[Chem. Pharm. Bull. 29(10)2966—2973(1981)]

Inhibitory Effects of Stomachic Crude Drugs on Digestive Enzymes[1)]

KATSUHIRO YAMAZAKI,*,[a] HIROSHI YOKOYAMA,[a] YOSHIKI NUNOURA,[a] CHISAE UMEZAWA[b] and CHIKATARO KAWASAKI[b]

Osaka Prefectural Institute of Public Health,[a] Nakamichi, Higashinari-ku, Osaka 537, Japan and School of Pharmacy, Kobe-Gakuin University,[b] Ikawadani, Tarumi-ku, Kobe 673, Japan

(Received February 26, 1981)

The effect on digestive enzymes of stomachic crude drugs that were often compounded in gastrointestinal OTC drugs was investigated. The β-amylase activity of takadiastase was slightly inhibited by cloves and extract of scopolia. Coptis root and extract of scopolia were weak inhibitors of saccharated pepsin. The proteolytic activity of pancreatin was inhibited by 50% in the presence of 5 mg of cinnamon, phellodendron or Japanese prickly ash berry under the conditions used. Cinnamon inhibited trypsin and chymotrypsin, proteases in pancreatin. The inhibitory activity of cinnamon varied from sample to sample; it was localized in the water-soluble and the residual fractions, and little inhibitory activity was found in the volatile oil fraction. Cinnamic acid, methyl cinnamate, cinnamaldehyde and cinnamyl alcohol were not inhibitory. Antiproteolytic activity was found in both the inner and outer solutions after dialysis of the 25% ethanol extract of cinnamon. Hide powder treatment removed inhibitory activity in the outer solution, while that in the inner solution was unchanged.

Keywords——stomachic crude drugs; cinnamon; inhibition of digestive enzymes; takadiastase; pancreatin; saccharated pepsin; trypsin; chymotrypsin

In Japan over a thousand gastrointestinal OTC drugs are now on the market. About 30% of these drugs are combination products with various kinds of digestive enzymes and stomachic crude drugs.[2)] Each enzyme protein is stable and active in a specific three-dimensional conformation. This native conformation is affected by interactions of the amino acid side chains with the solvent and with other solutes. The effect of drugs compounded with enzyme preparations, therefore, has to be duly considered. Studies along this line have been done mostly with single chemicals such as antacids, sulfa drugs, antibiotics and so on.[3)] Though protease inhibitors are known to be distributed widely in animals and plants, there are few reports on protease inhibitors in stomachic crude drugs. In this study the authors investigated the effects of several stomachic crude drugs on various digestive enzymes often compounded in gastrointestinal combination products. Among the proteaseinhibitory crude drugs, cinnamon was studied in detail as cinnamon ranks high in the list of imported crude drugs (in terms of amount)[4)] and is used widely not only as a stomachic crude drug but also as a spice in food.

Experimental

Materials——Pancreatin was obtained from Iwaki Pharmaceutical Co., Ltd., trypsin and α-chymotrypsin from Merck, takadiastase from Sankyo Pharmaceutical Co., Ltd., saccharated pepsin from the National Institute of Hygienic Sciences, subtilisin from Sigma and bromelain from Katayama Chemical Industries, Ltd. Crude drugs listed in Table I were obtained from the following companies; liquorice root (*Glycyrrhiza glabra* LINNE var *glandulifera* REGEL *et* HERDER) and phellodendron (*Phellodendron amurense* RUPRECHT) from Takasago Yakugyo Co., fennel (*Foeniculum vulgare* MILLER) from Tochimoto-Tenkaido, ginger root (*Zingiber officinale* ROSCOE) from Ito Kanpodo, cinnamon (*Cinnamomum cassia* BLUME) and extract of scopolia from Nihon-Hunmatsu, Japanese prickly ash berry (*Zanthoxylum piperitum* DE CANDOLLE), clove (*Eugenia caryophyllata* THUNBERG) and coptis root (*Coptis japonica* MAKINO) from Mikuni Co., Ltd. Cinnamon samples listed in Tables III and IV were obtained from Mikuni Co., Ltd. and from Tochimoto-Tenkaido. All the crude drugs were ground to a fine powder with a mortar and pestle, and sifted with a 300 μ mesh sieve.

