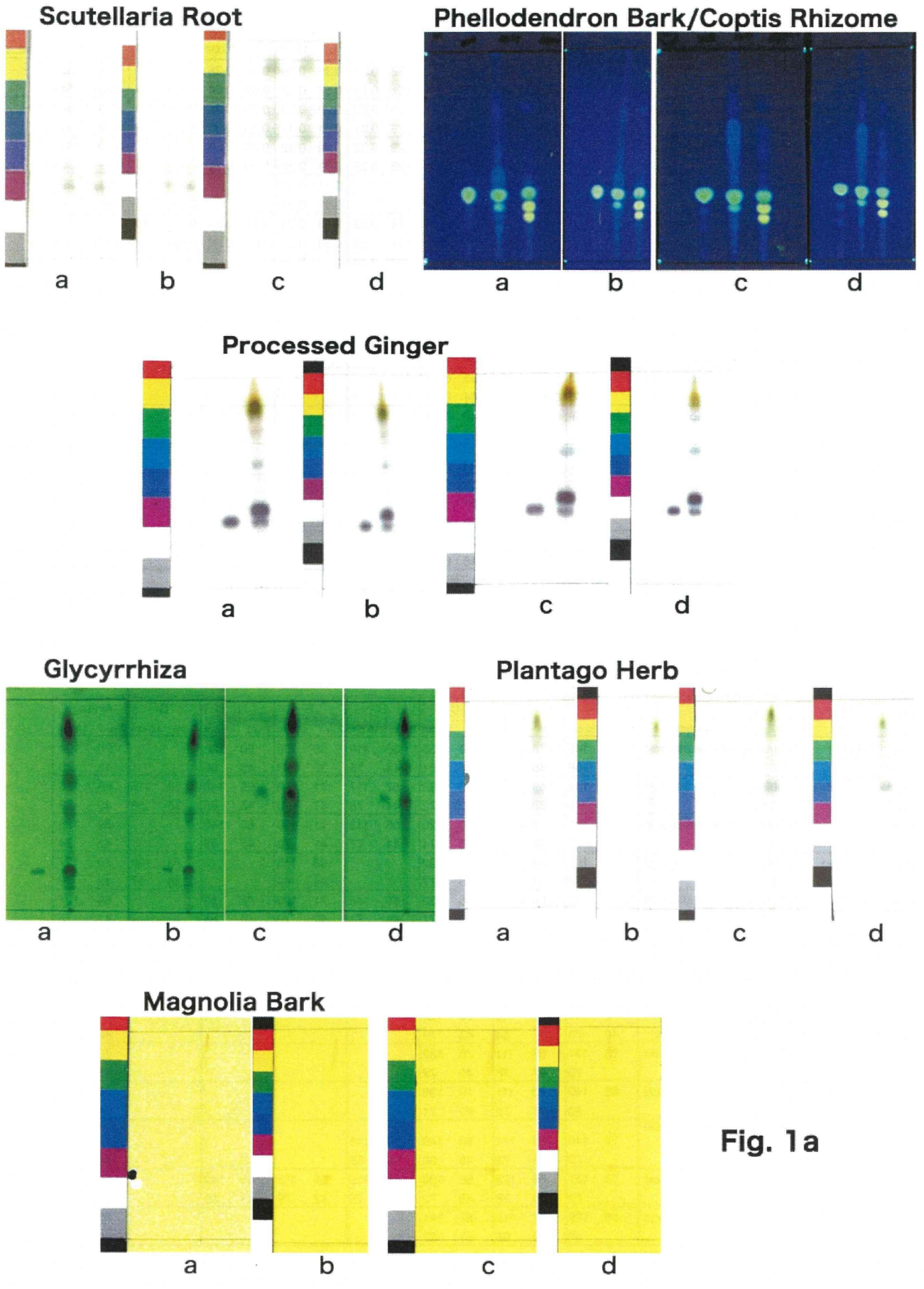
Scutellaria Root
a
b
c
d
Phellodendron Bark/Coptis Rhizome
a
b
c
d
Processed Ginger
a
b
c
d
Glycyrrhiza
a
b
c
d
Plantago Herb
a
b
c
d
Magnolia Bark
a
b
c
d

Fig. 1a

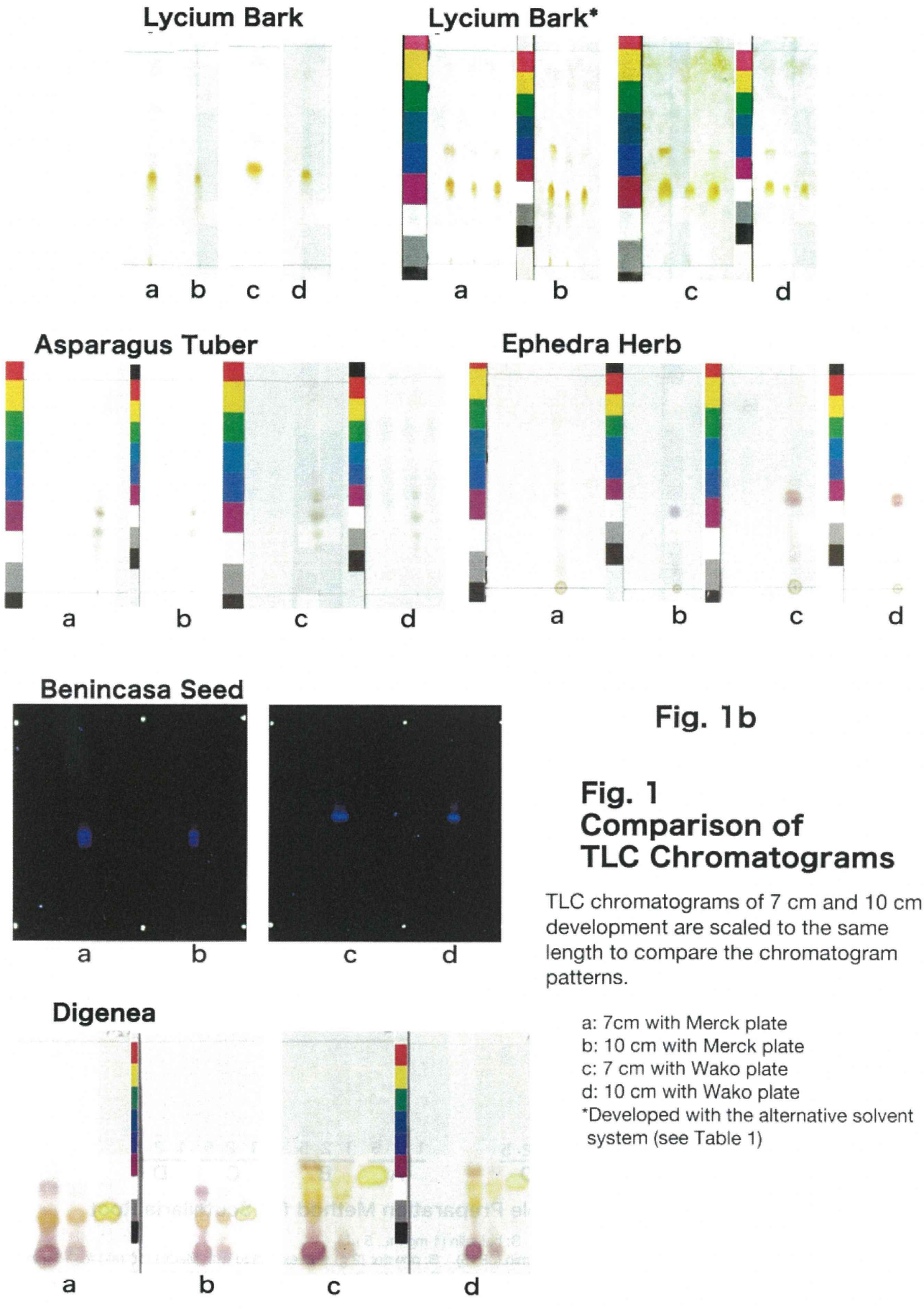

Fig. 1b

Fig. 1
Comparison of TLC Chromatograms

TLC chromatograms of 7 cm and 10 cm development are scaled to the same length to compare the chromatogram patterns.

a: 7cm with Merck plate
b: 10 cm with Merck plate
c: 7 cm with Wako plate
d: 10 cm with Wako plate
*Developed with the alternative solvent system (see Table 1)

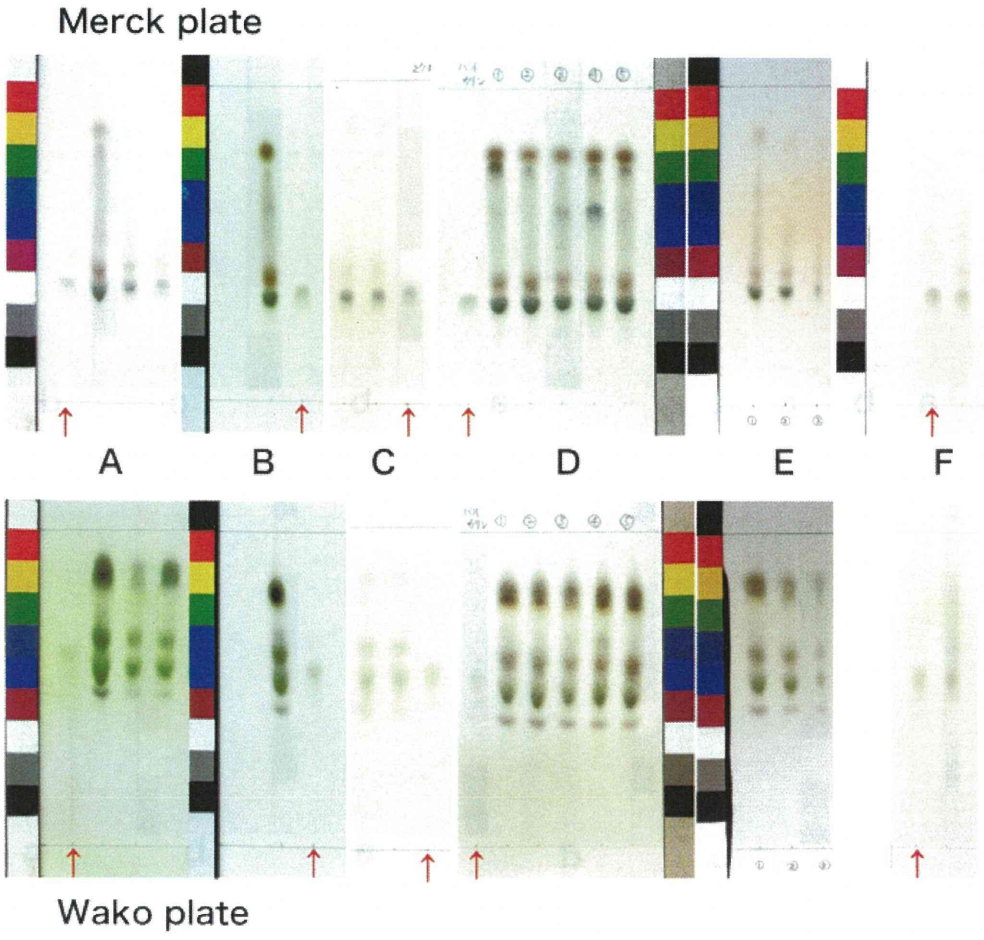

Fig. 2. Comparison of TLC Chromatograms of Scutellaria Root from Different Laboratories

