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CASEINOLYTIC ACTIVITY IN PLANT TISSUES (II)

By

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Abstract

Extracts from various plants were examined for caseinolytic activity. Very high activity was found in the extracts of snake gourds. Among them the activity of Karasuuri, *Trichosanthes cucumeroides Maxim.*, is higher than that of Kikarasuuri, *Trichosanthes Kirilowii Maxim. var. japonica Kitam.* Their proteases seem to be serine protease.

Introduction

A number of plant proteases have been studied, usually emphasizing the properties of such well-known enzymes as papain(1), ficin(2), and bromelain(3). In contrast to the above thiol proteases, relatively little is known about other types of proteases from plant sources.

As a successor to our previous paper(4), we describe here the protease screening test of various plants.

Experimental

Vegetables, fruits and cereals were purchased from greengrocers and other plants were collected locally in Kagoshima city. Casein was a product of E. Merck, Darmstadt, West Germany. Trichloroacetic acid was purchased from Wako Pure Chemical Industries Ltd.

Preparation of Sample Solution— Juice : A sarcocarp was ground with a grator made of synthetic resin. The homogenate was centrifuged for 20 min at $3000 \times g$, or filtered through a cotton cloth.

Extracts : Leaves and seeds were ground in equal weight of 0.02M phosphate buffer, pH 7.3, in a mortar, and the homogenate was stirred for 5 min and filtered through a cotton cloth.

Juices and extracts were diluted to the point of appropriate concentration for assay with 0.02M phosphate buffer, pH 7.3.

Assay of Protease— Proteolytic activity was measured by the method of Kunitz(5), with casein as a substrate. One ml of sample solution was preincubated for 10 min at 30°C , and

then added to 1 ml of a solution of 1%(w/w) casein containing 0.02M phosphate buffer, pH 7.3, at 30°C. After incubation for 30 min the reaction was terminated by the addition of 3 ml of 5 % trichloroacetic acid. After standing for 30 min at room temperature, the precipitate was removed by filtration through Toyo filter paper No. 5C and the absorbancy at 280 nm of the trichloroacetic acid-soluble peptides formed was determined with Hitachi spectrophotometer 100-60.

A unit of activity was defined as that amount which yielded 0.001 $A_{280 \text{ nm}}$ unit of change per min under the conditions mentioned above. The specific activity is expressed as the number of enzyme units per 1 ml of juice or extract.

Results and Discussion

The results of the screening test are shown in Table I.

Proteolytic activity was observed in several plants. The activities of snake gourds were prominent in the sample tested. These proteases were confirmed to be serine protease by further investigation. We had already isolated serine protease, cucumisin [EC 3.4.21.25] from the sarcocarp of prince melon(6). The proteases contained in the fruit of the *Cucurbitaceae* seem to be serine type, but a different quantity was observed for each variety of *Cucurbitaceae*.

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Table 1. Caseinolytic Activity of Extracts from Plant Tissues

Plant	Plant parts	Method of extraction	Activity (Units)
Ajisai, Hydrangea(<i>Hydrangea macrophylla</i> (Thunb) Ser.var. <i>macrophylla</i>)	Leaf	Ext	0
Akebi, <i>Akebia</i> (<i>Akebia quinata</i> Decne)	Sar	Ext	10
Amachazuru, (<i>Gynostemma pentaphyllum</i> Makino)	Berry	Ext	19
Asagao, Japanese morning glory (<i>Pharbitis Nil</i> Choisy)	Leaf	Ext	0
	Seed	Ext	15
Asebi, <i>Andromeda</i> (<i>Pieris japonica</i> (Thunb)D.Don)	Leaf	Ext	0
Azumagaya, (<i>Aspella longe-aristata</i> (Hachel) Ohwi)	Seed	Ext	28
	Leaf	Ext	31
Fudanso, Leaf beet(<i>Beta vulgaris</i> L.var. <i>Cicla</i> L.)	Leaf	Ext	0
Fuji, Japanese wistaria(<i>Wistaria floribunda</i> DC.)	Fruit	Ext	81
Fuki, Butter bur(<i>Petosites japonicus</i> (Sieb. et Zucc) Maxim.)	Lf,St	Ext	9
Hasu, East indian lotus(<i>Nelumbo nucifera</i> Gaertn)	Rhizome	Ext	5
Hassaku, Hassaku(<i>Citrus hassaku</i> Hort.ex Tanaka)	Sar	Pre	0
Hekusokazura, (<i>Paederia scandens</i> Merrill.)	Fruit	Ext	3
Higanbana, Cluster amaryllis (<i>Lycoris radiata</i> Herb.)	Stem	Ext	0
Higanzakura, Early flowering cherry(<i>Prunus subhirtella</i> Miq.)	Sar	Pre	0
	Seed	Ext	52
Horenso, Spinach(<i>Spinacia oleracea</i> L.)	Leaf	Pre	0
Hotokenoza, Henbit(<i>Lamium amplexicaule</i> L.)	Leaf	Ext	8
Ine, Rice plant(<i>Oryza sativa</i> L.)	Leaf	Ext	0
Inubiwa, (<i>Ficus</i> (sect. <i>Ficus</i>) <i>erecta</i> Thunb.)	Sar	Ext	11
Inumugi, Rescue grass(<i>Bromus unioloides</i> Humb. Bonpl et Kunth)	Seed	Ext	45
Irohakaede, Japanese maple(<i>Acer palmatum</i> Thunb.)	Leaf	Ext	0
Karasunoendo, Crow pea(<i>Vicia anqustifolia</i> L. var. <i>segetalis</i> (Thail) Koch)	Lf,St	Pre	0
Karasuuri, Snake gourd(<i>Tricosanthes cucumeroides</i> Maxim.)	Fruit	Pre	3,065
Karatachi, Trifoliate orange (<i>Poncirus trifoliate</i> Rafin.)	Sar	Ext	4