Enzyme Assay——a) Schwimmer's Method: β-Amylase activity in takadiastase was assayed by a modification of Schwimmer's method.[5] To 2 ml of 2% soluble starch solution in 0.02 M acetate buffer (pH 4.8), 1 ml of 0.1% takadiastase solution and 5 mg of a crude drug were added. After incubation at 30°C for 10 min, the reaction mixture was transferred to a test tube containing 10 ml of 0.05 N $K_3Fe(CN)_6$ in 0.2 M Na_2CO_3, and the test tube was heated for 20 min in a boiling water bath. The mixture was cooled with tap water, and 25 ml of acid-salt mixture were added. I_2 liberated after adding 0.5 g of KI was titrated with 0.05 N $Na_2S_2O_3$ (A ml). As a blank, the enzyme solution was replaced by 1 ml of water and treated similarly (B ml). The ratio of (A—B) with a crude drug to (A—B) without the crude drug was taken as the relative activity.

b) Casein-Folin Method: A test for proteolytic activity of pancreatin given in JP IX[6] was used. All the solutions were prepared according to JP IX.[6] One ml of the enzyme solution was transferred to a test tube, then 5 mg of crude drug and 5.0 ml of the casein solution were added and the reaction mixture was incubated at 40°C for 10 min. The enzyme reaction was stopped by adding the trichloroacetic acid solution, and the filtrate was used for the color reaction with Folin-Ciocalteau reagent. The optical density of the filtrate at 660 nm was designated as A_1. In another test tube, the enzyme solution, a crude drug and trichloroacetic acid solution were mixed, and then the casein solution was added. The filtrate from this test tube was treated in the same way, and the optical density of the filtrate at 660 nm was designated as A_2. The value obtained by subtracting A_2 from A_1 was taken as the relative activity.

In other experiments, pancreatin was replaced by trypsin (0.01%), chymotrypsin (0.002%), saccharated pepsin (0.06%), bromelain (0.01%) or subtilisin (0.001%). The pH of the casein solution was adjusted to 1.5 in the case of saccharated pepsin and to 4.5 for bromelain.

c) Cup Method: Casein-agar was prepared according to Okazaki *et al.*[3] Ten ml of the casein-agar were transferred to a Petri dish. Three open cylinders were placed on the surface of the casein-agar plate, then another 10 ml of the casein-agar were layered over and allowed to stiffen. The open cylinders were filled with 5 mg of a crude drug and 0.2 ml of the 0.25% pancreatin solution. After incubation at 37°C for 24 h, the open cylinders were removed from the plate and glacial acetic acid was applied to the surface of the plate. The remaining casein was coagulated and a clear circle was formed around each open cylinder. The diameter of the circle was measured and compared with that of the circle around the open cylinder without a cinnamon sample.

Dialysis——One g of cinnamon powder was extracted by refluxing it with 50 ml of 25% ethanol for 2 h. The extract was evaporated to dryness and the volume of the residue was made up to 50 ml with water (extract from 20 mg of cinnamon powder per ml). The extract was placed in dialysis tubing (visking tubing with 28.6 mm flat width; Niplon Product Co., Ltd.) and dialyzed against distilled water at 5°C. The outer solution was changed several times till the brown color did not come through the dialysis membrane. This usually took about 24 h. The inner and the combined outer solutions were each concentrated *in vacuo* to 50 ml and 1 ml of each solution was tested for its effect on pancreatin and trypsin.

Acid-insoluble ash,[7] loss on drying[7] and volatile oil content[8] were determined according to JP IX.

Hide Powder Treatment[9]——Dry hide powder was treated according to the direction.[9] Five ml of either the 25% ethanol extract of cinnamon, or the inner or outer solution after dialysis of the ethanol extract (each corresponding to 20 mg of original cinnamon powder per ml) were added to the hide powder (equivalent to 0.3 g of dry hide powder) in a test tube with a stopper. The test tubes were shaken for exactly 10 min. At the end of this time the content of the test tube was filtered and the filtrate was tested for inhibitory activity.

