

Inorganic Chemical Approaches to Pharmacognosy. VI.¹⁾ X-Ray Fluorescence Spectrometric Studies on the Inorganic Constituents of Crude Drugs. (4): *Coptidis Rhizoma* and *Phellodendri Cortex*

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Inorganic constituents of many *Coptidis Rhizoma* and *Phellodendri Cortex* (28 and 37 samples, respectively; almost all obtained commercially in Osaka market) were investigated using energy-dispersive X-ray fluorescence spectrometry.

The results can be summarized as follows:

(1) *Coptidis Rhizoma* contained Zn, Mn, Cu, and S at higher levels, and Ca and Sr at lower levels than those of *Phellodendri Cortex*. The analysis of Zn and Ca contents provided almost perfect distinction between the two drugs.

(2) *Coptidis Rhizoma* contained Zn at high levels, and the contents of Zn, Mn, and Rb were dependent on the producing district (Japan or China).

(3) The metals profile of each crude drug provides valuable information regarding the identification of not only the kind of drug but also the producing district or the botanical origin.

Keywords energy-dispersive X-ray fluorescence spectrometry; crude drug; multi-elemental analysis; inorganic constituent; metals profile; *Coptidis Rhizoma*; *Phellodendri Cortex*; identification; producing district; zinc

In spite of the importance of inorganic elements for human health, only a few studies²⁻⁴⁾ have been conducted on the inorganic constituents of crude drugs, in contrast to their organic constituents. We have elucidated the inorganic constituents of several crude drugs using X-ray fluorescence spectrometry,^{1,5-8)} which is a very powerful tool for the multi-elemental analysis of many samples. In the course of our studies, it has been demonstrated that metal profiles provide valuable information about not only the kind of crude drug but also the producing district or botanical origin.^{1,5,6)}

In this work, we have dealt with *Coptidis Rhizoma* (黄連) and *Phellodendri Cortex* (黄柏), which contain berberine type alkaloids as principal components. Both these crude drugs exhibit obviously different metal profiles, with typical extreme distinctions being a high Zn-content for *Coptidis Rhizoma* and a high Ca-content for *Phellodendri Cortex*. The analysis of these element-concentrations can provide almost perfect identification of these two drugs. In addition, the relationship between metals profiles and producing districts is described here.

Experimental

Apparatus X-Ray measurements were performed on a thin sample (48 mg/cm²) with a Rigaku-KeveX energy-dispersive X-ray spectrometer (ultra-trace system), consisting of a molybdenum anode X-ray tube, secondary targets (Ti, Ge, Mo, and Gd) and a filter assembly used to generate bichromatic radiation, a 30 mm² × 3 mm Si(Li) detector, an X-ray amplifier, and a conventional multi-channel analyzer.

The high-performance gel chromatographic analysis was carried out on a Waters ALC/GPC 206D high-performance liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.) equipped with TSK G3000PW column (7.5 mm i.d. × 65 cm). Zn concentration was detected by an atomic absorption spectrometer (Shimadzu model AA-670) with a zinc hollow-cathode lamp (at 213.9 nm).

Materials The crude drug samples were provided by Mikuni Co., Ltd., Koshiro Chuji Co., Ltd., Nihon Funmatsu Yakuhin Co., Ltd., and Shinwa Bussan Co., Ltd. (Osaka). The *Coptidis Rhizoma* samples [sample number (*n*)=28] consisted of those (*n*=24) for which the country of production was indicated clearly and those of unknown origin (*n*=4). The former samples were divided into Japanese (*n*=11), Chinese (*n*=10), and one sample each from Burma, Thailand, and Hong Kong. The producing countries of Moh-Ohren (毛黃連) samples (*n*=3) were unknown. The *Phellodendri Cortex* samples (*n*=37) consisted of those (*n*=32) for which the country of production was clearly indicated and those of unknown origin (*n*=5). The former were divided into Japanese (*n*=18), Chinese

(*n*=9), Korean (*n*=2), Hong Kong products (*n*=2), and Taiwanese. NBS orchard leaves (SRM 1571), one of the NBS standard reference materials, was purchased from National Bureau of Standards (Washington, D.C., U.S.A.). All other reagents were of the highest quality available.

