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Inorganic Chemical Approaches to Pharmacognosy. II.[1] X-Ray Fluorescence Spectrometric Studies on the Inorganic Constituents of Crude Drugs. (1). On the Licorice Root

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Inorganic constituents of many licorice roots (55 samples; almost all obtained commercially in Osaka market) were investigated using energy-dispersive X-ray fluorescence spectrometry. The results can be summarized as follows:

(1) Most of the elemental contents of each licorice root showed considerable variation.

(2) Many licorice roots contained Sr at a high level, and the contents of Sr and K were dependent on the geographical source of the root, *i.e.*, the producing district (Seihoku or Tohoku).

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

Keywords——energy-dispersive X-ray fluorescence spectrometry; crude drug; multi-elemental analysis; inorganic component; metal-contents profile; licorice root; identification; producing district

Humans and other living things require a well-balanced supply of many metal ions to maintain proper growth. The interaction of biomolecules with these metals has been an active area for research in biochemistry. Recently, it has been demonstrated that certain kinds of platinum complexes, *e.g.*, *cis*-dichlorodiamine platinum (II), have antitumor activity,[2] and that some transition metal ions play an important role in the activation of antibiotics such as bleomycin.[3] Furthermore, it has been shown that acrodermatitis enteropathica, a zinc deficiency disease, usually occurs when infants are weaned from human breast milk to cow's milk because the zinc in human milk is present in a form different from that found in cow's milk.[4] Metal ions or metal-containing compounds are theorefore important in the pharmaceutical field.

Metal constituents in crude drugs may also be important, because the presence of various metal ions is a feature of crude drugs (that originate from natural products), as compared to general drugs. In our previous paper,[1] we reported on a convenient multi-elemental analysis method for crude drugs using energy-dispersive X-ray fluorescence spectrometry. Each crude drug seems to have a characteristic metals profile on the basis of the analytical results for several kinds of crude drugs.

In the course of this series of studies to elucidate the significance of inorganic components in crude drugs, we first began research on the inorganic components of licorice root (Glycyrrhizae Radix), which is a well-known and very important crude drug in traditional oriental medicine, and is also contained in a large number of herbal medicines (the kanpo preparations). Many samples were analyzed in detail, and it was ascertained that licorice root has a characteristic metals profile, though the concentrations of elements varied considerably from sample to sample. The results also provide valuable information on the differences in metals profiles among the products from different districts or different original plants.

Experimental

Apparatus——X-Ray measurements were performed on a thin sample (48 mg/cm^2) 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 mm^2 × 3 mm Si (Li) detector, an X-ray amplifier, and a conventional multi-channel analyzer.

Materials——The crude drug samples were kindly provided by Mikuni Co., Ltd., Koshiro Chuji Co., Ltd., Nihon Funmatsu Co., Ltd., and Shinwa Bussan Co., Ltd. (Osaka). The licorice samples [sample number (n)=55] consisted of those (n=50) for which the region of production was indicated clearly and those of unknown origin (n=5). The former ones were divided into bark-less samples (n=6) and normal ones (n=44), and the normal samples were subdivided into Chinese (n=39) and others (n=5). Furthermore, Chinese samples were classified into Seihoku (n=16), Tohoku (n=20), and Shinkyo (n=3). All other reagents were of the highest quality available.

Procedure——The 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.[1] The results are given in Table I. The experiment on water-soluble elements in licorice root was carried out as follows: 20 g of the powdered licorice root was shaken occasionally with 100 ml of water for 2 d, then the mixture was filtered. The residue was shaken again with 60 ml of water for 12 h, and after filtration the resulting residue was dried *in vacuo*. Next, X-ray multi-elemental analysis was performed on both the original licorice root and the residue. Finally, the elution ratios were calculated from the analytical results.

