Kenji OHGUCHI, Toshiyuki TANAKA, Tadashi KIDO, Kimiye BABA, Munekazu IINUMA*, Kenji MATSUMOTO, Yukihiro AKAO and Yoshinori NOZAWA

Recently, polyhydroxystilbene compounds which are *trans*-resveratrol analogs have been demonstrated as potent tyrosinase inhibitors. However, their detailed inhibitory mechanisms are not clearly understood. In the present study, a variety of synthesized hydroxystilbene compounds were tested for their inhibitory effects against murine tyrosinase activity. The inhibitory potencies of the hydroxy-*trans*-stilbene compounds were remarkably elevated by increasing number of phenolic hydroxy substituent. Methylated hydroxy-*trans*-stilbene lost the inhibitory activity. Furthermore, hydrogenated hydroxystilbene or hydroxy-*cis*-stilbene exerted little or no inhibitory effect compared with the hydroxy-*trans*-stilbene on tyrosinase activity. The structure-activity relationships demonstrated in the present study suggest that the phenolic hydroxy group and the *trans*-olefin structure in a parent stilbene skeleton contribute to the inhibitory potency of hydroxystilbene for tyrosinase activity.

[*Biosci. Biotechnol. Biochem.*, **67**, 663-665 (2003)] [Lab. of Instrumental Center]

Gnetol as a Potent Tyrosinase Inhibitor from Genus *Gnetum*.

Kenji OHGUCHI, Toshiyuki TANAKA, Ibrahim ILIYA, Tetsuro ITO, Munekazu IINUMA*, Kenji MATSUMOTO, Yukihiro AKAO and Yoshinori NOZAWA

Melanin, which is a pigment of skin color, is produced by melanocytes. Synthesis of melanin starts from the conversion of the amino acid L-tyrosine to L-dopa and then the oxidation of L-dopa yields dopaquinone by tyrosinase, an enzyme catalyzing the rate-limiting step for melanin biosynthesis. This tyrosinase process is involved in abnormal accumulation of melanin pigment (hyperpigmentation). Gnetol (2,6,3',5'-tetrahydroxy-*trans*-stilbene), a naturally occurring compound particularly found in the genus *Gnetum*, had a strong inhibitory effect on murine tyrosinase activity. Gnetol (IC_{50} 4.5 μM) was stronger than kojic acid (IC_{50}, 139 μM) as a standard inhibitor for murine tyrosinase activity. Moreover, gnetol significantly suppressed melanin biosynthesis in murine B16 melanoma cells.

[*Carcinogenesis*, **24**, 1489-1497 (2003)] [Lab. of Instrumental Center]

Antitumor Effect of Resveratrol Oligomers against Human Cancer Cell Lines and the Molecular Mechanism of Apoptosis Induced by Vaticanol C.

Tetsuro ITO, Yukihiro AKAO, Hong YI, Kenji OHGUCHI, Kenji MATSUMOTO, Toshiyuki TANAKA, Munekazu IINUMA* and Yoshinori NOZAWA

Twenty resveratrol (Res) derivatives, which had been isolated from stem bark of *Vataica rassak* (Dipterocarpaceae), were evaluated for *in vitro* cytotoxicity against a panel of human tumor cell lines. Among them, seven compounds displayed marked cytotoxicity. Vaticanol C as a major component in the stem bark induced a considerable cytotoxicity in all cell lines tested and exhibited growth suppression in colon cancer cell lines at low dose. Vaticanol C caused two cell lines (SW80 and HL60) to induce cell death at four to seven times lower concentration compared with Res. The growth suppression by vaticanol C was found to be due to apoptosis, which was assessed by morphological findings (nuclear condensation and fragmentation) and DNA ladder formation in the colon cancer cell lines. The apoptosis in SW80 colon cancer cells was executed by the activation of caspase-3, which was shown by western blot and apoptosis inhibition assay.

[*Biosci. Biotechnol. Biochem.*, **67**, 1587-1589 (2003)] [Lab. of Instrumental Center]

Inhibitory Effects of Resveratrol Derivatives from Dipterocarpaceae Plants on Tyrosinase Activity.

