Comparative Study of Proteinase Inhibitors in Tropical Root Crops and Survey of Allelochemicals in the Edible Aroids

J. Howard Bradbury*,‡ and Brendon C. Hammer

Chemistry Department, Australian National University, Canberra, ACT, Australia

A comparative study was made of the amount of proteinase inhibitors present in tropical root crops. In *Alocasia macrorrhiza*, the amount of trypsin inhibitor exceeds that reported elsewhere in potato, barley, and legumes and is 10–100 times greater than the amount present in sweet potato and taro (*Colocasia esculenta*). The latter amounts to 1–10 times that found in *Cyrtosperma chamissonis*. No significant amounts of trypsin inhibitor are present in cassava, yam (*Dioscorea* spp.), and taro (*Xanthosoma sagittifolium*). The trypsin inhibitor molecule found in *A. macrorrhiza* is also a chymotrypsin inhibitor. Chymotrypsin inhibitor is absent from the other tropical root crops studied. For *A. macrorrhiza* and *C. chamissonis* there is a correlation between crude protein and trypsin inhibitor contents. Such a correlation is lacking for taro *Colocasia* and sweet potato, which unlike *A. macrorrhiza* and *C. chamissonis* have been subject to extensive selection/breeding. The content of allelochemicals (trypsin inhibitor, calcium oxalate raphides and acridity) present in five edible aroids is generally consistent with the "apparency" (defined as the susceptibility to discovery by parasites) of the plant part and its resistance to parasites in the South Pacific, except for taro *Xanthosoma* (relatively recently introduced to the region) which is more resistant to parasites than expected.

Proteinase inhibitors have been shown to be present in tropical root crops such as sweet potato (*Ipomoea batatas*) (Sugiura et al., 1973; Sumathi and Pattabiraman, 1975; Bradbury et al., 1984, 1985), taro (*Colocasia esculenta* var. *esculenta* and *Colocasia esculenta* var. *antiquorum*) (Ryan et al., 1968; Sumathi and Pattabiraman, 1975, 1979; Ogata and Makisumi, 1984, 1985; Hammer et al., 1989), giant taro (*Alocasia macrorrhiza*) (Sumathi and Pattabiraman, 1977; Hammer et al., 1989), and giant swamp taro (*Cyrtosperma chamissonis*) (Hammer et al., 1989). Trypsin inhibitors from three members of the taro family (edible aroids) were purified and characterized. The trypsin inhibitors each consisted of two probably identical subunits having molecular weights of about 20 000. The N-terminal sequences showed homology with one another and with Kunitz soybean trypsin inhibitor (Hammer et al., 1989). The inhibitor from *A. macrorrhiza*, molecular weight of about 40 000, inhibited both trypsin and chymotrypsin with 1:2 stoichiometry (Hammer, 1987).

It is important to know the amount of proteinase inhibitor present because such substances, particularly trypsin inhibitors, have been shown to cause diminished growth in rats, chickens, and other experimental animals (Liener and Kadade, 1980). Since these foods are cooked before human consumption, a study of the heat stability of their respective proteinase inhibitors is also important (Hammer, 1987; Bradbury and Holloway, 1988; Di Pietro and Liener, 1989). The heat stability of these inhibitors in dilute solution and in the root on cooking, as well as its importance for nutrition, will be considered in a subsequent publication.

Feeny (1976) has developed an ecological theory of the distribution of plant allelochemicals (chemicals that provide defense against attack by parasites) which relies upon assignment of a degree of "apparency" to plant parts and species. The degree of apparency increases in relation to the probability of parasite discovery for the plant part or species considered, while increased apparency should lead to the presence of increased levels of allelochemicals. In more apparent plants, a greater share of metabolic resources will be expended upon the production of allelochemicals.

In this paper we report on the content of trypsin and chymotrypsin inhibitors present in all major tropical root crops. The distribution of the inhibitor between root and leaves of taro and within the stems of the aroids has also been studied. The content of allelochemicals in five edible aroids is also considered in relation to the degree of apparency of the plant part and its resistance to parasites.

MATERIALS AND METHODS

Root crops (see Table I) were harvested in various countries of the South Pacific and cleaned and their fresh weights recorded. They were consigned by air freight to Canberra, where they were stored at 15 °C until processed, usually within about 1 week of harvesting. Roots were weighed and peeled. For smaller specimens the whole root was used, and for larger roots a representative cross section near the center of the root was taken for analysis. The sample was assayed in the fresh state or else diced and dried at 40 °C in a ventilated oven to constant weight. The sample dried at 40 °C was ground to a fine powder by using an electric grinder. A small sample of the root crop was dried at 100 °C to constant weight, and the percentage moisture in the root crop as harvested was calculated (Holloway et al., 1989).

Bovine pancreatic trypsin and bovine pancreatic α-chymotrypsin (type II) were obtained from Sigma Chemical Co. Synthetic substrates *p*-tosyl-L-arginine methyl ester hydrochloride (TAME), *N*-benzoyl-L-tyrosine ethyl ester (BTEE), *N*-α-benzoyl-D,L-arginine β-naphthylamide hydrochloride (BANA), and *N*-α-benzoyl-D,L-arginine *p*-nitroanilide hydrochloride (BAPNA) were also obtained from Sigma Chemical Co.

