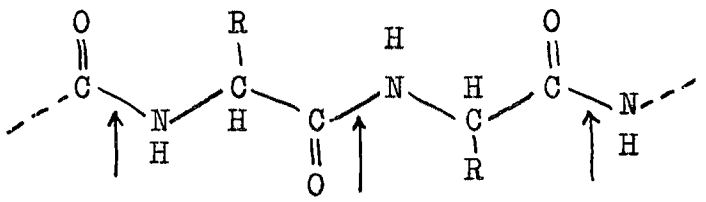
I N T R O D U C T I O N

INTRODUCTION

All organisms that can use proteins as sources of nitrogen, are equipped with proteolytic enzymes, which catalyze the hydrolysis of peptide bonds. In protein molecules, amino acids are linked to one another in long chains through peptide linkages, in which the α-amino group of one amino acid is bound as a substituted amide to the carboxyl group of the next amino acid. It is the function of the proteolytic enzymes to cleave these peptide bonds (1) :



Points of attack by hydrolytic enzymes

Till now, numerous proteolytic enzymes have been purified and characterized. These may be classified into two groups according to the position of the hydrolysable peptide bond in the substrate protein (2) :-

(a) Endopeptidases,- which hydrolyze internal peptide bonds, e.g., pepsin and rennin (from gastric juices of animals); trypsin, chymotrypsin and enterokinase (from pancreatic juices of animals); thrombin and plasmin or fibrinolysin (from blood); cathepsins A-C (from animal tissues); papain (from latex of Carica papaya fruits); ficin (from fig); bromelin (from pineapple); gelatinase (from a bacteria : B. subtilis), etc.

(b) Exopeptidases, - which hydrolyze terminal peptide linkages, e.g., carboxy peptidases (from pancreatic juices of animals); amino-peptidases, dipeptidases, aminotripeptidases and prolidases (from intestinal juices of animals), and other amino-peptidases and dipeptidases (from pineapple and a bacteria : Ps. aeruginosa, respectively).

Every proteolytic enzyme discriminates between various types of peptide bonds. This specificity comes into play in the course of physiological digestion of proteins. According to Waldschmidt-Leitz (3) proteolytic enzymes do not distinguish between the peptides of different amino acids, but rather those of different chain lengths. According to Max Bergmann (154), proteolytic enzymes are classified into three groups viz.,

A. Peptidases : dipeptidases and polypeptidases, - e.g., amino-polypeptidase, tryptic carboxypolypeptidase and chatheptic carboxypolypeptidase; these attack and hydrolyze the peptide bonds of di-, tri-, or polypeptides.

B. Proteinases : e.g., pepsin, trypsin, papain and cathepsin; all these act on high molecular proteins and peptones; and

C. Other proteolytic enzymes : e.g., prolinase, prolidase, dehydrodipeptidase, gelatinase, yeast-trypsin, etc.

Proteolytic enzymes in general, exhibit two types of specificity :

(a) Homospecificity,- exhibiting differences of backbone, e.g., trypsin, beef spleen trypsinase, etc., and

(b) Heterospecificity,- exhibiting differences of the side group specificity, as is the case of trypsin when compared with chymotrypsin.

According to the mode of action, proteolytic enzymes may be of three categories (1) :

(a) Pepsinases which attack protein cations;

(b) Tryptases which digest protein anions, and

(c) Papain(ases) which act upon protein zwitterions (4).

Plant bodies are rich sources of proteolytic enzymes. Amongst the plants, proteolytic enzymes are obtained from dicotyledonous plants like figs, papaya, milkweed, euphorbia, etc., and from monocotyledonous plants like pineapple, cereals, etc. According to biochemical properties, plant proteinases may be divided into two groups (5) :

A. Proteinases requiring free -SH group for activity, - having their optimum activity at pH ~ 7; these strongly digest haemoglobin, casein and egg albumin, and exhibit strong milk clotting activity, and

B. Proteinases lacking the active -SH group for activity, - they have a more alkaline optimum pH and an inferior milk clotting power.

Both these groups are heat resistant and remain active yet at 60^{o}-70^{o}.