Kenji OHGUCHI, Toshiyuki TANAKA, Tetsuro ITO, Munekazu IINUMA*, Kenji MATSUMOTO, Yukihiro AKAO and Yoshinori NOZAWA

Melanin production is principally responsible for skin color and plays an important role in prevention of sun-induced skin injury. Melanin is produced by melanocytes in the basal layer of the epidermis. Stilbene derivatives, which are resveratrol oligomers ranging from monomer to tetramer and have been isolated from Dipterocarpaceae plants were tested for their inhibitory effects against murine tyrosinase activity. The structure-activity relationships obtained in this study suggest that the double bond in a stilbene skeleton is critical for the inhibition, and also that the molecular size is important for inhibitory potency.

[*J. Health Sci.*, **49**, 475-480 (2003)] [Lab. of Instrumental Center]

Anti-Platelet and Membrane-Rigidifying Flavonoids in Brownish Scale of Onion.

Miyuki FURUSAWA, Hironori TSUCHIYA, Motohiko NAGAYAMA, Toshiyuki TANAKA, Ken-ichi NAKAYA and Munekazu IINUMA*

The bio-activity of the brownish scale of onion (*Allium cepa*) was studied together with identifying the active components and addressing the mode of action. A crude MeOH extract (0.5-1.0 ml) showed the inhibitory effect on human platelet aggregation induced by collagen, ADP, thrombin and epinephrine. The anti-platelet extract (1.0 μg/ml) rigidified liposome membrane by acting on the hydrocarbon core more intensively than the surface of membrane lipid bilayers. Serial solvent extractions and chromatographic purifications provided four isolates which were structurally identified as different quercetin dimers, quercetin and quercetin-4'-glucoside. The interaction with membrane lipids to modify membrane fluidity appears to be partly responsible for the anti-aggregatory and disaggregatory effects on human platelets. Although the inedible scale of onion is usually regarded as waste, it has the possibility to be a medicinal resource.

[*Helv. Chim. Acta*, **86**, 3394-3401 (2003)] [Lab. of Instrumental Center]

A New Dimeric Stilbenoid with a Five-Membered Lactone Ring from *Shorea hemsleyana*.

Testuro ITO, Toshiyuki TANAKA, Munekazu IINUMA,* Ken-ichi NAKAYA, Yoshikazu TAKAHASHI, Hikaru NAKAMURA, Hiroshi NAGANAWA and Soedarsono RIWAN

From the stem bark of *Shorea hemsleyana*, a new dimeric stilbenoid, shorealactone was isolated. The absolute configuration was determined by means of 2D NMR techniques and X-ray crystal-structure analysis of its 4-bromobenzoyl derivative by means of anomalous scattering of the Br-atom. The skeleton of shorealactone consists of two resveratrol units and an aliphatic moiety. The aliphatic moiety forms a novel framework composed of a five-membered lactone and a fused tetrahydrofuran rings. This unique framework is unprecedented among naturally occurring phenols. Shorealactone is considered to be a new kind of a complex stilbenoid. Its stilbene unit has been shown to connect to other skeletons such as in the flavonostilbenes (stilbenoid and flavonoid). The different kinds of linkages to heterocyclic units are responsible for the variation of stilbenoid structures.

[*J. Exper. Therap. Oncol.*, 3, 283-288 (2003)] [Lab. of Instrumental Center]

Antitumor Effect of Stilbenoids from *Vateria indica* against Allografted Sarcome S-180 in Animal Model.

Satoshi MISHIMA, Kenji MATSUMOTO, Yoshihiro FUTAMURA, Yoko ARAKI, Testuro ITO, Toshiyuki TANAKA, Munekazu IINUMA,* Yoshinori NOZAWA and Yukihiro AKAO

Dipterocarpaceous plants contain various resveratrol oligomers that exhibit variety of biological activities such as antibacterial and antitumor effects. Previously we found that vaticanol C, a resveratrol tetramer, exhibited strong cytotoxicity against various tumor cell lines. In the present study we examined the antitumor activity of the ethanol extract from the stem bark of *Vateria indica*, which has been traditionally used for health and healing diseases as Ayurveda in India. The *in vivo* assay displayed the extract's anticancer activity against sarcoma 180 cells (IC50= 29.5μM). In the animal study, the tumor growth of sarcoma S-180 cells subcutaneouly allografted in DDY mice significantly retarded by oral administration of the extract (30 or 100 mg/kg body weight: $p<0.001$). The extract did not show significant toxicity to mice even at a dosage of 1000mg/kg body weight by daily oral administration for 28 days.

