Inorganic Chemical Approaches to Pharmacognosy. V.[1] X-Ray Fluorescence Spectrometric Studies on the Inorganic Constituents of Crude Drugs. (3). On the Cinnamomi Cortex

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Inorganic constituents of many Cinnamomi Cortices (64 samples; almost all obtained commercially on the Osaka market) were investigated using energy-dispersive X-ray fluorescence spectrometry.

The results can be summarized as follows: (1) The Cinnamomi Cortex contains K, Ca, and Fe (except for Japanese cinnamon) at lower levels than those of orchard leaves, while Mn and Sr are present at high levels. (2) A feature of the metals profile of Cinnamomi Cortex is high Mn-content. Especially, Chinese cinnamon (originated from *Cinnamomum cassia*) contains at extremely high levels, 300—900 ppm, whereas Mn concentrations of Java and Japanese cinnamons range from 100 to 300 ppm, and those of Ceylon cinnamon ranged from 50 to 150 ppm. (3) The contents of Mn and Rb depend on the kind of Cinnamomi Cortex, making identification possible.

Keywords energy-dispersive X-ray fluorescence spectrometry; crude drug; multi-elemental analysis; inorganic compound; metals profile; Cinnamomi Cortex; cinnamon; identification; producing district; manganese

Many mineral elements are closely related to our health. Since crude drugs generally contain mineral elements at various levels, the knowledge of not only their organic but also their inorganic constituents is indispensable for the complete understanding of the pharmacological effects of these drugs. Thus, we planned a series of studies on the significance of inorganic constituents of crude drugs, and earlier established a multi-elemental analysis method using energy-dispersive X-ray fluorescence spectrometry.[2] In a previous paper, the analytical results for many samples of licorice roots indicated that this crude drug has a characteristic metals profile, which provides valuable information regarding the identification of both the kind of drug and the original plant or the producing district.[3]

In this paper, we have dealt with Cinnamomi Cortex, one of the most widely used Chinese crude drugs. Inorganic constituents of many samples (64 specimens) were analyzed by X-ray fluorescence spectrometry, and it has been demonstrated that Cinnamomi Cortex as well as licorice root has a characteristic metals profile, that is, markedly high Mn content, the quantity of which depends on the original plants or the producing districts.

Experimental

Apparatus X-Ray measurements were carried out 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.

X-Band electron spin resonance (ESR) spectra of Cinnamomi Cortices (powder) were measured at 77 K using a JES-FE-3X spectrometer operating at 100 kHz magnetic field modulation.

Materials The crude drug samples were kindly provided by Mikuni Co., Ltd., Koshiro Chuji Co., Ltd., Nihon Funmatsu Yakuhin Co., Ltd., and Shinwa Bussan Co., Ltd. (Osaka). The samples (64 in number) consisted of Chinese cinnamon (so-called cassia bark, $n=23$) originated from *Cinnamomum cassia*, Japanese cinnamon ($n=14$) from *C. sieboldii*, Ceylon cinnamon ($n=12$) from *C. zeylanicum*, and Java cinnamon ($n=11$) from *C. burmanni*.

SRM 1571 orchard leaves, one of the NBS standard reference materials, was purchased from National Bureau of Standards (Washington, D.C., U.S.A.).

Procedure The sample preparation and X-ray fluorescence multi-element analysis (P, S, Cl, K, Ca, Ti, Cr, Mn, Cu, Zn, As, Br, Rb, Sr, Pb, Mo, I, and Ba) were performed according to previous procedures.[2] The experiment on water-soluble elements in Cinnamomi Cortex was carried out in the same way as previously reported.[3]

Results and Discussion

Analytical Results Cinnamomi Cortex is generally classified into Chinese cinnamon (so-called cassia bark; 広南桂皮 (Guangnan Guipi), 肉桂 (Rougui), 東興桂皮 (Dongxin Guipi), *etc.* in Chinese) which originated from *Cinnamomum cassia*, Japanese cinnamon (Nihon keihi in Japanese) which is the root bark of *C. sieboldii*, Ceylon cinnamon from *C. zeylanicum*, and Java cinnamon from *C. burmanni*.

