化 学 応 用 部 門

教 授　門 田 重 利（薬学博士）
助教授　手 塚 康 弘（薬学博士）11月１日から
助教授(前) 畑 中 保 丸（薬学博士）８月31日まで
助 手(前) 手 塚 康 弘（薬学博士）10月31日まで
技 官　幸 田 恭 治（薬学博士）４月１日から

◇研究目的

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

◇研究概要

Ⅰ．天然薬物成分の科学的研究

東南アジア（インドネシア，スリランカ，ベトナム，タイ，ミャンマー等）の薬用植物

Ⅱ．和漢薬成分の医薬化学

1．麝香から単離した新規成分ムスクライド類の合成および誘導体化
2．羅布麻，プロポリスから単離した生理活性成分の合成
3．肝臓病や骨粗鬆症に有効な天然薬物成分の開発研究
4．マトリックスメタロプロテアーゼ産生阻害を有する天然薬物成分の研究

Ⅲ．漢方製剤の品質評価法

1．通関丸，桃核承気湯，当帰飲子など
2．基源植物，修治生薬のLC-MSによる評価

Ⅳ．和漢薬成分の生物有機化学的研究

ベルベリン，アクチオサイド，テトラヒドロスベルチアノリン，キナ酸誘導体，カリクシン類，サポニン，花椒成分など

Ⅴ．天然薬物調査

ベトナム，ミャンマー，コンゴ民主共和国など

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

◇原　著

1）**Banskota A. H., Tezuka Y., Tran K. Q., Tanaka K., Saiki I., and Kadota S.: Thirteen Novel Cycloartane-type Triterpenes from *Combretum quadrangulare*. *J. Nat. Prod.*, 63, 57-64 (2000).**

Summary: Thirteen novel cycloartane-type triterpenes were isolated from *Combretum quadrangulare*, a Vietnamese medicinal plant. The structures of the novel triterpenes were determined by spectroscopic methods as well as by chemical transformations. Among those compounds, quadrangularic acids F (**1**), G (**2**), and H (**4**) and 24-epiquadrangularic acid G (**3**) are the first examples of cycloartane-type triterpenes bearing carboxylic acid groups at both C-4 and C-20. Furthermore, norquadrangularic acid A (**13**) is the first example of a trinorcycloartane-type triterpene isolated from the genus *Combretum*.

2）**Hamasaki N., Ishii E., Tominaga K., Tezuka Y., Nagaoka T., Kadota S., Kuroki T., and Yano I.: Highly selective antibacterial activity of novel alkyl quinolone alkaloids from a Chinese herbal medicine, Gosyuyu (Wu-Chu-Yu), against *Helicobacter pylori in vitro*. *Microbiol. Immunol.*, 44, 9-15 (2000).**

Summary: To elucidate the antibacterial activity of Gosyuyu, the crude extract from the fruit of *Evodia rutaecarpa,* a Chinese herbal medicine, has been fractionated chromatographically, and each fraction was assayed for antibacterial activity against *Helicobacterpylori* (*H. pylori*) *in vitro*. As the result, a single spot having marked antibacterial activity against *H. pylori* was obtained and the chemical structure was analyzed. The isolated compound was revealed to be a novel alkyl quinolone alkaloid based on the solubility, IR spectra, NMR analysis and mass spectrometric data after purification by TLC of silica. We compared the antimicrobial activity of this compound with that of other antimicrobial agents and examined susceptibility of various intestinal pathogens. As the result, the new quinolone compounds obtained from Gosyuyu extracts were found to be a mixture of two quinolone alkaloids, 1-methyl-2-[(Z)-8-tridecenyl]-4-(1H)-quinolone and 1-methyl-2-[(Z)-7-tridecenyl]-4-(1H)-quinolone (MW: 339), reported previously. The minimum inhibitory concentration (MIC) of these compounds against reference strains and clinically isolated *H. pylori* strains were less than 0.05 microg/ml, which was similar to the MIC of amoxicillin and clarithromycin that are used worldwide for the eradication of *H. pylori*, clinically. Furthermore, it was noted that the antimicrobial activity of these compounds was highly selective against *H. pylori* and almost non-active against other intestinal pathogens. The above results showed that these alkyl methyl quinolone (AM quinolones) alkaloids were useful for the eradication of *H. pylori* without affecting other intestinal flora.