Results

As crude drugs were often compounded as fine powders, all the crude drugs were used as powders in the following experiments. Table I shows the effect of various stomachic crude drugs on digestive enzymes commonly compounded in gastrointestinal combination products. The β-amylase activity of takadiastase was weakly inhibited by cloves and extract of scopolia, but was rather stimulated by phellodendron and coptis root. Other crude drugs were without effect on the β-amylase activity of takadiastase. The activity of saccharated pepsin was weakly inhibited by coptis root and extract of scopolia, but no inhibition was observed with other crude drugs. The proteolytic activity of pancreatin, however, was decreased to about 50% of the control (without crude drug) on adding phellodendron, cinnamon or Japanese prickly ash berry. Liquorice root was also inhibitory to the proteolytic activity of pancreatin.

Among the crude drugs which inhibited the protease activity of pancreatin, cinnamon was chosen for the previously mentioned reasons and its protease-inhibitory activity was investigated in more detail. To find whether the protease inhibitory activity of cinnamon

TABLE I. Effects of Crude Drugs on Digestive Enzymes

Crude drug[a]	Activity (%)[b]		
	Pancreatin (3)[c]	Takadiastase (3)[c]	Saccharated pepsin (3)[c]
Control (No crude drug added)	100.0±0[d]	100.0±0[d]	100.0±0[d]
Liquorice root	60.6±3.5	105.0±0.6	97.2±0.2
Fennel	104.8±8.8	97.0±0.4	111.9±2.6
Ginger root	89.2±11.1	98.0±0.3	112.5±6.3
Phellodendron	45.8±2.7	124.1±0.7	105.9±4.6
Cinnamon	54.1±5.0	88.6±1.4	100.9±4.3
Japanese prickly ash berry	51.4±5.2	92.6±8.5	98.7±11.6
Cloves	117.4±4.3	80.5±0.7	100.9±1.1
Coptis root	80.6±5.7	124.1±0.7	75.1±7.4
Extract of scopolia	99.9±1.7	81.9±6.6	79.4±9.3

a) 5 mg of crude drug were added.
b) Enzyme activities were determined by the following methods.
Pancreatin (proteinase activity)……Casein-Folin method.
Takadiastase (β-amylase activity)……Schwimmer's method.
Saccharated pepsin (proteinase activity)……Casein-Folin method.
Each value represents the relative remaining activity (%).
c) Numbers in parentheses indicate numbers of determinations.
d) Means±S.D.

TABLE II. Properties of Cinnamon Samples listed in Tables III and IV

Sample	Part of plant	Shape	Place of origin	Year of processing	Loss on drying (%)	Acid-insoluble ash (%)	Volatile oil content (ml)
A	Bark	Medium powder	Kuangnan	Not known	9.9	3.4	1.79
B	Bark	Medium powder	Vietnam	Not known	10.0	3.2	1.31
C	Bark	Fine powder	Not known	1965	9.9	2.2	0.06
D	Bark	Medium powder	Not known	1976	8.9	3.5	*a*)
E	Bark	Fine slice	Not known	Not known	10.3	2.3	*a*)
F	Bark	Medium slice	Vietnam	1976	9.9	4.5	1.00
G	Bark	Medium slice	Kuangnan	1976	9.0	3.5	2.13
H	Bark	Medium slice	Kuangnan	1978	8.7	1.1	0.60
I	Bark	Medium slice	Kuanhsi	Not known	11.0	2.2	0.87
J	Bark	Coarse slice	Kuanhsi	Not known	10.7	2.4	0.83
K	Bark	Coarse slice	Kuanhsi	Not known	10.6	1.9	1.46
O	Branch	Fine slice	Vietnam	Not known	8.8	1.1	0.02
P	Tip	Fine slice	Vietnam	Not known	8.3	2.0	*a*)
Q	Petiole	Fine slice	Vietnam	Not known	10.2	5.8	*a*)
R	Bark from small branch	Medium slice	Vietnam	Not known	9.8	1.8	1.05

a) Volatile oil content could not be determined because the amounts of samples were insufficient.