Procedures Sample preparation and X-ray fluorescence multi-element analysis (P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, As, Br, Rb, Sr, Pb, Mo, I, and Ba) were carried out according to the previous procedures.⁵⁾ The experiments on water-soluble elements in the two crude drugs were performed the manner reported previously.⁶⁾

The sample solution of *Coptidis Rhizoma* for high-performance gel chromatography was carried out as follows: 2 g of powdered *Coptidis Rhizoma* was extracted with 200 ml of distilled water for 3 h at 50 °C, and after filtration the resulting solution was evaporated to near dryness *in vacuo*. The residue was dissolved in 3 ml of 0.2M NaCl solution. After filtration with a membrane filter (0.45 μm), 100 μl of the solution was injected to the column with a microsyringe.

Results and Discussion

Analytical Results Japanese *Coptidis Rhizoma* is the rhizoma of *Coptis japonica* (Ranunculaceae), and the Chinese product originates from the rhizoma of *Coptis chinensis*, *C. deltoidea*, *C. teetoides*, etc. *Phellodendri Cortex* is the bark of *Phellodendron amurense* (Rutaceae). Both crude drugs contain berberine type alkaloids. In the powdered state, these bitter and yellow powders are similar to each other but distinguishable by the existence of mucous, through microscopic examination⁹⁾ and by other means.

The analytical results for *Coptidis Rhizoma* (sample number, *n*=28), Moh-Ohren (hair root of *C. japonica*, *n*=3), and *Phellodendri Cortex* (*n*=37) are summarized in Table I. The results for *Coptidis Rhizoma* and *Phellodendri Cortex* were compared with those of NBS orchard leaves (SRM 1571) (Fig. 1). In contrast to *Glycyrrhizae Radix*⁶⁾ and *Cinnamomi Cortex*,¹⁾ only Br varied considerably, while the other elements deviated within reasonable narrow ranges. It was therefore not difficult to determine the characteristic metals profile for each crude drug (*C. Rhizoma* or *P. Cortex*). *Coptidis Rhizoma* contained higher levels of S, Mn, and Zn and the levels of Ca, Sr, and K were lower than those of orchard leaves. *Phellodendri Cortex* contained Ca and Ba at higher levels, but K, S, Cl, Fe, Mn, Cu etc. at lower levels. Appreciable differences in the metals profile between the two drugs were as follows: *Phellodendri Cortex* contained higher levels of Ca and Sr

TABLE I. Analytical Results (ppm) for Coptidis Rhizoma and Phellodendri Cortex by X-Ray Fluorescence Spectrometry

Element	Coptidis Rhizoma				Phellodendri Cortex		
	Total (n=28)	Japanese (n=11)	Chinese (n=10)	Moh-Ohren (n=3)	Total (n=37)	Japanese (n=18)	Chinese (n=9)
P	0.16 (6)%	0.15 (6)%	0.15 (3)%	900 (900)	0.21 (4)%	0.21 (4)%	0.22 (3)%
S	0.30 (13)%	0.23 (4)%	0.37 (15)%	0.22 (4)%	0.10 (4)%	0.10 (2)%	0.10 (2)%
Cl	500 (400)	500 (200)	400 (600)	700 (300)	400 (200)	350 (200)	420 (170)
K	0.47 (16)%	0.40 (16)%	0.51 (13)%	0.82 (9)%	0.52 (15)%	0.52 (16)%	0.59 (11)%
Ca	0.30 (8)%	0.29 (5)%	0.27 (7)%	0.5 (2)%	2.5 (6)%	2.5 (6)%	2.6 (3)%
Ti	50 (40)	50 (30)	40 (20)	170 (120)	40 (20)	50 (20)	33 (13)
Cr	8 (8)	5 (3)	7 (3)	11 (9)	3 (2)	2 (2)	4 (2)
Mn	170 (89)	210 (80)	110 (60)	160 (20)	16 (7)	16 (8)	15 (7)
Fe	500 (500)	400 (400)	400 (200)	0.3 (2)%	80 (60)	60 (30)	100 (40)
Ni	ND	ND	ND	ND	ND	ND	ND
Cu	19 (7)	16 (7)	22 (7)	13 (3)	5 (2)	5 (1)	5 (2)
Zn	500 (300)	700 (300)	230 (140)	160 (30)	5 (8)	5 (8)	4 (2)
As	ND	ND	ND	3 (2)	ND	ND	ND
Br	70 (130)	4 (3)	160 (150)	30 (20)	20 (50)	ND	40 (70)
Pb	8 (9)	10 (9)	2 (2)	20 (20)	2 (3)	1 (2)	2 (3)
Rb	18 (16)	9 (4)	24 (16)	30 (10)	11 (6)	11 (4)	12 (10)
Sr	28 (10)	29 (9)	21 (5)	56 (26)	170 (70)	190 (70)	150 (70)
Mo	ND	ND	ND	ND	ND	ND	ND
I	ND	ND	ND	ND	ND	ND	ND
Ba	70 (50)	80 (60)	30 (18)	160 (130)	140 (80)	160 (90)	90 (40)