Trypsin Inhibitor Assay. A weighed fresh or dry sample of root crop was made up to a fixed volume in a 100-mL measuring cylinder with buffer solution (0.04 M Tris, 0.01 M $CaCl_2$, pH adjusted to 8.1) and homogenized for 1 min by using a Polytron (Kinematica, GmbH, Switzerland). The homogenate was

‡ Present address: Botany Department, Australian National University, Canberra, ACT, Australia.

0021-8561/90/1438-1448$02.50/0

allowed to stand for 5 min before centrifugation for 30 min at 7500g and 5 °C. Aliquots of the supernatant were diluted to exactly 5 mL with the pH 8.1 buffer solution to give a range of four or five solutions of different inhibitor concentrations.

The incubation of trypsin inhibitor with trypsin and subsequent analysis of results follows Bradbury et al. (1984), using an extinction coefficient at 247 nm for the TAME hydrolysis product, p-tosyl-L-arginine of 0.540 cm^2 μmol^{-1} (Rick 1974). In all assays the same source of trypsin was used, with an activity (turnover of TAME) of 300 ± 10 μmol of TAME min^{-1} (mg of trypsin)$^{-1}$. Control experiments, using extracts from all root crops studied and conducted in the absence of trypsin, gave no change in absorbance with time. Each experiment used 0.05 mg of trypsin, which in the absence of inhibitor hydrolyzed 15 μmol of TAME/min (15 units of trypsin); hence, an operational unit was defined, viz., a trypsin inhibitor unit (TIU), which was the amount of inhibitor required to 50% inhibit the hydrolysis of 0.05 mg of trypsin. Since 1 IU has been defined as the amount of proteinase inhibitor to decrease the enzyme-catalyzed hydrolysis of substrate by 1 μmol/min (Fritz et al., 1974), then it is seen that 1 TIU = 7.5 IU. From the known amount of root crop used to produce the aqueous extract of trypsin inhibitor and its moisture content, the amount of trypsin inhibitor (C_{TI}) in TIU per gram of fresh sample was calculated. Average error of duplicate samples was 2%; the limit of detection was about 0.2 TIU/g fresh weight.

Chymotrypsin Inhibitor Assay. The extraction of enzyme inhibitor from the root crop followed the method already described above for trypsin inhibitor except that a different buffer solution was used, viz., 0.08 M Tris and 0.10 M $CaCl_2$ adjusted to pH 7.80 with 6 M HCl. Aliquots of the supernatant solution from extraction of the root crop were diluted to 5 mL with pH 7.80 buffer solution to give four or five solutions with different inhibitor concentrations. Chymotrypsin, 0.100 mL of a solution of α-chymotrypsin (1.00 mg/mL in 10^{-3} M HCl), was added to each solution followed by shaking and incubation at 25 °C for 10 min.

After incubation, 0.100-mL aliquots of these solutions were added in turn to preincubated quartz cuvettes which contained 1.4 mL of 1.00×10^{-3} M BTEE solution in 50% methanol/water (w/w) plus 1.5 mL of pH 7.8 buffer. The increase in absorbance at 256 nm was followed for 5 min, and a linear relationship was always obtained. Control experiments using supernatant solutions from the different root crops, but in the absence of chymotrypsin, gave no change of absorbance over 5 min.

The data treatment for the four or five solutions was analogous to that with trypsin and followed the method of Bradbury et al. (1985). All assays used the same sample of chymotrypsin which hydrolyzed 49 ± 3 μmol of BTEE min^{-1} (mg of chymotrypsin)$^{-1}$. Each experiment used 0.10 mg of chymotrypsin, which in the absence of inhibitor hydrolyzed 4.9 μmol of BTEE/min (4.9 units of chymotrypsin); hence, an operational chymotrypsin inhibitor unit (CIU) was defined as the amount of inhibitor required to 50% inhibit 4.9 units of chymotrypsin. Since 1 IU was defined as the amount of proteinase inhibitor to decrease the enzyme-catalyzed hydrolysis of substrate by 1 μmol/min (Fritz et al., 1974), then it is clear that 1 CIU = 2.45 IU. From the known amount of root crop used to produce the inhibitor solution and its moisture content, the amount of chymotrypsin inhibitor (C_{CI}) present in CIU/g fresh weight sample was calculated. Average error on duplicate samples was 3% and lower limit of detection about 0.5 CIU/g fresh weight.

Diffusion Assay for Trypsin Inhibitor. This is an adaptation of a method of Gatehouse and Gatehouse (1979). Agarose (3.75 g), type 2, Sigma, was dissolved in 190 mL of boiling water, and 190 mL of cold buffer (0.04 M Tris, 0.01 M $CaCl_2$ at pH 8.1) solution was added with stirring.