3）**Hasegawa H., Lee K.-S., Nagaoka T., Tezuka Y., Uchiyama M., Kadota S., and Saiki I.: Pharmacokinetics of Ginsenoside Deglycosylated by Intestinal Bacteria and its Transformation to Biologically Active Fatty Acid Esters. *Biol. Pharm. Bull.*, 23, 298-304 (2000).**

Summary: Five new triterpene glucosides, quadranosides I-V (**1-5**), have been isolated from a MeOH extract of the seeds of *Combretum quadrangulare*, together with thirteen known compounds. The structures of compounds **1-5** were elucidated on the basis of spectroscopic analysis. Among the new triterpene glucosides, three compounds (**1, 2, 5**) showed significant hepatoprotective effects against D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes.

4）**Banskota A. H., Tezuka Y., Tran K. Q., Tanaka K., Saiki I., and Kadota S.: Methyl Quadrangularates A-D and Related Triterpenes from *Combretum quadrangulare*. *Chem. Pharm. Bull.*, 48, 496-504 (2000).**

Summary: From the MeOH extract of leaves of *Combretum quadrangulare*, fifteen new cycloartane-type triterpenes, methyl quadrangularates A-D (**1-4**) and N-P (**8, 6, 12**), methyl 24-epiquadrangularate C (**5**), quadrangularic acid E (**9**), 23-deoxojessic acid (**10**), 1-*O*-acetyl-23-deoxojessic acid (**11**), quadragularols A (**7**) and B (**13**) and

norquadrangularic acids B (**14**) and C (**15**) were isolated together with two known cycloartane-type triterpenes, methyl 23-deoxojessate (**16**) and 4β,14α-dimethyl-5α-ergosta-9β,19-cyclo-24(31)-en-3β-hydroxy-4α-carboxylic acid (**17**). Betulinic acid (**18**), β-sitosterol (**19**), kamatakenin (**20**), isokaempferide (**21**), 5,7,4'-tirhydroxy-3,3'-dimethoxyflavone (**22**) and 5,4'-dihydroxy-3,7,3'-trimethoxyflavone (**23**) were also obtained from the same extract. The structures of the new compounds were elucidated on the basis of spectral analysis and chemical conversions. All the isolated compounds were tested for their cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells, and the cycloartane-type triterpenes showed various degrees of cytotoxicity, whereas all the flavonoids possessed strong cytotoxicity with ED50 values equal to or less than 6 μM.

5) **Banskota A. H., Tezuka Y., Adnyana I K., Xiong Q., Hase K., Tran K. Q., Tanaka K., Saiki I., and Kadota S.: Hepatoprotective Effect of Leaves of *Combretum quadrangulare and Its Constituents. Biol. Pharm. Bull.*, 24, 456-460 (2000).**

Abstract: The MeOH extract of leaves of Combretum quadrangulare showed significant hepatoprotective effect on D-galactosamine (D-GalN)/lipopolysaccharide (LPS)-induced experimental liver injury in mice and on D-GalN/ tumor necrosis factor-a (TNF-a)-induced cell death in primary cultured mouse hepatocytes. Phytochemical investigation led to the isolation of thirty cycloartane-type triterpenes together with betulinic acid, b-sitosterol, b-sitosterol glucoside, 4 flavones (34(37), and 3 flavone C-glucosides (38(40). These compounds showed various potencies of hepatoprotective effect on D-GalN/TNF-a-induced cell death in primary cultured mouse hepatocytes. Quadrangularol B (29), methyl quadrangularate I (33), kamatakenin (34), 5,7,4'-trihydroxy-3,3'-dimethoxyflavone (35), 5,4',-dihydroxy-3,7,3'-trimethoxyflavone (36) and isokaempferide (37) showed strong inhibitory effect on TNF-a-induced cell death with IC50 values of 34.3, 33.7, 13.3, 22.4, 13.4 and 22.8 mM, respectively, whereas clinically-used silibinin had an IC50 value of 39.6 mM and glycyrrhizin showed very weak inhibitory effect. Methyl quadrangularates A (30) and N (32), norquadrangularic acid B (31) and vitexin (40) also showed potent inhibition on TNF-a-induced cell death with IC50 values of 45.7, 89.3, 67.6 and 40.1 mM, respectively. The flavonoids and some of the cycloartane-type triterpenes appeared to be the hepatoprotective principles of the leaves of C. quadrangulare.