Standard deviations are given in parentheses. ND: not detected.

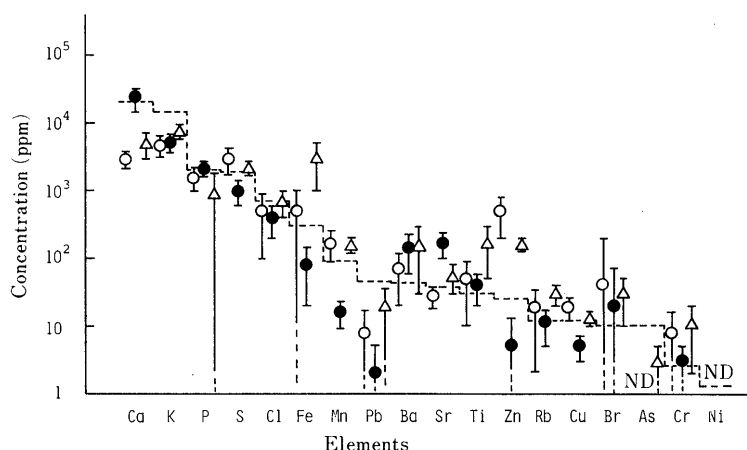


Fig. 1. Analytical Results for Coptidis Rhizoma and Phellodendri Cortex by X-Ray Fluorescence Spectrometry
 -----, orchard leaves; ○, Coptidis Rhizoma (n=28); ●, Phellodendri Cortex (n=37); △, Moh-Ohren (n=3).

and lower levels of S, Mn, Zn, and Cu than Coptidis Rhizoma. These features thus seem to be the respective characteristics of the metals profiles of these two groups. The large difference in Ca content is consistent with the anatomical observation that Phellodendri Cortex contains numerous plate-like crystals of calcium oxalate in its crystal fibers, while Coptidis Rhizoma contains only a limited amount of small crystals of calcium oxalate. Itokawa *et al.*²⁾ and Matsuda *et al.*³⁾ also examined the contents of several metals in these crude drugs (sample number: 1 or 2) by atomic absorption spectrometry. Nine elements (K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, and Pb) among those they tested were common with those tested in our study. The analytical values of the nine elements seem to be in reasonable agreement with the present results (Table I).

Application of X-Ray Fluorescence Spectrometry to the Identification of Coptidis Rhizoma or Phellodendri Cortex We have already demonstrated that each crude drug

has a characteristic metals profile, although the concentrations of most of the elements in each drug vary considerably among individual samples.^{5,6)} Figure 2 shows the relationship between Ca and Zn contents in Coptidis Rhizoma and Phellodendri Cortex. It is interesting to note that all the points of each crude drug are located in non-coincident pseudo-ellipsoids, suggesting that the analytical values of the two elements can provide valuable information for the identification of the two drugs. The each relationship in concentration between S and Sr or Cu and Mn, elements whose contents seem to differ from each other, is shown in Figs. 3 and 4, respectively. In these cases, most of the points of each crude drug were apparently distributed in independent regions. The difference in contents of these elements can provide more definite identification of the two drugs. In addition to the existence or lack of mucous, and microscopic examination (anatomical characters),⁹⁾ analysis of the metals profile is thus a