Calculation of Similarity to the Average Metals Profile of Licorice Root——For each sample, the degree of similarity of the metals contents with the standard profile (Seihoku or Tohoku; shown in Table I) was expressed by the following equations:

$$D_{all}=\sum Dj \quad (1)$$

$$Dj=\alpha(1-|xj-\bar{x}j|/\sigma) \quad (2)$$

where Dj is the degree of similarity for element j, xj is the concentration of element j, and $\bar{x}j$ and σ are the mean value and standard deviation for many crude drug samples (for Seihoku or Tohoku Kanzo), respectively. In addition, α is a weighting coefficient taking account of the large difference in element concentrations ($\alpha=1$ for major elements, P, K, Ca, Fe, *etc.*, $\alpha=1/2$ for trace elements, Mn, Cu, Zn, Rb, and Ba, and $\alpha=1/4$ for ultra-trace elements, Cr and Ni). If Dj is less than -1 for the elements except for P, S, Cl, K, and Ca, it can be described by the equation

$$Dj=-\sqrt{|Dj|} \quad (3)$$

Results and Discussion

Analytical Results

Licorice root is prepared from roots and stolons of the genus *Glycyrrhiza* (Leguminosae). Most Japanese commercial licorice root is imported from China, and licorice root obtained from other countries is mainly used as a source for the isolation of glycyrrhizin.Chinese licorice root is generally classified into Sipei licorice (Seihoku Kanzo in Japanese), Tongpei licorice (Tohoku Kanzo), and Sinking licorice (Shinkyo Kanzo) according to their trade names. It is considered that Seihoku and Tohoku Kanzo originate from *Glycyrrhiza glabra* L. var. *glandulifera* REG. *et* HERD. and *Glycyrrhiza uralensis* FISCH. *et* DC., respectively, though the original plant of Sinkyo Kanzo is obscure.

First, four samples, A, B, C, and D, which differed in size (see Fig. 1), were analyzed. As can be seen in Fig. 1, elements such as Ca, Sr, Ti, and Zn were found in the larger parts (A and B) at higher levels, but no significant difference among the four parts was observed among other metals. Furthermore, comparison of the metals profiles of the big licorice root samples (d=*c.a.* 1.8 cm, n=3) with those of the small ones (d=*c.a.* 0.8 cm, n=3) indicated that the concentration of each element was essentially independent of the size of the sample. In addition, no apparent difference in metals profile between root and stolon was observed in the analytical results for the root and the stolon of a given lot. Figure 2 shows the difference in metals profile between the bark (periderm) and content (periderm-less) for both Seihoku and Tohoku Kanzo. The concentrations of Ca, Ti, and especially Fe in the bark were high

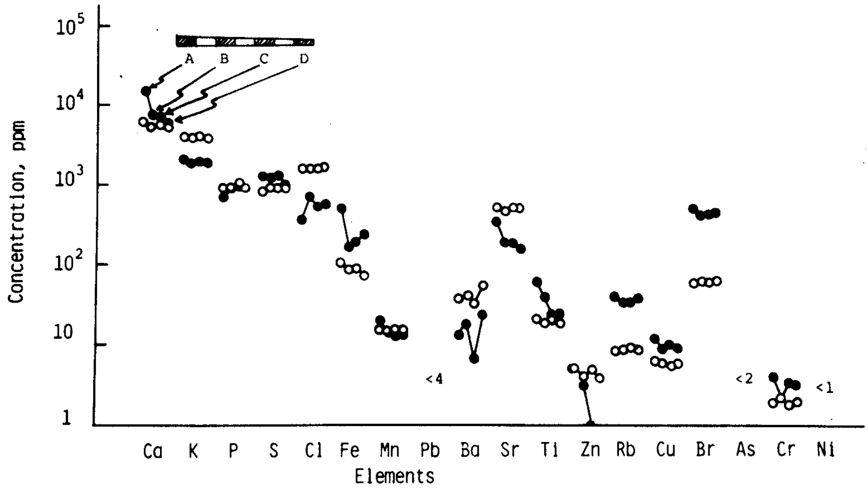

Fig. 1. Analytical Results for Licorice Root by X-Ray Fluorescence Spectrometry
○, Seihoku; ●, Tohoku.