was universal, various cinnamon samples differing in shape, part of the plant, place of origin and year of processing were tested (Table II). The results of general tests for crude drugs provided in JP IX are summarized in Table II. Loss on drying of all samples fell in a fairly narrow range (8.3—11.0%). Acid-insoluble ash was between 1.1 and 5.8%. Volatile oil content fluctuated, and samples containing extremely low volatile oil contents (such as samples C and O) showed almost no inhibitory effect on the proteolytic activity of pancreatin. As shown in Table III, the protease inhibitory activity of cinnamon varied from sample to sample and the proteolytic activity of pancreatin was lowered in the presence of cinnamon samples to 35 to 92% of the activity without cinnamon.

Protease activity of pancreatin was determined according to JP IX by measuring the amount of tyrosine produced from hydrolyzed casein with Folin-Ciocalteau reagent. Each cinnamon sample contained a different amount of Folin-Ciocalteau reagent-positive substances and a sample with a high A_2 value tended to have a low relative proteolytic activity. To avoid the effect of Folin-Ciocalteau reagent-positive substances in cinnamon samples, the cup method was also used. Though more than 20% difference was observed between the data obtained by the casein-Folin method and the cup method in 2 samples (samples H and O), in other samples data obtained by the two different methods agreed quite well (Table III). In other words, samples which showed strong inhibitory activity by the casein-Folin method were also found to be highly inhibitory by the cup method. The 50% inhibitory concentrations of all cinnamon samples (IC_{50}) are also indicated in Table III.

TABLE III. Comparison of the Casein-Folin Method and the Cup Method

Sample[a]	Casein-Folin method (6)[d] Activity (%)[b]	Cup method (6)[d] Activity (%)[c]	IC_{50}[f] (mg/ml)
Control (No crude drug added)	100.0±0[e]	100.0±0[e]	
A	67.7±3.3	71.9±3.0	1.3
B	55.8±5.3	57.3±4.2	0.9
C	90.8±5.1	84.1±7.8	4.5
D	76.5±10.5	68.0±6.2	1.8
E	50.1±7.0	49.8±4.2	0.8
F	84.2±7.0	72.0±3.1	2.6
G	78.2±7.3	62.6±6.1	1.9
H	89.8±5.8	63.4±4.8	4.1
I	48.3±3.9	47.5±3.4	0.8
J	65.8±4.3	53.2±1.9	1.2
K	57.6±5.6	50.3±4.5	1.0
O	92.3±5.0	68.7±4.1	5.4
P	68.9±5.4	62.0±5.6	1.3
Q	53.7±7.5	48.4±3.7	0.9
R	35.3±4.5	42.0±3.6	0.6

a) 5 mg of cinnamon sample were added per tube.
b) Calculated as activity in Table I.
c) $\frac{\text{Diameter of a transparent circle with a cinnamon sample}}{\text{Diameter of a transparent circle without a cinnamon sample}} \times 100$
d) Numbers in parentheses indicate numbers of determinations.
e) Means±S.D.
f) Determined by the casein-Folin method.

As pancreatin contains trypsin, chymotrypsin and carboxypeptidase as proteases, the effects of cinnamon on trypsin and chymotrypsin preparations were investigated. As shown in Table IV, samples which showed strong inhibition of the proteolytic activity of pancreatin were also strong inhibitors of trypsin and chymotrypsin. When a cinnamon sample with inhibitory activity comparable to that of sample I was used, bromelain (9%) and subtilisin (33%) were also inhibited. Carboxypeptidase A, another pancreatic protease, was, however, hardly inhibited by the cinnamon sample (87%).