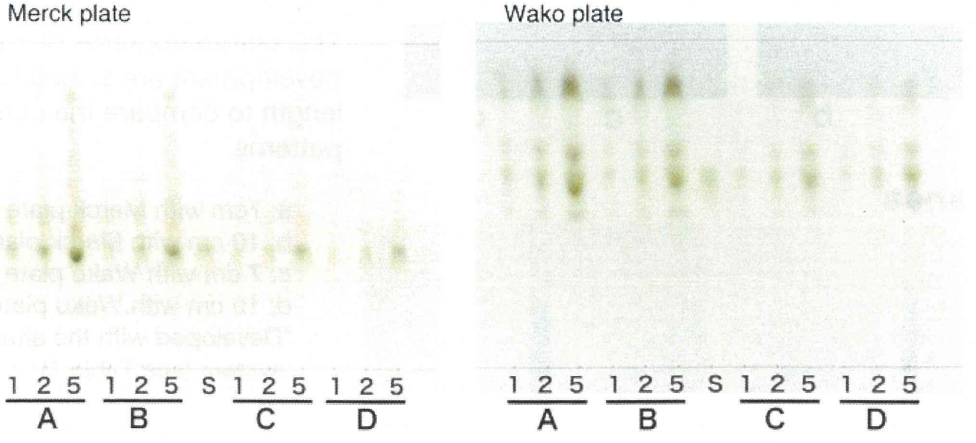

Fig. 3. Comparison of Sample Preparation Method for Scutellaria Root

amount of spotted sample: 1=1 μL, 2=2 μL, 5=5 μL. S: baicalin (1 mg/mL, 5 μL)
A: powder (2 g) was heated in MeOH (10 mL) for 3 min (JP15). B: powder (2 g) was extracted with MeOH (10 mL) for 15 min.
C: powder (1 g) was heated in MeOH (25 mL) for 3min. D: powder (1 g) was extracted with MeOH (25 mL) for 15 min.

R_f 値が規定されている．今回検討した生薬のうち，JP15 に R_f 値が規定されているコウボク,[4] ジコッピ,[5] シャゼンソウ,[6] テンモンドウ,[7] トウガシ[3] 及びマオウ[8] について，JP15 に規定された R_f 値と実際に得られた値を比較すると（Table 1），JP15 に規定された値は Merck のプレートで得られた値とほぼ一致していた．しかし，ジコッピとシャゼンソウについては，JP15 規定値と実際に得られた値とがかなり異なるため，日局の規定を訂正する必要があるものと思われる．

2．展開距離と R_f 値の再現性に関する検討

薄層クロマトグラフィーでは，1-ブタノール/水/酢酸(100)のような高極性溶媒を使用する場合，非常に長い展開時間を必要とする．展開に要する時間は，展開距離が長くなるにつれて加速度的に長くなるため，展開距離を少し短くすることにより，展開に要する時間を大幅に短縮できると考えられる．そこで，現在 JP15 の各条で 10 cm と規定されている展開距離を 7 cm に短縮することにより，指標スポットの R_f 値並びに分離パターンが変化するかを検討した．

同一機関で行った展開距離 7 cm と 10 cm の TLC の画像を，原線から溶媒先端までの長さを揃えて比較したものを Fig. 1 に示す．また，指標成分の R_f 値を Table 1 にまとめた．Fig. 1 から明らかなように，展開距離を 7 cm とした場合と 10 cm とした場合の間には，クロマトグラムのパターンにほとんど差がなく，展開距離を 7 cm としても生薬の確認試験でのスポットの確認には全く支障がないことが明らかとなった．また，指標成分スポットの R_f 値を比較しても，展開距離の短縮による R_f 値の変化はほとんど見られなかった (Table 1)．一方，展開に必要な時間について見ると (Table 2)，Merck のプレートを用いた場合，いずれの生薬に於いても 10 cm 展開するのに必要な時間が 120—130 分であるのに対し，7 cm の展開に必要な時間は 70 分程度であり，展開距離を 30%短くすることにより，展開に要する時間を 55%程度に短縮できることが明らかとなった．なお，Wako の薄層板は展開に要する時間が短く (Table 2), Merck の薄層板を用いた場合の半分程度で展開が完了した．

3．オウゴンの確認試験条件の再検討

オウゴンの確認試験では，バイカリンの標準物質のスポットの位置と，サンプル中のバイカリンのスポットの位置がずれる傾向が見られた (Fig. 2)．この R_f 値のずれは，スポットするバイカリンの量の差に起因し，サンプル中のバイカリンの濃度が標準物質の濃度に較べて高いためと思われたことから，サンプルの調製条件を検討した．即ち，オウゴンの粉末並びにメタノールの量と抽出条件を，A：粉末 2 g にメタノール 10 mL を加え，3 分間加温抽出（JP15 の規定[9]），B：粉末 2 g にメタノール 10 mL を加え，15 分間振盪抽出，C：粉末 1 g にメタノール 25 mL を加え，3 分間加温抽出及び D：粉末 1 g にメタノール 25 mL を加え，15 分間振盪抽出とし，スポットするサンプル量を 1 μL，2 μL，5 μL に変えて検討した結果，サンプル量を減らし，室温で振り混ぜる抽出法に変える（条件 D）ことにより，標準物質とサンプルの R_f 値の違いを解消することができることが明らかとなった (Fig. 3)．

考察

薄層クロマトグラフィーは，特別な装置を必要とせず簡便に行えることから，日局の生薬の確認試験として多用されている．一般に薄層クロマトグラフィーでは，温度等の条件によって展開槽内の溶媒蒸気の状態が影響されやすいため，R_f 値の再現性については頑健性が乏しいとされているが，今回，日局の一般試験法＜*2.03*＞薄層クロマトグラフィーの規定を厳密に守って実験を行ったところ，かなり良好な R_f 値の室間再現性が得られ，日局の規定によって R_f 値の再現性が担保されていることが確認された．

日局の一般試験法＜*2.03*＞薄層クロマトグラフィーでは，通例として使用する薄層板の作製法を規定しており，自作の薄層板を使用することを前提としているが，現在では，通常市販の薄層板が使用されている．そこで，現在生薬の確認試験に用いられている Merck 社製と Wako 社製の薄層板を用い，展開結果を比較した結果，両者の間には酸性物質の R_f 値に大きな差が見られた．生薬の TLC による確認試験の中には，標準物質を一緒に展開せず，確認スポットの R_f 値と色調を示してある場合がある．今回検討した生薬について見ると，JP15 に規定された R_f 値は，Merck のプレートを用いた場合のものとよく一致しており，R_f 値が大きく出る傾向にある Wako のプレートを用いると，確認試験が正しい結果を与えない場合があることが明らかになった．また，ジコッピとシャゼンソウでは，実際に観察された R_f 値と JP15 の規定とにずれが見られたことから，日局の規定を見直す必要がある．更に，オウゴンの確認試験ではサンプルの調製法等に問題があると思われたことから，その改良法を見出した．

薄層クロマトグラフィーは，迅速に行える分析法である

が，1-ブタノール/水を含む溶媒系などでは，展開に長時間を必要とする．生薬の確認試験でも1-ブタノール/水/酢酸(100)混液のような溶媒系がしばしば用いられているが，このような溶媒系では10 cmの展開に2時間程度が必要であり，時間の短縮が望まれる．今回，1-ブタノール/水/酢酸(100)混液を展開溶媒として用いる確認試験について，展開距離を7 cmとして現行の10 cmの展開と比較した結果，分離パターン並びに R_f 値に影響を与えること無く，展開時間を半分近くまで短縮できることが明らかとなった．この結果から，現行では10 cmとなっている日局のTLCによる生薬の確認試験の展開距離を7 cmに変更することにより，1-ブタノール/水/酢酸(100)混液を展開溶媒として用いる確認試験の大幅な効率化が図れるものと思われる．

謝辞

本研究は，厚生労働科学研究費補助金（医薬品・医療機器等レギュラトリーサイエンス総合研究事業）の補助を受けて実施した．

References and Notes

1) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006); The Japanese Pharmacopoeia Fifteenth Edition Supplement 1 (Ministry Notification No. 316 of Sep. 28, 2007); The Japanese Pharmacopoeia Fifteenth Edition Supplement 2 (Ministry Notification No. 425 of Sep. 30, 2009), The Ministry of Health, Labour and Welfare, Japan.

2) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, p. 35.

3) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, p. 1244.

4) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, pp. 1209-1210.

5) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, p. 1222.

6) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, p. 1225.

7) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, pp. 1243-1244.

8) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, pp. 1272-1273.

9) The Japanese Pharmacopoeia Fifteenth Edition (Ministry Notification No. 285 of Mar. 31, 2006), The Ministry of Health, Labour and Welfare, Japan, p. 1183.

J Nat Med (2011) 65:293–300
DOI 10.1007/s11418-010-0489-3

ORIGINAL PAPER

Evaluation of the taste of crude drug and Kampo formula by a taste-sensing system (4): taste of Processed Aconite Root

Naoko Anjiki · Junko Hosoe · Hiroyuki Fuchino · Fumiyuki Kiuchi · Setsuko Sekita · Hidekazu Ikezaki · Masayuki Mikage · Nobuo Kawahara · Yukihiro Goda

Received: 24 August 2010 / Accepted: 25 October 2010 / Published online: 11 December 2010

Abstract It is difficult to describe the taste of Processed Aconite Root (PAR) because it contains toxic compounds, and tasting poses some risk to the examiner. Therefore, there is no description of the taste of PAR in the latest Japanese Pharmacopoeia, although the taste of crude drugs has been regulated as a criterion for judgment. In this study, we revealed the objective taste of PAR by using a taste-sensing system. The PAR samples examined were classified into four types by how the samples were processed: PAR1 processed by autoclaving; PAR2-a processed by autoclaving after rinsing in salt (sodium chloride) solution; PAR2-h processed by heating after rinsing in calcium chloride solution; PAR3 processed by treating with hydrated lime after rinsing in salt solution. The most characteristic taste factor of PAR is an aftertaste of cationic bitterness, which was detected in all PAR sample solutions, even at the concentration of 0.1 mg/ml. In addition, anionic bitterness and saltiness were detected in all sample solutions at 1 mg/ml. Furthermore, umami was detected in the PAR1, PAR2-a, and PAR3 sample solutions at 1 mg/ml. Detailing the analyses of the four taste factors on the four sample types, we found each type has its own characteristic taste pattern. On the basis of these results, we proposed a method for discriminating one PAR type from another by using the system.