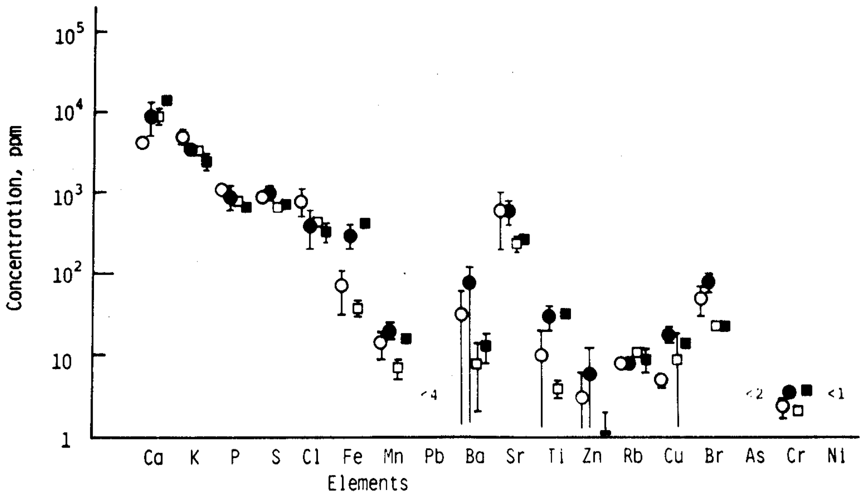

Fig. 2. Analytical Results for Licorice Root by X-Ray Fluorescence Spectrometry
○, Seihoku (contents, $n=3$); ●, Seihoku (bark, $n=3$); □, Tohoku (contents, $n=3$); ■, Tohoku (bark, $n=3$).

compared with those in the content, but K and Cl seem to be contained at high levels in the content as compared with the bark.

The analytical results of the normal (Kawatsuki Kanzo in Japanese, $n=49$), bark-less (Kawasari Kanzo, $n=6$), Chinese ($n=39$), and non-Chinese samples ($n=5$) are summarized and compared in Fig. 3. The concentrations of individual elements, especially, Cl, Sr, and Br, appear to vary considerably. The characteristics of the metals profiles can be represented by comparison with that of NBS orchard leaves, SRM 1571. The concentrations of major elements such as Ca, K, P, and S and trace elements such as Mn and Ba were less than those of the orchard leaves, whereas, the Sr and Br concentrations were higher. These features appear to be characteristic of the metals profile of the licorice root. No significant difference in metals profiles was observed between licorices from different producing countries, or between licorices with and without bark because of the large individual variations. Itokawa *et al.*[5] and Okitsu *et al.*[6] have also examined the contents of several metals in licorice root (sample number: 1 or 2) by atomic absorption spectrometry. Nine elements (K, Ca, Cr, Mn, Fe, Ni, Cu, Zn, and Pb) among the elements tested by them were common with those tested in our

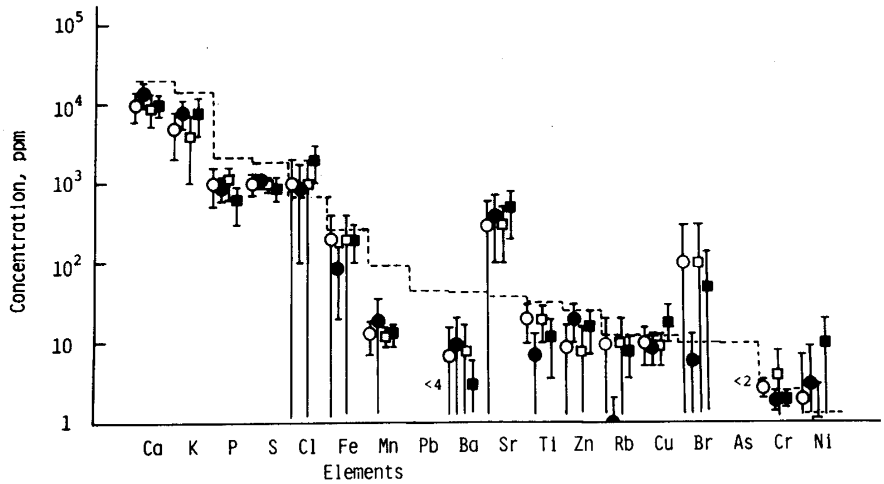

Fig. 3. Analytical Results for Licorice Root by X-Ray Fluorescence Spectrometry

----, orchard leaves; ○, normal ($n=49$); ●, bark-less ($n=6$); □, Chinese ($n=39$); ■, others ($n=5$).