6) **Xiong Q., Fan W., Tezuka Y., Adnyana I K., Stampoulis P., Hattori M., Namba T., and Kadota S.: Hepatoprotective Effect of *Apocynum venetum* and Its Active Constituents. *Planta Medica*, 66, 127-133 (2000).**

Abstract: The leaves of Apocynum venetum L. are used as a tea material in north China and Japan. A water extract (500 μg/kg/day, one week administration) of the leaves of A.*venetum* showed protective effects against carbon tetrachloride (CCl_4, 30μl/mouse) or D-galactosamine (D-GalN, 700μg/kg) / lipopolysaccharide (LPS, 20μg/kg)-induced liver injury in mice. Tumor necrosis factor - α (TNF- α) secreted from LPS-stimulated macropharges is the most crucial mediation in the D-GalN/LPS-induced liver injury model. The extract had no significant inhibition on the increase of serum TNF- α (1169±132 pg/ml vs.1595±314 pg/ml of control), but exhibited a complete inhibition at the concentration of 100 μg/ml on TNF- α (100 ng/ml)-induced cell death in D-GalN (0.5 mM)-sensitized mouse hepatocytes. Further activity-guided fraction resulted in the isolation of fifteen flavonoids viz. (-)-epicatechin (**1**), (-)-epigallocatechin (**2**), isoquercetin (**3**), hyperin (**4**), (+)-catechin (**5**), (+)-gallocatechin (**6**), kaempferol-6'-O-acetate (**7**), isoquercetin-6'-O-acetate (**8**), catechin-[8, 7-*e*]-4 α - (3, 4-dihydroxpyhenyl)-dihydro-2 (3*H*)-pyranone (**9**), apocynin B (**10**), apocynin A (**11**), cinchonain Ia (**12**), apocynin C (**13**), apocynin D (**14**) and quercetin (**15**). All the compounds showed potent inhibitory effects on TNF-a-induced cell death with IC_{50} values of 37.5, 14.5, 31.2, 55.1, 71.9 and 41.2 μM, respectively. In contrast, the clinically used **5** and its analogues **1, 2** and **6** showed apparent activity only at 80 μM. These flavonoids appeared to be the hepatoprotective principles of the leaves of A.*venetum*. The hepatoprotective effects exhibited by the extract and its constituents suggest a validation of the leaves as a tea material.

7) **Adnyana I K., Tezuka Y., Banskota A. H., Xiong Q., Tran K. Q., and Kadota S.: Quadranosides I-V, New Triterpene Glucosides from the Seeds of *Combretum quadrangulare*. *J. Nat. Prod.*, 63, 496-500 (2000).**

Summary: Five new triterpene glucosides, quadranosides I-V (**1-5**), have been isolated from a MeOH extract of the seeds of *Combretum quadrangulare*, together with thirteen known compounds. The structures of compounds **1-5** were elucidated on the basis of spectroscopic analysis. Among the new triterpene glucosides, three compounds (**1, 2, 5**) showed significant hepatoprotective effects against D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes.

8) **Kimura I., Islam Md. A., Honda R., Nojima H., Tezuka Y., and Zhao W.: Blood-Pressure Lowering, Positive Chronotropy and Inotropy by the Veratrum Alkaloids Germidine and Germerine but Negative Chronotropy by Veratrodine in Mice. *J. Asian Nat. Prod. Res.*, 2, 134-144 (2000).**

9) **Fan W., Tezuka Y., and Kadota S.: Prolyl Endopeptidase Inhibitory Activity of Fourteen Kampo Formulas and Inhibitory Constituents of Tokaku-joki-to (桃核承気湯). *Chem. Pharm. Bull.*, 48, 1055-1061 (2000).**

