Physicochemical Studies on Decoctions of Kampo Prescriptions. I. Transfer of Crude Drug Components into the Decoctions

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To evaluate the profile of the crude drug components transferred into the decoction, decoctions of about 30 kampo prescriptions, comprising 13 quantitatively assayable crude drugs, were prepared; the quantities (mg/daily dose) and ratios (%) of these components transferred from the drug into the decoctions were determined by HPLC. Then, a chromatographic substitute for the hydrophobic parameter was determined in various crude drug components, and its relationship with the quantities and ratios transferred was examined. The transfer of stable crude drug components into the decoctions was found to be regulated by the hydrophobicity of the components, which is related to their transfer ratio, rather than the amount transferred. In addition, the transfer ratio and the value of the hydrophobic parameter did not exhibit a simple linear relationship.

Keywords transfer ratio; crude drug component; kampo decoction; partition coefficient; HPLC; capacity factor

Many people who are involved with kampo medicine, including clinicians, are interested in the content and proportion of crude drug components in kampo decoctions. However, numerous components are present in the decoctions and, therefore, the measurement of each individual component is impractical. In the present study, we examined the transfer profile of all crude drug components into the decoctions by clarifying the fundamental mechanisms of this transfer rather than evaluating the transfer of individual components.

There have been many reports describing the transfer of components from crude drugs into kampo decoctions, but few on the mechanism. The dissolution of ephedrine, a component of Ephedra Herba, has been reported to be promoted by pasted starch.[1] Yata and co-workers also found that the saponin of bisdesmoside, which is readily soluble in water, promoted the dissolution of monodesmosides, slightly soluble saponins.[2] On the other hand, "negative compounds" inhibit dissolution, that is particular components in a crude drug are removed from a decoction by adsorption[3] and the components once eluted onto the residue of the drug are readsorbed.[4,5] There are also reports of special reactions such as the conversion of saikosaponins a and d, which are components of Bupleuri Radix, into saikosaponins b,[6,7] and precipitation reactions between berberine-type alkaloids in Coptidis Rhizoma or Phellodendri Cortex and glycyrrhizin, a component of Glycyrrhizae Radix,[5,8] and between alkaloids and tannin.[9] However, all these reports relate to individual components or their interrelationships, and no investigation has been made on the comprehensive behavior of all the components in a decoction. Pattern analysis by thin-layer chromatography[10] is qualitative, although it may be possible to evaluate all organic components comprehensively.

Not all components in a bulk crude drug are completely transferred into its decoction. Only part of each component is transferred either unchanged or after chemical changes during the process of decoction. Although it is generally supposed that only the components readily soluble in water are transferred into a decoction, even a high percentage of organic components poorly soluble in water can be transferred.[6,11] We also found that about 70% of 6,7-dimethylesculetin (**18**), which is a poorly water-soluble component of Artemisiae Capillaris Spica, was transferred to a signification extent into the decoction of Inchinko-to.[12]

The ratio of the content of the components in a decoction to those in the bulk drug is called the transfer ratio (r_T, %). Noguchi has speculated that this transfer ratio is dependent on the physicochemical properties of each component and that components with analogous properties exhibit similar behavior.[13] However, he did not examine the different types of properties or the factory regulating the transfer ratio. We studied the relationship between the transfer ratio of crude drug components and their water solubility, which seems to be one of the properties determining the transfer ratio. The hydrophobicity of organic compounds with especially high lipophilicity is known to be closely related to their solubility in water.[14] Therefore, we speculated that some of those properties which regulate the transfer ratio may be clarified by studying the relationship between the quantities or ratios of crude drug components transferred to the decoction and the values of a hydrophobic parameter of the components.

In this study, we prepared decoctions in beakers on the basis of kampo prescriptions involving 13 crude drugs containing assayable components (Gardeniae Fructus, Paeoniae Radix, Puerariae Radix, Saposhnikoviae Radix, Astragali Radix, Artemisiae Capillaris Spica, Zingiberis Rhizoma, Zingiberis Siccatum Rhizoma, Alpiniae Officinarum Rhizoma, Schisandrae Fructus, Alismatis Rhizoma, Magnoliae Cortex and Trichosanthis Semen) and determined the quantity and ratio of those components transferred to the decoctions. Since the capacity factor ($\log k'$) determined by reverse-phase HPLC has been used as a substitute for the hydrophobic parameter[15–18] of compounds when the determination of their partition coefficient is practically difficult, we also measured the