The analytical results for several kinds of Cinnamomi Cortex, that is, total ($n=64$), Chinese cinnamon ($n=23$), Japanese cinnamon ($n=14$), Ceylon cinnamon ($n=12$), and Java cinnamon ($n=11$) are summarized in Table I, together with the branch bark of *C. sieboldii* ($n=4$) which was not included in the group of Cinnamomi Cortex because only the root bark is used as a crude drug. The metals profiles for the four groups except the branch bark of *C. sieboldii* are compared in Fig. 1. The concentrations of individual elements, especially, Cl, Zn, and Br appear to vary considerably. The Fe content of Japanese cinnamon seems high and to vary remarkably, but this is because the root bark contains Fe at higher levels than branch bark as will be mentioned later. The characteristics of the metals profile can be represented by comparison with that of NBS orchard leaves, SRM 1571. The concentrations of major elements such as Ca, K, P, S, and Cl, and trace elements, Cu and Fe (except for Japanese cinnamon) were less than those of the orchard leaves, but on the other hand, trace elements such as Mn and Sr were at higher levels. Itokawa *et al.*[4] and Matsuda *et al.*[5] have also examined the contents of several metals in Cinnamomi Cortex (sample number, $n=1$ 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. These analytical values seem to be in reasonable agreement with the present results, although the value of Cu content, 46 ppm, reported by Matsuda *et al.*[5] seems abnormally high.

In the case of Cinnamomi Cortex, K and Sr contents were almost independent of the original plant or producing district. This is not in agreement with our results for licorice root, in which the contents of both elements were de-

TABLE I. Analytical Results (ppm) for Cinnamomi Cortex by X-Ray Fluorescence Spectrometry

Element	Total[a] ($n=64$)	Chinese cinnamon ($n=23$)	Japanese cinnamon ($n=14$)	Ceylon cinnamon ($n=12$)	Java cinnamon ($n=11$)	Branch bark of *C. siboldii* ($n=4$)
P	0.11 (5)%	0.10 (5)%	800 (400)	0.11 (3)%	0.16 (5)%	0.15 (2)%
S	0.12 (6)%	0.12 (4)%	800 (200)	0.19 (9)%	0.10 (2)%	900 (100)
Cl	200 (300)	70 (200)	300 (300)	300 (300)	200 (100)	30 (30)
K	0.6 (3)%	0.7 (3)%	0.7 (2)%	0.6 (2)%	0.6 (2)%	0.6 (2)%
Ca	1.2 (6)%	1.2 (7)%	0.9 (4)%	1.2 (4)%	1.7 (8)%	1.4 (2)%
Ti	50 (40)	50 (30)	60 (20)	40 (60)	30 (30)	30 (30)
Cr	1 (1)	1 (1)	2 (1)	2 (1)	2 (1)	3 (2)
Mn	300 (300)	600 (200)	220 (50)	140 (100)	170 (90)	360 (10)
Fe	200 (300)	70 (40)	500 (500)	60 (40)	100 (100)	200 (100)
Ni	ND	ND	ND	ND	ND	ND
Cu	7 (3)	5 (1)	9 (5)	9 (3)	5 (1)	7 (1)
Zn	10 (20)	4 (3)	20 (20)	20 (20)	10 (6)	40 (10)
As	ND	ND	ND	ND	ND	ND
Br	10 (10)	10 (20)	5 (5)	10 (10)	20 (20)	ND
Rb	20 (10)	30 (10)	9 (6)	20 (9)	20 (10)	3 (1)
Sr	70 (30)	50 (30)	70 (30)	80 (30)	60 (40)	80 (30)
Pb	4 (10)	ND	9 (15)	ND	ND	8 (6)
Mo	ND	ND	ND	ND	ND	ND
I	2 (6)	1 (3)	4 (10)	3 (4)	ND	ND
Ba	100 (70)	140 (80)	80 (50)	60 (40)	50 (70)	55 (8)

Standard deviations are given in parentheses. *a*) Except for the branch bark of *Cinnamomum sieboldii*. ND: not detected.