[*Redox Rep.*, 8, 193-197 (2003)] [Lab. of Clinical Pharmaceutics]

Apomorphine Attenuates 6-Hydroxydopamine-Induced Apoptotic Cell Death in SH-SY5Y Cells.

Hirokazu HARA*, Mitsuhiro OHTA, Kiyoe OHTA, Sadako KUNO and Tetsuo ADACHI

We investigated the effect of apomorphine on 6-hydroxydopamine (6-OHDA)-induced apoptotic cell death using the human dopaminergic neuroblastoma cell line, SH-SY5Y. The co-treatment of cells with apomorphine significantly attenuated 6-OHDA-induced ROS generation, JNK phosphorylation, DNA fragmentation and subsequent apoptotic cell death. In addition, pretreatment with apomorphine for 24 h and the following concomitant treatment enhanced the protective effects against 6-OHDA-induced toxicity except for the attenuation of JNK phosphorylation. These findings suggested that apomorphine acts principally as a radical scavenger to suppress the level of ROS and ROS-stimulated apoptotic signaling pathway.

[*Mol. Brain Res.*, 119, 125-131 (2003)] [Lab. of Clinical Pharmaceutics]

Increase of Antioxidative Potential by *tert*-Butylhydroquinone Protects against Cell Death Associated with 6-Hydroxydopamine-Induced Oxidative Stress in Neuroblastoma SH-SY5Y Cells.

Hirokazu HARA*, Mitsuhiro OHTA, Kiyoe OHTA, Sadako KUNO and Tetsuo ADACHI

tert-Butylhydroquinone (tBHQ) is known as a strong inducer of phase II detoxification enzymes which have antioxidative functions. We investigated the neuroprotective effect of tBHQ against 6-hydroxydopamine (6-OHDA)-induced cell death using human neuroblastoma SH-SY5Y cells. The pretreatment of SH-SY5Y cells with tBHQ significantly reduced 6-OHDA-induced generation of reactive oxygen species (ROS), JNK phosphorylation, and subsequent cell death. tBHQ increased the intracellular glutathione levels and induced the expression of NAD(P)H:quinone oxidoreductase (NQO1) mRNA. In addition, tBHQ dose-dependently activated the antioxidant responsive element. These results indicate that an increase of intracellular antioxidative potential in SH-SY5Y cells by tBHQ treatment protects cells from 6-OHDA-induced oxidative stress.

[*Clin. Chim. Acta*, 328, 113-119 (2003)] [Lab. of Clinical Pharmaceutics]

Blood Extracellular Superoxide Dismutase Levels in Hemodialysis Patients Pre- and Post-Hemodialysis and Its Association with Lipoprotein Lipase Mass and Free Fatty Acid.

Hiroji SHIMOMURA, Eisuke MAEHATA, Tomi TAKAMIYA, Tetsuo ADACHI* and Tugikazu KOMODA

Hemodialysis (HD) is a method of chronic therapy for patients with renal failure. Diabetic nephropathy is the most common underlying disease among HD patients in Japan. A characteristic problem associated with this condition is endothelial cell damage. We have been using extracellular superoxide dismutase (EC-SOD) attached to the heparan sulfate on the endothelial cell surface as a marker of vascular damage. Pre-HD blood samples showed highly significant correlations between EC-SOD and lipoprotein lipase (LPL) mass and between EC-SOD and free fatty acid (FFA). EC-SOD, LPL mass and FFA were remarkably high among the patients who had been placed on HD treatment for over 20 years. Because EC-SOD and LPL mass represent heparin-binding proteins, these results were considered to reflect severe vascular damage.