TABLE I. Kampo Prescriptions

Kampo prescription	Compounded crude drugs (g)
Anchu-san (安中散)	Cinnamomi Cortex (4), Corydalis Tuber (3), Ostreae Testa (3), Foeniculi Fructus (1.5), Glycyrrhizae Radix (1), Amomi Semen (1), Alpiniae Officinari Rhizoma (0.5)
Bofu-tsusho-san (防風通聖散)	Angelicae Radix (1.2), Paeoniae Radix (1.2), Cnidii Rhizoma (1.2), Gardeniae Fructus (1.2), Forsythiae Fructus (1.2), Menthae Herba (1.2), Zingiberis Rhizoma (0.3), Schizonepetae Spica (1.2), Saposhnikoviae Radix (1.2), Ephedrae Herba (1.2), Rhei Rhizoma (1.5), Natrii Sulfus (1.5), Atractylodis Rhizoma (2), Platycodi Radix (2), Scutellariae Radix (2), Glycyrrhizae Radix (2), Gypsum Fibrosum (2), Talcum Crystallium (3)
Boi-ogi-to (防已黄耆湯)	Astragali Radix (5), Sinomeni Caulis et Rhizoma (5), Atractylodis Lanceae Rhizoma (3), Zizyphi Fructus (3), Glycyrrhizae Radix (1.5), Zingiberis Rhizoma (1)
Choto-san (釣藤散)	Gypsum Fibrosum (5), Aurantii Nobilis Pericarpium (3), Ophiopogonis Tuber (3), Pinelliae Tuber (3), Hoelen (3), Uncariae Uncis Cum Ramulus (3), Ginseng Radix (2), Saposhnikoviae Radix (2), Chrysanthemi Flos (2), Glycyrrhizae Radix (1), Zingiberis Rhizoma (1)
Goshaku-san (五積散)	Atractylodis Lanceae Rhizoma (3), Aurantii Nobilis Pericarpium (2), Angelicae Radix (2), Pinelliae Tuber (2), Hoelen (2), Glycyrrhizae Radix (1), Platycodi Radix (1), Aurantii Fructus Immaturus (1), Cinnamomi Cortex (1), Magnoliae Cortex (1), Paeoniae Radix (1), Zingiberis Rhizoma (1), Cnidii Rhizoma (1), Zizyphi Fructus (1), Angelicae Dahuricae Radix (1), Ephedrae Herba (1)
Hachimi-jio-gan (八味地黄丸)	Rehmanniae Radix (6), Corni Fructus (3), Dioscoreae Rhizoma (3), Alismatis Rhizoma (3), Hoelen (3), Moutan Cortex (2.5), Cinnamomi Cortex (1), Aconiti Calefactum Tuber (0.5)
Hange-byakujutsu-temma-to (半夏白朮天麻湯)	Aurantii Nobilis Pericarpium (3), Pinelliae Tuber (3), Atractilodis Rhizoma (3), Hoelen (3), Gastrodiae Tuber (2), Hordei Fructus Germinatus (2), Astragali Radix (1.5), Alismatis Rhizoma (1.5), Ginseng Radix (1.5), Phellodendri Cortex (1), Zingiberis Siccatum Rhizoma (1), Zingiberis Rhizoma (0.5)
Hange-koboku-to (半夏厚朴湯)	Pinelliae Tuber (6), Hoelen (5), Magnoliae Cortex (3), Perillae Herba (2), Zingiberis Rhizoma (1)
Heii-san (平胃散)	Atractylodis Lanceae Rhizoma (4), Magnoliae Cortex (3), Aurantii Nobilis Pericarpium (3), Zizyphi Fructus (2), Glycyrrhizae Radix (1), Zingiberis Rhizoma (0.5)
Hochu-ekki-to (補中益気湯)	Astragali Radix (4), Atractylodis Lanceae Rhizoma (4), Ginseng Radix (4), Angelicae Radix (3), Bupleuri Radix (2), Zizyphi Fructus (2), Aurantii Nobilis Pericarpium (2), Glycyrrhizae Radix (1.5), Cimicifugae Rhizoma (1), Zingiberis Rhizoma (0.5)
Inchin-gorei-san (茵蔯五苓散)	Alismatis Rhizoma (6), Atractylodis Lanceae Rhizoma (4.5), Polyporus (4.5), Hoelen (4.5), Cinnamomi Cortex (2.5), Artemisiae Capillaris Spica (4)
Inchinko-to (茵蔯蒿湯)	Gardeniae Fructus (3), Rhei Rhizoma (1), Artemisiae Capillaris Spica (4), or Gardeniae Fructus (6), Rhei Rhizoma (2), Artemisiae Capillaris Spica (8)
Jinso-in (参蘇飲)	Pinelliae Tuber (3), Hoelen (3), Aurantii Nobilis Pericarpium (2), Puerariae Radix (2), Platycodi Radix (2), Peucedani Radix (2), Zingiberis Rhizoma (1.5), Zizyphi Fructus (1.5), Ginseng Radix (1.5), Glycyrrhizae Radix (1), Aurantii Fructus Immaturus (1), Perillae Hebra (1), Saussureae Radix (1)