Keywords Processed Aconite Root · Taste evaluation · Japanese Pharmacopoeia · Discrimination method · Taste-sensing system using artificial lipid membrane sensor

N. Anjiki · H. Ikezaki
Intelligent Sensor Technology, Inc., 5-1-1 Onna, Atsugi, Kanagawa 243-0032, Japan

N. Anjiki · M. Mikage
Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

J. Hosoe · N. Kawahara · Y. Goda (✉)
National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
e-mail: goda@nihs.go.jp

H. Fuchino · F. Kiuchi · S. Sekita · N. Kawahara
Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, 1-2 Hachimandai, Tsukuba, Ibaraki 305-0843, Japan

Present Address:
F. Kiuchi
Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan

Present Address:
S. Sekita
Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University, 1314-1 Shido, Sanuki, Kagawa 769-2193, Japan

Introduction

Aconitum plants, such as *A. carmichaeli* Debeaux (*Ranunculaceae*), which contain diester-type aconite alkaloids (DAs) in their entire bodies, particularly in their roots, are known as a poisonous herb in Europe. In Asia, however, the tuberous root of *Aconitum* plants has a long history of use as a poison and also as a crude drug; for example, it is listed in the low-grade item portion of *Shen-nong-ben-cao-jing*, which is the earliest known book of Chinese materia medica [1].

Depending on their toxicity, crude drugs derived from the tuberous root of *Aconitum* plants are divided into two types. One is the raw type, which is the dried tuberous root, and the other is the processed type, which is appropriately

processed and then dried. The former is highly toxic because of its high content of DAs. Hikino et al. [2] reported that LD50s to mice by oral administration of the raw tuberous root of *A. carmichaeli* are 1.61 g/kg (collected in Hokkaido, Japan) and 5.49 g/kg (collected in China), although the severity of toxicity in this type varies widely depending on the production region and the time of collection. Thus, prior to medicinal use, the tuberous root of *Aconitum* plants is generally processed by heating, immersing in salt (sodium chloride) solution, or coating with hydrated lime to attenuate the toxicity and control the medicinal effect. Almost all crude drugs in Japan made from the tuberous root of *Aconitum* plants are now classified as this processed type. The root is used for its analgesic properties, cardiotonic action, and alleviating coldness of the extremities [3–7] and is blended into many Kampo formulae, such as Hachimijiogan, Maobushisaishinto, Shinbuto, and so on.

In Japan, the second edition of the Japanese National Formulary (*Kokumin Iyakuhinshu*) adopted the processed type of the tuberous root of *Aconitum* plants in the monograph of Aconiti Sinensis Tuber in 1955 [8], and the seventh edition of the Japanese Pharmacopoeia (JP7) adopted its contents without modification in 1961 [9]. In 1966, however, the monograph was deleted in the revised version of the JP7, probably because of this crude drug's ambiguous toxicity [10]. Then a variety of studies were performed on component characteristics and quality evaluation of both the raw and processed types of the crude drugs because of the medicinal importance of the tuberous root as a crude drug [2, 11–20]. Subsequently, in 2004, Processed Aconite Root (PAR) was newly adopted in Supplement II of the JP14, with strict control of the DAs by a purity test [21].

PAR in the JP is subdivided into three categories (PAR1–PAR3) in accordance with how it is processed: PAR1 processed by autoclaving; PAR2 processed by heating or autoclaving after rinsing in sodium chloride, rock salt, or calcium chloride solution; PAR3 processed by treating with hydrated lime (calcium hydroxide) after rinsing sodium chloride solution. The JP set an upper PAR limit for individual and total contents of aconitine, jesaconitine, hypaconitine, and mesaconitine, which are representative DAs, by high-performance liquid chromatography (HPLC) analysis. In addition, the amounts of total alkaloids are controlled by colorimetric titration assay.

In the course of our successive studies for quality control of crude drugs [22–37], we used a taste-sensing system to objectively evaluate the taste of crude drugs and Kampo formulae [38–40], as the taste of crude drugs has been regulated as a criterion for judgment by the JP. In the JP7, for Aconiti Sinensis Tuber, the taste was described as "slightly salty, then prolonged numbing" [9]. However, in modern books on medicinal plants and crude drugs, there are no descriptions of the taste of the tuberous root of *Aconitum* plants and PAR. In the JP15, the latest JP edition, the taste of PAR is also not described, because tasting poses some risk to the examiner due to the intake of DAs [41].

In the 1990s, a taste-sensing system was developed by Toko et al. [42–44] for objective taste measurement based on the concept of modeling the mechanism of human taste recognition. The system is composed of a sensor unit, which consists of various artificial lipid membrane sensor probes and a personal computer. Taste data are obtained from the change in electrical potential of the artificial lipid membranes when the taste substances interact electrically with, or are absorbed to, the membrane.

In recent years, using the taste-sensing system, we have examined the taste of PAR collected by the official working group for the establishment of the monograph of PAR in the JP14 Supplement II (PAR-WG). The PAR samples provided were previously classified into four types by how they were processed: PAR1, PAR3, and PAR2-a (autoclaving type) and PAR2-h (heating type). In this study, we report the results on taste characteristics of the four types of PAR samples measured by the taste-sensing system. In addition, based on these results, we propose a method for discriminating one PAR type from another using the system.

Materials and methods

Materials

Processed aconite root samples

Forty-seven PAR samples were provided by the PAR-WG. All major companies handling PAR in Japanese markets have participated in the PAR-WG, and the PAR samples were collected by the PAR-WG in 2001 and 2002. Therefore, these samples are thought to be representatives of PAR in the Japanese markets. The number of PAR samples classified by typical processing was as follows: PAR1, 13; PAR2, 23; PAR3, 11. PAR2 samples were further subdivided into PAR2-a (12 samples) and PAR2-h (11 samples) by how the samples were processed: PAR2-a is autoclaved after being rinsed in sodium chloride solution, whereas PAR2-h is heated (steamed) after being rinsed in calcium chloride solution. All samples were stored at the Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation (NIBIO), Tsukuba, Japan. Brick and cut type PAR samples were pulverized using an electric mill and passed through a no. 50 sieve (300 μm).

Chemicals and reagents

A solution of 30 mM potassium chloride in 0.3 mM tartaric acid was the reference solution in the taste measurement. A 30% ethanol aqueous solution containing 100 mM hydrochloric acid was used as the washing solution for negatively charged artificial lipid membrane sensor probes and a 30% ethanol aqueous solution containing 100 mM potassium chloride and 10 mM potassium hydroxide for positively charged probes. Potassium chloride, tartaric acid, ethanol (99.5 v/v%), and potassium hydroxide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Hydrochloric acid (35–37%) was purchased from Nakalai Tesque, Inc. (Kyoto, Japan). Ultrapure water was obtained by an EQS-10L system (Nihon Millipore K.K., Tokyo, Japan). The internal solution for the artificial lipid membrane sensor probes containing 3.3 M potassium chloride in saturated silver chloride (AgCl) aqueous solution was obtained from Intelligent Sensor Technology, Inc. (Atsugi, Japan).

Preparation of sample solutions for taste measurement

A pulverized and sieved PAR sample was weighed and suspended in ultrapure water at concentrations of 1 or 0.1 mg/ml and then extracted by sonication for 10 min at 25°C. After centrifuging at 1,710×*g* for 10 min at 25°C, the supernatant was filtered with cotton. Potassium chloride and tartaric acid were added to the filtrate at concentrations of 10 mM and 0.1 mM, respectively. This solution was applied to the taste-sensing system for taste measurement. Aqueous solution containing 10 mM potassium chloride and 0.1 mM tartaric acid was used as a blank control.

Taste measurement

The objective tastes of the PAR samples were measured fundamentally according to our previous report [38] using the taste-sensing system SA402B (Intelligent Sensor Technology, Inc.) equipped with a sensor unit, which consists of artificial lipid membrane sensor probes of anionic bitterness, astringency, saltiness, umami, and catatonic bitterness (C00, AE1, CT0, AAE, and AN0, respectively) (Table 1).

The artificial lipid membrane sensor probe is composed of silver-wire electrode, the surface of which is coated with Ag/AgCl, with a sensor body made of polypropylene, and artificial lipid membranes made by mixing lipids (which play an important role in taste sensing) with a polymer. The internal cavity of the artificial lipid membrane sensor probe is filled with the internal solution. The reference solution was measured by the sensor unit for 30 s, and the electric potential at the endpoint of measurement (Vr) was recorded. Then, the sample solution was measured by the sensor unit in the same manner (Vs). After measuring the sample solution, the sensor unit was briefly rinsed in the reference solution, then the reference solution is measured again by the sensor unit for 30 s (Vr′). Finally, the sensor unit was washed by the washing solution and then rinsed by the reference solution for the next measurement. All measurement procedures were carried out at the room temperature. Electrical potential changes between the sample solution and the reference solution, which occurs before the sample solution measurement (Vs − Vr) is called the relative potential and used to calculate the initial tastes. The change in electric potentials of the reference solution between before and after sample solution measurement (Vr′ − Vr) is called the change of membrane potential caused by adsorption (CPA) value and used to calculate the aftertaste [45].

Table 1 Characteristics of taste information on taste sensors

Sensor probes	Taste information
C00	Anionic bitterness (initial taste)
	Aftertaste of anionic bitterness[a] (aftertaste)
AE1	Astringency (initial taste)
	Aftertaste of astringency[a] (aftertaste)
AAE	Umami (initial taste)
CT0	Saltiness (initial taste)
AN0	Aftertaste of cationic bitterness[a] (aftertaste)

[a] Converted from change of membrane potential caused by absorption (CPA) values of each sensor probe; others converted from relative potentials

This system detects two types of taste: the initial taste and aftertaste. The initial taste means the taste a person senses when food and/or drink is in the mouth, whereas aftertaste means the taste a person senses after swallowing food and/or drink. Estimate of taste intensity from the outputs of the artificial lipid membrane sensor probes is based on the Weber–Fechner's law that the intensity of the perception is proportional to the logarithm of stimulus intensity. For taste, it is said that humans normally recognize a change of taste if the concentration of the taste substance is changed by 20% [46, 47], and this 20% change was defined as one unit of taste intensity [44].

In this study, relative potentials (Vs − Vr) obtained from the C00, AE1, AAE, and CT0 sensor probes were used to measure selective initial taste. CPA values (Vr′ − Vr) obtained from C00, AE1, and AN0 sensor probes were used to measure selective aftertastes.

Statistical analysis

Values are shown as mean ± standard deviation (SD). Mann–Whitney *U* test was applied to test the difference between two independent groups. Kruskal–Wallis test was applied to test the difference among four independent groups, and when significance was noted, Mann–Whitney

U test was applied to each independent groups. For each test, a value of $P < 0.01$ was considered to be significant.

Results

Taste patterns of PAR obtained by the taste-sensing system

Seven taste factors were measured by the artificial lipid membrane sensor probes used in this study. Anionic bitterness, astringency, umami, and saltiness were measured as taste factors of the initial taste, whereas the aftertastes of anionic bitterness, astringency, and cationic bitterness were measured as taste factors of the aftertaste (Table 1). Figure 1 and Table 2 show the taste patterns of water extract from the PAR samples and the taste intensities of each taste factor in each type of PAR sample. The aftertaste of cationic bitterness was detected in all PAR sample solutions, even at a concentration of 0.1 mg/ml. In addition, anionic bitterness and saltiness were detected in all PAR sample solutions at 1 mg/ml. Furthermore, umami was detected in PAR1, PAR2-a, and PAR3 sample solutions at 1 mg/ml. In contrast, astringency and the aftertastes of anionic bitterness and astringency were not detected in most of PAR sample solutions.