In a separate experiment 50 mL of Sepharose 4B (Pharmacia Fine Chemicals) was activated and coupled to trypsin (March et al., 1974). The enzymic activity of the trypsin–Sepharose 4B was checked by shaking the washed beads with a saturated solution of BAPNA in pH 8.1 buffer, which produced a yellow solution after a few seconds.

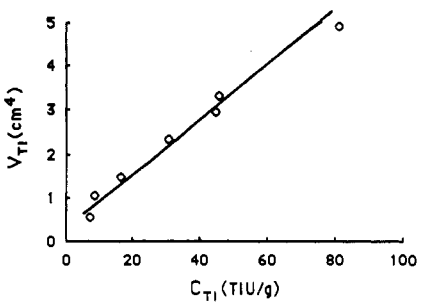

Figure 1. Trypsin inhibitor activity of samples of taro *C. esculenta* from Fiji determined by spectrophotometric assay C_{TI} (TIU per gram) and by the diffusion assay V_{TI}.

The trypsin–Sepharose 4B slurry (1 mL) diluted to 3 mL with distilled water was added to the agarose–buffer solution with rapid stirring and the mixture poured into a leveled tray 40 × 40 × 5 cm to a depth of about 2.5 mm. The gel layer set, after which a grid of 8 × 8 wells about 3.5 mm in radius was made in the gel by using a stainless steel cork borer. Aliquots (0.1 mL) of the supernatant solution containing trypsin inhibitor (see Trypsin Inhibitor Assay) were added to the wells, and the tray was covered and left for 20 h at 20 °C. The contents of the wells were absorbed by the gel matrix within 2 h. Each solution was run in three separate wells, and the results were averaged. The development of the gel was made with a solution prepared in the following way. The diazonium chloride salt of o-dianisidine (Koch Light Laboratories) (500 mg) was dissolved in 500 mL of 0.10 M sodium acetate and 375 mL of 0.14 M NaCl added. BANA (375 mg in 50 mL of methanol) was added with stirring followed by 50 mL of 0.02 M aqueous potassium cyanide by use of a fume cupboard. The developing solution was immediately poured onto the surface of the gel, and the tray was rocked for about 2 min to facilitate even diffusion of reagent into the gel. After 3 h, it was found that regions which contained active trypsin–Sepharose beads stained an orange color and there were unstained circular regions which surrounded those wells that had been loaded with trypsin inhibitor. The gel was rinsed twice to remove free cyanide with 500 mL of ferrous sulfate (0.001 M) in 0.001 M sodium hydroxide and then three times with distilled water. The diameter of each circular unstained region was carefully measured by using vernier calipers, and triplicate results were averaged.

After the development of each gel, a calibration curve was constructed to determine absolute trypsin inhibitor levels for the samples loaded. This was accomplished by determining trypsin inhibitor through spectrophotometric assay in five of the samples which had given gel results covering the full range of activity. For these five samples the concentration of trypsin inhibitor determined by spectrophotometric assay C_{TI} (TIU per gram) was plotted against the parameter V_{TI}, which is calculated by the equation of Hammer (1987)

$$V_{TI} = (2R^3 - 3r^2R + r^3)\pi R/3$$

where R is the radius of unstained circular region (centimeters) and r is the radius of well (0.35 cm). This equation gave a linear relationship between C_{TI} and V_{TI} and was applicable over the range shown in Figure 1. The relation of Gatehouse and Gatehouse (1979) was based on the assumption that the area of the unstained region was proportional to the amount of inhibitor loaded and did not give a linear response.

From Figure 1 C_{TI} could be read from the value of V_{TI} determined by the diffusion assay technique. The lower limit of quantitative detection for this method was about 5 TIU/g and the error ±3 TIU/g. If low or zero values were obtained by this method, then the measurement was normally repeated by using the much more sensitive trypsin inhibitor assay (see above). Because of the much greater trypsin inhibitor activity of giant taro as compared with taro (see Table I), the method was modified in this case by use of 3.75 mL of trypsin–Sepharose 4B slurry instead of 1 mL (see above) and use of smaller wells (2.5-mm radius) which were loaded with only 0.050 mL of supernatant.

Table I. Concentration of Trypsin Inhibitor (C_{TI}) and Chymotrypsin Inhibitor (C_{CI})[a] in Tropical Root Crops (Standard Deviation in Parentheses)