Ji-zuso-ippo (治頭瘡一方)	Cnidii Rhizoma (3), Atractylodis Lanceae Rhizoma (3), Forsythiae Fructus (3), Saposhnikovia Radix (2), Lonicerae Folium Cum Caulis (2), Glycyrrhizae Radix (1), Schizonepetae Spica (1), Carthami Flos (1), Rhei Rhizoma (0.5)
Jumi-haidoku-to (十味敗毒湯)	Platycodi Radix (3), Bupleuri Radix (3), Cnidii Rhizoma (3), Hoelen (3), Quercus Cortex (3), Araliae Cordatae Rhizoma (1.5), Saposhnikoviae Radix (1.5), Glycyrrhizae Radix (1), Schizonepetae Spica (1), Zingiberis Rhizoma (1)
Juncho-to (潤腸湯)	Rehmanniae Radix (6), Angelicae Radix (3), Scutellariae Radix (2), Aurantii Fructus Immaturus (2), Armeniacae Semen (2), Magnoliae Cortex (2), Rhei Rhizoma (2), Persicae Semen (2), Glycyrrhizae Radix (1.5), Cannabis Fructus (2)
Juzen-taiho-to (十全大補湯)	Astragali Radix (3), Cinnamomi Cortex (3), Rehmanniae Radix (3), Paeoniae Radix (3), Cnidii Rhizoma (3), Atractylodis Lanceae Rhizoma (3), Angelicae Radix (3), Ginseng Radix (3), Hoelen (3), Glycyrrhizae Radix (1.5)
Kakkon-to (葛根湯)	Puerariae Radix (8), Zingiberis Rhizoma (4), Zizyphi Fructus (4), Ephedrae Herba (4), Cinnamomi Cortex (3), Paeoniae Radix (3), Glycyrrhizae Radix (2)
Kami-shoyo-san (加味逍遥散)	Bupleuri Radix (3), Paeoniae Radix (3), Atractylodis Lanceae Rhizoma (3), Angelicae Radix (3), Hoelen (3), Gardeniae Fructus (2), Moutan Cortex (2), Glycyrrhizae Radix (1.5), Zingiberis Rhizoma (1), Menthae Herba (1)
Keishi-ka-ryukotsu-borei-to (桂枝加竜骨牡蠣湯)	Cinnamomi Cortex (4), Paeoniae Radix (4), Zizyphi Fructus (4), Ostreae Testa (3), Fossilia Ossis Mastodi (3), Glycyrrhizae Radix (2), Zingiberis Rhizoma (1.5)
Oren-gedoku-to (黄連解毒湯)	Coptidis Rhizoma (2), Phellodendri Cortex (1.5), Scutellariae Radix (3), Gardeniae Fructus (2)
Saikan-to (柴陥湯)	Bupleuri Radix (5), Pinelliae Tuber (5), Scutellariae Radix (3), Zizyphi Fructus (3), Ginseng Radix (2), Glycyrrhizae Radix (1.5), Zingiberis Rhizoma (1), Trichosanthis Semen (3), Coptidis Rhizoma (1.5)
Seihai-to (清肺湯)	Scutellariae Radix (2), Platycodi Radix (2), Mori Cortex (2), Armeniacae Semen (2), Gardeniae Fructus (2), Asparagi Radix (2), Fritillariae Bulbus (2), Aurantii Nobilis Pericarpium (2), Zizyphi Fructus (2), Bambusae Caulis (2), Hoelen (3), Angelicae Radix (3), Ophiopogonis Tuber (3), Schisandrae Fructus (1), Zingiberis Rhizoma (1), Glycyrrhizae Radix (1)
Seisho-ekki-to (清暑益気湯)	Atractylodis Lanceae Rhizoma (3.5), Ginseng Radix (3.5), Ophiopogonis Tuber (3.5), Astragali Radix (3), Aurantii Nobilis Pericarpium (3), Angelicae Radix (3), Phellodendri Cortex (1), Glycyrrhizae Radix (1), Schisandrae Fructus (1)
Shichimotsu-koka-to (七物降下湯)	Paeoniae Radix (4), Angelicae Radix (4), Astragali Radix (3), Rehmanniae Radix (3), Cnidii Rhizoma (3), Uncariae Uncis Cum Ramulus (3), Phellodendri Cortex (2)
Shimbu-to (真武湯)	Hoelen (4), Paeoniae Radix (3), Atractylodis Lanceae Rhizoma (3), Zingiberis Rhizoma (1.5), Aconiti Calefactum Tuber (0.5)
Shoma-kakkon-to (升麻葛根湯)	Puerariae Radix (5), Paeoniae Radix (3), Cimicifugae Rhizoma (2), Zingiberis Rhizoma (2), Glycyrrhizae Radix (1.5)