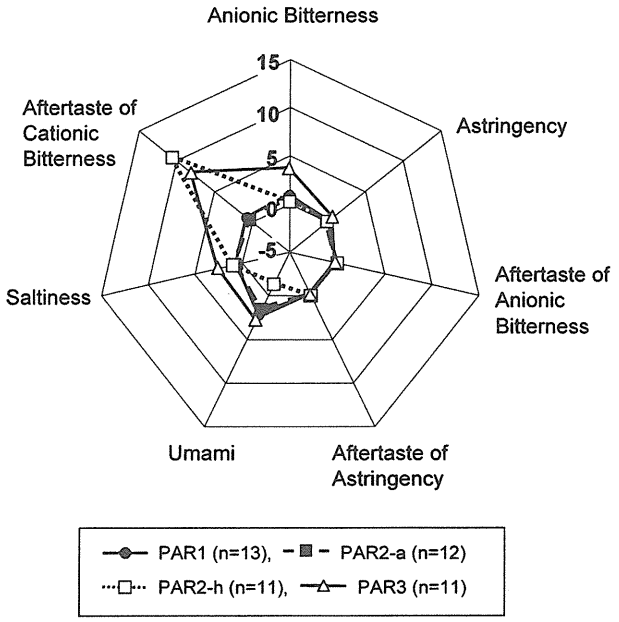

Fig. 1 Taste patterns of each type of Processed Aconite Root (PAR). Data were obtained by the taste-sensing system SA402B. Aqueous solution containing 10 mM potassium chloride and 0.1 mM tartaric acid was measured as the blank control. Taste values of aftertaste of cationic bitterness were obtained from 0.1 mg/ml sample solutions and the others from 1 mg/ml. Details of each taste value in a radar chart are described in Table 2

Differences in taste intensities among four PAR types

Next, we investigated the detailed characteristics of the four taste factors detected in the water extracts of four types of PAR samples, namely, the aftertaste of cationic bitterness, umami, anionic bitterness and saltiness. As shown in Fig. 2, at 0.1 mg/ml concentration, PAR2-h and PAR3, both the nonautoclaved type, had higher intensity of aftertaste of cationic bitterness than PAR1 and PAR2-a, both autoclaved types (Fig. 2a). In addition, the taste intensity of the aftertaste of cationic bitterness was significantly higher for PAR1 than for PAR2-a, even at 0.1 mg/ml concentration. The tendency was more clearly at the 1 mg/ml concentration (Fig. 2b). As for umami, PAR2-h showed negative taste intensity, meaning that the taste value was lower than that of the blank control, whereas the other PAR types showed positive taste intensity at 1 mg/ml (Fig. 3). For anionic bitterness and saltiness, PAR3 showed the highest taste intensity (Fig. 4a, b). These data clearly suggested that each PAR type has specific taste features.

Discussion

Taste of PAR obtained by the taste-sensing system

In the measurements by the taste-sensing system, the aftertaste of cationic bitterness was detected in all four

Table 2 Taste intensities of each taste factor in each type of Processed Aconite Root (PAR) samples

Processed Aconite Root samples Abbreviation (number)	Taste intensities of each taste factor (mean ± standard deviation)						
	Anionic bitterness	Astringency	Aftertaste of anionic bitterness	Aftertaste of astringency	Umami	Saltiness	Aftertaste of cationic bitterness
PAR1 ($n = 13$)	0.87 ± 0.16	0.22 ± 0.14	0.07 ± 0.08	0.07 ± 0.03	2.18 ± 0.38	0.51 ± 0.09	5.55 ± 1.06 0.60 ± 0.29[a]
PAR2-a ($n = 12$)	0.50 ± 0.10	−0.01 ± 0.05	0.03 ± 0.07	0.01 ± 0.04	1.61 ± 0.28	0.66 ± 0.12	1.47 ± 0.37 0.19 ± 0.06[a]
PAR2-h ($n = 11$)	0.19 ± 0.23	0.01 ± 0.03	0.09 ± 0.05	0.04 ± 0.02	−1.33 ± 0.85	0.99 ± 0.15	10.53 ± 2.38[a]
PAR3 ($n = 11$)	3.61 ± 1.57	0.75 ± 0.20	−0.10 ± 0.09	−0.02 ± 0.03	2.80 ± 0.23	2.50 ± 0.45	7.94 ± 0.66[a]

[a] Taste values obtained from 0.1 mg/ml sample solutions; the others were obtained from 1 mg/ml

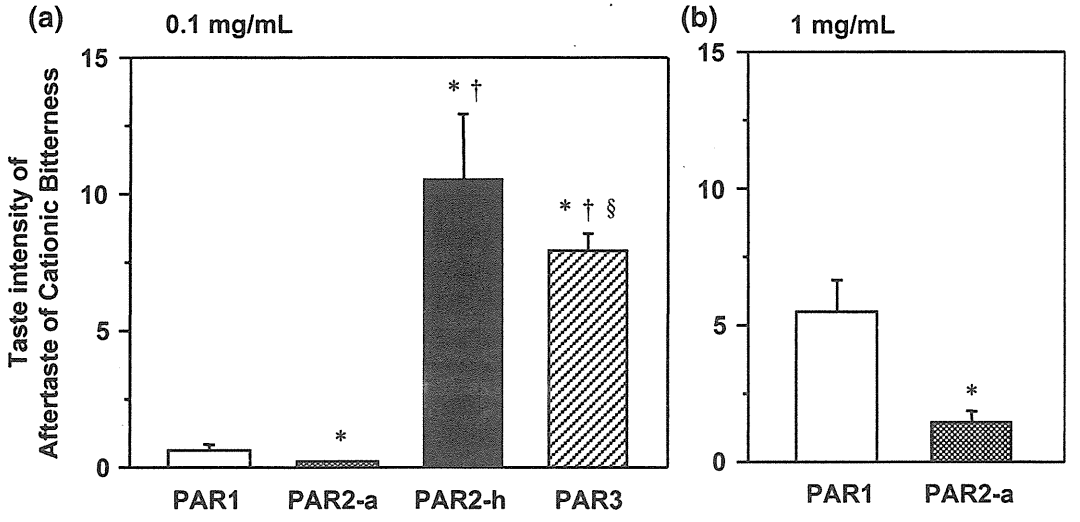

Fig. 2 Taste intensities of aftertaste of cationic bitterness in each type of Processed Aconite Root (PAR). Data were obtained by the taste-sensing system SA402B. Sample solution concentrations were 0.1 mg/ml (**a**) and 1 mg/ml (**b**). Each value represents the mean ± standard deviation (SD). Details of each value are described in Table 2. $^{*}P < 0.01$ compared with PAR1, $^{\dagger}P < 0.01$ compared with PAR2-a, and $^{\S}P < 0.01$ compared with PAR2-h

PAR types, whereas umami, anionic bitterness, and saltiness were detected in some PAR types. In contrast, astringency and the aftertastes of anionic bitterness and astringency were not detected in any PAR sample solutions (Fig. 1; Table 2).

Considering PAR components, we thought that the high intensity of the aftertaste of cationic bitterness may derive from alkaloids. It is of interest to analyze the contents of alkaloids in the tested PAR samples and to deduce the correlation between their contents and the taste intensities of the aftertaste of cationic bitterness. Results of the related experiments will be reported elsewhere.

As far as we know, there is no description for umami in the JP, probably because the Pharmacopoeia is not the standard for food. Therefore, our data suggest that the taste of PAR could be described as "strongly bitter with saltiness" if a description of taste is needed in the JP. As mentioned above, the taste of Aconiti Sinensis Tuber was described in the JP7 as "slightly salty, then prolonged numbing" [9]. It is of interest that numbing is not a taste and could not be measured by the taste-sensing system. Although the way of processing by which Aconiti Sinensis Tuber in the JP7 was prepared seemed to be different from those of the PAR examined, some taste factors, such as bitterness in Aconiti Sinensis Tuber, might be masked in the sensory test by its strong numbing qualities.

The AAE sensor probe used for umami detection responds to amino acids. PAR includes higenamine (demethyl coclaurine), a benzylisoquinoline-type alkaloid, as well as various terpenoid-type alkaloids, such as aconitine [3, 48]. This means that amino acids such as tyrosine, a precursor of higenamine, could exist in PAR. In addition,

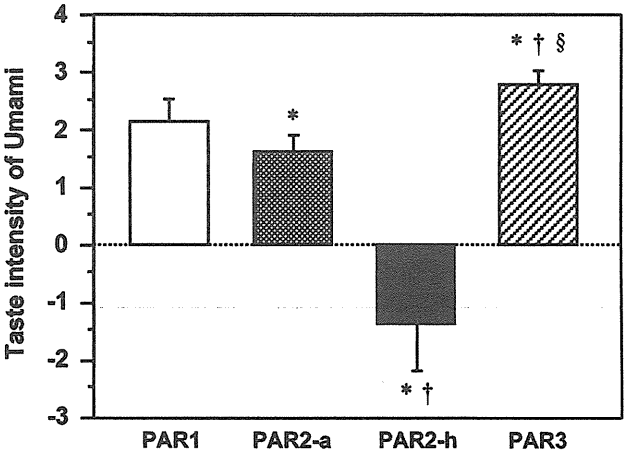

Fig. 3 Taste intensities of umami in each type of Processed Aconite Root (PAR). Data were obtained by the taste-sensing system SA402B. Sample solution concentration was 1 mg/ml. Each value represents mean ± standard deviation (SD). Details of each value are described in Table 2. $^{*}P < 0.01$ compared with PAR1, $^{\dagger}P < 0.01$ compared with PAR2-a, and $^{\S}P < 0.01$ compared with PAR2-h

Matsui et al. [49] reported that Kako-Bushi-Matsu originating from *A. japonicum* contained many kinds of amino acids. Thus, it is plausible that PAR also contains substantial amounts of amino acids and shows some intensities of umami, although the original plant of most tested PAR samples is *A. carmichaeli*.