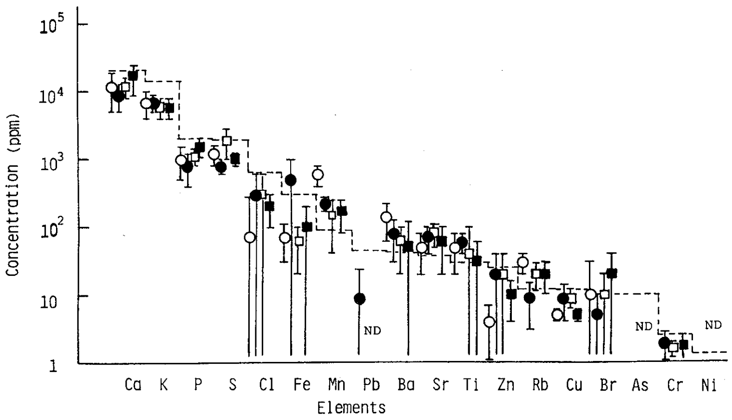

Fig. 1. Analytical Results for Cinnamomi Cortex by X-Ray Fluorescence Spectrometry
----, orchard leaves; ○, Chinese cinnamon ($n=23$); ●, Japanese cinnamon ($n=14$); □, Ceylon cinnamon ($n=12$); ■, Java cinnamon ($n=11$).

pendent on the producing districts (Seihoku or Tohoku kanzou).[3] On the other hand, the Mn, Rb, P, Ca, and Cu contents appear to vary appreciably according to the original plant or the producing district. For instance, Chinese, Japanese, Ceylon, and Java cinnamons contained 300—900, 100—300, 50—150, and 50—300 ppm of Mn, respectively. Note that orchard leaves and licorice root (sample number, $n=ca.$ 50) contained only 91 ppm and less than 30 ppm of Mn, respectively.

Application of X-Ray Fluorescence Spectrometry to the Identification of Cinnamomi Cortex Determination of the originating plant of a crude drug or the producing district is an important factor from the standpoint of evaluating the drug. The relationship between the Rb and Mn contents is shown in Fig. 2. Of interest is the fact that most of the points of Chinese, Japanese, and Ceylon cinnamons are located in individual non-overlapping pseudo-ellipsoids, clearly suggesting that the analytical values of the two elements can provide valuable information for the identification of this crude drug. Because Rb and Mn contents of Java cinnamon resemble those of Ceylon cinnamon (Table I), it seems difficult to distinguish between them. Thus, a new elements set of Ca and Cu was chosen to obtain the preferred separation for Java and Ceylon cinnamons. As seen in Fig. 3, the two groups of crude drugs can be distinguished by the contents of these two elements.

Obvious difference in constituents of essential oil[6] and microscopic anatomical characters[7] have been observed among various kinds of Cinnamomi Cortex samples. Our study of inorganic constituents of Cinnamomi Cortex has indicated that the metals profile as well as both essential oil components and anatomical characters provides valuable information for identification of the kinds of Cinnamomi Cortex.

It is not clear that such difference in metals profiles arises from the character of the plant itself or inorganic components of the soil in the producing district. In addition to these two factors, another one, namely, the part used, may cause different metals profiles in this case because Chinese and Java cinnamons are the branch barks of *C. cassia* and

C. brumanni, respectively. On the other hand, Ceylon cinnamon is the inner bark (branch) of *C. zeylanicum*, and Japanese cinnamon is the root bark of *C. sieboldii*. To clarify this, a comparison of the metals profile between the root bark and branch bark of *C. sieboldii* was first carried out. The analytical results are shown in Fig. 4. The branch

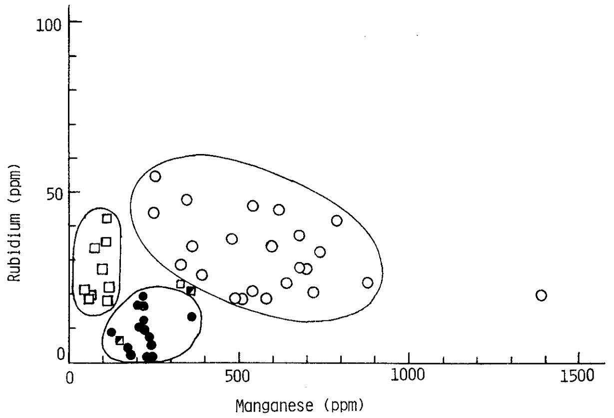

Fig. 2. Relationship between Rubidium and Manganese Contents in Cinnamomi Cortex

○, Chinese cinnamon; ●, Japanese cinnamon; □, Ceylon cinnamon; ◩, Ceylon cinnamon from a certain variety of *Cinnamomum zeylanicum*.