TABLE I. (continued)

Kampo prescription	Compounded crude drugs (g)
Sho-seiryu-to (小青竜湯)	Pinelliae Tuber (6), Glycyrrhizae Radix (2), Cinnamomi Cortex (3), Schisandrae Fructus (3), Asiasari Radix (3), Paeoniae Radix (3), Ephedrae Herba (3), Zingiberis Siccatum Rhizoma (3)
Toki-inshi (当帰飲子)	Angelicae Radix (5), Rehmanniae Radix (4), Paeoniae Radix (3), Cnidii Rhizoma (3), Saposhnikoviae Radix (3), Tribuli Fructus (3), Polygoni Multiflori Radix (2), Astragali Radix (1.5), Schizonepetae Spica (1.5), Glycyrrhizae Radix (1)
Toki-shakuyaku-san (当帰芍薬散)	Paeoniae Radix (4), Atractylodis Lanceae Rhizoma (4), Alismatis Rhizoma (4), Hoelen (4), Cnidii Rhizoma (3), Angelicae Radix (3)

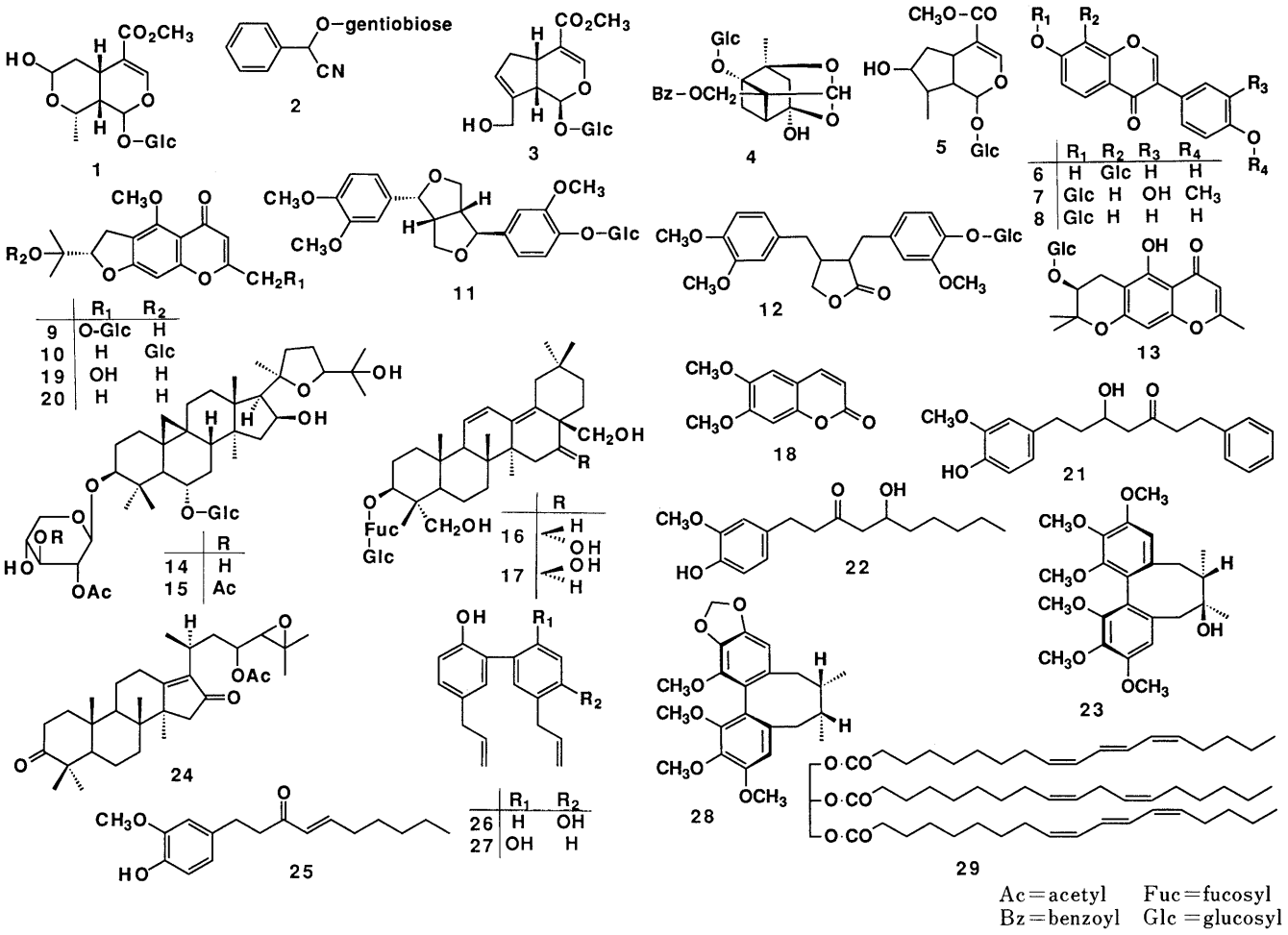

Chart 1

$\log k'$ of crude drug components and examined its relationship to the transfer ratio. The components assayed in this study were stable under the usual conditions for the preparation of decoctions.

Experimental

A. Measurement of the Quantity and Ratio of Crude Drug Components Transferred to Kampo Decoctions. Crude Drugs Crude drugs, other than Aconiti Calefactum Tuber, cut for dispensing were purchased from Uchida Wakanyaku, Co., Ltd. Aconiti Calefactum Tuber was purchased from Sanwa Shoyaku, Co., Ltd. Schizandrae Fructus was crushed in an iron mortar to break the seeds.

Kampo Prescriptions Kampo prescriptions were prepared as described by "The Guidebook for General Kampo Prescriptions[19]" (Table I). The compound ratios varied in some of the prescriptions.

Standard Compounds The following compounds used, except **18** which was synthesized, were isolated from each crude drug and spectrophotometrically identified or determined (Chart 1): Geniposide (**3**, Gardeniae Fructus), paeoniflorin (**4**, Paeoniae Radix), puerarin (**6**, Puerariae Radix), 7-glucosyloxy-3′-hydroxy-4′-methoxyisoflavone (**7**, Astragali Radix), *prim-O*-glucosylcimifugin (**9**, Saposhnikoviae Radix), 6,7-dimethylesculetin (**18**, Artemisiae Capillaris Spica), cimifugin (**19**, Saposhnikoviae Radix), 5-*O*-methylvisamminol (**20**, Saposhnikoviae Radix), 5-hydroxy-7-(4″-hydroxy-3″-methoxyphenyl)-1-phenylhept-3-one (**21**, Alpiniae Officinarum Rhizoma), [6]-gingerol (**22**, Zingiberis Rhizoma or Zingiberis Siccatum Rhizoma), schizandrin (**23**, Schisandrae Fructus), alisol C monoacetate (**24**, Alismatis Rhizoma), [6]-shogaol (**25**, Zingiberis Rhizoma; Zingiberis Siccatum Rhizoma), honokiol (**26**, Magnoliae Cortex), magnolol (**27**, Magnoliae Cortex), gomisin N (**28**, Schisandrae Fructus), and 1,3-ditrichosanoyl-2-linoleoylglycerol (**29**, Trichosanthis Semen).

Preparation of Decoctions The crude drugs in each prescription were placed in a 1-liter beaker and decocted with 600 ml water on an electric heater (National NK-685SG; 300—600 W) for 1—1.5 h until the volume was reduced to about 300 ml. For the investigation, the decoction was filtered through 2 layers of gauze while hot and adjusted, after cooling, to a volume of exactly 250 ml with water.

Preparation of Sample Solutions for HPLC Exactly 50 ml of each decoction following the addition of 50 ml butanol was evaporated to dryness under reduced pressure. The residue was extracted with 50 ml methanol for 30 min under reflux and filtered. This procedure was repeated again. The filtrates obtained were combined, concentrated under reduced pressure, and adjusted exactly to a predetermined volume with methanol and then subjected to HPLC analysis.

In the case of **3**, **4** and **6**, the decoctions were used directly for HPLC. Component **29** was extracted with a mixture of chloroform and methanol (1 : 1), and the volume was adjusted with the same mixed solvent.

Preparation of Standard Solutions for HPLC One-fifth of each pulverized crude drug in each kampo prescription was accurately measured. The components were extracted by refluxing with 50 ml methanol for 30 min and, after filtration, the residue was treated in a similar manner with 50 ml methanol. The extracts were combined, concentrated under reduced pressure, adjusted exactly to a predetermined volume with methanol and used as a standard solution for HPLC.