It is interesting that PAR2-h showed negative taste intensity in umami (Fig. 3). All PAR2-h samples in this study were processed by steaming after rinsing with calcium chloride solution. Therefore, PAR2-h probably contains calcium salt. The artificial lipid membrane sensor

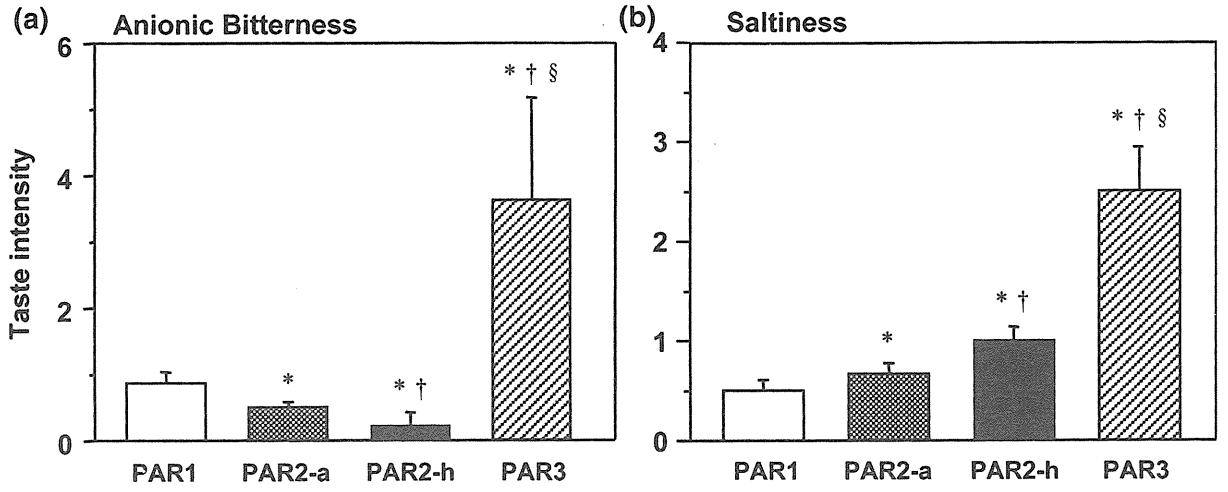

Fig. 4 Taste intensities of anionic bitterness (**a**) and saltiness (**b**) in each type of Processed Aconite Root (PAR). Data were obtained by the taste-sensing system SA402B. Sample solution was 1 mg/ml. Each value represents mean ± standard deviation (SD). Details of each value are described in Table 2. $^{*}P < 0.01$ compared with PAR1, $^{\dagger}P < 0.01$ compared with PAR2-a, and $^{\S}P < 0.01$ compared with PAR2-h

probes of the taste-sensing system were designed by emulating the biological membrane, and the sensor probe for umami detection includes phospholipid as a component of the sensor membrane. Furthermore, it is known that phospholipids in the biological lipid bilayer membrane respond to calcium ion [50]. Considering these facts, we thought that an excess amount of residual calcium ion in PAR2-h might affect sensor output and result in the negative taste intensity for umami. In the early processing stage, PAR3 was coated with hydrated lime (calcium hydroxide). However, it is known that calcium hydroxide is removed in subsequent processes, and we think this is the reason PAR3 showed the positive intensity of umami.

PAR1, PAR2-a, and PAR2-h are produced after a heating process, whereas PAR3 is not. This may contribute to the difference in anionic bitterness taste intensity between the former three types and that of PAR3 (Fig. 4a).

For saltiness (Fig. 4b), it is logical that PAR1 showed significantly lower taste intensity than any other PAR types, because PAR1 was not treated with any salts during the processing. The differences of the taste intensities of saltiness among PAR2-a, PAR2-h, and PAR3 may be attributed to variety in the salt treatment procedure, including the amounts and treating time of salts. Unfortunately, we could not obtain detailed information to clarify this point.

Discrimination among four types of PAR by taste characteristics

It is possible to discriminate each type of PAR from the others by their appearance when PAR exists as a whole crude drug. However, it is very difficult to know how the PAR was processed when PAR exists as small cuts or powders. As mentioned above, we found that the taste intensities of each type of PAR measured by the taste-sensing system showed specific features. Therefore, we propose a procedure for discriminating these four types of PAR by using water extract of PAR and the taste-sensing system. The scheme is shown in Fig. 5. First, sample extracts at the concentration of 0.1 mg/ml (solution A) and 1 mg/ml (solution B) are prepared. Then, the taste intensity of the aftertaste of cationic bitterness of solution A is measured. When the taste intensity is less than +1, the measurement is repeated with the solution B. If the taste intensity of this measurement is not less than +4, the tested PAR is identifiable as PAR1, while if the intensity is less than +4, it is identifiable as PAR2-a. When the taste intensity of the aftertaste of the cationic bitterness of solution A is not less than +1, the taste intensity of umami of solution B is measured. If the taste intensity of umami is observed as a negative value, the tested PAR is identifiable as PAR2-h, while if the taste intensity is observed as a positive value, this PAR is identifiable as PAR3. Discrimination of PAR2-h from PAR3 is also possible by comparing the taste intensity of anionic bitterness or saltiness.

It was found that PAR2-a and PAR2-h have characteristic taste patterns, even though they are classified as being in the same category in the JP15 monograph. This suggests the possibility that these two types of PAR have different composition patterns. Further studies are needed to separate PAR2 into two different categories in future JP monographs.

In this study, we investigated the objective taste of four PAR types collected by PAR-WG by using the taste-sensing system. These PAR samples are thought to be representative of PAR in Japanese markets, and we found

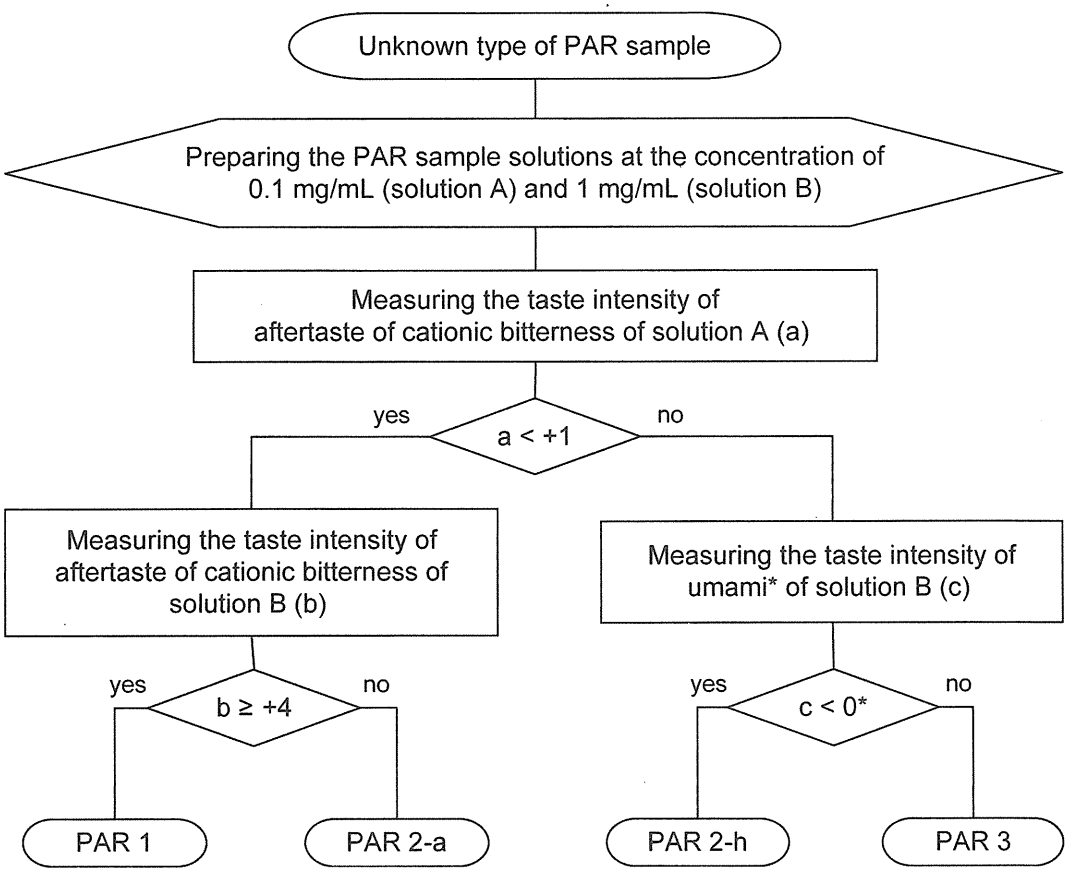

Fig. 5 A flow chart for discrimination on the Japanese Pharmacopoeia (JP)-stipulated Processed Aconite Root (PAR) by the taste intensities. *For discrimination of PAR2-h and PAR3 by the taste intensity of anionic bitterness or saltiness of solution B, the borderline value (c) is $< +1.5$

PAR taste was undoubtedly affected by its own processing method.

Acknowledgements The authors are grateful to members of PAR-WG for kindly providing PAR samples and information for aconite processing. This work was supported in part by a Health and Labour Sciences Research Grant.

References

1. Matsumoto K (ed) (1984) Shinkoku-koho Shinnohonzokyo. Shobundo, Tokyo, pp 185–186
2. Hikino H, Yamada C, Nakamura K, Sato H, Ohizumi Y, Endo K (1977) Change of alkaloid composition and acute toxicity of *Aconitum* roots during processing. Yakugaku Zasshi 97:359–366
3. Kosuge T, Yokota M (1976) Studies on cardiac principle of aconite root. Chem Pharm Bull 24:176–178
4. Higashi K (1996) A case of postherpetic neuralgia successfuly controlled with 3 Kampo medicines which Bushi commonly. Jpn J Orient Med 47:267–270
5. Toriizuka K (ed) (2003) Monographs of pharmacological research on traditional herbal medicines. Ishiyaku Publishers, Inc., Tokyo, pp 401–413
6. Nakae H (2009) Efficacy of aconite tuber powder in patients with arthralgia and somatic pain. Kampo Med 60:81–85
7. Chino A, Ishida A, Sekiya N, Ohno K, Hirasaki Y, Kasahara Y, Namiki T, Miyazaki M, Terasawa K (2010) A case of multiple intractable skin ulcers of bilateral legs due to arteriovenous fistula successfully treated with Kampo medicines. Kampo Med 61:325–330
8. The Ministry of Health and Welfare Notification No. 65, March 15, 1955
9. The Ministry of Health and Welfare Notification No. 76, April 1, 1961
10. The Ministry of Health and Welfare Notification No. 163, April 1, 1966
11. Hikino H, Shiota S, Takahashi M, Murakami M (1983) Seasonal dynamics of the accumulation of aconite alkaloids in *Aconitum carmichaeli* roots. Jpn J Pharmacog 37:68–72
12. Kitagawa I, Chen ZL, Yoshihara M, Yoshikawa M (1984) Chemical studies on crude drug processing. IV. Aconiti Tuber (3), Quantitative determination of aconitine alkaloids in aconiti tuber by means of high performance liquid chromatography. Yakugaku Zasshi 104:867–872
13. Mori T, Murayama M, Bando H, Kawahara N (1991) Studies on the constituents of Aconitum species. XII. Syntheses of Jesaconitine derivatives and their analgestic and toxic activities. Chem Pharm Bull 39:379–383
14. Taki M, Omiya Y, Suzuki Y, Ikeda Y, Noguchi M, Matuba T, Kubo M, Niitu K, Komatsu Y, Okada M (1998) Quality and pharmacological investigation of processed aconiti tuber (TJ-3022). Nat Med 52:343–352