root crop and source	names of cultivars	C_{TI}, TIU/g	F_I[b]
taro, *C. esculenta*			
Fiji corms, mean of 10 cvs.	Samoa green, Samoa hybrid, Samoa, Samoa oriori, Dalo ni Toga,Toakulu, Tausala ni Samoa, Tausala ni mumu, Vavai dina, Hawaii	27 (26)	0.04 (0.04)
Western Samoa, corms, mean of 4 cvs.	Manua, Pa'epa'e, Niue, Fa'ele'ele	8.8 (2)	0.02 (0.02)
Solomon Islands, corms, mean of 14 cvs.	Akalomamale, Luma'abu, Mudi mudi, Sasagiha, PD1(20), PD1(32), PD11(5), PD12(11), PD41(1), PD41(4), PD41(8), PD41(7), PD51(9), PD51(27)	5.1 (6)	0.01 (0.01)
Fiji, suckers,[c] mean of 2 cvs.	Samoa hybrid, Samoa	12 (10)	
Fiji, leaves, 3 cvs.	Tausala ni mumu, Tausala ni Samoa, Samoa	0	
taro, *X. sagittifolium*			
Tonga corms, mean of 3 cvs.	Futuna, Maheleuli, Tea	0.3 (0.3)	
giant taro, *A. macrorrhiza*			
Western Samoa, 1984, mean of 6 cvs.	Lau penitala, Faitama, Sega, Niukini, Toga, Fui	340 (190)	0.23 (0.06)
Western Samoa, 1985, mean of 6 cvs.	Toga, Laufola, Niu kini, Fui, Sega, Uli	200 (100)	0.14 (0.05)
giant swamp taro, *C. chamissonis*			
Kiribati, mean of 6 cvs.	Ikaraoi kairoro, Atimainiku, Ikaraoi ikauraura, Katutu uraura, Katuta kairori, Ikaraoi natutebubua	2.5 (2.6)	0.01 (0.01)
sweet potato			0.003–0.02[e]
Solomon Islands, mean of 9 cvs.	Santa cruz, Three months, Western, Toni, TIS 2498,[d] Nawaro, 108, 107, 131	25 (17)	
Tonga, mean of 2 cvs.	Melefakahau, Halasika	22 (26)	
Papua New Guinea Highlands, mean of 13 cvs.	Simbul sowar, Tomun, Po, Parabea, Harbare, Wanmun, Kariap, Sapel, Soii, Mame, Bau, Paipa, Padua	3.5 (3.7)	
Fiji, mean of 4 cvs.	TIB2, TIS1499, TIS3017, TIS3030[d]	2.2 (2.0)	
cassava			
Solomon Islands, 9 cvs.	WSH9, Tikopia, Malaita red, WSH1, Betikama, Maliae 2, Curry gizo, WSH5, WSH2	0	
yam, *D. alata*			
Papua New Guinea, mean of 5 cvs.	Kpmora, Du kupmi, Takua yaimbi, Tolai, Yavovi	0.6 (0.3)	
yam, *D. esculenta*			
Solomon, Islands, 4 cvs.	NGP4, RFP1, GUP5, NGP8	0	

[a] The chymotrypsin inhibitor content C_{CI} (CIU per gram) was 57 (22) for giant taro, 1.0 (0.3) for sweet potato cvs. from Solomon Islands but zero for sweet potato cvs. from Papua New Guinea (Bradbury et al., 1985), and zero for taro *Colocasia* from Fiji, taro *Xanthosoma*, giant swamp taro, and yam (*D. esculenta*). [b] F_I, fraction of inhibitor to total crude protein present. [c] Suckers or cormels grow around the base of the corm. [d] TIS and TIB cultivars imported from IITA, Nigeria. [e] Bradbury et al. (1984).

Nitrogen Analysis and Crude Protein Content. Total nitrogen was determined by the Dumas method using a Carlo Erba 1106 CHN analyzer by the Analytical Group, Research School of Chemistry, Australian National University (Bradbury et al., 1985). Results obtained by the Dumas method agreed satisfactorily with analyses for organic nitrogen by the Kjeldahl method using a Kjeltec digestion and distillation apparatus, although the latter method was found to give more reproducible results (Bradbury & Holloway, 1988). The crude protein content was calculated by multiplying the %N by 6.25 (Bradbury et al., 1984).

Calculation of Fraction F_I of Inhibitor to Total Crude Protein. It was found in an earlier study using Worthington trypsin (activity 180 μmol of TAME min^{-1} mg^{-1}) that 0.012 mg of soybean trypsin inhibitor (STI) had the inhibitory activity of 1 TIU; i.e., it caused 50% inhibition of 0.05 mg or complete inhibition of 0.025 mg of Worthington trypsin (Bradbury et al., 1984). Because of the 1:1 stoichiometry of STI and trypsin and their similar molecular weights, the purity of the Worthington trypsin was seen to be 48%. On the basis of the greater activity of the Sigma trypsin used in this study (300 μmol of TAME min^{-1} mg^{-1}) it is calculated that the Sigma trypsin is 80% pure. Thus, the amount of active trypsin in 0.025 mg of Sigma trypsin (completely inhibited by 1 TIU of inhibitor) is 0.020 mg. Since one molecule of trypsin reacts with one aroid trypsin inhibitor subunit of about the same molecular weight (Hammer, 1987; Hammer et al., 1989), 0.02 mg of trypsin reacts with 0.02 mg of trypsin inhibitor, which is equivalent to 1 TIU. From the concentration C_{TI} of inhibitor in TIU per gram of root crop it is thus possible to calculate the amount of inhibitor present per gram of root crop and express this as a fraction of the total crude protein determined from the %N (see above). Because of the accumulation of errors in this calculation, the accuracy of F_I is only about 25%.