Abstract: Prolyl endopeptidase (PEP, EC 3.4.21.26) is an enzyme playing a role in the metabolism of proline-containing neuropeptides which have been suggested to be involved in learning and memory processes. In screening for PEP inhibitors from fourteen traditional Kampo formulas, we found that Tokaku-joki-to (桃核承気湯) shows a significant inhibitory activity. Examination of the constituents of the Kampo formula resulted in the isolation of two new compounds, *cis*-3,5,4'-trihydroxystilbene 4'-*O*-β-D-(6-*O*-galloyl) glucopyranoside (**10**) and 4-(4-hydroxyphenyl)-2-butanone 4'-*O*-β-D-(6-*O*-galloyl-2-*O*-cinnamoyl)glucopyranoside (**16**), along with twenty-five known compounds, cinnamic acid (**1**), protocatechuic acid (**2**), gallic acid (**3**), torachrysone 8-*O*-β-D-glucoside (**4**), emodin(**5**), emodin 8-*O*-β-D-glucoside (**6**), 3,5,4'-tri-hydroxystilbene 4'-*O*-β-D-glucopyranoside (**7**), 3,5,4'-trihydroxystilbene 4'-*O*-β-D-(2-*O*-galloyl)-glucopyranoside (**8**), 3,5,4'-trihydroxystilbene 4'-*O*-β-D-(6-*O*-galloyl)glucopyranoside (**9**), 4-(4-hydroxyphenyl)-2-butanone 4'-*O*-β-D-glucopyranoside (**11**), isolindleyin (**12**), lindleyin (**13**), 4-(4-hydroxyphenyl)-2-butanone 4'-*O*-β-D-(2,6-di-*O*-galloyl)glucopyranoside (**14**), 4-(4-hydroxyphenyl)-2-butanone 4'-*O*-β-D-(2-*O*-galloyl-6-*O*-cinnamoyl)glucopyranoside (**15**), 1-*O*-galloylglucose (**17**), 1,2,6-tri-*O*-galloylglucose (**18**), gallic acid 4-*O*-β-D-(6-*O*-galloyl)-glucopyranoside (**19**), liquiritigenin (**20**), liquiritigenin 4'-*O*-β-D-glucoside (**21**), liquiritigenin 7,4'-diglucoside (**22**), liquiritigenin 4'-*O*-β-D-apiofuranosyl-(1→2)-β-D-glucopyranoside (**23**), licuroside (**24**), (-)-epicatechin (**25**), (-)-epicatechin 3-*O*-gallate (**26**) and (+)-catechin (**27**). Among these compounds, twelve (**7-10, 14-16, 18, 19, 24-26**) showed noncompetitive inhibition with an IC50 of 22.9, 3.0, 14.9, 2.8, 10.5, 0.69, 8.2, 0.44, 9.39, 26.5, 28.1 and 0.052 μM, respectively.

10) **Xiong Q., Tezuka Y., Kaneko T., Li H., Tran L. Q., Hase K., Namba T., and Kadota S.: Inhibition of Nitric Oxide by Phenylethanoids in Activated Macrophages. *Eur. J. Pharmacol.*, 400, 137-144 (2000).**

Abstract: Nitric oxide (NO) is one of the pro-inflammatory molecules. Some phenylethanoids have been previously shown to possess anti-inflammatory effects. Seven phenylethanoids from the stems of *Cistanche deserticola, viz.* isoacteoside, tubuloside B, acteoside, 2'-*O*-acetylacteoside, echinacoside, cistanoside A and tubuloside A, were tested for their effect on NO radical generation by activated murine macrophages. At the concentration of 100-200 μM, all the phenylethanoids reduced (6.3-62.3%) nitrite accumulation in lipopolysaccharide (0.1μg/ml)-stimulated J774.1 cells. At 200 μM, they inhibited by 32.2-72.4% nitrite accumulation induced by lipopolysaccharide (0.1 μg/ml)/interferon-γ (100 U/ml) in mouse peritoneal exudate macrophages. However, these compounds did not

affect the expression of inducible nitric oxide (iNOS) mRNA, the iNOS protein level, or the iNOS activity in lipopolysaccharide-stimulated J774.1 cells. Instead, they showed a clear scavenging effect (6.9-43.9%) at the low concentrations of 2-10 μM of about 12 μM nitrite generated from a NO donor, 1-propanamine-3-hydroxy-2-nitroso-1-propylhydrazino (PAPA NONOate). These results indicate that the phenylethanoids have NO radical-scavenging activity, which possibly contributes to their anti-inflammatory effects.

11) Tezuka Y., Ali M. S., Banskota A. H., and Kadota S.: Blepharocalyxins C-E; three novel antiproli-ferative diarylheptanoids from the seeds of *Alpinia blepharocalyx*. *Tetrahedron Lett.*, 41, 5903-5907 (2000).