15. Nose M, Arai T, Zhao CH, Kojima K, Ogihara Y, Sekita S, Satake M (2001) Quantitative determination of aconitine alkaloids in aconiti tuber and Kampo prescription containing aconiti tuber commercially available. Nat Med 55:124–133
16. Taki M, Terabayashi S, Matsuba T, Sasaki H, Fukuchi M, Okada M (2002) Quality investigation of aconiti tuber in China and Japan. Nat Med 56:163–172
17. Okada K, Kawaguchi K (2004) The effect of tuberous root size on growth and alkaloid content of aconite (Aconitum subcuneatum). Nat Med 58:49–54
18. Taki M, Matsuba T, Fukuchi M, Aburada M, Okada M (2004) Comparison of seasonal variations on growth of *Aconitum carmichaeli* DEBX. and constituents of root tubers cultivated in Hokkaido and Ibaraki prefecture. Nat Med 58:55–63
19. Okada K, Kawaguchi K (2005) Change in chemical component characters within and among years of aconite. Nat Med 59:36–41
20. Nakamura Y, Yomura K, Kammoto T, Ishimatsu M, Kikuchi Y, Niitsu K, Terabayashi S, Takeda S, Sasaki H, Arimoto K, Okada M, Sekita S, Satake M, Goda Y (2006) Physicochemical quality evaluation of natural compounds isolated from crude drugs. Standard compounds for the official specification and testing method of "Processed Aconite Root" and "Powdered Processed Aconite Root" in the Japanese Pharmacopoeia. J Nat Med 60:285–294. doi:10.1007/s11418-006-0005-y
21. The Ministry of Health, Labour and Welfare Ministerial Notification No. 461, December 28, 2004. http://jpdb.nihs.go.jp/jp14supp2/YAK2T.pdf
22. Sato M, Anetai M, Goda Y (2005) Analysis of organophosphorus pesticide residues in crude drugs. Pharm Regul Sci 36:83–97
23. Yamamoto K, Yamamoto T, Kondo S, Tamura M, Shibata Y, Umeda K, Akiba S, Kawakami T, Saito F, Sugimoto T, Isomi Y, Nakada T, Takao M, Nakashima K, Tahara M, Hayashi K, Sudo M, Nakanishi K, Isozaki O, Kawahara N, Goda Y (2005) Assay of ginsenoside Rg_1 and ginsenoside Rb_1 in ginseng and red ginseng by high-performance liquid chromatography. Pharm Regul Sci 36:211–222
24. Kawahara N, Kim IH, Goda Y (2006) Content of sulfur dioxides in herbal materials obtained form the Japanese market. Jpn J Food Chem 13:105–108
25. Sato M, Anetai M, Goda Y (2006) Organophosphorus pesticide residues in decoctions of crude drugs. Pharm Regul Sci 37:245–250
26. Kawahara N, Anjiki N, Kim IH, Mikage M, Goda Y (2007) Studies on the relationship between color and content of sulfur dioxides in crude drugs obtained from the Japanese market. Jpn J Food Chem 14:140–144
27. Maruyama T, Sugimoto N, Kuroyanagi M, Kim IH, Kamakura H, Kawasaki T, Fujita M, Shimada H, Yamamoto Y, Tada A, Yamazaki T, Goda Y (2007) Authentication and chemical study of Isodonis Herba and Isodonis extracts. Chem Pharm Bull 55:1626–1630
28. Maruyama T, Kamakura H, Miyai M, Komatsu K, Kawasaki T, Fujita M, Shimada H, Yamamoto Y, Goda Y (2008) Authentication of the traditional medicinal plant *Eleutherococcus senticosus* by DNA and chemical analyses. Planta Med 74:787–789. doi:10.1055/s-2008-1074537
29. Sato M, Anetai M, Kamakura H, Goda Y (2008) Analysis of organophosphorus pesticide residues in crude drugs (Part 2). Pharm Regul Sci 39:203–222
30. Tokumoto H, Shimomura Y, Katsuki S, Goda Y (2008) Morphological discrimination of *Curcuma longa* L. and *Curcuma aromatica* Salisb. Jpn J Pharmacog 62:54–65
31. Goda Y, Kawahara N, Kiuchi F, Hirakura K, Kikuchi Y, Nishimura H, Marumoto M, Kitazaki H (2009) A guanidine derivative from seeds *of Plantago asiatica*. J Nat Med 63:58–60. doi:10.1007/s11418-008-0275-7
32. Kawahara N, Anjiki N, Hosoe J, Kim IH, Ikezaki H, Mikage M, Goda Y (2009) Studies on relationship between taste and content of sulfur dioxide in crude drugs obtained from the Japanese market. Pharm Regul Sci 40:129–135
33. Kondo K, Shiba M, Yotsuyanagi Y, Nishimura N, Maruyama T, Goda Y (2009) Discrimination between *Atractylodes* Rhizome (Byaku-jutsu) and *Atractylodes lancea* Rhizome (So-jutsu) by the PCR-RFLP analysis of ITS region on nrDNA. J Jpn Bot 84:356–359
34. Terabayashi S, Sakai E, Yamaji H, Kondo K, Kawahara N, Goda Y (2009) Authentication and standardization of botanical origin and morphology of Coix Fruit in the Japanese Pharmacopoeia. J Jpn Bot 84:77–84
35. Maruyama T, Miyai M, Kamakura H, Nakajima I, Kawasaki T, Komatsu K, Fujita M, Yamamoto Y, Shibata T, Goda Y (2010) The authentication and the purity test of Eleutherococcus Senticosus Rhizome based on the genetic approach. Jpn J Pharmacog 64:15–20
36. Sato M, Anetai M, Kamakura H, Goda Y (2010) Analysis of organophosphorus pesticide residues in crude drugs (Part 3). Pharm Med Dev Regul Sci 41:324–337
37. Maruyama T, Kondo K, Yotsuyanagi Y, Yamamoto Y, Kawasaki T, Shiba M, Terasaka K, Yamane M, Zhu S, Sakata K, Fujita M, Akiyama H, Nishimura N, Komatsu K, Mizukami H, Goda Y (2010) The inter-laboratory validation study for the purity test of crude drugs based on a PCR-RFLP. Jpn J Pharmacog 64:96–101
38. Anjiki N, Kawahara N, Goda Y (2005) Evaluation of the taste of Kampo formulae by taste-sensing system (1). Nat Med 59:164–170
39. Anjiki N, Suzuki A, Kawahara N, Goda Y (2006) Evaluation of the taste of a Kampo formula by a taste-sensing system (2), taste of Kakkonto. Jpn J Pharmacog 60:21–27
40. Anjiki N, Yoshino C, Kawahara N, Goda Y (2007) Evaluation of the taste of a Kampo formula by a taste-sensing system (3), the taste of Ryokeijutsukanto. Jpn J Pharmacog 61:6–13
41. The Ministry of Health, Labour and Welfare Ministerial Notification No. 285, March 31, 2006. http://jpdb.nihs.go.jp/jp15/YAKKYOKUHOU15.pdf
42. Toko K (2000) Biomimetic sensor technology. The Press Syndicate of The University of Cambridge, Cambridge
43. Toko K, Uchida T (2007) Taste.modification technology of food and medicine. CMC Publishing Co., Ltd., Tokyo, pp 219–252
44. Habara M, Toko K (2009) Biomimetic membrane for taste sensing, Chap. 6. In: Ariga K, Nalwa HS (eds) Bottom-up nanofabrication, vol 6. American Scientific Publishers, Los Angeles, pp 91–109
45. Ikezaki H, Taniguchi A, Toko K (1998) Increase in information by improvement of measuring method in a multichannel taste sensor. TIEE Japan 118-E:506–512
46. Pfaffmann C (1959) Neurophysiology. In: Field J (ed) Handbook of physiology, vol 1. American Physiological Society, Washington, DC, pp 507–534
47. Schutz HG, Pilgrim ES (1957) Differential sensitivity in gustation. J Exp Psychol 54:41–48
48. Bai G, Yang Y, Shi Q, Liu Z, Zhang Q, Zhu YY (2008) Identification of higenamine in radix aconiti *Lateralis Preparata* as a beta2-adrenergic receptor agonist. Acta Pharmacol Sin 29:1187–1194
49. Matsui M, Bando H, Murayama M, Miura T (1999) Constituent of "KAKO-BUSHI-MATSU" II Components of amino acid and sugars. Nat Med 53:313–315
50. Ohnishi S (1975) A spin-label study of biological membranes with special emphasis on calcium-induced lateral phase separation. Adv Biophys 8:35–82

生薬学雑誌 65 (2), 103-107 (2011)

漢方薬抽出自動包装機を用いた湯液品質の経時変化 (1) —大黄甘草湯について—

堂井　美里[a,b], 安食菜穂子[a,c], 伊奈小百合[a], 吉光見稚代[a,b], 川原　信夫[d], 合田　幸広[e], 垣内　信子[f], 御影　雅幸[a]*

[a] 金沢大学大学院自然科学研究科資源生薬学研究室, [b] 株式会社ウチダ和漢薬, [c] 株式会社インテリジェントセンサーテクノロジー, [d] 独立行政法人医薬基盤研究所薬用植物資源研究センター, [e] 国立医薬品食品衛生研究所, [f] 九州保健福祉大学薬学部生薬学講座

Temporal Change in Quality of a Kampo Decoction Packed by a Decocting Machine (1) —On Daiokanzoto—

Misato Doui[a,b], Naoko Anjiki[a,c], Sayuri Ina[a], Michiyo Yoshimitsu[a,b], Nobuo Kawahara[d], Yukihiro Goda[e], Nobuko Kakiuchi[f] and Masayuki Mikage[a]*

[a] *Herbal Medicine and Natural Resources, Division of Pharmaceutical Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa, Ishikawa 920-1192, Japan*

[b] *Uchida Wakanyaku Co., Ltd., 4-4-10 Higashi-nippori, Arakawa-ku, Tokyo 116-8571, Japan*

[c] *Intelligent Sensor Technology, Inc., 5-1-1 Onna, Atsugi, Kanagawa 243-0032, Japan*

[d] *Research Center for Medicinal Plant Resources, National Institute of Biomedical Innovation, 1-2 Hachimandai, Tsukuba, Ibaraki 305-0843, Japan*

[e] *National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*

[f] *Department of Pharmacognosy, School of Pharmaceutical Sciences, Kyusyu University of Health and Welfare, 1714-1 Yoshino-cho, Nobeoka, Miyazaki 882-8508, Japan*

(Received November 18, 2010)

In recent years, decocting machines have come into practical use. These machines can provide one month's packages of decoction at one operation. Thus, patients can have their Kampo formula decocted by pharmacists with stable quality. However, it is necessary to clarify the change in quality of decoctions after long storage. Therefore in this report, we preserved Daiokanzoto produced by a decocting machine at 4, 25 or 40℃ and elucidated suitable preservation conditions and quality assurance periods on the basis of color, taste, and principal compound contents. The color of Daiokanzoto was maintained for 1 week at 4℃, 2 weeks at 40℃ and 2 months at 25℃. However, the color of the decoction changed after preserving for 2 months at 25℃; hence we consider 1 month to be a reasonable period for preservation in terms of color. Sennoside A content after storage for 6 months at 4℃ and 2 months at 25℃; glycyrrhizin content, and taste hardly showed any change. Thereby we concluded the packed decoction of Daiokanzoto produced by a decocting machine maintained the color, taste, and principal compound contents for a month at 25℃, i.e., room temperature.