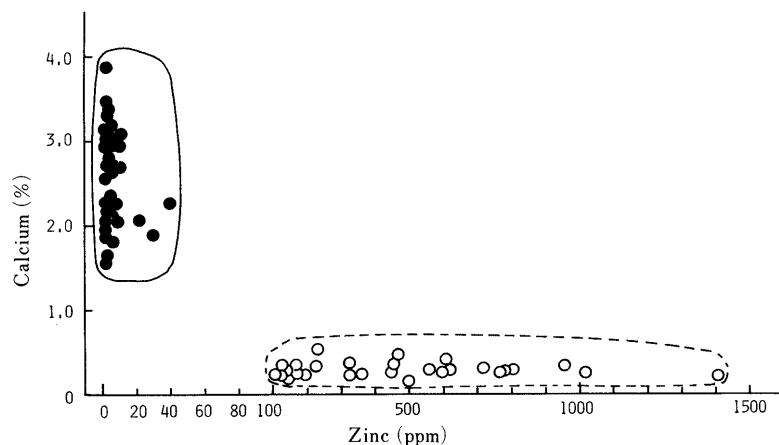


Fig. 2. Relationship between Calcium and Zinc Contents in Coptidis Rhizoma and Phellodendri Cortex
○, Coptidis Rhizoma; ●, Phellodendri Cortex.

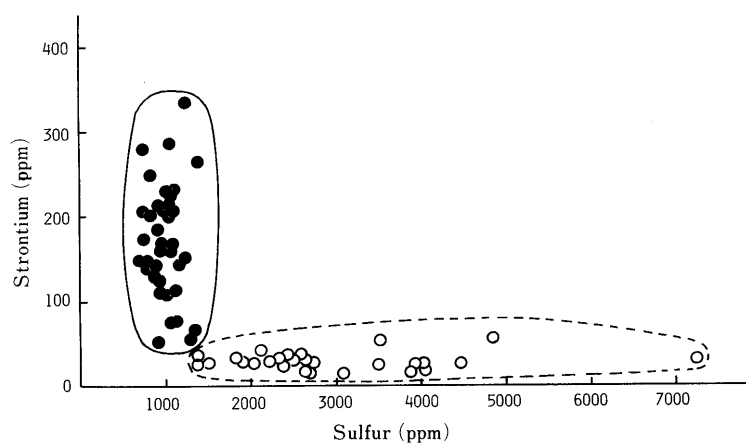


Fig. 3. Relationship between Strontium and Sulfur Contents in Coptidis Rhizoma and Phellodendri Cortex
○, Coptidis Rhizoma; ●, Phellodendri Cortex.

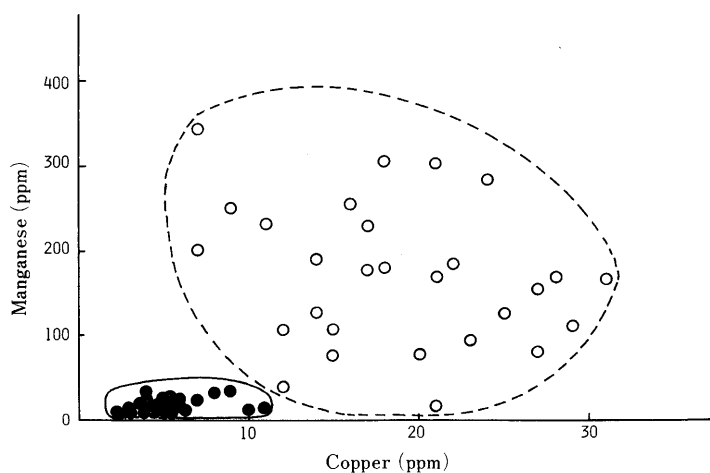


Fig. 4. Relationship between Manganese and Copper Contents in Coptidis Rhizoma and Phellodendri Cortex
○, Coptidis Rhizoma; ●, Phellodendri Cortex.

useful method of identification. A comparison of Coptidis Rhizoma with Moh-Ohren (hair-root) revealed that Moh-Ohren contained higher levels of K and Fe but a lower level of Zn than did Coptidis Rhizoma. In this case, however, it is difficult to rule out the possibility of soil contamination.