Table I. Analytical Results (ppm) for Licorice Root by X-Ray Fluorescence Spectrometry

Element	Normal[a] ($n=49$)	Bark-less ($n=6$)	Chinese[b] ($n=39$)	Others[c] ($n=5$)	Seihoku ($n=16$)	Tohoku ($n=20$)	Shinkyo ($n=3$)
P	0.10 (5)%	0.09 (3)%	0.11 (5)%	600 (300)	0.15 (5)%	0.08 (4)%	0.08 (5)%
S	0.10 (3)%	0.11 (2)%	0.10 (2)%	900 (300)	0.10 (1)%	0.10 (3)%	0.11 (3)%
Cl	0.1 (1)%	0.09 (8)%	0.1 (1)%	0.2 (1)%	0.13 (5)%	0.05 (5)%	0.3 (4)%
K	0.5 (3)%	0.8 (3)%	0.4 (3)%	0.8 (4)%	0.6 (2)%	0.2 (1)%	0.88 (6)%
Ca	1.0 (4)%	1.4 (5)%	0.9 (4)%	1.0 (3)%	1.0 (4)%	0.7 (2)%	1.3 (2)%
Ti	20 (10)	7 (6)	20 (10)	12 (8)	13 (6)	19 (9)	30 (30)
Cr	2.7 (7)	2.0 (6)	4 (4)	2.0 (5)	2.6 (4)	3.0 (8)	3.1 (3)
Mn	13 (6)	20 (20)	12 (3)	13 (4)	12 (3)	12 (3)	15 (7)
Fe	200 (200)	90 (70)	200 (200)	200 (100)	160 (60)	200 (100)	500 (500)
Ni	2 (5)	3 (6)	1 (2)	10 (10)	1 (3)	1 (1)	1 (1)
Cu	10 (5)	9 (4)	9 (4)	20 (10)	10 (4)	9 (3)	12 (3)
Zn	9 (8)	20 (10)	8 (8)	16 (9)	12 (9)	4 (3)	22 (6)
As	ND	ND	ND	ND	ND	ND	ND
Br	100 (200)	6 (7)	100 (200)	50 (90)	200 (200)	80 (90)	13 (9)
Rb	10 (10)	1 (1)	10 (10)	8 (4)	20 (20)	11 (7)	3 (2)
Sr	300 (300)	400 (300)	300 (200)	500 (300)	400 (200)	120 (60)	600 (200)
Pb	ND	ND	ND	ND	ND	ND	ND
Mo	ND	ND	ND	ND	ND	ND	ND
I	ND	ND	ND	ND	ND	ND	ND
Ba	7 (9)	10 (10)	8 (9)	3 (3)	10 (10)	7 (7)	6 (10)

Standard deviations are given in parentheses.
a) Except for bark-less.
b) The sum of Seihoku, Tohoku, and Shinkyo Kanzo.
c) Samples obtained from other countries.
ND: not detected.

study. The analytical values of the nine elements seem to be in reasonable agreement with the present results (Table I).

In Fig. 4, the analytical results for the Chinese crude drugs are summarized by producing district. Three elements (S, Mn, and Cu) were contained at a similar level regardless of producing district; that is, the concentration of S is 600—1400 ppm, and those of Mn and Cu