[*Diabetes Care*, **26**, 1246-1250 (2003)] [Lab. of Clinical Pharmaceutics]

Serum Extracellular Superoxide Dismutase in Patients with Type 2 Diabetes: Relationship to the Development of Micro- and Macrovascular Complications.

Fumiaki KIMURA, Goji HASEGAWA, Hiroshi OBAYASHI, Tetsuo Adachi, * Hirokazu HARA, Mitsuhiro OHTA, Michiaki FUKUI, Yoshihiro KITAGAWA, Hyohun PARK, Naoto NAKAMURA, Koji NAKANO and Toshikazu YOSHIKAWA

The aim of this study was to determine the distribution of serum extracellular superoxide dismutase (EC-SOD) concentrations in patients with type 2 diabetes and to assess whether increased EC-SOD concentration is associated with the development of diabetic vascular complications. We observed a strong relationship between the serum concentration of EC-SOD and the severity of both micro- and macrovascular diabetic complications. These findings suggest that serum EC-SOD concentration levels may be a marker of vascular injury, possibly reflecting hyperglycemia-induced oxidative injury to the vascular endothelium and decreased binding of EC-SOD to the vascular wall.

[*J. Analytical Bio-Science*, **26**, 169-174 (2003)] [Lab. of Clinical Pharmaceutics]

Plasma Levels of Human Brain Natriuretic Peptide (BNP) in Diabetics: Its Correlations with the Albumin Excretion Index (AEI) and Extracellular (EC) Superoxide Dismutase (SOD).

Eisuke MAEHATA, Ikuo HATAKEYAMA, Toshio IMAI, Tetsuo ADACHI,* Minoru YAMAKADO and Minoru INOUE

Plasma BNP level has been included in the ACC/AHA guidelines because its reflects the increase in left ventricular end-diastolic pressure. In view of the propensity of diabetics to develop vascular complications, we examined the albumin excretion index (AEI) and EC-SOD, and confirmed the presence of significant correlations between BNP and these markers of vascular injury. Based on its observed effectiveness as a bedside marker of heart failure, we considered the plasma BNP assay may be indispensable in the management of aged patients with critical conditions of lifestyle-related diseases.

[*Hum. Immunol.*, **64**, 302-309 (2003)] [Lab. of Clinical Pharmaceutics]

TNF, TNF Receptor Type 1, and Allograft Inflammatory Factor-1 Gene Polymorphisms in Japanese Patients with Type 1 Diabetes.

Masataka NISHIMURA, Hiroshi OBAYASHI, Ikuko MIZUTA, Hirokazu HARA*, Tetsuo ADACHI, Mitsuhiro OHTA, Hisataka TEGOSHI, Michiaki FUKUI, Goji HASEGAWA, Hirofumi SHIGETA, Yoshihiro KITAGAWA, Koji NAKANO, Ryuji KAJI and Naoto NAKAMURA

We investigated AIF-1, TNF, TNF receptors type 1 (TNFR1) and LT-alpha gene polymorphisms in 165 patients with type 1 diabetes. Although there were significant differences between type 1 diabetes patients and controls in the distributions of TNF promoter polymorphisms at position -1031 and -857, and LT-alpha gene NcoI polymorphism, none of them was independently associated with the disease after two-locus analysis with HLA class II alleles. We detected the significantly increased frequency of the -383C allele, located in the TNFR-1 promoter region, in diabetes patients compared with controls. In addition, the -383C allele was found to be associated with higher expression of the TNFR1 gene than that of -383A allele in *in vitro* expression.

[*Phytochemistry*, **62**, 1243-1246 (2003)] [Lab. of Medicinal Informatics]

Eryvarins F and G, Two 3-Phenoxychromones from the Roots of *Erythrina variegata*.