For the analysis of **29**, extraction was carried out using a mixture of chloroform and methanol (1 : 1) instead of methanol and the volume was adjusted with the same mixed solvent.

TABLE II. HPLC Conditions for Quantitative Analysis of Crude Drug Components in Kampo Decoctions

Component	Mobile phase	Detection
3	CH_3CN–CH_3OH–H_2O (1 : 1 : 9)	UV 254 nm
4	CH_3OH–H_2O (3 : 7)	UV 240 nm[a]
6	CH_3CN–H_2O (3 : 17)	UV 254 nm
7	THF–CH_3CN–CH_3OH–H_2O (1 : 1 : 1 : 30)	UV 254 nm[a,b]
9, **10**, **19**	CH_3CN–CH_3OH–1%CH_3CO_2H (1 : 1 : 7)	UV 254 nm[a]
18	THF–CH_3CN–H_2O (1 : 1 : 10)	UV 254 nm
21	CH_3CN–1%CH_3CO_2H (1 : 2)	UV 280 nm[a]
22, **25**	CH_3CN–CH_3OH–H_2O (1 : 1 : 2)	UV 280 nm[a]
23, **28**	CH_3CN–CH_3OH–H_2O (11 : 11 : 18) 8 min→(10 : 10 : 10)	UV 254 nm
24	THF–CH_3CN–CH_3OH–H_2O (1 : 1 : 1 : 4)	UV 254 nm
26, **27**	CH_3CN–CH_3OH–H_2O (2 : 4 : 3)	UV 254 nm
29	THF–CH_3CN–H_2O (5 : 5 : 1)	UV 254 nm

Equipment, ALC/GPC 244 (Waters); column, μ Bondapak C_{18} (10 μm, 3.9 mm i.d. × 30 cm, Waters); flow rate, 1 ml/min. *a*) Equipment, Trirotar III (JASCO). *b*) Column, Nucleosil 5C_{18} (5 μm, 4 mm i.d. × 15 cm, Sumitomo Chemicals).

HPLC Assay After passing sample and standard solutions for HPLC through a 0.45 μm filter, a fixed volume of each was subjected to HPLC. Table II shows the mobile phase and the detection wavelength used. The quantity (mg/daily dose, corresponding to daily dose) transferred to the decoction was determined by calculating the peak area of the component and comparing it with the absolute calibration curve. The transfer ratio (%) was determined by comparison of the peak area with that from the corresponding standard solution for HPLC.

B. Determination of log k' for Standard Compounds. Standard Compounds The following compounds were used to determine log k' (Chart 1). Morroniside (**1**), amygdalin (**2**), **3**, **4**, loganin (**5**), **6**, **7**, daidzin (**8**), **9**, 4′-*O*-β-D-glucosyl-5-*O*-methylvisamminol (**10**), phillyrin (**11**), arctiin (**12**), *sec*-*O*-glucosylhamaudol (**13**), astragaloside II (**14**), astragaloside I (**15**), saikosaponin b_2 (**16**), saikosaponin b_1 (**17**), **18**, **19**, **21**, **22**, **23**, **24**, **25**, **26**, **27**, **28**, and **29**.

HPLC Assay HPLC was performed using a μ Bondapak C_{18} column with acetonitrile as the mobile phase because of its low specificity for the characteristic chemical structures of the solutes.[16] To prevent direct interaction between the silanol residues of the solid phase and the solutes, 0.01 M ammonium acetate was added to the mobile phase.[17] The ratio between acetonitrile and 0.01 M ammonium acetate was varied by 5% (v/v) and the capacity factor (log k') was determined for each mobile phase. A differential refractometer was used to detect those components having no significant UV absorption, such as **14** and **15**. The HPLC conditions were as shown below;

Equipment, ALC/GPC 244 (Waters); column, μ Bondapak C_{18} (10 μm, 3.9 mm i.d. × 30 cm, Waters); detection, UV 254 nm; flow rate, 1 ml/min.

Calculation of Extrapolated log k' To estimate the HPLC capacity factor (log k') to be used as a hydrophobic parameter, part of the curves describing the log k' of various compounds was regarded as a quadratic curve over a limited range (Fig. 1) and the value in the case where the mobile phase contained no organic solvent was approximated by extrapolation using a quadratic regression equation (Table III). In the quadratic regression, data are adopted in the order of higher log k' so that the standard deviation may become 0.05 or less, and the values obtained are regarded as the extrapolated log k'. This argument does not mean that the elution behavior of these compounds is actually in accordance with this extrapolated log k' when water alone is used as the mobile phase.