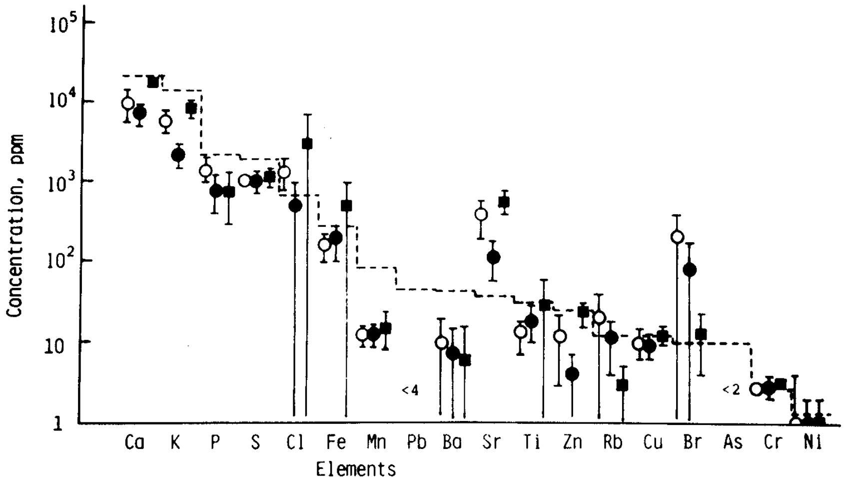

Fig. 4. Analytical Results for Licorice Root by X-Ray Fluorescence Spectrometry

----, orchard leaves; ○, Seihoku ($n=16$); ●, Tohoku ($n=20$); ■, Shinkyo ($n=3$).

TABLE II. Elution of Inorganic Elements from Licorice Root

Element	Amount (μg)		Elution ratio (%)
	Origin (1 g)	Extract[a]	
P	4200	1800	43
S	1700	900	53
Cl	1800	1730	96
K	6600	5500	83
Ca	8400	1000	12
Ti	14	1	7
Cr	2.1	0.6	29
Mn	9.2	4.4	48
Fe	172	11	6
Ni	ND	ND	—
Cu	5.5	2.2	40
Zn	3.2	1.6	50
As	ND	ND	—
Br	30	23	77
Rb	7.5	6.0	80
Sr	260	27	10
Pb	ND	ND	—
Mo	ND	ND	—
I	ND	ND	—
Ba	17	10	53

a) The water-extract mentioned in Experimental.
ND: not detected.

are 6—28 ppm. In contrast, the Sr and K contents appear to depend on the district of production.

Water-Soluble Elements in Licorice Root

A knowledge of the water-soluble elements in licorice root is indispensable for an understanding of the effects of its inorganic components on health, since this crude drug, like many other crude drugs, is usually administered in the form of a decoction or extract. Table II shows the analytical results for the original licorice root and the aqueous extract. These results

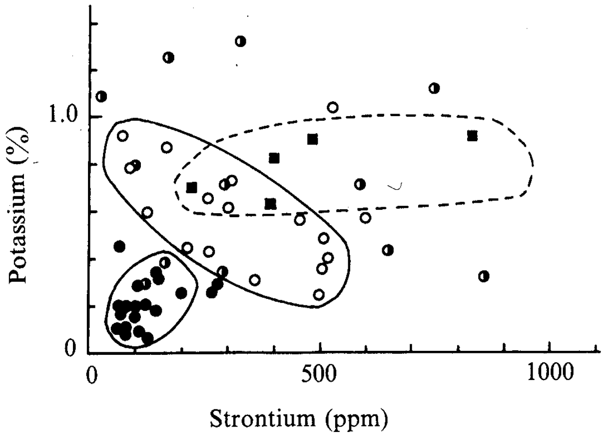

Fig. 5. Relationship between Potassium and Strontium Contents in Licorice Root

○, Seihoku; ●, Tohoku; ■, Shinkyo; ◑, others.