Hitoshi TANAKA, Miyuki HIRATA, Hideo ETOH, Hiroshi SHIMIZU, Magoichi SAKO,* Jin MURATA, Hiroko MURATA, Dedy DARNAEDI, Toshio FUKAI

Erythrina variegata L. (Leguminosae) is used as antibacterial, anti-inflammatory, antipyretic, and antiseptic agents in many tropical and subtropical regions and as a collyrium in China. Phytochemical study on the non-alkaloidal secondary metabolites of the genus *Erythrina* has revealed the presence of one cinnamoylphenol and several isoflavonoid compounds in this plant, some of which exhibit antibacterial and anti-inflammatory activities and inhibit the Na^+/H^+ exchange system. Our recent phytochemical study on the *E. variegata* allowed the isolation of five isoflavonoids (eryvarins A-E) from the wood and the roots. On this line, novel types of 3-phenoxychromones, *i.e.*, 3-(2,4-dihydroxyphenoxy)-7-hydroxy-6,8-bis(3,3-dimethylallyl)chromen-4-one (eryvarin F; having two isoprenoid groups in the molecule) and 3-(2,4-dihydroxyphenoxy)-8-(3,3-dimethylallyl)-2,2-dimethylpyrano[5,6:6,7]-chromen-4-one (eryvarin G), were isolated from the roots of this plant collected in Indonesia.

[*Phytochemistry*, 63, 597-602 (2003)] [Lab. of Medicinal Informatics]

An Arylbenzofuran and Four Isoflavonoids from the Roots of *Erythrina poeppigiana*.

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Masaru SATO, Toshio FUKAI, Yoichi TATEISHI

The genus *Erythrina* is widely distributed in tropical and subtropical regions of the world and has been used in indigenous folk medicine for the treatment of microbial infections. *Erythrina poeppigiana* is distributed in Central and South America and is cultivated in Okinawa prefecture, Japan as an ornamental plant and a street tree. Recent phytochemical study of this plant allowed the structural determination of five isoflavonoids (erypoegins A-E) isolated from the roots. During the continuation of this study, an arylbenzofuran (erypoegin F) and four new isoflavonoids (erypoegins G-J) were isolated, together with six known compounds (cristacarpin, demethylmedicarpin, erysubin F, eryvarin D, folitenol and oriantanol C), from the roots. Erypoegin F is a rare 2-arylbenzofuran, possessing a formyl group in the molecule, as a natural source. Additionally, erypoegin I is the first naturally occurring isoflavonoid having a 2-oxo-3-methylbutyl group in the molecule.

[*Tetrahedron Lett.*, 44, 7303-7306 (2003)] [Lab. of Medicinal Informatics]

Oxidative Hydrolysis of a Cyclic 1,N^2-Propano-2'-deoxyguanosine, an Adduct of 2'-Deoxyguanosine with Acetaldehyde or Crotonaldehyde.

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Current attention has been paid to the characteristic chemical reactivity of cyclic 1,N^2-propanoguanine adducts, chemo- and regio-selectively produced in the reactions of DNA and 2'-deoxyguanosine with excessive acetaldehyde (AA) or crotonaldehyde (CA) in relation to genotoxic, mutagenic, and carcinogenic effects of these aldehydes and their precursors. The $SO_4^{\cdot-}$-oxidation of cyclic 1,N^2-propano-2'-deoxyguanosine resulted in the smooth formation of guanine-ring opened products, (4-hydroxy-5-hydroxymethyltetrahydrofuran-2-ylimino)-(4-hydroxy-6-methyltetrahydropyrimidin-2-ylideneamino)acetic acid, and 3-(4-hydroxy-5-hydroxymethyltetrahydrofuran-2-yl)-6-methyl-3*H*-1,3,4,5,8a-pentaazacyclopenta[*b*]naphthalen-9-one, together with a small amount of 2'-deoxyguanosine, even under neutral conditions. The formation of the guanine-ring opened products during the reaction appears to closely relate to the mechanisms for the point-mutations of DNA such as G-A transition and G-T transversion by AA and CA.

[*Phytochemistry*, 64, 753-758 (2003)] [Lab. of Medicinal Informatics]

Isoflavonoids from Roots of *Erythrina zeyheri*.