Distribution of Trypsin Inhibitor within the Stem. Taro, giant taro, and giant swamp taro stems were used, and two disk-shaped segments were cut toward each end of the stem. After peeling, four circular sections were cut from the center outward and the trypsin inhibitor content of each section was determined after activation of the trypsin inhibitor by previous immersion in distilled water at 80 °C for 5 min (Hammer, 1987; Bradbury et al. 1989).

RESULTS AND DISCUSSION

Inhibitor Content in Root Crops. The content of trypsin inhibitor and chymotrypsin inhibitor present in the various root crops is summarized in Table I. There is a large amount of trypsin inhibitor in giant taro which as a fraction of the total crude protein present (0.14–0.23) exceeds that from other inhibitor-rich plants such as potato *Solanum* (0.10), barley (0.075), and legumes (0.06) (Hammer, 1987). Also, it has been found that the inhibitor activity for giant taro is greatly increased by pretreating the root crop by heating at 80 °C for 5 min (Hammer, 1987), which has the effect of increasing the fraction for giant taro from about 0.2 to 0.5. This is about 10–100 times the amount present in sweet potato and taro *Colocasia*, which is 1–10 times greater than in giant swamp taro. Taro *Colocasia* suckers have about the same trypsin inhibitor content as the parent corms. There is no detectable amount of trypsin inhibitor in taro leaves. Small amounts of trypsin inhibitor were found in taro *Xanthosoma* and yam (*D. alata*), while no trypsin inhibitor was found in either yam (*D. esculenta*) or in cassava. On the other hand, Sumathi and Pattabiraman (1975) have reported the absence of inhibitor in yam (*D. alata*) while observing low

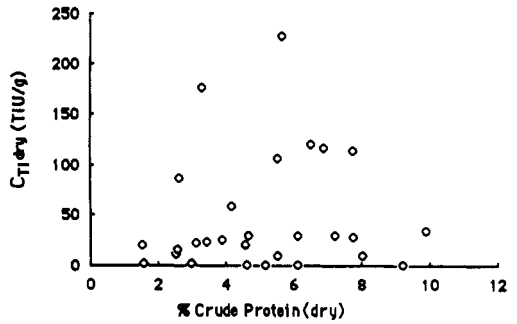

Figure 2. Graph of the trypsin inhibitor content (C_{TI}) of dry taro *Colocasia* corms against the percent crude protein content.

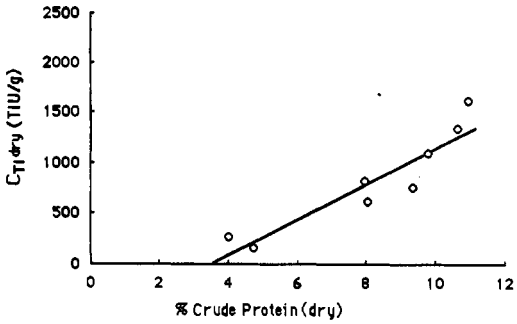

Figure 3. Trypsin inhibitor content (C_{TI}) of dry giant taro plotted against the percent crude protein.

levels in cassava, but these discrepancies are minor when one considers the very small amounts reported.

The chymotrypsin inhibitor content of giant taro is very large and equals or exceeds that found in other rich sources of chymotrypsin inhibitor such as navy bean, French bean, winged bean, and peas (Hammer, 1987). It was found by Hammer (1987) that, for a series of giant taro cultivars, a graph of the trypsin inhibitor content (C_{TI}) against the chymotrypsin inhibitor content (C_{CI}) gave a linear relationship, which would be expected if the antitryptic and antichymotryptic activity of giant taro are due to one bifunctional inhibitor. This has been shown to be the case, whereas the inhibitors present in taro *Colocasia* and giant swamp taro inhibit only trypsin (Hammer, 1987). A small amount of chymotrypsin inhibitor was found in sweet potato from Solomon Islands (Table I), but none was present in cultivars from the Highlands of Papua New Guinea (Bradbury et al., 1985). Chymotrypsin inhibitor was absent from taro *Colocasia*, taro *Xanthosoma*, giant swamp taro, and yam (*D. esculenta*).

Correlation between Crude Protein and Trypsin Inhibitor Contents. For sweet potato it was found that in general there was no positive correlation between trypsin inhibitor and crude protein content, which indicated the possibility of selection/breeding for high protein content with low levels of trypsin inhibitor (Bradbury et al., 1984, 1985). However, it was found that within any particular cultivar there was some correlation and also that a range of cultivars grown in the same environment gave a poorer correlation (Bradbury et al., 1985).