On the other hand, plant peptidases are divided into three types :

(a) Peptidase molecules attaching themselves to the amino groups of their substrates;

(b) Peptidase molecules attaching themselves to the carboxyl groups of their substrates, and

(c) Peptidase molecules attaching themselves to the amino and carboxyl groups of their substrates, simultaneously.

Plants are sources of varied types of proteolytic enzymes. There are numerous proteolytic enzymes on record which have been prepared from plants. Some of these deserve mention (6, 7) (Table I).

As regards proteolytic enzymes of higher plants (Table I), crystalline papain has a molecular weight of about 20,500 and an apparent isoelectric point of pH 8.8. It readily forms a mercuric complex which contains 1 gram atom of Hg/43,000 grams of protein. Approximately, two third of the 180 amino acid residues of crystalline mercuripapain can be removed by means of purified aminopeptidase without loss of potential enzymic activity (32-34). Other plant papainases like ficin and bromelin act over a wide pH range and exhibit

TABLE I

Proteolytic Enzymes Obtained from Plants

Enzymes	Source	References
Papain	Latex of *Carica papaya*	(8)
Chymopapain	Latex of *Carica papaya*	(9)
Ficin	Latex of *Ficus* sp.	(10)
Asclepain	Roots of *Asclepias* sp.	(11)
Bromelin	Pineapple	(5)
Pinguinain	*Pinguicula* sp.*	(5, 12-14)
Mexicanain	Latex of *Argemone mexicana*	
Tabermontanain	*Taberni montana*	
Euphorbain	*Euphorbia* sp.	
Solanain	*Solanum* sp.	
Proteinases	*Drosera* sp.*, squash juice and *Aspergillus* sp.	
Carboxypeptidase	*Saccharomyces* sp.	(15)
Y -glutamyl-carboxypeptidase	*Saccharomyces* sp.	(16-18)
Leucinamino-peptidase (cathepsin-III)	*Saccharomyces* sp.	(19-22)
Glycylglycine dipeptidase	Some higher plants	(23)
Asparaginase	*Saccharomyces* sp. and higher plants	(24)
Allantoinase	Higher plants	(25)
Dihydropyrimi-dinase	Higher plants	(26)
Urease	Seeds of *Canavalia* sp.	(27)
Peptidases	Moulds and *Camellia sinensis*	(28, 29)
Proteases	Germinating lettuce seeds and *Bacillus subtilis var. amylosaccharitícus*	(30, 31)

* Insectivorous plants.

maximum activity near pH 6.0. Papain and ficin hydrolyze the amide bonds of benzoyl-L-argininamide and of carbobenzoxy-L-methioninamide. Papain hydrolyzes carbobenzoxy-L-glutamic acid- α -amide and benzoylglycinamide. Papain hydrolyzes also ester linkages e.g., benzoylglycinethyl ester and thiol esters e.g., benzoylglycylethanethiol (35).

According to the nature of the source, plant proteolytic enzymes may be grouped into three classes :

A. Proteolytic enzymes obtained from latex or juices, e.g., papain, euphorbain, asclepain, etc.

B. Proteolytic enzymes obtained from glands of insectivorous plants, e.g., pinguiculin, etc., and

C. Proteolytic enzymes obtained from any other sources, e.g., from lettuce seeds, etc.

The work on plant proteolytic enzymes has principally centered on the study of the enzymes contained in the latex of various plant species, and has concerned also, to some extent the enzymes of certain seeds.

There is a group of plants, cosmopolitan in distribution, which has acquired special devices for the capture of animalcules in terms of lure, for protein diet. Insectivorous plants are one of the most important and extraordinary sources of proteolytic enzymes, known since the time of Charles Darwin. Insectivorous

or carnivorous plants obtain their nitrogenous nutrients from small animals like insects. This is a case of feeding on animal protein. So far as their carbohydrate nutrition is concerned, insectivorous plants are autotrophs since they possess the proper chlorophyll apparatus.

There are more than 450 sps. of insectivorous plants, representing 15 genera and belonging to 6 families. Customarily, these plants are divisible into two groups viz., Choripetalae and Sympetalae according to the morphology of their flower petals. A general account of insectivorous plants is represented in Table II.