(continued on the following page)

(from the Table I)

Kikarasuuri, (Trichosanthes Kirilowii Maxim.var. japonica (Miq.) Kitam.)	Fruit	Pre	978
Kimigayoran, Curveleaf yucca (Yucca recurvifolia Salisb.)	Leaf	Ext	27
Kiranso, Ground pine (Ajuga decumbens Thunb.)	Leaf	Ext	0
Konara, (Quercus serrata Thunb.)	Nut	Ext	27
Kuri, Chestnut (Castanea crenata Sieb. et Zucc.)	Nut	Ext	31
Kusagi, Glory bower (Clerodendron trichotomum Thunb.)	Leaf	Ext	0
Kuzu, Arrowroot (Pueraria Thunbergiana Benth.)	Leaf	Ext	0
Kyanberu, Cambell early (Vitis labruscara L.H.Bailey)	Sar	Pre	0
Mamushigusa, Indian turnip (Arisaema serratum Scott forma Thunbergii Makino)	Leaf	Ext	0
Masaki, (Euonymus japonicus Thunb.)	Leaf	Ext	0
Mitsuba, (Cryptotaenia japonica Hassk.)	Lf, St	Pre	2
Murasakishikibu, Japanese beauty berry (Callicarpa japonica Thunb.)	Berry	Ext	0
Nashi, Pear (Pyrus serotina Rehder)	Sar	pre	0
Natsume, Jujube tree (Zizyphus Jujuba Mill var. inermis Rehd.)	Sar	Ext	0
Nobudo, Wild grape (Ampelopsis brevipedunculata Trautv. var. maximowiczii Rend.)	Sar	Pre	0
Neomasukatto, Neo muscat (Vitis vinifera L.)	Sar	Pre	0
Nezumimochi, Japanese privet (Ligustrum japonicum Thunb.)	Berry	Ext	0
Ogatamanoki, (Michelia compressa (Maxim.) Serq.)	Leaf	Ext	0
Okumayanagi, (Berchemia recemosa Sieb. et Zucc. var. magna Makino)	Seed	Ext	0
Omoto, (Rohdea japonica (Thunb.) Roth.)	Berry	Ext	4
Onamomi, Cocklebur (Xanthium strumarium L.)	Seed	Ext	0
Oshiroibana, Marvel of Peru (Mirabilis jalapa L.)	Seed	Ext	0
Rakkyo, Baker's garlic (Allian bakeri Regel)	Bulb Germ	Ext Ext	0 15
Sansho, Japanese pepper (Xanthoxyum piperitum DC.)	Fruit	Ext	0
Sarusuberi, Grape myrtle (Lagerstroemia indica L.)	Sar	Ext	0
Seitakaawadachiso, Tall goldenrod (Solidago serotina Ait)	Root	Ext	0

(continued on the following page)

(from the Table I)

Plant	Plant parts	Method of extraction	Activity (Units)
Sendan, Bead tree(<i>Malia azedarach</i> L. var. <i>japonica</i> Makino)	Sar	Ext	0
Suzumenoteppo, Foxtail(<i>Alopecurus aequalis</i> Sobol. var. <i>amurensis</i> (Komar.) Ohwi)	Seed	Ext	125
Suzuran, Lily of the valley (<i>Convallaria majalis</i> L. var. <i>Keiskei</i> Makino)	Leaf	Ext	0
Tabirako, Nipplewort(<i>Lapsana apogonoides</i> Maxim.)	Leaf	Ext	0
Tabunoki, Perseu boehmer(<i>Machilus thunbergii</i> Sieb. et Zucc.)	Leaf	Ext	0
Tasai, Chinese cabbage(<i>Brassica chinensis</i> L.)	Leaf	Pre	9
Takana, Chinese cabbage(<i>Brassica juncea</i> Czern. et Coss. var. <i>integrifolia</i> Sinskaia)	Leaf	Pre	8
Tsubaki, Camellia(<i>Camellia japonica</i> L.)	Sar	Ext	0
Ume, Japanese apricot(<i>Prunus mume</i> Sieb. et Zucc.)	Seed	Ext	6
Yosai, Ensai, Water convolvulus (<i>Ipomea aquatica</i> Forsk.)	Leaf	Ext	15
Zakuro, Pomegranate(<i>Punica granatum</i> L.)	Sar	Pre	0

Ext: Extract, Lf: Leaf, Pre: Pressed juice, Sar: Sarcocarp, St: Stem