Abstract: Three novel diarylheptanoids, blepharocalyxins C-E (**1-3**), were isolated from an EtOH extract of seeds of Alpinia blepharocalyx, and their structures have been elucidated by the use of spectroscopic techniques. Blepharocalyxins D (**2**) and E (**3**) exhibited potent antiproliferative activity against murine colon 26-L5 carcinoma and human HT-1080 fibrisarcoma cells with an ED50 value of 3.61 μM and 9.02 μM, respectively.

12) Adnyana I K., Tezuka Y., Awale S., Banskota A. H., Tran K. Q., and Kadota S.: Quadranosides VI-XI, Six New Triterpene Glucosides from the Seeds of *Combretum quadrangulare*. *Chem. Pharm. Bull.*, 48, 1114-1120 (2000).

Abstract: Six new triterpene glucosides, quadranosides VI-XI (**1-6**), belonging to three different [ursane- (**1-4**), oleanane- (**5**) and lupane-type (**6**)] triterpene classes, have been isolated from a MeOH extract of the seeds of *Combretum quadrangulare* Kurz (Combretaceae), together with nine known compounds, rosamutin (**7**), 28-*O*-β-D-glucopyranosyl-6β,23-dihydroxytormentic acid (**8**), arjunetin (**9**), arjunglucoside II (**10**), combreglucoside (**11**), chebuloside II (**12**), vitexin (**13**), (+)-catechin (**14**) and (-)-epigallocatechin (**15**). The structures of these compounds were elucidated on the basis of spectroscopic analysis.

13) Sekiya K., Tezuka Y., Tanaka K., Prasain J. K., Namba T., Katayama K., Koizumi T., Maeda M., Kondo T., and Kadota S.: Distribution, Metabolism and Excretion of Butylidenephthalide of Ligustici Chuanxiong Rhizoma in Hairless Mouse after Dermal Application. *J. Ethnopharmacol.*, 71, 401-409 (2000).

Summary: The absorption, distribution and excretion of butylidenephthalide after dermal application to hairless mouse have been examined with [8-14C] butylidenephthalide. By the investigation of the whole body autoradiogram and liquid scintillation analysis, it was indicated that the transdermally applied butylidenephthalide quickly permeate into peripheral circulation system without accumulation in the skin and then distribute into lung, liver, bile and kidney. The total radioactivity, however, was decreased due to excretion into urine, and in the case of i.v.-administration, 80% of the administered butylidenephthalide was excreted into urine within 24 h, while only 5% was excreted into feces within 24 h. Then, the metabolite in urine was determined to be a cysteine conjugate by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method. Thus, it has been concluded that after dermal application butylidenephthalide quickly permeates through skin into peripheral circulation system; distributes to lung, liver, bile and kidney; and then excreted into urine as a cysteine adduct.

14) Banskota A. H., Tezuka Y., Adnyana I K., Midorikawa K., Matsushige K., Message D., Huertas A. A. G., and Kadota S.: Cytotoxic, hepatoprotective and free radical scavenging effects of propolis from Brazil, Peru, the Netherlands and China. *J. Ethnopharmacol.*, 72, 239-246 (2000).

Abstract: Propolis is a resinous hive product collected by honeybees from various plant sources. The composition of the propolis depends upon the time, vegetation and the area of collection. Thus, quality evaluation of the propolis

is important, before to be used in food and beverage. For this propose we carried out three different biological activities, *i.e.,* 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity, cytotoxicity and hepatoprotective activity, of MeOH and water extracts of nine different propolis from Brazil, Peru, the Netherlands and China. The results showed that water extracts of six Brazilian and a Chinese propolis possessed stronger DPPH free radical scavenging activity than the corresponding MeOH extract, whereas in the case of Netherlands and Peruvian propolis MeOH extract exhibited stronger DPPH free radical scavenging activity. The MeOH extracts of all propolis possessed stronger cytotoxicity than the corresponding water extract towards murine colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cells. The result of hepatoprotective activity of Brazilian propolis on D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes were found in accordance with the grade setted up by beekeepers in Brazil.

15) Banskota A. H., Tezuka Y., Midorikawa K., Matsushige K., and Kadota S.: Two Novel Cytotoxic Benzofuran Derivatives from Brazilian Propolis. *J. Nat. Prod.*, 63, 1277-1279 (2000).