Data for taro *Colocasia*, giant taro, and giant swamp taro are given in Figures 2–4, respectively. It is clear from Figure 2 that for *Colocasia* there is no correlation between trypsin inhibitor and crude protein content; hence, it should be possible to select/breed taro *Colocasia* of high protein content and low inhibitor content. With giant taro the relation as shown in Figure 3 is fitted by the equation

$$C_{TI} = 182\ (\%\ \text{crude protein}) - 668$$

with a correlation coefficient $r = 0.93$. Since 1 TIU is

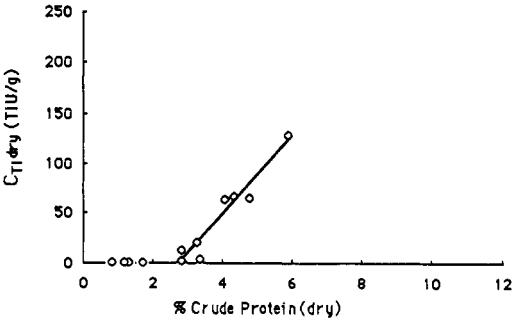

Figure 4. Graph of the trypsin inhibitor content (C_{TI}) of dry giant swamp taro against the percent crude protein.

related to a known weight of inhibitor (see above), it is possible to convert the ordinate in the graph to amount of trypsin inhibitor (grams). It is concluded that, above the 3% level of crude protein, about 40% of the additional protein produced by the giant taro corm would be trypsin inhibitor (Hammer, 1987). The results for giant swamp taro in Figure 4 similarly indicate that the trypsin inhibitor content is zero for corms that contain less than 3% crude protein (dry basis), while above 3% dry protein there is a linear relation fitted by the equation

$$C_{TI} = 39.8\ (\%\ \text{crude protein}) - 113$$

(all on a dry basis) with $r = 0.97$. For giant taro and giant swamp taro it appears that the resources of the plant available for protein synthesis have been dedicated to the manufacture of proteins which are more essential to the survival of the plant than is trypsin inhibitor, until a threshold protein content is reached (see Figures 3 and 4). Above this threshold of protein content giant taro allocates over one-third of its protein-directed resources to production of trypsin inhibitor and giant swamp taro over one-tenth of its resources.

These results are in direct contrast to the findings with taro (Figure 2) and sweet potato (Bradbury et al., 1984, 1985), where there is no correlation between protein content and trypsin inhibitor activity. While taro and sweet potato have been subjected to selection and breeding for many hundreds of years, giant taro and giant swamp taro are minor and reserve foods except in limited areas of the South Pacific (Bradbury and Holloway, 1988) and have only been selected to a minor degree in these countries. Selection of cultivars for desirable traits (high yield, resistance to organisms, etc.) may inadvertently cause loss or enhancement of other important characters, including a specific resistance factor such as trypsin inhibitor (Herklots, 1972; Broadway, 1989). This could produce the "scrambling" effect of trypsin inhibitor content observed with taro and sweet potato.

Distribution of Trypsin Inhibitor within the Corm. In the longitudinal direction corms of giant taro showed no change in trypsin inhibitor content, whereas with taro *Colocasia* and giant swamp taro the inhibitor content was lower toward the base of the corm. Changes observed in the radial direction obtained from measurements on cross sections (see above) are shown in Figures 5 and 6. There is not much change in inhibitor content across corms of taro *Colocasia* and giant swamp taro (Figure 6). With giant taro, however, there is a large concentration of inhibitor near the center of the corm which reduces to about zero near the skin. By contrast, the crude protein content is lowest at the center of the giant taro corm and increases by 20–50% near the skin (Hammer, 1987). Thus, the fraction of inhibitor/total protein changes from a value approaching unity at the center of the corm to about zero

Table II. Data on the Possible Protective Role of Chemicals Present in Aroids

aroid and plant part	resistance to parasites and diseases of plant in South Pacific[a]	chemicals present, mean concentration, and gradient within the corm		
		trypsin inhibitor F_I	calcium oxalate[b] (mg/100 g)	acridity[a,c]
taro *Colocasia*, corm	large number of pests and diseases	0.01–0.04, no gradient (Figure 6)	43, gradient, skin high, center low[d]	edible cvs. are low, some high in acridity
taro *Colocasia*, leaf	large number of parasites and diseases	0	400	edible cvs. are low, nonedible cvs. are high in acridity
taro *Xanthosoma*, corm	less subject to parasites and diseases than *Colocasia*	0.001	23, gradient skin higher, center lower[b]	similar to *Colocasia* corms
giant taro *A. macrorrhiza*	hardy plant, resistant to parasites and diseases	0.14–0.23[e] gradient skin low, center high (Figure 5)	37, gradient skin high, center low[b]	high acridity often concentrated in surface layers
giant swamp taro *C. chamissonis*	resistant to parasites and diseases	0.008, no gradient (Figure 6)	399	some acridity may be present in skin
elephant foot yam *A. campanulatus*	resistant to parasites and diseases	very low[f]	382	edible cvs. are low, but wild forms are high in acridity

[a] See Bradbury and Holloway (1988). [b] Holloway et al. (1988). [c] Nixon (1987). [d] Sunell and Healey (1979). [e] This trypsin inhibitor also strongly inhibits chymotrypsin. [f] Sumathi and Pattabiraman (1975). [g] Sakai (1983).

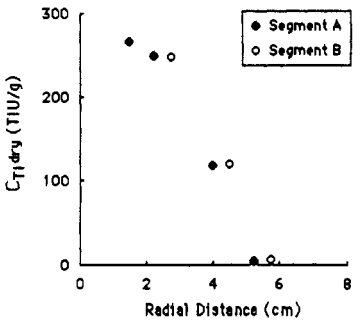

Figure 5. Trypsin inhibitor content (C_{TI}) plotted against the radial distance from the center of the corm for giant taro, cv. Toga. Cross sections taken near top (◆) and bottom (◇) of corm.