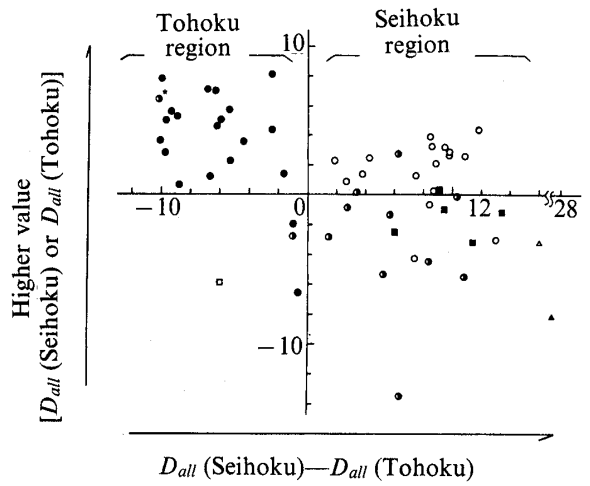

Fig. 6. Similarity of Licorice Root and Other Crude Drugs to the Standard Metals Profiles of Seihoku and Tohoku Kanzo

○, Seihoku; ●, Tohoku; ■, Shinkyo; ◑, others; △, *Rheum palmatum* (root); ▲, Rhei Japonici Rhizoma; □, Arecae Semen; *, *Glycyrrhiza uralensis* FISCH. *et* DC. (root, Tsumura specimen).

The seven samples with values less than −15 (ordinate) were as follows: Cinnamomi Cortex, −26; Sennae Folium, −26; Cardamomi Fructus, −29; Ephedrae Herba, −34; Moschus, −72; Carthami Flos, −77; Digenea, −93.

indicated that Cl, K, Br, and Rb were eluted easily, whereas Ca, Ti, Fe, and Sr dissolved only slightly in water. Thus, major inorganic elements in the extract were as follows: $K \gg P > Cl > Ca > S$. At present, it is difficult to discuss the relationship between these soluble elements and the pharmacological effects of the crude drug because little information is available regarding their chemical forms and the ease of their uptake by the human body. Investigations on their chemical forms and uptake are in progress.

Application of X-Ray Fluorescence Spectrometry to the Identification of Licorice Roots

Judgment of the plant of origin of a crude drug or the producing district is an important problem from the standpoint of the evaluation of crude drugs, because the contents of principal constituents are frequently dependent on such factors. A procedure for the identification of licorice roots on the Japanese market is also desirable. Although methods such as a high-performance liquid chromatographic method[7] and the paper-electrophoresis fingerprint method[8] have been reported, classical organoleptic and morphological evaluations are still predominantly used as present.

From the above results (Fig. 4 and Table I), the K and Sr contents in Seihoku Kanzo seem to differ from those in Tohoku Kanzo. The relationship between the K and Sr contents is shown in Fig. 5. It is interesting to note that most of the points of Seihoku and Tohoku Kanzo are located in non-coincident pseudo-ellipsoids, suggesting that the analytical values of the two elements can provide valuable information for the identification of this crude drug. Next, the identification of the licorice roots from the overall metals profiles was attempted. Figure 6 shows the degree of similarity of the licorice roots ($n=55$) and other crude drugs ($n=10$)[1] to the standard profiles of Seihoku and Tohoku Kanzo (the average of elemental concentrations of Seihoku Kanzo ($n=16$) and Tohoku Kanzo ($n=20$) are shown in Table I). On the abscissa, positive and negative values indicate similarity to Seihoku and Tohoku Kanzo, respectively. On the ordinate, a higher value represents greater similarity. Note that the points for most of the licorice samples were distributed in the region of more than −3 (ordinate), whereas all the other kinds of crude drugs tested except for Rhei Rhizoma fell in the region of less than -5. Of special interest is the fact that Seihoku and Tohoku Kanzo were distributed in the expected region without exception. These results strongly suggest that the metals profiles of samples provide valuable information regarding not only the kind of a crude drug but also the producing district or original plant.

Conclusions

The present analytical results on many samples of the licorice root may be summarized as follows:

(1) The concentrations of most of the elements tested varied considerably among individual licorice root samples, and the variation in Br and Cl were especially marked.

(2) The licorice root contains K, Ca, Mn, and Ba at low levels compared to those of orchard leaves, whereas Sr and Br are present at high levels.

(3) The contents of Sr and K were dependent on the producing district.

(4) Each crude drug has a characteristic metals profile, and the profile provides valuable information regarding the kind of a crude drug. In the case of licorice root, it is also useful for identifying the producing district or the original plant.

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