薬効解析センター

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◇研究目的

本センターは，世界各地の民族薬物に関する資料の収集及び整理，薬効の評価及び解析並びにデータベースの構築を行い，世界の伝統薬物及び薬用植物に関する共同研究を推進することを目的としている。

◇研究概要

Ⅰ．伝統薬物に関するデータベース（ETHMED）の構築

民族薬物資料館保有生薬に関するデータベース（生薬名，生薬画像，原植物名，原植物画像，原植物科名，薬用部位，産地情報，入手先情報等）への入力，生薬目録の作成，Web化を目的にしたソフトの開発を行った。

Ⅱ．伝統薬物の薬効の評価と解析に関する研究

1. 難治性神経疾患に対する伝統薬物の有効性の検討とそれらの薬理作用機序に関する研究

ヒト神経モデル細胞の神経突起伸展に及ぼす効果を検討し，八味地黄丸，地黄，コーヒー豆中のtrigonellineに活性を見出した。

2. 抗そう痒作用を有する伝統薬物の開発研究

Substance P誘発掻き動作を有意に抑制した白虎加人参湯，及び抑制傾向を示した他の4方剤をアトピー性皮膚炎モデルとされるNCマウスに投与した結果，白虎加人参湯と加味逍遥散が掻き動作を抑制した。2方剤の痒み抑制機序の一つに体表面温度の低下が示唆された。

Ⅲ．生薬の品質評価に関する研究

1. 遺伝子解析による生薬の同定法開発に関する研究

三七人参（*Panax notoginseng*の根）の遺伝的多型性を18S rRNA遺伝子，*mat*K遺伝子の塩基配列を検討して明らかにした。

2. 生薬の基源と品質に関する研究

丹参市場品の基源を*Salvia*属7種の根の比較組織学的研究により確証した後，水溶性成分による品質評価を行った。インド薬物Mamiraは*Coptis teeta*，雲南黄連とミャンマー黄連は*C. teeta* subsp. *lohitensis*の根茎であることを証明し，両者のアルカロイド組成を比較した。

Ⅳ．世界の伝統医薬学の調査研究

ベトナム南部で伝統薬物（南薬）及びベトナム人参，桂皮，沈香の調査を行った。

◇原　著

1) **Sasamura T., Sasaki M., Tohda C. and Kuraishi Y.: Existence of capsaicin-sensitive glutamatergic terminals in rat hypothalamus. Neuroreport, 9 : 2045–2048, 1998.**

Summary : Capsaicin has been suggested to act not only on thin primary afferents but also on the hypothalamus, but the neurotransmitters of central capsaicin-sensitive neurons are unknown. The present study was conducted to determine whether any central, especially hypothalamic, glutamatergic terminals were sensitive to capsaicin. Capsaicin evoked glutamate release from slices of hypothalamus and lumbar dorsal horn, but not cerebellum. Such capsaicin action was Ca^{2+} dependent and inhibited by the capsaicin antagonist capsazepine. Vanilloid receptor subtype 1 mRNA was widely distributed in the brain, with a marked level in the hypothalamus and cerebellum, but not in the spinal cord. The results suggest that there are glutamatergic terminals sensitive to capsaicin in the hypothalamus.

2) **Sasaki M., Tohda C. and Kuraishi Y.: Region-specific increase in glutamate release from dorsal horn of rats with adjuvant inflammation. Neuroreport, 9 : 3219–3222, 1998.**

Summary : Glutamate is considered an important pain transmitter and responsible for inflammatory hyperalgesia, but quantitative and topographical changes in glutamate release in the dorsal horn during peripheral inflammation have not been characterized. To address this issue, image analysis with a confocal laser scanning microscope was performed for quantitatively mapping capsaicin-evoked glutamate release from the lumbar cord slice of rats following unilateral adjuvant inoculation to the hind-paw. Capsaicin induced glutamate release from laminae I, II and X in the spinal cord of the adjuvant-treated and untreated sides, without apparent release from laminae III-V. The concentration of released glutamate in laminae I, II and X was higher on the adjuvant-treated side than on the untreated side. The results suggest that adjuvant inflammation increases glutamate release from capsaicin-sensitive primary afferents in laminae I, II and X.