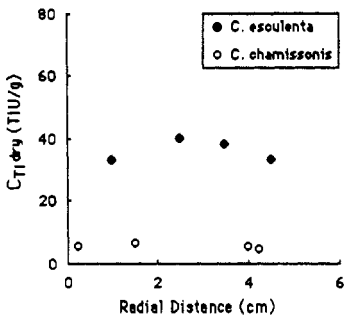

Figure 6. Trypsin inhibitor content (C_{TI}) graphed against the radial distance from the center of the corm for taro *Colocasia* cv. Toakula (◆) and giant swamp taro cv. Via (◇).

near the skin. This interesting result for inhibitor distribution contrasts with the calcium oxalate distribution in giant taro corms in which there is a large amount concentrated near the skin and much less at the center of the corm (Holloway et al., 1988).

Allelochemicals in Aroids. The potential physiological roles that have been proposed for proteinase inhibitors in the plant are as a protein store, as regulators of endogenous proteinases, and to provide protection of the plant from attack by parasites (Ryan, 1973; Richardson, 1977; Liener and Kakade, 1980). We will consider the possible protective role of proteinase inhibitors in conjunction with other potential allelochemicals present in aroids, viz., calcium oxalate and irritant acridity. In Table II this information is summarized together with data on resistance to South Pacific pests and diseases. Calcium oxalate, present in the form of barbed crystalline needles known as raphides, is thought to act as a deterrent to large parasites with mouth tissue being penetrated by the needles which carry an acrid irritant on their surface (Nixon, 1987; Bradbury and Holloway, 1988).

Each of the species listed have subterranean stems except giant taro, where the stem forms a trunk above and below the surface. Thus, the giant taro stem is more apparent than those of the other species, since it is exposed to a wider range of parasites, including airborne microbial spores and larger organisms which do not go below ground. Accordingly, giant taro exhibits high levels of acridity as well as exceptionally high levels of trypsin inhibitor. Giant taro appears to have a layered defense, with the highest concentrations of raphides near the stem surface and the highest inhibitor content near the core. Giant taro is very resistant to parasites. Giant swamp taro is also very resistant to pests, yet here the stem contains far lower levels of trypsin inhibitor and lower acridity but higher calcium oxalate content compared with giant taro; this finding is consistent with the lower apparency of the subterranean stem. A similar pattern of allelochemical levels was found in the subterranean stem of elephant foot yam (*Amorphophallus campanulatus*).

With taro *Colocasia* the difference in apparency between plant parts above and below ground may be reflected in the very high levels of calcium oxalate found in the leaves as opposed to that in the stem. Calcium oxalate, trypsin inhibitor, and acridity are all low in domestic cultivars, and it is not surprising that taro *Colocasia* is prone to parasitic attack. An explanation for this loss of pest resistance has been given above. Taro *Xanthosoma* shows greater resistance to pests yet contains very little trypsin inhibitor and calcium oxalate, and its acridity is comparable to that of taro *Colocasia*. While this result may appear inconsistent with those given for the other species studied, it should be noted that taro *Xanthosoma* has been only recently introduced to the Pacific region from America. It represents an ecologically remote member of the aroid family and may contain allelochemicals that differ from those studied here. Also, unlike *Colocasia*, it produces a large number of smaller stems (suckers), which cluster around a central mother stem. We used these suckers for our assays, since the mother corm is not customarily used for consumption, because of its generally higher acridity.

ACKNOWLEDGMENT

We thank J. E. Wilson (Western Samoa), P. Sivan and G. V. H. Jackson (Fiji), P. Linton and R. Liloqula (So-

lomon Islands), B. Thistleton, M. Levett, and G. King (Papua New Guinea), P. Taufatofua (Tonga), B. Ratieta, deceased (Kiribati), and M. F. Quin for their collaboration and provision of samples of root crops throughout the ACIAR/ANU Program on Nutrition of Tropical Root Crops in the South Pacific. We also thank The Australian Centre for International Agricultural Research (ACIAR) for financial support and G. Persley of ACIAR for useful suggestions, interest, and support.

LITERATURE CITED

Bradbury, J. H.; Holloway, W. D. *Chemistry of tropical root crops: significance for nutrition and agriculture in the Pacific*; Australian Centre for International Agricultural Research: Canberra, 1988; 201 pp.

Bradbury, J. H.; Baines, J.; Hammer, B.; Anders, M.; Millar, J. S. Analysis of sweet potato from the Highlands of Papua New Guinea: relevance to the incidence of enteritis necroticans. *J. Agric. Food Chem.* **1984**, *32*, 469–473.

Bradbury, J. H.; Hammer, B.; Nguyen, T.; Anders, M.; Millar, J. S. Protein quantity and quality and trypsin inhibitor content of sweet potato cultivars from the Highlands of Papua New Guinea. *J. Agric. Food Chem.* **1985**, *33*, 281–285.