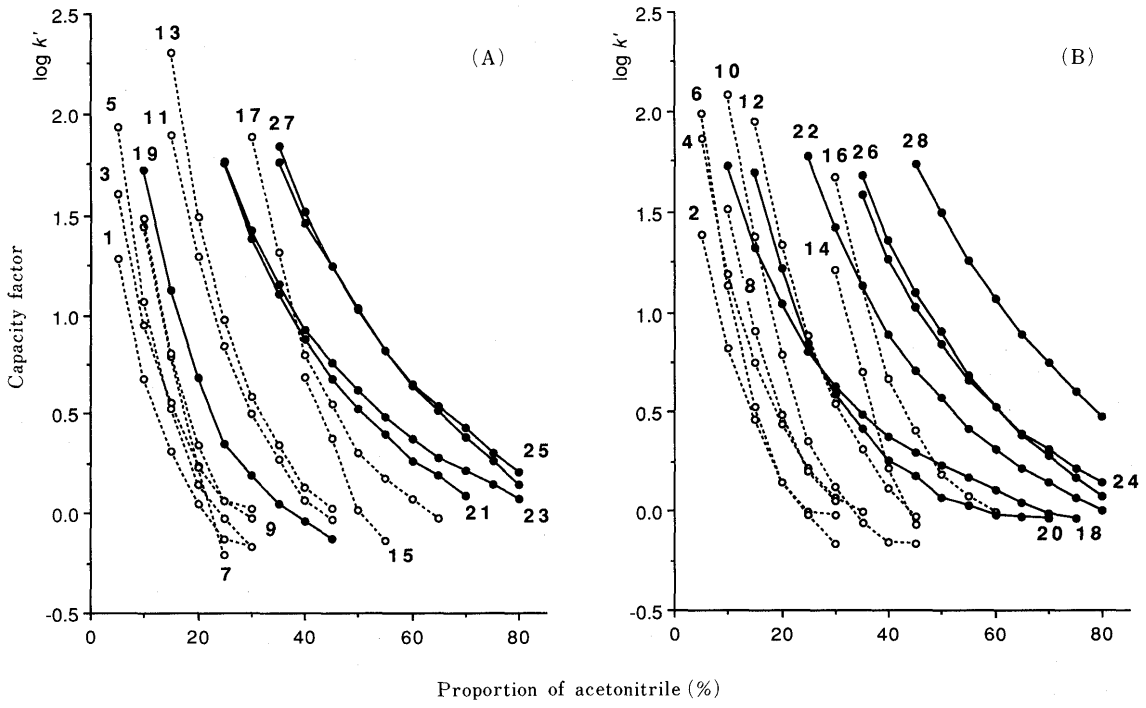

Fig. 1. Relationship between the Capacity Factor and the Proportion of Acetonitrile in the Mobile Phase

HPLC conditions: see Experimental. Component: ○, glycoside; ●, non-glycoside.

TABLE III. Extrapolated Capacity Factors of Crude Drug Components

Component	Quadratic equation[a]	Standard deviation	Adopted region[b]	Extrapolated $\log k'$
Glycoside				
1	$y=0.0025207x^2-0.14528x+1.9225$	0.027	5—30	1.92
2	$y=0.0025664x^2-0.14595x+2.0441$	0.017	5—30	2.04
3	$y=0.0028229x^2-0.16071x+2.3160$	0.024	5—30	2.32
4	$y=0.0021895x^2-0.14744x+2.4983$	0.038	5—35	2.50
5	$y=0.0045115x^2-0.23204x+2.9696$	0.022	5—25	2.97
6	$y=0.0046343x^2-0.23911x+3.0650$	0.005	5—25	3.07
7	$y=0.0022000x^2-0.18700x+3.0970$	0.003	10—25	3.10
8	$y=0.0030030x^2-0.19270x+3.1331$	0.005	10—30	3.13
9	$y=0.0039145x^2-0.23122x+3.3973$	0.005	10—30	3.40
10	$y=0.0029271x^2-0.21709x+3.9663$	0.005	10—30	3.97
11	$y=0.0024253x^2-0.20193x+4.3715$	0.007	15—35	4.37
12	$y=0.0024767x^2-0.20554x+4.4722$	0.008	15—35	4.47
13	$y=0.0026007x^2-0.21984x+4.8486$	0.005	20—35	4.85
14	$y=0.0015839x^2-0.20821x+6.0368$	0.039	30—55	6.04
15	$y=0.0015920x^2-0.20726x+6.4398$	0.033	40—55	6.44
16	$y=0.0022281x^2-0.25275x+7.2452$	0.017	30—55	7.25
17	$y=0.0023374x^2-0.26644x+7.7693$	0.031	30—55	7.77
Non-glycoside				
18	$y=0.0012357x^2-0.10439x+2.6343$	0.016	10—35	2.63
19	$y=0.0024471x^2-0.17570x+3.2225$	0.021	10—35	3.22
20	$y=0.0015395x^2-0.14159x+3.4502$	0.023	15—45	3.45
21	$y=0.0007183x^2-0.10270x+3.8464$	0.022	25—65	3.85
22	$y=0.0007128x^2-0.10192x+3.8526$	0.024	25—65	3.85
23	$y=0.0008629x^2-0.11101x+3.9934$	0.014	25—55	3.99
24	$y=0.0005828x^2-0.09717x+4.2438$	0.017	35—75	4.24
25	$y=0.0005758x^2-0.09821x+4.4859$	0.014	35—65	4.49
26	$y=0.0007288x^2-0.11441x+4.7831$	0.017	35—55	4.78
27	$y=0.0008058x^2-0.12301x+5.1537$	0.013	35—55	5.15
28	$y=0.0005377x^2-0.10128x+5.2064$	0.005	45—70	5.21

a) x, acetonitrile/0.01 M ammonium acetate % (v/v); y, $\log k'$. *b*) Proportion of acetonitrile to 0.01 M ammonium acetate in mobile phase.

Results and Discussion

The Quantities and Ratios of the Components Transferred to Kampo Decoctions The quantity (mg/daily dose) of each crude drug component transferred to the decoction from the daily doses of various kampo prescriptions and the transfer ratio (%) were examined (Fig. 2). The quantities of components transferred showed large variations among prescriptions. However, no notable differences in the transfer ratio were found among the components tested. For example, while the quantities of **18** transferred in Inchinko-to and Inchin-gorei-san was 6.4 and 61.5 mg/daily dose, respectively, a 10-fold difference, the transfer ratio was 56.8—77.8%. The quantities of **27** transferred in Goshaku-san, Juncho-to, Hange-koboku-to and Heii-san covered a 4-fold range from 0.95 to 4.06 mg/daily dose, but they had a similar transfer ratio, 7.1—10.1%. Among the glycosides, the quantities transferred were high in **4** and **6** but low in **7**, while the transfer ratio of these components remained within a narrow range. Therefore, under the present decocting conditions, the transfer of the components to decoctions was regulated by some properties related to the transfer ratio and had no relationship with the contents of components or the combination ratio of the crude drugs in these preparations.