Table IV. Effects of Various Cinnamon Samples on Trypsin and Chymotrypsin Activities

Sample[a]	Trypsin[b] (3)[c]	Chymotrypsin[b] (3)[c]
Control (No crude drug added)	100.0±0[d]	100.0±0[d]
A	39.4±2.1	67.0±1.9
B	29.9±4.6	38.2±3.8
C	76.2±6.8	92.7±3.8
D	54.5±5.1	63.9±4.9
E	29.2±6.2	17.8±1.1
F	70.3±3.8	86.1±5.3
G	65.2±3.3	66.1±1.3
H	56.0±2.5	68.5±1.7
I	13.7±2.3	21.3±2.6
J	9.4±1.4	33.6±2.5
K	24.7±1.3	37.5±5.1
O	84.1±6.9	83.0±4.5
P	26.5±10.8	36.4±5.6
Q	21.1±3.2	37.3±9.2
R	3.4±3.1	10.5±1.2

a) 5 mg of cinnamon sample were added.
b) Enzyme activity was determined by the casein-Folin method. Each value represents the relative remaining activity (%).
c) Numbers in parentheses indicate numbers of determinations.
d) Means±S.D.

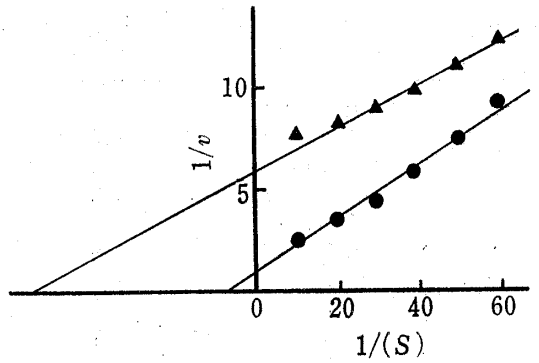

Fig. 1. Lineweaver-Burk Plots of Proteolytic Activity of Pancreatin in the Presence of Cinnamon

—●—: control (no crude drug was added).
—▲—: 5 mg of cinnamon (Sample B in Table II) were added per tube.

In Fig. 1, the kinetics of inhibition of proteolysis were plotted according to Lineweaver and Burk. Proteolytic activity of pancreatin was inhibited by cinnamon in an uncompetitive fashion, both K_m and V_{max} values being reduced in the presence of cinnamon. We also tested whether the interaction of cinnamon with pancreatin was specific or not (Table V). In the standard casein-Folin method, cinnamon and pancreatin were brought into contact first and then casein was added to start the enzyme reaction. In the modified method, cinnamon was brought into contact first with casein and then pancreatin was added. As shown in Table V, no inhibitory activity of cinnamon was observed in the modified casein-Folin method. This result suggests that the inhibitor (s) in cinnamon has non-specific interactions with proteins.

Table V. Effect of Preincubation of Cinnamon with Casein on the Proteolytic Activity of Pancreatin

	Assay method	Activity (%)[a]
Control	Standard casein-Folin method	100
With cinnamon[b]	Standard casein-Folin method	48.6
	Modified casein-Folin method[c]	88.9

a) Calculated as activity in Table I.
b) 5 mg of a cinnamon sample were added per tube.
c) Cinnamon was preincubated with casein for 5 min at 40°C and then pancreatin was added.

As protease inhibitors appeared to be present in cinnamon, isolation of the inhibitors was attempted using sample R in Table II. The volatile oil fraction was collected according to JP IX[8] by boiling for 5 h. After the volatile oil fraction had been separated, the water layer was centrifuged and the supernatant was used as the water-soluble fraction. The precipitate was suspended in a certain volume of water and the suspension was used as the residual fraction. As shown in Table VI, protease inhibitory activity was found in both the water-soluble fraction and the residual fraction. Though it was predicted from Tables II and III that the volatile oil might be responsible for the protease-inhibitory activity of cinnamon, the results shown in Table VI ruled out this possibility. As expected, cinnamaldehyde, a main component of the volatile oil of cinnamon, and related compounds were without inhibitory effect on the proteolytic activity of pancreatin at the 0.1 mg per tube level (Table VII).

Table VI Effect of Each Fraction of Cinnamon on the Proteolytic Activity of Pancreatin

Added[a]		Amount added Activity (%)[b]		
		5 mg	10 mg	25 mg
None	(3)[c]	100 ±0[d]	100 ±0[d]	100 ±0[d]
Volatile oil fraction	(3)	100 ±0	100 ±0	97.6±6.5
Water-soluble fraction	(3)	75.9±2.5	64.7±3.1	55.9±3.9
Residual fraction	(3)	80.7±7.4	70.9±4.4	57.5±2.3

a) Each fraction corresponding to 5, 10 or 25 mg of original cinnamon sample was added to the enzyme assay mixture.
b) Calculated as activity in Table I.
c) Numbers in parentheses indicate numbers of determinations.
d) Means±S.D.