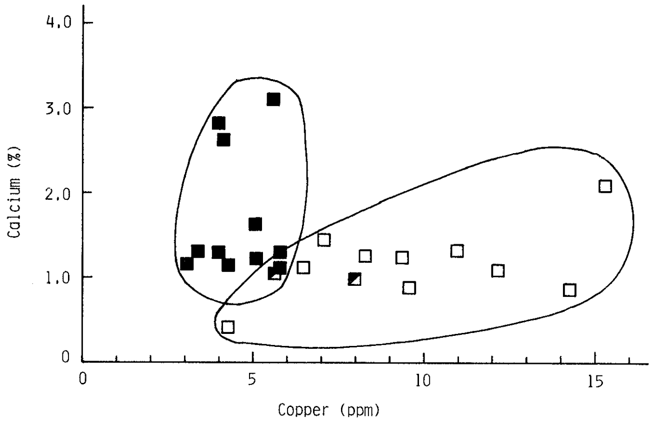

Fig. 3. Relationship between Calcium and Copper Contents in Java and Ceylon Cinnamons

■, Java cinnamon; □, Ceylon cinnamon; ◩, Ceylon cinnamon from a certain variety of *C. zeylanicum*.

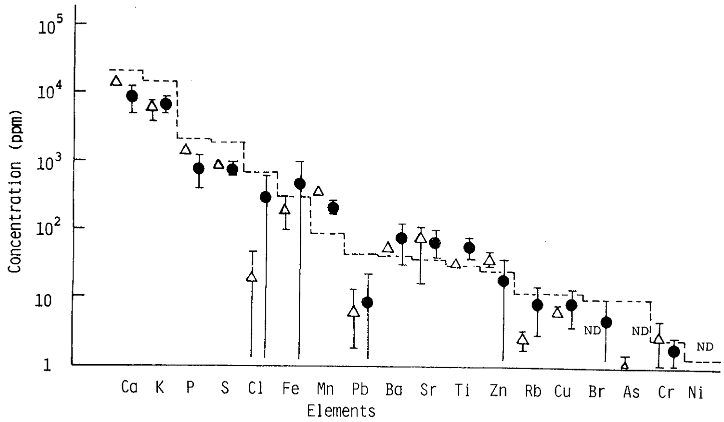

Fig. 4. Analytical Results for Japanese Cinnamon by X-Ray Fluorescence Spectrometry

----, orchard leaves; △, branch bark of *C. sieboldii*; ●, Japanese cinnamon (root bark of *C. sieboldii*).

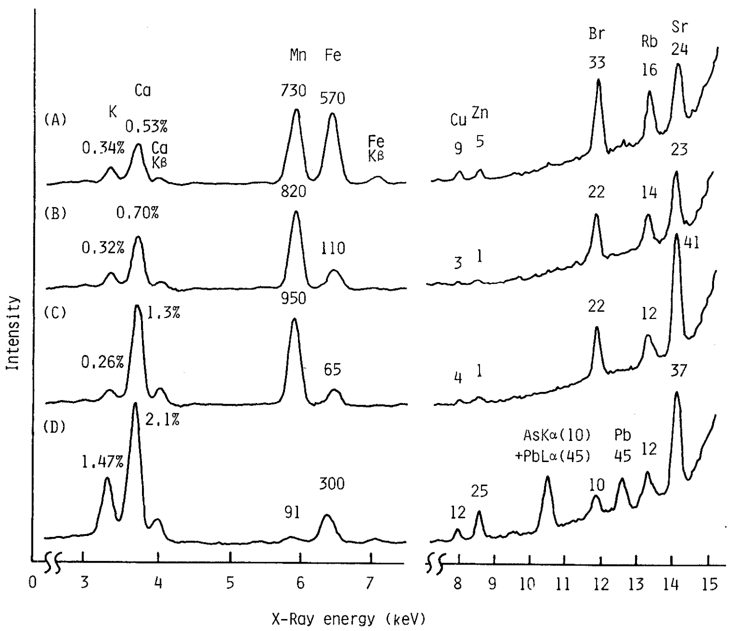

Fig. 5. X-Ray Fluorescence Spectra of Chinese Cinnamon (Guangnan Guipi)