Keywords: Daiokanzoto; a decocting machine; color; taste; sennoside

緒　　言

漢方湯液の薬効発現には煎じ時間，水の量など煎じ方が重要な役割を果たしている[1~3]．中国や韓国では，薬局で薬剤師が煎液を調製して患者に手渡しているが，日本では，煎じる作業を患者に任せることが多く，正しく煎じられていない可能性がある[4]．それ故，近年，漢方薬抽出自動包装機が開発され，実用化が進められている．本装置では，漢方湯液約1ヶ月分を一度に煎じて，1服分ずつアルミパック包装できる．しかし，長期保存による品質変化については十分な検討がされていない．

そこで本研究では，色彩，味，化学成分量を指標として，本装置で調製した湯液の適切な保存条件と品質保証期間を検討する目的で，第15改正日本薬局方[5]に収載される大黄甘草湯について分析した．なお，これまでに湯液の保存による経時変化に関する報告はない．

実験材料及び方法

1.　生薬材料

日本薬局方ダイオウ（雅黄，中国四川省産，ウチダ和漢薬 Lot. US262303）及びカンゾウ（西北甘草，中国寧夏回族自治区産，同 Lot. SU452905）．

2.　試　　薬

Sennoside A及び glycyrrhizin は和光純薬工業株式会社から生薬試験用を入手した．HPLC には HPLC 用試薬を，その他は試薬特級を用いた．

3.　大黄甘草湯の調製方法

漢方薬抽出自動包装機（ハニルパートナー EXT-500S，株式会社ウチダ和漢薬）の抽出タンクに生薬45日分（ダイオウ180 g，カンゾウ45 g）と水道水（金沢市）【45日分（100 mL×3回/日×45日）＋1.5 L（蒸発量）】を加え，30分間煎じて，アルミパック包装135包を得た．

4.　試料の保存方法

アルミパック包装した大黄甘草湯を4℃，25℃，40℃の恒温器内で，2，7，14，30，60，90，120，150，180日間保存した．なお，保存方法は厚生労働省ICHガイドライン（http: //www.pmda.go.jp/ich/ich_index.html）を参考にした．

5.　色彩の測定方法

近藤ら[6]の方法に従い，遠心分離（3000 rpm, 10分間）した煎液の上清を光路長10 mmのガラスセルに入れ，標準C光を照射した透過光を色彩色差計 CT-210（コニカミノルタホールディングス株式会社）で測定した．L^*値（明度），a^*値（赤み），b^*値（黄み）及びΔE^*ab値（色差）により評価した．なお，ΔE^*ab値はL^*値，a^*値，b^*値の色差を元に算出され，値が12以上の時，別の色系統に変わると評価される．

6.　味の測定方法

安食ら[7]の方法に従い，遠心分離（3000 rpm, 10分間）後の煎液の上清を超純水で20倍に希釈し，塩化カリウムと酒石酸をそれぞれ10 mM及び0.1 mMとなるように添加した液を試料溶液とした．これを味認識装置 SA402B（株式会社インテリジェントセンサーテクノロジー）で6種類のセンサ（AC0，C00，AE1，AAE，CT0，CA0）を用いて測定し，ウェーバーの法則に基づいてヒトが感じる味強度の違いを推定し，得られた推定値を各味要素の数値とした．本法則によると，ヒトが味の変化を認識できるのは味の測定値が20%以上変化したときとされており，この20%の差を推定値1としている．今回は，酸性苦味，酸性苦味後味，塩基性苦味後味，旨味，旨味後味，塩味，酸味，渋味及び渋味後味の強度を推定した．なお，煎液を20倍希釈したのは，センサの安定性を考慮したためである．

7.　成分含量の測定方法

検出器：L-2400，ポンプ：LC-6A，カラムオーブン：CTO-6A，レコーダー：C-R6A（島津製作所）を用いてHPLC法で測定した．

7.1　Sennoside A

7.1.1　試料溶液の調製

Sep Pak Accell Plus QMA カートリッジ（Waters）に水/メタノール混液（1: 1）10 mL，煎液5 mL，50%メタノール約1 mL，酢酸（100）/メタノール混液（5.8→1000）約10 mLを通した後，0.5 Mリン酸二水素ナトリウム/メタノール混液（1: 1）で溶出し10 mLにメスアップした．これをメンブランフィルター（0.45μm）でろ過し，試料溶液とした．

7.1.2　HPLC分析条件

カラム：RP-18 GP（4.6×250 mm，関東化学株式会社），移動相：水：アセトニトリル：リン酸（2520: 480: 1），流量：0.7 mL/min，検出波長：380 nm，カラム温度：40℃，注入量：10μL．

7.2　Glycyrrhizin

7.2.1　試料溶液の調製

荒木ら[8]の方法に従い，Sep Pak Accell Plus QMA カートリッジに0.5 Mリン酸緩衝液（pH 7.5）10 mL，煎液5 mLを吸着させた後，50%エタノール3 mLで洗い，0.5 Mリン酸二水素ナトリウム/エタノール（99.5%）混液（1: 1）で溶出し10 mLにメスアップした．これをメンブランフィルター（0.45μm）でろ過し，試料溶液とした．

7.2.2　HPLC分析条件

カラム：YMC-Pack ODS（6.0×150 mm，株式会社ワイエムシィ），移動相：酢酸（1→15）：アセトニトリル（13: 7），流量：1.0 mL/min，検出波長：254 nm，カラム温度：室温，注入量：10μL．

実　験　結　果

同様の実験を2回行い，平均値をグラフに示した．なお，各温度における色彩，味，成分含量の変化は2回ともほぼ等しかった．

1.　色彩の経時変化

漢方薬抽出自動包装機で作製し，アルミパック保存した大黄甘草湯は，保存温度に関わらずL^*値（明度）及びb^*

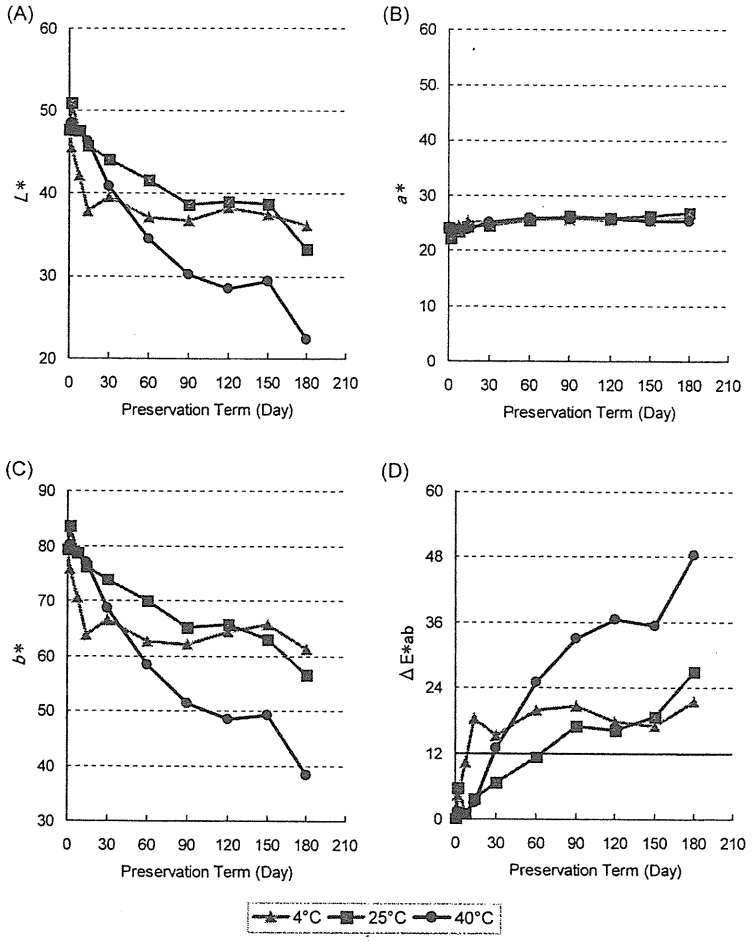

Fig. 1. Temporal change of color value on Daiokanzoto. (A) L^*, (B) a^*, (C) b^*, (D) ΔE^*ab.

値（黄み）の減少が認められた（Fig. 1-A, C）．これらの値は，4℃では10日後までに，40℃では保存期間の長さに伴って顕著に減少した．一方，a^*値（赤み）は変化なかった（図1-B）．以上のΔE^*ab（色差）は保存温度4℃で10日後，40℃で1ヶ月後，25℃で2ヶ月後に値が12となり，それ以降は別の色系統に変わった（Fig. 1-D）．

2. 味の経時変化

大黄甘草湯の煎液を20倍希釈した溶液の味は塩基性苦味後味及び酸性苦味を強く有することが明らかになった（Fig. 2-A）．塩基性苦味後味は温度に関わらず，保存開始から10日目までに増加し，その後減少する傾向が認められたが，味強度1以上の変化はなく，ヒトが感じる程度ではないと考えられる（Fig. 2-B）．一方，酸性苦味はほとんど変化なかった（Fig. 2-C）．なお，その他の味要素（酸味，渋味，酸性苦味後味，渋味後味，旨味，塩味，旨味後味）は経時変化が認められなかった（データ省略）．

3. 成分の経時変化

Sennoside A含量は40℃で保存開始直後から，25℃で60日後以降減少した（Fig. 3-A）．一方，glycyrrhizin含量には顕著な変化は認められなかった（Fig. 3-B）．

考　察

1. 本研究は，色彩，味，成分を指標に漢方薬抽出自動包装機で製した大黄甘草湯湯液の最適保存条件と品質保持期間の検討を目的とした．その結果，色彩は保存温度4℃で1週間，40℃で2週間，25℃で2ヶ月間は同系統の色を維持した．しかし，25℃では2ヶ月を超えると別系統の色になることから，維持期間は1ヶ月が適当であると考えられる．また，sennoside A含量は保存温度4℃で6ヶ月間，25℃で2ヶ月間はほぼ一定であった．一方，味及びglycyrrhizin含量に顕著な変化はなかった．以上から，漢方薬抽出包装機で製した大黄甘草湯は今回検討した色彩，味，含有化学成分量については，25℃すなわち室温保存で1ヶ月間，大きな変化なく維持できると判断した．

2. 大黄甘草湯の湯液の色は，保存期間の長さに伴い，明度を表すL^*値及び黄色を表すb^*値が減少したことから，暗くくすむ方向に変化した．また，煎液は保存温度が4℃，40℃，25℃の順に変色した．すなわち，大黄甘草湯は室温と離れた温度で保存することで変色が加速するといえる．