Since a judgement of the original plant of a crude drug

or the producing district is important from the standpoint of evaluation of the drug, the metals profiles of Japanese and Chinese Coptidis Rhizoma were compared (Fig. 5): significant difference between them was observed. Figures 6 and 7 show the relationships between Zn and Rb, and Zn and Mn contents, respectively, with regard to the two

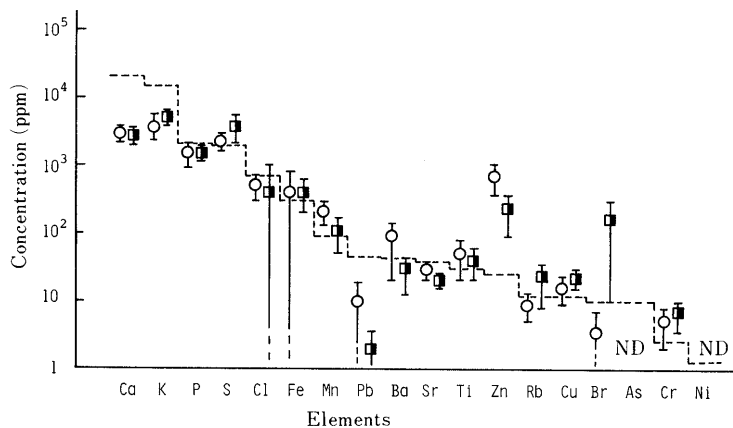


Fig. 5. Analytical Results for Coptidis Rhizoma by X-Ray Fluorescence Spectrometry

○, Japanese products; ■, Chinese products.

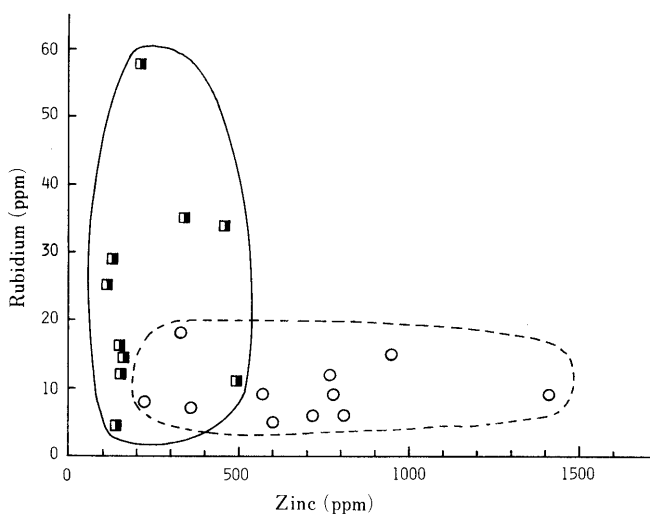


Fig. 6. Relationship between Rubidium and Zinc Contents in Coptidis Rhizoma

○, Japanese products; ■, Chinese products.

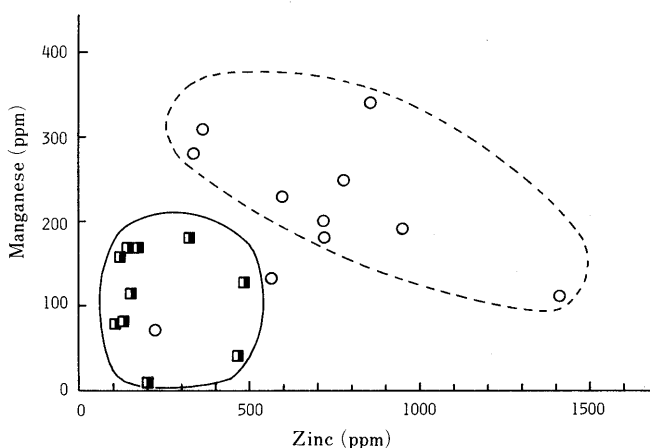


Fig. 7. Relationship between Manganese and Zinc Contents in Coptidis Rhizoma

○, Japanese products; ■, Chinese products.

producing countries. Zn contents ranged from 100 to 500 ppm in Chinese products and from 200 to 1500 ppm in Japanese products. Rb concentrations of the Chinese products ranged from 5 to 60 ppm, while the concentrations

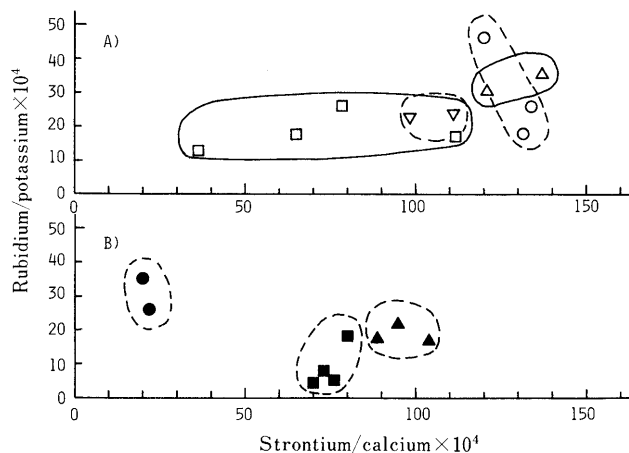


Fig. 8. Relationship between Rubidium/Potassium and Strontium/Calcium in Coptidis Rhizoma and Phellodendri Cortex