Table VII. Effects of Various Components of Cinnamon on the Proteolytic Activity of Pancreatin

Added[a]		Activity (%)[b]
None	(3)[c]	100 ±0[d]
Cinnamic acid	(3)	101.9±2.3
Methyl cinnamate	(3)	90.6±4.5
Cinnamaldehyde	(3)	97.2±5.8
Cinnamyl alcohol	(3)	105.3±4.1

a) Each compound was added at the 0.1 mg/tube level.
b) Calculated as activity in Table I.
c) Numbers in parentheses indicate numbers of determinations.
d) Means±S.D.

To get further information on the protease-inhibitory substance, the 25% ethanol extract of cinnamon powder was dialyzed against distilled water at 5°C for 24 h. As shown in Table VIII, trypsin- and pancreatin-inhibitory activities were distributed in both the inner and outer solutions. Under these conditions, most of the Folin-Ciocalteau reagent positive substances were dialyzed through the Visking tubing. As shown in Fig. 2, the inner and outer solutions after dialysis showed similar types of inhibition (uncompetitive inhibition) of pancreatin.

Hide powder is known to have highly specific affinity for tannin and is used for the quantitative determination of tannins in various specimens.[9] Antiproteolytic activities of dialyzed cinnamon samples were tested before and after hide powder treatment (Table IX). Standard tannic acid was also treated with hide powder to make sure that tannic acid was completely removed by this treatment. As expected, all solutions including tannic acid became Folin-

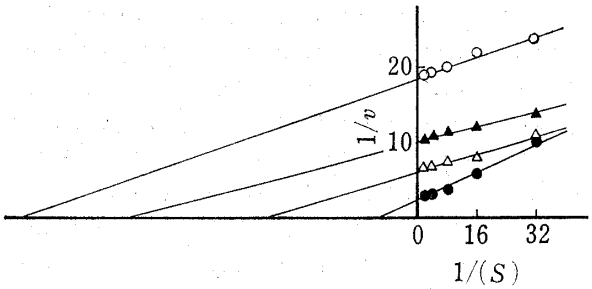

Fig. 2. Lineweaver-Burk Plots of Proteolytic Activity of Pancreatin in the Presence of Dialyzed Cinnamon Samples

A portion of sample solution corresponding to 20 mg of original cinnamon sample was added to the enzyme assay mixture.
—●—: Control (no crude drug was added).
—○—: 25% alcohol extract.
—▲—: Inner solution.
—△—: Outer solution.

Table VIII. Localization of Antiproteolytic Activity of Cinnamon after Dialysis

Added[a]		Tryptic activity (%)[b]	Pancreatic activity (%)[b]
None	(3)[c]	100.0±0[d]	100.0±0[d]
25% Alcohol extract	(3)	0.3±1.1	6.6±2.3
Inner solution	(3)	4.5±2.0	37.5±1.4
Outer solution	(3)	3.4±1.2	67.6±5.2

a) Each solution, corresponding to 20 mg of original cinnamon sample, was added to the enzyme assay mixture.
b) Calculated as activity in Table I.
c) Numbers in parentheses indicate numbers of determinations.
d) Means±S.D.

Table IX. Effect of Hide Powder Treatment of Dialyzed Cinnamon Samples on the Proteolytic Activity of Pancreatin

Added[a]	Activity (%)[b]	
	Without hide powder treatment	With hide powder treatment
None	100.0±0	100.0±0
25% alcohol extract	27.9±2.5	32.0±1.6
Inner solution	44.8±5.6	46.4±1.1
Outer solution	79.9±0.8	96.9±4.0
Tannic acid[c]	92.0±7.5	91.5±4.7

a) Each solution, corresponding to 20 mg of original cinnamon sample, was added to the enzyme assay mixture.
b) Calculated as activity in Table I.
c) One mg of tannic acid was added to the enzyme assay mixture.