一方，40℃または25℃で保存した場合，大黄の主成分

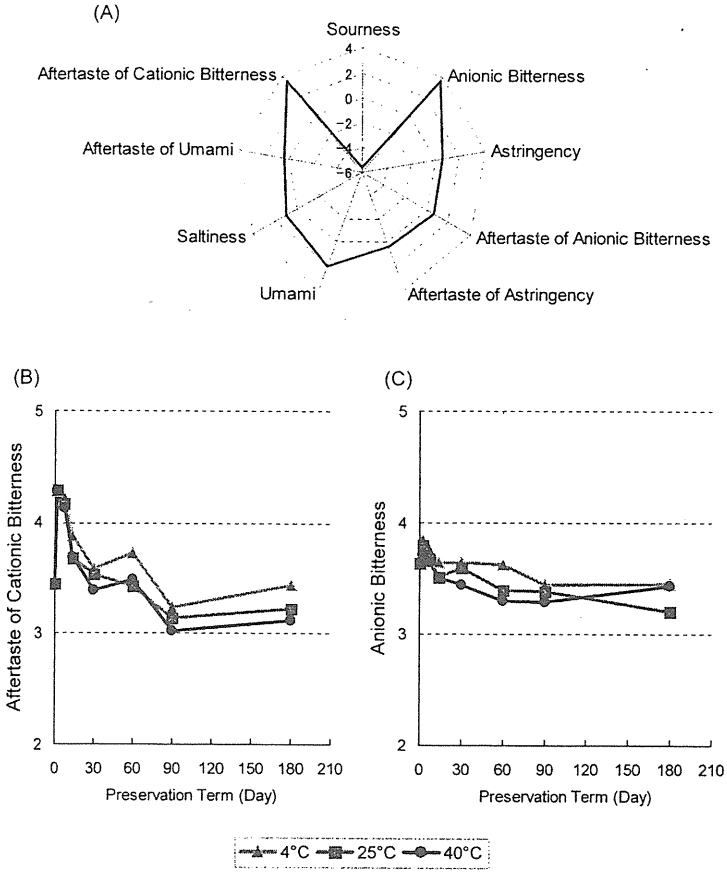

Fig. 2. Estimate taste value of twentyfold diluted decoction on Daiokanzoto.
(A) Taste pattern of unpreserved decoction, (B) Temporal change of aftertaste of cationic bitterness, (C) Temporal change of anionic bitterness.

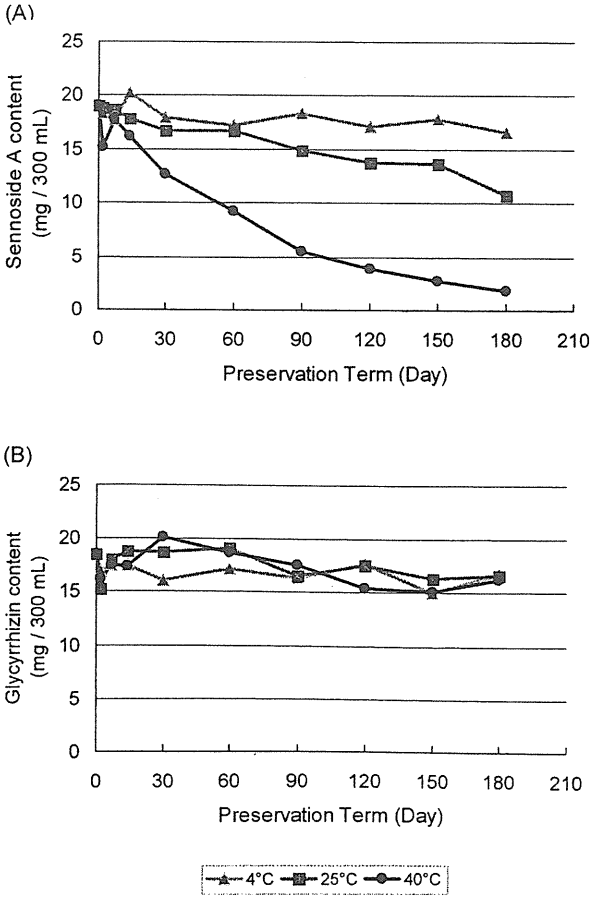

Fig. 3. Temporal change of principal compound contents on Daiokanzoto.
(A) Sennoside A, (B) Glycyrrhizin.

である sennoside A 含量は減少した．大黄を 180℃で加熱した時，sennoside は熱分解され anthraquinone 類が増加することが報告されているが[9]，本実験での 40℃及び 25℃でも，分解が進行すると考えられる．

これらのことから，大黄甘草湯の場合，一般に指示されている冷蔵保存では，含有成分量は維持できるが，色の変化が認められると患者に適切に説明することが重要である．

3. 冷蔵保存した湯液は通常，温めてから服用する．アルミパックにて冷蔵保存した大黄甘草湯湯液は，加温により色が更に変化する可能性もあり，この点についても検討する必要がある．

4. 大黄甘草湯（20 倍希釈溶液）の塩基性苦味後味は温度に関わらず保存開始後に増加して，その後減少する傾向が認められたが，ウェーバーの法則に基づく推定値 1 以上の変化はなく，ヒトが感じる程度ではなかった．しかし，味認識装置による測定には 20 倍希釈溶液を用いており，また本装置では甘味及び辛味の測定ができないことから，今後，官能試験を行い，味の変化がないことを確認する必要があると考えられる．

5. 保存温度 25℃及び 40℃で b^* 値の減少に伴い sennoside A 含量が減少したが，保存温度 4℃ではこの傾向が認められなかった．一方，保存に伴い湯液中の沈殿量が増加し，色彩が変化した可能性もある．これらのことから，b^* 値の減少には，sennoside の減少と共に沈殿量の変化が関連している可能性が考えられる．今後，tannin 類などの沈殿物質についても定量する必要があろう．

6. 保存期間 150 日目以降，色彩及び sennoside A 含量が大きく変化した．このことから，一定期間保存後に品質が大きく変化することが示唆され，長期保存した湯液を服用しないように患者に服薬指導する必要がある．

7. 本研究は大黄甘草湯に関するものであるが，配合される生薬の性質によって，漢方湯液の品質を維持できる期間が異なることが示唆され，今後，処方別での検討が必要である．

文　献

1) Takaishi K., Kuwajima H., Tokuda T., Endo M., Orita H., Tsuji E., *Shoyakugaku Zasshi,* **36** (3), 245-249 (1982).
2) Luo YY., Tani T., *Wakaniyaku-gakkaishi,* **5** (3), 396-397 (1988).
3) Fang Z.Z., Yoshizaki F., Ando T., Hisamichi S., *Shoyakugaku Zasshi,* **45** (2), 142-144 (1991).
4) Iwai T., Tani T., Arichi S, *Kampo Med.,* **39** (3), 201-205 (1989).
5) The Minidtry of Health, Labour and Welfare, The Japanese Phamacopoeia 15th Edition (2006).
6) Kondo S., Mikage M., Takano A., Tsuda Y., *Shoyakugaku Zasshi,* **46** (2), 174-178 (1992).
7) Anjiki N., Kawahara N., Goda Y., *Nat. Med.,* **59** (4), 164-170 (2005).
8) Araki N., Kanaya T., Yoshikawa Y., Nunoura Y., *Iyakuhin Kenkyu,* **28** (4), 297-300 (1997).
9) Yoshida A., Kondo S., Mikage M., *Nat. Med.,* **55** (6), 294-299 (2001).

日本薬局方における生薬等の成分定量用試薬を利用した定量 NMR (qNMR) のバリデーション試験

細江 潤子[*1], 杉本 直樹[*1], 末松 孝子[*2], 山田 裕子[*3], 早川 昌子[*4], 勝原 孝雄[*5], 西村 浩昭[*5], 合田 幸広[*1, #]

医薬品医療機器レギュラトリーサイエンス
Vol. 43, No. 2 別刷 (2012 年)
一般財団法人 医薬品医療機器レギュラトリーサイエンス財団

投稿/原著

医薬品医療機器レギュラトリーサイエンス
Pharmaceutical and Medical Device Regulatory Science
Vol. 43, No. 2, 182～193 (2012)

日本薬局方における生薬等の成分定量用試薬を利用した定量NMR (qNMR) のバリデーション試験

細江 潤子[*1], 杉本 直樹[*1], 末松 孝子[*2], 山田 裕子[*3], 早川 昌子[*4],
勝原 孝雄[*5], 西村 浩昭[*5], 合田 幸広[*1, #]

(受付：平成23年8月18日, 受理：平成23年11月28日)

Validation Studies of qNMR for Chemical Reagents Used as Reference Standards for Quantitative Analyses of Crude Drugs in the Japanese Pharmacopoeia

Junko HOSOE[*1], Naoki SUGIMOTO[*1], Takako SUEMATSU[*2],
Yuko YAMADA[*3], Masako HAYAKAWA[*4], Takao KATSUHARA[*5],
Hiroaki NISHIMURA[*5] and Yukihiro GODA[*1, #]

Summary

Quantitative NMR (qNMR) qualifies as an absolute quantification method and is theoretically able to determine the purity of any compound with SI-traceability. Therefore, we are trying to introduce qNMR to the Japanese Pharmacopoeia for the specification of reagents, using marker compounds for the quantitative analyses of crude drugs. In this study, we performed validation studies of qNMR by using two chemical reagents (magnolol: Mw 266.34; and geniposide: Mw 388.37) in five independent laboratories. The weighed amount of each sample was 5 mg ± 10% and each participant prepared three sample solutions. The absolute purity of each sample was measured by qNMR three times. The total averages (the averages of the participant averages) ± SD of absolute quantification results for magnolol and geniposide were 98.97± 0.19% and 96.09±0.28%, respectively. These data suggested that the variabilities in each NMR measurement (the average of all the SD of each sample average) and each sample liquid preparation (the average of all the SD of each participant average) were about 0.08% and 0.07% (magnolol), and 0.17% and 0.14% (geniposide), respectively. These data indicate that the purity of these compounds can be determined by qNMR with an accuracy of two significant digits when the molecular weight of the target reagent is around 300 with a weighed amount of about 10 mg.

Key words

Quantitative NMR, Validation study, the Japanese Pharmacopoeia, Chemical reagents, Magnolol, Geniposide, Marker compounds, Crude drugs

*1 国立医薬品食品衛生研究所　東京都世田谷区上用賀 1-18-1 (〒158-8501)
National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
*2 日本電子株式会社　東京都昭島市武蔵野 3-1-2 (〒196-8558)
JEOL Ltd., 3-1-2 Musashino, Akishima, Tokyo 196-8558, Japan
*3 和光純薬工業株式会社　埼玉県川越市大字的場 1633 (〒350-1101)
Wako Pure Chemical Industries, Ltd., 1633 Matoba, Kawagoe, Saitama 350-1101, Japan
*4 和光純薬工業株式会社　東京都中央区日本橋本町 4-5-13 (〒103-0023)
Wako Pure Chemical Industries, Ltd., 4-5-13 Nihonbashi-Honcho, Chuo-ku, Tokyo 103-0023, Japan
*5 株式会社ツムラ　茨城県稲敷郡阿見町吉原 3586 (〒300-1192)
TSUMURA & CO., 3586 Yoshiwara, Ami-machi, Inashiki-gun, Ibaraki 300-1192, Japan
責任著者 Corresponding author