A), Coptidis Rhizoma; B), Phellodendri Cortex. □, Hyogo Prefecture; ▽, Niigata Prefecture; △, Fukui Prefecture; ○, Tottori Prefecture; ●, 湖北省 (Hupei Province); ■, 黒竜江省 (Heilung Chiang Province); ▲, Hokkaido Prefecture.

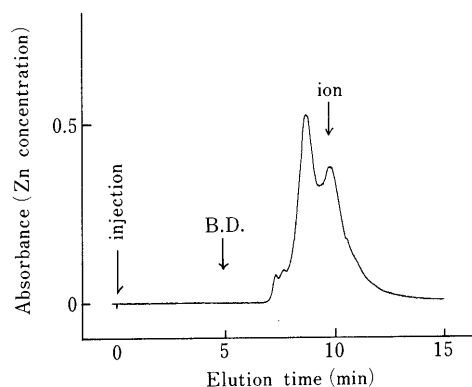
of the Japanese were less than 20 ppm. Mn contents ranged from 10 to 180 ppm for the Chinese, and from 100 to 350 ppm for the Japanese. Figure 7 indicates clearly that the analysis of Zn and Mn for Coptidis Rhizoma makes it possible to distinguish these crude drugs from each other.

On the other hand, no significant difference was observed between Chinese and Japanese products in Phellodendri Cortex. A detailed study of the analytical results, however, determined that the producing district was closely reflected by Rb/K and Sr/Ca. Figure 8 shows the relationship between Rb/K and Sr/Ca for the samples whose district was known: as seen, the points for Rb/K and Sr/Ca are located in the same region. In general, the physicochemical properties of Rb and Sr are similar to those of K and Ca, respectively.¹⁰⁾ So, the values of these two ratios of a crude drug are considered to reflect the inorganic constitution of the soil on which the original plant was grown. The results shown in Fig. 8 clearly indicate that Rb/K and Sr/Ca provide detailed information on the districts of these crude drugs, namely, the prefecture in Japan or the province in China. Because the organic constituents are not useful in making an exact identification of a district and the metals profile is thought to be directly related to the inorganic constitution

TABLE II. Elution of Inorganic Elements from *Coptidis Rhizoma* or *Phellodendri Cortex*

Element	<i>Coptidis Rhizoma</i>			<i>Phellodendri Cortex</i>		
	Amount (μg)		Elution ratio (%)	Amount (μg)		Elution ratio (%)
	Origin (1 g)	Extract		Origin (1 g)	Extract	
P	1500	1200	80	1150	ND	—
S	2500	900	36	600	150	25
Cl	320	300	94	230	160	70
K	3300	3200	97	3800	3700	97
Ca	3000	1500	50	19600	6200	32
Ti	30	8	27	14	ND	—
Cr	3	1	33	1	0.5	50
Mn	190	130	68	17	8.4	50
Fe	210	36	17	23	ND	—
Ni	ND	ND	—	ND	ND	—
Cu	11	5	45	4.2	1.9	45
Zn	870	400	46	3.8	2.1	55
As	2	1	50	ND	ND	—
Br	ND	ND	—	ND	ND	—
Rb	11	9	82	9	8.5	95
Sr	37	10	27	170	47	28
Pb	15	ND	—	1	ND	—
Mo	ND	ND	—	ND	ND	—
I	ND	ND	—	ND	ND	—
Ba	65	5	8	130	67	52

ND: not detected.

Fig. 9. High-Performance Gel Chromatogram of the Aqueous Extract of *Coptidis Rhizoma*

B.D., peak position of blue dextran; ion, peak position of Zn ion (as $\text{Zn}(\text{NO}_3)_2$). Column, TSK G3000PW (7.5 mm i.d. \times 65 cm); Mobile phase, 0.2M NaCl aqueous solution; Flow rate, 2.0 ml/min; Detection, Zn concentration (atomic absorption spectrometer; at 213.9 nm).

of the producing soil, the inorganic constituents of a crude drug are important factors in determining the producing district.