Broadway, R. M. Tryptic inhibitory activity in wild and cultivated crucifers. *Phytochemistry* **1989**, *28*, 755–758.

Di Pietro, C. M.; Liener, I. E. Heat inactivation of the Kunitz and Bowman-Birk soybean protease inhibitors. *J. Agric. Food Chem.* **1989**, *37*, 39–44.

Feeny, P. Plant apparency and chemical defense. *Recent Adv. Phytochem.* **1976**, *10*, 1–40.

Fritz, H.; Trautschold, I.; Werle, E. Protease inhibitors. In *Methods of Enzymatic Analysis*, 2nd ed.; Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; Vol. 2, p 1064.

Gatehouse, J. A.; Gatehouse, A. M. R. Two simple quantitative assays for trypsin inhibitors using immobilised trypsin. *Anal. Biochem.* **1979**, *98*, 438–444.

Hammer, B. C. A study of proteinase inhibitors in the edible aroids. Ph.D. thesis, Australian National University, 1987.

Hammer, B. C.; Shaw, D. C.; Bradbury, J. H. Isolation and characterization of trypsin inhibitors from *Colocasia esculenta*, *Alocasia macrorrhiza* and *Cyrtosperma chamissonis*. *Phytochemistry* **1989**, *28*, 3019–3026.

Herklots, G. A. C. *Vegetables in South-East Asia*; Unwin and Allen: London, 1972.

Holloway, W. D.; Argall, M. E.; Jealous, W. T.; Lee, J. A.; Bradbury, J. H. Organic acids and calcium oxalate in tropical root crops. *J. Agric. Food Chem.* **1989**, *37*, 337–341.

Liener, I. E.; Kakade, M. L. Protease Inhibitors. In *Toxic constituents of plant foodstuffs*, 2nd ed.; Liener, I. E., Ed.; Academic Press: New York, 1980; pp 7–71.

March, S. C.; Parikh, I.; Cautrecasas, P. A. Simplified method for cyanogen bromide activation of agarose for affinity chromatography. *Anal. Biochem.* **1974**, *60*, 149–152.

Nixon, R. Acridity in Araceae. Honours Thesis, Australian National University, 1987.

Ogata, F.; Makisumi, S. Isolation and characterization of trypsin inhibitors from tubers of taro, *Colocasia antiquorum* var. *nymphaifolia*. *J. Biochem.* (*Tokyo*) **1984**, *96*, 1565–1574.

Ogata, F.; Makisumi, S. Subunit compositions of trypsin inhibitors from tubers of taro *Colocasia antiquorum* var. *nymphaifolia*. *J. Biochem.* (*Tokyo*) **1985**, *97*, 589–597.

Richardson, M. The proteinase inhibitors of plants and microorganisms. *Phytochemistry* **1977**, *16*, 159–169.

Rick, W. Trypsin. In *Methods of Enzymatic Analysis*, 2nd ed.; Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; Vol. 2, p 1013.

Ryan, C. A. Proteolytic enzymes and their inhibitors in plants. *Annu. Rev. Plant Physiol.* **1973**, *24*, 173–196.

Ryan, C. A.; Huisman, O. O.; van Denburgh, R. W. Transitory aspects of a single protein in tissues of *Solanum tuberosum* and its coincidence with the establishment of new growth. *Plant Physiol.* **1968**, *43*, 589–596.

Sakai, W. S. Aroid root crops: *Alocasia*, *Cyrtosperma* and *Amorphophallus*. In *Handbook of Tropical Foods*; Chan, H. T., Ed.; Dekker: New York, 1983; pp 29–83.

Sugiura, M.; Ogiso, T.; Takeuti, K.; Tamura, S.; Ito, A. Studies on trypsin inhibitors in sweet potato. *Biochim. Biophys. Acta* **1973**, *328*, 407–417.

Sumathi, S.; Pattabiraman, T. N. Natural plant enzyme inhibitors: Part 1 – protease inhibitors of tubers and bulbs. *Indian J. Biochem. Biophys.* **1975**, *12*, 383–385.

Sumathi, S.; Pattabiraman, T. N. Natural plant enzyme inhibitors V. A trypsin chymotrypsin inhibitor from *Alocasia macrorrhiza* tubers. *Biochim. Biophys. Acta* **1977**, *485*, 167–178.

Sumathi, S.; Pattabiraman, T. N. Natural plant enzyme inhibitors VI. Studies on trypsin inhibitors of *Colocasia antiquorum* tubers. *Biochim. Biophys. Acta* **1979**, *566*, 115–127.

Sunell, L. A.; Healey, P. L. Distribution of calcium oxalate idioblasts in corms of taro *Colocasia esculenta*. *Am. J. Bot.* **1979**, *66*, 1029–1032.

Received for review May 11, 1989. Revised manuscript received November 13, 1989. Accepted March 9, 1990.

Registry No. Trypsin inhibitor, 9035-81-8; chymotrypsin, 9004-07-3; calcium oxalate, 563-72-4.