3) **Kurokawa M., Hozumi T., Basnet P., Nakano M., Kadota S., Namba T., Kawana T. and Shiraki K.: Purification and characterization of eugeniin as an anti-herpesvirus compound from *Geum japonicum* and *Syzygium aromaticum*. J. Pharmacol. Exp. Ther., 284 : 728–735, 1998.**

Summary : The hot-water extract of *Geum japonicum* has been shown to exhibit prophylactic and therapeutic anti-herpes simplex virus (HSV) activity in murine infection models. Eugeniin was purified as an anti-HSV compound from the extract and also was isolated from another herbal extract (*Syzygium aromaticum*) that had exhibited anti-HSV activity in mice. Thus the anti-HSV action of eugeniin was characterized. The effective concentration (5.0 μg/ml) for 50% plaque reduction of eugeniin for wild HSV type 1 (HSV-1) on Vero cells was 13.9-fold lower than its 50% cytotoxic concentration determined by a yield-reduction assay. Eugeniin also inhibited the growth of acyclovir-phosphonoacetic acid-resistant

HSV-1, thymidine kinase-deficient HSV-1 and wild HSV type 2. Eugeniin as well as phosphonoacetic acid inhibited viral DNA and late viral protein syntheses in their infected Vero cells, but not cellular protein synthesis at its inhibitory concentrations. Purified HSV-1 DNA polymerase activity was inhibited by eugeniin noncompetitively with respect to dTTP. Its apparent Ki value for euginiin was 8.2- and 5.8-fold lower than the Ki values of purified human DNA polymerases alpha and beta, respectively. Thus one of the major target sites of inhibitory action of eugeniin is viral DNA synthesis; the inhibitory action for viral DNA polymerase activity was novel compared with anti-HSV nucleoside analogs.

4) **Prasain J. K., Tezuka Y., Li J-X., Tanaka K., Basnet P., Dong H., Namba T. and Kadota S.: Novel diarylheptanoids from the seeds of *Alpinia blepharocalyx*: Revised structure of calyxin A. J. Chem. Res. (S) : 22–23, 1998.**

Summary : Calyxins A (1), E (2) and F (3), 6-hydroxycalyxin F (4), Calyxin G and epicalyxin G (5 and 6), novel diarylheptanoids having a chalcone or a flavanone moiety, were isolated from *Alpinia blepharocalyx* K. SCHUM. and their structures, including the corrected one of calyxin A (1), were elucidated by spectroscopic methods.

5) **Kasimu R., Tezuka Y., Tanaka K., Gong Z-N., Li J.-X., Basnet P., Namba T. and Kadota S.: Liquid chromatography-mass spectrometry analysis of diterpenoid constituents of seventeen *Salvia* plants. J. Trad. Med., 15 : 109–115, 1998.**

Summary : The MeOH extracts of the seventeen *Salvia* plants, including ten species used as resources of the Chinese crude drug, Dhan-shen (丹参, Radix Salviae miltiorhizae, Tan jin in Japanese), were comparatively examined by the liquid chromatography-mass spectrometry (LC-MS) method using thirteen diteropeniods as standards. The principle component analysis (PCA) on the relative intensity of the protonated molecular ion of standard diterpenoids in LC-MS showed the presence of several groups in the genus *Slavia* with regard to the diterpeniods, which means that ten species used as Dan-shen resources were not the same. Their use as a Dan-shen resource, thus, should be based on their activity and/or active constituents.

6) **Prasain J. K., Tezuka Y., Hase K., Basnet P., Dong H., Namba T. and Kadota S.: Inhibitory effect of diarylheptanoids on nitric oxide production in activated murine macrophages. Biol. Pharm. Bull., 21 : 371–374, 1998.**

Summary : Thirteen novel diarylheptanoids bearing a chalcone or a flavanone moiety (1-13), a new curcumin derivative, 1,2-dihydrobis(de-*O*-methyl)curcumin (14), and two known flavonoids (15 and 16) isolated from the seeds of *Alpinia blepharocalyx* K. SCHUM. were tested for their inhibitory effects on nitric oxide (NO) production in lipopolysaccharide (LPS)-activated murine macrophages J774.1 *in vitro*. All the tested compounds inhibited NO production in a concentration-dependent manner (IC_{50}=36-568 μM). Among the compounds examined, blepharocalyxin B (13) was the most potent inhibitor of NO production (IC_{50}=36