Elution Behavior of Crude Drug Components in Reverse-Phase HPLC The elution behavior of each crude drug component in reverse-phase HPLC is shown by the relationship between the composition of the mobile phase and the capacity factor (Fig. 1). The elution behavior and slope of the quadratic curve from the estimated extrapolated $\log k'$ were similar for all the glycosides tested (Table III). For non-glycosides, the slope of the quadratic curve was generally smaller than that for the glycosides and a change in composition had little effect on the capacity factor. These findings suggest that the relationship between the transfer ratio and extrapolated $\log k'$ must be evaluated separately for glycosides and non-glycosides.

Correlation between the Transfer Ratio and the Hydrophobic Parameter The relationship between the transfer ratios of various crude drug components in each kampo decoction and the extrapolated $\log k'$ as a substitute for the hydrophobic parameter of these components, was examined (Fig. 3). In the case of the glycosides, the transfer ratio decreased slightly with an increase in the hydrophobicity of the component. In the case of the non-glycosides, also, the transfer ratio clearly decreased with an increase in the hydrophobicity of the component, suggesting a relationship between the transfer ratio and extrapolated $\log k'$. The relationship was not markedly reduced even in highly hydrophobic non-glycosides such as **27** and **28**, and the transfer ratio was 0.8% even in the case of **29**, an oily fat, which is too hydrophobic for its extrapolated $\log k'$ to be determined. Therefore, these observations suggest that the relationship cannot be expressed as a simple linear function but is more complex in nature.

This study showed that the transfer of stable crude drug components (organic compounds), unaffected by factors

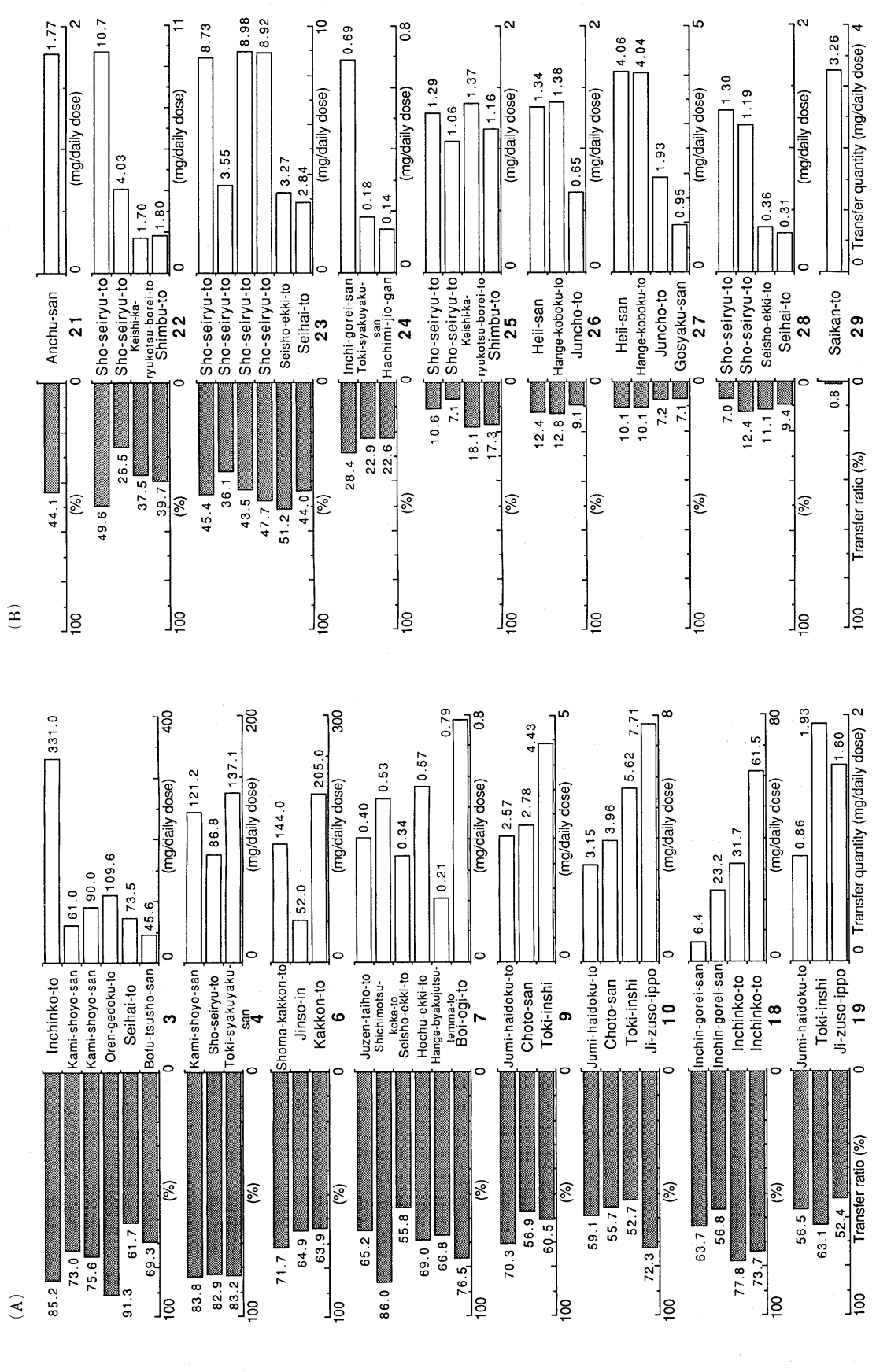

Fig. 2. Quantities and Transfer Ratios of Crude Drug Components transferred to Kampo Decoctions