The right and left spectra were obtained by using Ge and Ti as a secondary target, respectively. (A), periderm; (B), outer bark, (C), inner bark; (D), orchard leaves.

bark contained Mn and Ca at higher levels, but contained Rb, Ti, and Fe at lower levels than those of the root bark. If the branch bark of *C. sieboldii* were used as Japanese cinnamon, the Mn content would be slightly close to that of Chinese cinnamon, but the Rb content would be quite different from Chinese cinnamon. Therefore, the difference in the part (branch or root) is not thought to be the main cause of the apparent distinction in Mn and Rb contents between the Japanese cinnamon and the other Cinnamomi Cortices. Next, to elucidate the difference in metals profile among the periderm (mainly the cork layer), outer bark, and inner bark, these three parts prepared from a certain Chinese cinnamon (Guangnan Guipi) were analyzed. Figure 5 shows the X-ray fluorescence spectra of the three parts with the spectrum of orchard leaves as a reference. As seen, Mn content is almost independent of the part used, but Fe content of periderm is at higher levels than both outer and inner barks. The Ca content of inner bark is higher than that of the other two parts. This is presumably due to the anatomical difference in distribution of the crystal of calcium oxalate observed by microscope.[7] A remarkable difference exists in Mn content between Chinese and Ceylon cinnamon. Little variation among these three parts strongly suggests that the large difference in Mn content between these two cinnamons is mainly related to the original plant or the producing district rather than the part used.

Water-Soluble Elements in Cinnamomi Cortex A knowledge of the water-soluble elements in Cinnamomi Cortex is indispensable for an understanding of the effects of its inorganic components on health. Table II shows the analytical results for the original Cinnamomi Cortex (Guangnan Guipi) and the aqueous extract. S, K, Br, and Rb were eluted easily, whereas Ca, Ti, Fe, and Cu dissolved only slightly in water. Thus, the major inorganic elements in the extract were K > Ca > S > Mn. At present, it is difficult to discuss these pharmacological effects on health; however, it should be noted that Mn is a characteristic metal ion in this crude drug, especially in Chinese cinnamon, because there are few other such drugs containing Mn at such a high level. Rather high Mn contents have been reported for Caryophylli Flos, Kumazasa (Folium), Bankyo (Herba), Akebiae Caulis, *etc.* and several crude drugs (Semen, Fructus, or Rhizoma) originating from Zingiberaceae. Chinese cinnamon is certainly a unique example of a high Mn-containing "Cortex" crude drug.

Mn is one of the 27 elements now known to be essential for life. The number of Mn metalloenzymes is very limited, and include pyruvate carboxylase and superoxide dismutase which functions as a defense against the deleterious reactivities of the free radical O_2^-. On the contrary, enzymes that can be activated by Mn are numerous; they include hydrolases, kinases, decarboxylases, and transferases.[8] The common foods in human diets are highly variable in Mn concentration. In Peterson and Skinner's report,[9] average concentrations ranged from 20—23 ppm for the three groups with highest Mn content: nuts, whole cereals, and dried fruits, to as low as 0.2—0.5 ppm for the three groups with lowest Mn content: dairy products, poultry and poultry products, and fish and seafood. The 300—900 ppm of Mn content for Chinese cinnamon is more than 10 fold that for common foods. Although Mn deficiency has not often been observed in man, in such a case, Mn in Cinnamomi Cortex might provide optimal supplementation and bring about improvement in manifestations of Mn deficiency, namely, impaired growth, skeletal abnormalities, disturbed or depressed reproductive function, ataxia of the newborn, or defects in lipid and carbohydrate metabolism. In this connection, it is also interesting that Chinese cinnamon used as a drug, even if incidentally, contains Mn at higher levels than Ceylon cinnamon used as a flavoring.