Elution of Inorganic Elements from *Coptidis Rhizoma* and *Phellodendri Cortex* Water-soluble elements are important in understanding the biological effect of inorganic constituents of crude drugs. The analytical results for the original crude drugs and their aqueous extracts are shown in Table II. For *Coptidis Rhizoma* K, Cl, P, and Rb were eluted easily, whereas Fe, Sr, Ba, and Ti dissolved only slightly in water. Thus, major inorganic elements in the extract were: $\text{K} > \text{Ca} > \text{P} > \text{Zn}$. In *Phellodendri Cortex*, K, Cl, and Rb were eluted easily, while Fe, Ti, Sr, S, and Ca dissolved only slightly in water. Because Ca is known to exist predominantly as insoluble crystals of calcium oxalate in

Phellodendri Cortex, a low Ca elution ratio can be predicted. In fact, the ratio (32%) for *Phellodendri Cortex* was lower than that (50%) for *Coptidis Rhizoma*, although a larger amount of Ca in the former actually eluted with water. The major inorganic elements in the extract of *Phellodendri Cortex* were: $\text{Ca} > \text{K} \gg \text{S} \approx \text{Cl}$. At present, it is difficult to discuss the relationship between these inorganic components and the pharmaceutical effects of the crude drugs. However, it should be emphasized that one feature of the *Coptidis Rhizoma* extract is its extremely high Zn-content, because to date only a limited number of crude drugs have been reported as having this, for instance, *Cardamomi Fructus* (590 ppm), *Zedoariae Rhizoma* (259 ppm), and *Geranii Herba* (215 ppm).²⁾ In animal crude drugs, *Bezoar Bovis* has also been indicated to contain Zn at very high levels of 1000–2000 ppm.¹¹⁾

One of the essential elements, Zn is contained in many metalloenzymes and plays an important role in mammals. Zn deficiency results in growth retardation, impaired appetite, alopecia and gross skin lesions, and impaired development of the primary and secondary sex organs in males.¹²⁾ An optimal supplement of Zn may counteract these symptoms.

In general foods, the richest sources of Zn are oysters (which may contain on the order of 1000 ppm) and to a lesser extent other seafoods and muscle meats and nuts, which usually contain within the range of 30–50 ppm Zn in a single edible portion. The poorest sources are white sugar, pome and citrus fruits, nonleafy vegetables and tubers, and vegetable oils, which generally contain less than 1 ppm Zn.¹³⁾ Therefore, *Coptidis Rhizoma* is certainly a unique, high Zn-containing vegetable drug.

To elucidate the fundamental property of the Zn-containing component, high-performance gel chromatography was performed. Figure 9 shows a chromatogram obtained by the combination of high-performance liquid chromatography with TSK PW3000 column and atomic absorption spectrometry as Zn-concentration monitor. This high-resolution chromatogram indicates that there are multiple Zn-containing components, namely, two small components (molecular weight (M.W.): ca. 3000) and one major component (M.W.: ca. 1500). A third peak is in the same position as the retention time of free Zn ion (as $\text{Zn}(\text{NO}_3)_2$). Since no peak was observed in the rechromatography of the main peak, the Zn-containing component was considered to be an unstable compound, in other words, a certain organic compound coordinates to the Zn ion with weak affinity. Further detailed investigation will be needed to determine the Zn-containing component in *Coptidis Rhizoma*.

Conclusions

Coptidis Rhizoma is a unique, high Zn-containing vegetable drug. The experiment of high-performance gel chromatography has suggested that the Zn-containing compound in *Coptidis Rhizoma* is considerably unstable. *Coptidis Rhizoma* and *Phellodendri Cortex* were distinguishable from each other by their metals profile: *Phellodendri Cortex* contains higher levels of Ca and Sr and lower levels of Zn, Mn, S, and Cu than *Coptidis Rhizoma*. In *Coptidis Rhizoma*, a significant difference in content of Zn, Mn, and Rb was observed between Japanese

and Chinese products. Furthermore, the values of Sr/Ca and Rb/K have been suggested to be a good indication of the producing district.

General relationships between metals profile and species, genus, family, portion used, or type of soil on which plants are grown are under investigation.

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