Abstract: Two novel benzofuran derivatives, propolis-benzofurans A (**1**) and B (**2**), were isolated from the MeOH extract of Brazilian propolis, together with two known isoprenylated compounds (*E*)-3-[2,3-dihydro-2-(1-methylethenyl)-7-prenyl-5-benzofuranyl]-2-propenoic acid and (*E*)-3-[4-hydroxy-3-[(*E*)-4-(2,3-dihydrocinnamoyloxy)-3-methyl-2-butenyl]-5-prenylphenyl]-2-propenoic acid. Structures of these compounds were elucidated on the basis of spectral analysis. Both the new compounds possessed mild cytotoxicity towards highly liver-metastatic murine colon 26-L5 carcinoma and human HT-1080 fibrosarcoma cells.

16) Tezuka Y., Stampoulis P., Banskota A. H., Awale S., Tran K. Q., Saiki I., and Kadota S.: Constituents of Vietnamese Medicinal Plant *Orthosiphon stamineus*. *Chem. Pharm. Bull.*, 48, 1711-1719 (2000).

Summary: From the MeOH extract of the aerial part of Vietnamese *Orthosiphon stamineus*, five new isopimarane-type diterpenes [orthosiphols F-J (**1-5**)] and two new diterpenes [staminols A (**6**) and B (**7**)] with a novel carbon-framework, to which we proposed the name "staminane", and three new highly-oxygenated staminane-type diterpenes [staminolactones A (**8**) and B (**9**) and norstaminol A (**10**)] were isolated. Moreover, staminolactone A (**8**) is 8,14-secostaminane-type and staminolactone B (**9**) is 13,14-secostaminane-type, while norstaminol A (**10**) is 14-norstaminen-type. Together with these new diterpenes, sixteen known compounds were also isolated and identified to be: 7,3',4'-tri-O-methylluteolin (**11**), eupatorin (**12**), sinensetin (**13**), 5-hydroxy-6,7,3',4'-tetramethoxyflavone (**14**), salvigenin (**15**), ladanein (**16**), tetramethylscutellarein (**17**), 6-hydroxy-5,7,4'-trimethoxyflavone (**18**), vomifoliol (**19**), aurantiamide acetate (**20**), rosmarinic acid (**21**), caffeic acid (**22**), oleanolic acid (**23**), ursolic acid (**24**), betulinic acid (**25**), and β-sitosterol (**26**). All the isolated compounds were tested for their cytotoxicity towards highly liver metastatic murine colon 26-L5 carcinoma cells, and the new diterpenes, except for 4, and flavonoids (**11, 12, 16, 18**) showed cytotoxicity with an ED50 value between 10 and 90 μg/ml.

17) Adnyana I K., Tezuka Y., Banskota A. H., Tran K. Q., and Kadota S.: Hepatoprotective Constituents of the Seeds of *Combretum quadrangulare*. *Biol. Pharm. Bull.*, 24, 1328-1332 (2000).

Abstract: Hepatoprotective effect of MeOH, MeOH-H2O (1:1) and H2O extracts of *Combretum quadrangulare* seeds were examined on D-galactosamine (D-GalN)/tumor necrosis factor-α (TNF-α)-induced cell death in primary cultured mouse hepatocytes. The MeOH extract showed the strongest inhibitory effect on D-GalN/TNF-α-induced cell death (IC50, 56.4 μg/ml). Moreover, the MeOH extract also significantly lowered the serum glutamic pyruvic transaminase (sGPT) level on D-GalN/lipopolysaccharide (LPS)-induced liver injury in mice. Bioguided separation of the MeOH extract led to the isolation of thirty-eight compounds of various classes including triterpene glucosides,

lignans and catechin derivatives. Among the isolated triterpene glucosides, lupane-type (**1-3**; IC50, 63.1, 59.8 and 76.2 μM, respectively) and ursane-type (**11**, mixture of **12** and **14**; IC50, 30.2 and 34.6 μM, respectively) compounds exhibited strong hepatoprotective activity. 1-*O*-Galloyl-6-*O*-(4-hydroxy-3,5-dimethoxy)benzoyl-β-D-glucose (**26**; IC50, 7.2 μM), methyl gallate (**28**; IC50, 19.9 mM), and (-)-epicatechin (**31**; IC50, 71.2 μM) also had a potent hepatoprotective effect on D-GalN/TNF-α-induced cell death in primary cultured mouse hepatocytes.