μM). Analysis of the structure activity relationship among these novel diarylheptanoids led to the conclusion that the position of attachment of a chalcone or a flavanone to a diarylheptanoid does not affect their inhibitory potency although their presence in association causes a substantial enhancement of the inhibitory activity. Moreover, a conjugated double bond in a chalcone moiety potentiated the inhibitory activity. On the other hand, hexamethoxydeoxycalyxin A (17) and pentamethoxycalyxin B (18), a methylated product of calyxin A (1) and an epimeric mixture of calyxin B, showed greatly reduced activity suggesting that phenolic hydroxyl groups are involved in the inhibitory activity.

7) **Kasimu R., Tanaka K., Tezuka Y., Gong Z. N., Li J-X., Basnet P., Namba T. and Kadota S.: Comparative study of seventeen *Salvia* plants: Aldose reductase inhibitory activity of water and MeOH extracts and liquid chromatography-mass spectrometry (LC-MS) analysis of water extracts. Chem. Pharm. Bull., 46 : 500–504, 1998.**

Summary : The dry root and rhizome of *Salvia miltiorhiza* (Lamiaceae) are used as a crude drug Danshen, while those of *S. deserta* (Xinjiang-Danshen) are mixed in Danshen at Xinjiang province when the former is in short supply. The water and MeOH extracts of *S. deserta* showed strong aldose reductase (AR) inhibitory activity, and their active constituents were determined to be polar compounds different from "tanshinones" of *S. miltiorhiza*, i.e., lithospermic acid B (1), salvianolic acid K (2), salviaflaside (3), and rosmarinic acid (4) (IC50, 2.63-3.91 μM). We also examined the AR inhibitory activity of water and MeOH extracts of seventeen *Salvia* plants, including ten species of Danshen resources (*S. bowleyana, S. deserta, S. miltiorhiza, S. miltiorhiza* var. *miltiorhiza* f. *alba, S. paramiltiorhiza, S. paramiltiorhiza* f. *purpureo-rubra, S. przewalskii, S. przewalskii* var. *mandarinorum, S. sinica* f. *purpurea, S. trijuga*), and their water extracts were also analyzed by liquid chromatography-mass spectrometry (LC-MS). The results indicated that there were four types with regard to the AR inhibitory activity and three types with regard to the amount of 1. Ten species used as Danshen resources showed good correlation between the AR inhibitory activity and the morphological classification. However, the intensities of their AR inhibitory activity varied, and they contained 1 in varying amounts. These facts suggested that the ten species were not the same, and thus their use as a Danshen resource should be based on their activity and/or active constituents.

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

Summary : *Salvia deserta* SCHANG. (Lamiaceae) is a plant grown in Xinjiang province of China, and its dried roots are called Xinjiang-Danshen. This plant has not been used as a medicine or a food, but recently it was reported that Xinjiang-Danshen is mixed in Danshen (root of *Salvia miltiorhiza* BUNGE), a well-known Chinese crude drug, at Xinjing province

when later was in short supply. We examined the constituents of roots of *S. deserta* (Xinjiang-Danshen) and identified a new caffeic acid trimmer {salvianolic acid K (1)}, along with two known caffeic acid dimmers {salviaflaside (2), rosmaric acid (3)}, a known caffeic acid tetrammer {lithospermic acid (4)}, seven known abietene-type diterpene {6,7-dehydroroyleanone (5), royleanone (6), taxadione (7), ferruginol (8), 7-*O*-methylhorminone (9), 7-*O*-acetylhorminone, horminone (10)}, and a known steroid {daucosterol (12)}.

Five of the diterpenes (5, 6, 9-11) were "royleanones" and the main caffeic acid derivative was the trimmer 1. These differed from the constituents of roots of *S. miltiorhiza*, which contains tanshinones as diterpines and magnesium lithospermate B as the main caffeic acid derivative. Thus, the mixing of Xinjiang-Danshen with Danshen is not appropriate and two should be considered different drugs.