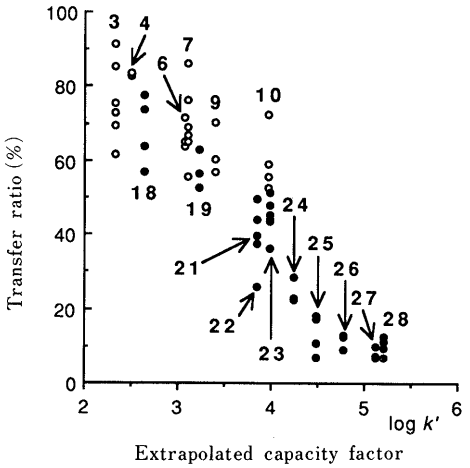

Fig. 3. Relationship between the Extrapolated Capacity Factors of Crude Drug Components and Their Transfer Ratios to Kampo Decoctions

Symbols are the same as in Fig. 1.

such as chemical changes, evaporation and pH, is regulated primarily by hydrophobicity as for as the physicochemical properties of the components are concerned and this relate to the ratio rather than the quantity transferred to the kampo decoction. Therefore, the transfer of crude drug components into decoctions may be estimated by evaluating the hydrophobic parameter of the components concerned.

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References

1) K. Takaishi, Y. Torii, *Yakugaku Zasshi*, **89**, 538 (1969); K. Takaishi, Y. Watanabe, *ibid.*, **91**, 1092 (1971).
2) N. Yata, O. Tanaka, *J. Traditional Sino-Japan. Med.*, **4**, 68 (1983).
3) S. Tashiro, Y. Akazawa, *J. Med. Pharm. Soc. WAKAN-YAKU*, **1987**, 476.
4) M. Noguchi, M. Kubo, Y. Naka, *Yakugaku Zasshi*, **98**, 923 (1978).
5) T. Tomimori, M. Yoshimoto, *Shoyakugaku Zasshi*, **34**, 138 (1980).
6) S. Arichi, T. Tani, M. Kubo, *Med. J. Kinki Univ.*, **4**, 59 (1979).
7) A. Yamaji, Y. Maeda, M. Oishi, Y. Hirotani, H. Kishi, E. Hiraoka, K. Yoneda, *Yakugaku Zasshi*, **104**, 812 (1984).
8) M. Noguchi, M. Kubo, T. Hayashi, M. Ono, *Shoyakugaku Zasshi*, **32**, 104 (1978).
9) T. Okuda, K. Mori, M. Shioda, *Yakugaku Zasshi*, **104**, 854 (1984).
10) Y. Hiraga, K. Hosoyama, K. Takahashi, S. Shibata, *Shoyakugaku Zasshi*, **33**, 38 (1979).
11) Y. Kano, K. Saito, T. Sakurai, S. Kanemaki, M. Tanabe, M. Yasuda, *Shoyakugaku Zasshi*, **40**, 333 (1986); T.-K. Huang, *Chinese Traditional Herbal Drugs*, **17**, 323 (1986); A. Akahori, K. Kagawa, Abstracts of Papers, Proc. Symp. WAKAN-YAKU 10, Toyama, August 1977, p. 61.
12) I. Imazeki, H. Taguchi, K. Nakajima, M. Aburada, S. Takeda, "Shoyaku-Bunseki No Giho," ed. by M. Noguchi, Osaka Shoyaku Kyokai, Osaka, 1980, pp. 142—156; H. Taguchi, T. Endo, K. Nakajima, M. Aburada, Abstracts of Papers, Proc. Symp. WAKAN-YAKU 9, Toyama, August 1975, p. 85.
13) M. Noguchi, *J. Traditional Sino-Japan. Med.*, **3**, 102 (1982).
14) Y. C. Martin, "Quantitative Drug Design," translated by T. Esaki, Chijin Shokan, Tokyo, 1980, pp. 48—53.
15) T. Kubota, M. Yamakawa, H. Terada, M. Yoshimoto, "Kagaku No Ryoiki, Extra No.122: Structure-Activity Relationships," ed. by the Conversazione of Structure-Activity Relationships, Nankodo, Tokyo, 1979, pp. 73—94; H. Terada, Z. Taira, "Kagaku No Ryoiki, Extra No. 136: Structure-Activity Relationships. II," ed. by the Conversazione of Structure-Activity Relationships, Nankodo, Tokyo, 1982, pp. 18—24.
16) T. Hanai, "Chromatography Separation System," ed. by S. Hara, S. Mori, T. Hanai, Maruzen, Tokyo, 1981, pp. 136—149.
17) T. Yamana, A. Tsuji, "LC Family," Vol. 15, Japan Spectroscopic Co., Ltd., Tokyo, 1980, pp. 4—6; T. Yamana, A. Tsuji, E. Miyamoto, O. Kubo, *J. Pharm. Sci.*, **66**, 747 (1977).
18) M. Morita, H. Nakanishi, S. Mihashi, H. Itokawa, K. Kawahara, Abstracts of Papers, The 4th Symposium on the Naturally Occurring Drug Materials, Osaka, July 1982, pp. 25—27.
19) Supervision by the Pharmaceutical and Supply Bureau of the Welfare Ministry of Japan (ed.), "Ippanyo Kampo Syoho No Tebiki," Yakugyo Jihosya, Tokyo, 1975.