TABLE II. Elution of Inorganic Elements from Cinnamomi Cortex

Element	Amount (μg) Origin (1 g)	Amount (μg) Extract	Elution ratio (%)
P	760	150	20
S	1010	470	47
Cl	ND	ND	—
K	4160	3210	77
Ca	7800	800	10
Ti	50	1	2
Cr	ND	ND	—
Mn	790	170	22
Fe	86	6	8
Ni	ND	ND	—
Cu	5.5	0.4	7
Zn	4.3	ND	—
As	ND	ND	—
Br	27	15	56
Rb	42	32	76
Sr	47	16	34
Pb	ND	ND	—
Mo	ND	ND	—
I	ND	ND	—
Ba	114	11	10

ND: not detected.

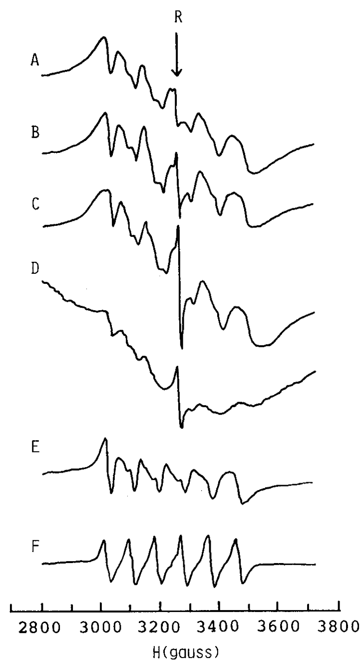

Fig. 6. ESR Spectra for Cinnamomi Cortex (Powder) at 77 K

(A), Chinese cinnamon; (B), Ceylon cinnamon; (C), Java cinnamon; (D), orchard leaves; (E), Mn (II)-conconavalon A; (F), $Mn(H_2O)_6^{2+}$; R, radicals. (E) and (F) were cited from the report of Reed and Cohn.[10]

ESR Spectra for Cinnamomi Cortex Because the feature of the metals profile of this crude drug is a high content of Mn, one might be interested in the chemical species of the Mn-containing compound. A preliminary ESR spectroscopic experiment was carried out for several kinds of Cinnamomi Cortex. The typical ESR spectra of the crude drugs are shown in Fig. 6, together with those of orchard leaves, Mn(II) (H_2O), and concanavalin A (jack bean phytohemagglutin).[10] The ESR spectra of all Cinnamomi Cortex tested exhibited an obvious typical Mn(II) ($S=5/2$) hyper fine pattern with the absorption by free radicals, compared with that of orchard leaves. In general, the understanding of an ESR spectrum of Mn is difficult. However, Reed and Cohn[10] have described that the reduction in the amplitude of Mn(II)-concanavalin A spectrum compared to that of Mn(II) free ion may be caused by a restriction of motion freedom of the bound Mn(II) ion. In addition, the coordination environment around Mn ion is approximately a cubic symmetry. The ESR spectra of these crude drugs are similar to that of Mn(II)-concanavalin A, and entirely different from that of free Mn(II) ion. This similarity suggests that the rotation motion of the Mn(II) in Cinnamomi Cortex is highly restricted, as is true in Mn(II)-concanavalin A. Further detailed investigations on the Mn-containing compound are under way.

Conclusions

The present analytical results on many samples of the Cinnamomi Cortex may be summarized as follows:

(1) The concentrations of most of the elements tested varied considerably among individual Cinnamomi Cortex, and the variations in Cl and Br were especially marked.

(2) The Cinnamomi Cortex contains K, Ca, and Fe (except for Japanese cinnamon) at low levels compared to those of orchard leaves, whereas Mn and Sr are present at high levels.

(3) The contents of Mn and Rb were dependent on the original plant or the producing district, which makes it possible for us to identify the kind of Cinnamomi Cortex.

(4) Preliminary ESR measurements suggested that the Mn-containing compound in Cinnamomi Cortex has Mn(II) ($S=5/2$) ion whose motion freedom is highly restricted.

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