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

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

10) **Malla K.J., Komatsu K., Tamura T., Kurihara S. and Takano A.: A study visit for the observation of an endemic medicinal plant, *Coptis teeta* WALL. in Mayodia, Dibang Valley District, Arunachal Pradesh, India. Bull. Showa Coll. Pharm. Sci., 32 : 25–30, 1998.**

Summary : *Coptis teeta* WALL. of family Ranunculaceae is considered as an indigenous plant of India, the distribution being confined only in some district of Arunachal Pradesh at an elevation of 2,000-3,000m. The rhizome of this plant has been found to be used as an important medicine by Mishmis, the ethnic tribe of Dibang Valley. It also has got an important place in modern pharmacy. In this area this plant is commonly known as "Mishmi teeta," where the tribal name of this plant is "Aro" or "Aron." The name "Mishmi teeta" has been given to it because its natural habitat is confined in Mishmi hills of Dibang Valley and its bitter taste (tita in Hindi). This plant has been listed as a threatened plant due to excessive collection in the past years. Arunachal Pradesh has strict regulation to collect teeta from wild and export it as a raw material. But, it has been found traded in Indian and Nepalese market. Therefore, it has been a quest about where this marketable teeta comes from and whether this is the same teeta from Arunachal Pradesh or somewhere else. It has been a matter of high concern for scientists to know the difference between the *Coptis teeta* and other species of *Coptis* growing in other parts of the world. The main objective of this study visit

is to locate the aforesaid plant in its natural habitat for futher study.

◇総　説

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「生薬からの抗そう痒活性成分の単離，同定」，1997～
「麻黄附子細辛湯の鎮痛効果における構成生薬の作用解析」，1998～

服部征雄：富山医科薬科大学和漢薬研究所細胞資源工学部門,
「痴呆脳に対するコーヒーの作用」，1998～

◇非常勤講師

1）小松かつ子：金沢大学教養的・総合科目「ヒマラヤ風土記」第10回中国ヒマラヤの自然と文化，第11回チベット医学と仏教，1998，11/5，12/17，金沢.

◇研究費取得状況

1）文部省科学研究費，国際学術研究・学術調査（第2年度），（分担：小松かつ子）「ベトナ

ム，タイ，ミャンマーにおける伝統医学並びに天然薬物資源の調査研究」，300万

2）文部省科学研究費，基盤研究（B）（分担：小松かつ子）「腸内細菌による代謝活性化を利用した新しい薬物の開発」，280万

3）文部省科学研究費，奨励研究（A）（代表：東田千尋）「アトピー性皮膚炎の痒みに関連した因子の単離，同定および機能解析」，50万

4）文部省科学研究費，基盤研究（B）（分担：東田千尋）「アレルギー性の痒みの発生機序」，550万円

5）全日本コーヒー協会（分担：小松かつ子，東田千尋）「痴呆脳に対するコーヒーの作用」，200万

◇研究室在籍者

大学院前期1年（派遣）：菅原裕之

研究機関研究員：伏見裕利（富山医科薬科大学，1997，4～1999，3）

研究生：朱 姝

外国人客員研究員：李 暁波，曹 暉，劉 玉萍，P.M. Unnikrishnan，Suresh Awale

技術補佐員：佐藤利江

◇民族薬物資料館記録

1）一般公開

平成10年10月25日に第1回の民族薬物資料館一般公開を実施した．予約制とし，10時，11時，14時，15時，16時からの5回に分けて各1時間，生薬の解説を加えながら館内を案内した．13時～14時に資源開発部門谿　忠人教授による―和漢薬との「つきあい方」～生活“悪”習慣病を例にして―の講演会を同時に行った．参加者は51名．

2）見学者記録（1997年4月1日～1998年3月31日）

来館者総数：474名（日本人　441名，外国人　33名）

案内総回数：78回（日本人　69回，外国人　9回）

外国人の国名（人数）：中国（7），韓国（8），タイ（6），ベトナム（3），ミャンマー（3），アメリカ合衆国（2），ドイツ，インド，ネパール，ブラジル（各1）．