Ciocalteau reagent-negative after this treatment. Inhibitory activities in the 25% ethanol extract and the inner solution were not removde by the hide powder treatment, while that in the outer solution was eliminated.

Discussion

In the presence of crude drugs such as cinnamon, the proteolytic activities of pancreatin, trypsin, chymotrypsin and some other proteases of different types were found to be inhibited. Pepsin, an acid protease, was not inhibited by cinnamon. Among the protease inhibitors of high and low molecular weights so far studied, only a few inhibitors are inhibitory to more than one type of protease.[10] On the basis of this fact and the data shown in Table V, the inhibitory substance in cinnamon was supposed to have nonspecific affinity for protein.

Tannin is known to combine easily with protein and this property has been utilized to manufacture albumin tannate for semiacute or acute intestinal catarrh and other types of diarrhea and as a ligand for affinity chromatography.[11] Tannic acid was permeable to dialysis tubing, was Folin-Ciocalteau reagent-positive and had high affinity for hide powder. These properties resemble those of our dialyzable protease-inhibitory substance.

During a search for anti-inflammatory substances, Furusawa *et al.*[12] examined various crude drugs and found trypsin-inhibitory activity in cinnamon. They found that all the trypsin-inhibitory activity was dialyzable, while in our study antiproteolytic activity was

present in both the inner and outer solutions after dialysis. The inhibitory substance in the outer solution is possibly a tannin-related compound, as Furusawa *et al.* postulated. The inhibitory substance in the inner solution was, however, supposed to be non-tannic, since the inner solution retained its inhibitory activity after the hide powder treatment. The inhibitory substance appeared to be heat-stable, as inhibition was observed in the water soluble fraction after volatile oil extraction (130—150°C, 5 h).

Our results indicated that the casein-Folin method described in JP IX could not be recommended for determining the activities of proteases compounded with crude drugs such as cinnamon because of the variability of protease activity with different amounts of crude drugs and because of the high blank value due to the Folin-Ciocalteau reagent-positive substances.

Whether these inhibitory substances in crude drugs act similarly on enzymes in the gastrointestinal tract remains to be investigated. This type of study should be useful as a preliminary to a reexamination of gastrointestinal combination products containing stomachic crude drugs.

References and Notes

1) A part of this work was presented at the 29th and the 30th meetings of the Kinki Branch, Pharmaceutical Society of Japan, Kyoto, November 1979 and Osaka, November 1980.
2) Drugs in Japan (OTC Drugs), 1st Ed., Yakugyojiho-sha, Tokyo, 1978, p. 181.
3) K. Okazaki, *Yakuzaigaku*, **17**, 33 (1957); K. Okazaki, J. Yamamoto, and Y. Chiba, *Yakuzaigaku*, **22**, 98 (1962).
4) Osako Iyakuhinkyokai Nempo 29, Osaka Iyakuhinkyokai, Osaka, 1979, p. 485.
5) S. Schwimmer, *Cereal. Chem.*, **24**, 167 (1947); M. Ito, "Koso-Kenkyuho," Vol. 2, Ed. by S. Akabori, Asakura-Shoten, Tokyo, 1966, p. 120.
6) Pharmacopoeia Japonica, Editio Nona, 1976, p. 1100.
7) Pharmacopoeia Japonica, Editio Nona, 1976, p. 658.
8) Pharmacopoeia Japonica, Editio Nona, 1976, p. 660.
9) *J. Amer. Leather Chem. Assoc.*, **49**, 174 (1954).
10) T. Ikenaka, *Metabolism and Disease*, **14**, 1057 (1977); H. Umezawa, T. Aoyagi, and T. Takeuchi, *ibid.*, **14**, 1071 (1977); N. Aoki and M. Moroi, *ibid.*, **14**, 1099 (1977).
11) I. Chibata, T. Tosa, T. Mori, and T. Watanabe, *Protein, Nucleic Acid and Enzyme*, **22**, 76 (1980).
12) Y. Furusawa, Y. Kurosawa, and I. Chuman, *Nippon Nogei Kagaku Kaishi*, **47**, 359 (1973).