18) Tezuka Y., Terazono M., Kusumoto I. T., Hatanaka Y., Kadota S., Hattori M., Namba T., Kikuchi T., Tanaka K., and Supriyatna S.: Helicterins A-F, Six New Dimeric (7.5',8.2')-Neolignans, from Indonesian Medicinal Plant *Helicteres isora*. *Helv. Chim. Acta*, 83, 2908-2919 (2000).

Abstract: During a chemical study of Indonesian medicinal plants, we examined the constituents of fruits of *Helicteres isora* L. (Sterculiaceae), one of the famous Jamu medicines. From a water extract of the fruits, we isolated six new neolignans, helicterins A-F (**1-6**), and elucidated their structures by spectral analyses. Helicterins A-F (**1-6**) are dimeric (7.5',8.2')-neolignans with a bicyclo[2.2.2]octene C-framework, and showed mild inhibitory activity against reverse transcriptase from avian myelobrastosis virus.

19) Tezuka Y., Honda K., Banskota A. H., Thet M. M., and Kadota S.: Kinmoonosides A-C, Three New Cytotoxic Saponins from the Fruits of *Acacia concinna*, a Medicinal Plant Collected in Myanmar. *J. Nat. Prod.*, 63, 1658-1664 (2000).

Abstract: Three genuine saponins, named kinmoonosides A-C (**1-3**), have been isolated together with a new monoterpenoid (**4**) from a methanolic extract of the pods of *Acacia concinna*. The structures of kinmoonosides A-C were elucidated on the basis of spectral analysis as 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-{(6*R*),(2*E*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2'E)-6'-hydroxyl-2'-hydroxymethyl-6'-methyl-2',7'-octadienoyl-β-D-quinovopyranosyl]-2,7-octadienoyl}acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester (1), 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-{(6*S*),(2*E*)-2-hydroxymethyl-6-methyl-6-*O*-[4-*O*-(2'*E*)-6'-hydroxyl-2'-hydroxymethyl-6'-methyl-2',7'-octadienoyl-β-D-quinobopyranosyl]-2,7-octadienoyl} acacic acid 28-*O*-α-L-arabinofuranosyl(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranosyl ester (2), and 3-*O*-{α-L-arabinopyranosyl(1→6)-[β-D-glucopyranosyl(1→2)]-β-D-glucopyranosyl}-21-*O*-[(2*E*)-6-hydroxyl-2-hydroxymethyl-6-methyl-2,7-octadienoyl]acacic acid 28-*O*-α-L-arabinofuranosyl-(1→4)-[β-D-glucopyranosyl(1→3)]-α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl ester (3), respectively. The new monoterpenoid 4 was determined as 4-*O*-[(2*E*)-6-hydroxyl-2-hydroxy-methyl-6-methyl-2,7-octadienoyl]-D-quinovopyranose. Compounds 1-3 showed significant cytotoxicity against human HT-1080 fibrosarcoma cells.

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◇研究室在籍者

大学院前期１年：Tepy Usia（2000. 4～）
大学院後期１年：Suresh Awale（2000. 4～），長岡武馬（2000. 4～），上田純也（2000. 4～）
大学院後期２年：Tran Le Quan，朴　鍾集
大学院後期３年：I Ketut Adnyana，范　文哲，Mohammad Shawkat Ali
受託研究員：緑川　淑
外国人客員研究員：Maung Maung Thet（マンダレー伝統医療専門学校，2000. 1. 25～2000. 12. 25）
　　李　俊（広西師範大学，2000. 7. 17～2001. 1. 10）
非常勤研究員：Arjun H. Banskota

◇学位（修士・博士）取得者

修士：
晴山　亨：「中国産牛膝（*Achyranthes bidentata* blume）の成分研究」
本田和之：「ミャンマー産　*Acacia concinna* Wall. の成分研究」
Stampoulis Pavlos：「Novel Diterpenes from Medicinal Plant *Orthosiphon stamineus* Benth」

課程博士：
Arjun H. Banskota：「Cytotoxic and Hepatoprotective Constituents from the Leaves of *Combretum quadrangulare* Kurz」
熊　泉波：「Study of the Antioxidant and Hepatoprotective Effects of Phenylethanoids from *Cistanche deserticola*」