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Fujio ASAI, Toshio FUKAI, Masaru SATO, Jin MURATA, Yoichi TATEISHI

The genus *Erythrina* (Leguminosae), widely distributing in tropical and subtropical regions of the world, is often used for the folklore medicinal treatment of microbial infections. Two isoflavonoids, *i.e.*, erycristagallin and orientanol, isolated from the roots of *E. variegata*, have been shown to exhibit potent anti-methicillin-resistant *Staphylococcus aureus* (MRSA). During the continuation of our screening of anti-MRSA compounds from the *Erythrina* plants, five isoflavonoids [(±)-7,2',4'-trihydroxy-8,3'-di(γ,γ-dimethylallyl)isoflavanone (Eryzerin A), (3*R*)-7,4'-dihydroxy-2'-methoxy-6,8-di(γ,γ-dimethylallyl)isoflavanone (Eryzerin B), (3*R*)-7,2',4'-trihydroxy-6,8-di(γ,γ-dimethylallyl)isoflavan (Eryzerin C), 2',4'-dihydroxy-8-γ,γ-dimethylallyl-2",2"-dimethylpyrano-[5,6:6,7]isoflavan (Eryzerin D), and (6a*S*,11a*S*)-3,6a-dihydroxy-9-methoxy-4,10-di(γ,γ-dimethylallyl)pterocarpan (Eryzerin E)] were isolated from the roots of *E. zeyheri*, along with five known compounds. Among the newly isolated compounds (Eryzerin A-E), Eryzerin C exhibited highest antibacterial activities (MIC: 3.13-6.25 μg mL^{-1}) against MRSA.

[*Heterocycles*, **60**, 2767-2773 (2003)] [Lab. of Medicinal Informatics]

Four New Isoflavonoids and a New 2-Arylbenzofuran from the Roots of *Erythrina variegata*.

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Masaru SATO, Jin MURATA, Hiroko MURATA, Dedy DARNADI, Toshio FUKAI

Erythrina variegata is a member of the Leguminosae family exhibiting antibacterial activity. Phytochemical studies on the non-alkaloidal secondary metabolites of this plant resulted in the isolation of a cinnamylphenol, flavanons and isoflavonoids. During the continuation of our phytochemical study on the genus *Erythrina*, four new isoflavonoids (two isoflav-3-enes: eryvarins H and I; two pterocarpans: eryvarins J and K) and a new 2-arylbenzofuran (eryvarin L) together with five known compounds (bidwillon C, erysubin C, erythrabyssin II, orientanol B, and phaseollin), were isolated from the roots of *E. variegata* collected in Indonesia. Eryvarins H and I are rare naturally-occurring isoflav-3-enes. Among these newly isolated compounds (Eryzerin H-L), Eryzerin L exhibited weak antibacterial activities (MIC: 25 μg mL^{-1}) against MRSA and inhibited the growth of five strains of vancomycin-resistant *Enterococci* at 50 μg mL^{-1}.

[*Bioorg. Med. Chem. Lett.*, **13**, 3497-3498 (2003)] [Lab. of Medicinal Informatics]

Histones Accelerate the Cyclic 1,N^2-Propanoguanine Adduct-formation of DNA by the Primary Metabolite of Alcohol and Carcinogenic Crotonaldehyde.

Magoichi SAKO,* Shinsuke INAGAKI, Yukihiro ESAKA, Yoshihiro DEYASHIKI

Alcohol abusers are well known to have highly increased cancer-risks for the upper digestive tract and liver due to their excessive drinking of alcohol. The absorbed ethanol is metabolized by two types of dehydrogenases to acetaldehyde (AA) and then acetic acid. The primary metabolite AA is a highly reactive electrophile and also gradually polymerizes even in an aqueous solution to give the corresponding dimer (so-called, aldol), trimer (aldoxane), and tetramer (paraldol). AA and its polymers have been shown to react chemo- and regio-selectively with the exocyclic amino group of the guanine moiety in 2'-deoxyguanosine and DNA. The present chemical modifications of DNA by excessive AA and crotonaldehyde were significantly accelerated by the presence of histones, which are nuclear proteins very rich in the basic amino acids such as L-arginine and L-lysine, resulting in the smooth and selective formation of the corresponding cyclic 1,N^2-propanoguanine adducts under physiological conditions. Thus, histones have a very close connection with the genotoxic and carcinogenic effects of these aldehydes.

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