All or most of the insectivorous plants are provided with special entrapping organs, which are remarkable adaptations, rather metamorphosis of leaves for capturing insects and securing nitrogen from their body protein. Among the Choripetalae (Table II), greatest specialization of insect traps have been reached by Dionea sp. and Aldrovanda sp. Among Sympetalae (Table II), it is Utricularia sp. reaching specialization of highest order. The characters and occurrence of trapping systems of insectivorous plants have been tabulated below in Table III.

Carnivorous habit is supposed to have arisen among higher plants at two points in the course of evolution: possibly the majority, i.e., the widely distributed ones, are descendants

TABLE II

A General Account of Insectivorous Plants

Group	Family and Genus	No. of known species	Distribution
CHORIPETALAE	Sarraceniaceaê :		
	Heliamphora sp.	5	British Guiana, Venezuela
	Sarracenia sp.	9	Labrador to South Eastern parts of U.S.A.
	Darlingtonia sp. (or Chrysamphora)	1	North California and South Oregon
	Nepenthaceaê :		
	Nepenthes sp.	65	Eastern tropics to Ceylon and Malagasy Republic
	Droseraceaê :		
	Dionaea sp.	1	North and Northern parts of South Carolina
	Aldrovanda sp.	1	Africa, Australia, Europe, India, Japan and Queensland
	Drosophyllum sp.	1	S.Portugal, S.W. Spain and Morocco
	Drosera sp.	90	Ubiquitous
	Byblidaceaê :		
	Byblis sp.	2	N.W. to S.W. Australia
	Cephalotaceaê :		
	Cephalotus sp.	1	(Extreme) S.W. Australia
SYMPETALAE	Lentibulariaceaê :		
	Pinguicula sp.	30	N.Hemisphere, - in old and new worlds
	*Utricularia sp.	275	Ubiquitous
	Biovularia sp.	2	Cuba and Eastern part of South America
	Polypompholyx sp.	2 to 4	S. and S.W. Australia
	Genlisea sp.	10	Tropics of both of W.Africa and Eastern sector of S. America.

* In the present investigation, proteolytic enzymes have been isolated from a plant of this genus (Fig. 1).

TABLE III

Characters and Occurrence of Insect-traps of Insectivorous Plants

Trap Types	Trap Action	Name of genus
Pitfalls	Passive	Heliamphora, Sarracenia, Darlingtonia, Cephalotus, Nepenthes, etc.
Lobster pot	Passive	Genlisea
Bird-lime or Fly-paper traps	Passive	Byblis and Drosophyllum Pinguicula and Drosera
Steel trap	Active**	Dionea and Aldrovanda
Mouse trap	Active**	Utricularia*, Biovularia, and Polypompholyx

* In the present investigation, proteolytic enzymes have been isolated from a plant of this genus (Fig. 1).

**Display special movements necessary or contributory to the capture of prey.

of ancient forms (36) and some others are of endemic distribution (37). Perhaps these two types of plant groups are parallely evolved.

The insectivorous plants at least partly depend on animals. From purely physiological point of view, the insectivorous plants are concerned, in somewhat special way, in the procurement of nutrient substances containing protein, vitamins and salts of potassium and phosphorus, etc. These plants have gone to the extreme, exhibiting movements of traps for procuring their foods, - the animalcules. The modus operendi and points of lure to insects, displayed by insectivorous plants, have been expressed in Table IV. Beyond proteolytic enzymes the insectivorous plants often secrete sticky substances to catch and/or attract small insects. These sticky substances may be of three types, viz., aromatic oil, resins and mucilages.

The biochemical aspect of protein metabolism by the insectivorous plants is noteworthy. These plants undoubtedly gain by the insects captured in the traps (38). The digestion of insect protein by carnivorous plants, is a function carried out by the secretion of an appropriate enzyme or enzyme system secreted from the glands of the traps of the insectivorous plants, and partly is a result of metabolic action of bacterial flora of the traps, leading to decay and rotting of body-proteins of entrapped insects.

TABLE IV

Modus operendi and Points of Lure to Insects, Displayed by Insectivorous Plants

Plants	Points of lure to insects
Cephalotus sp. and Darlingtonia sp.	Colour and brightness
Dionea sp.	Movement of various degree of rapidity
Drosera sp.	Odour; secretion of mucilage and reflection of light from the same; movement of various degree of rapidity
Drosophyllum sp.	Honey
Nepenthes sp.	Necter
Pinguicula sp.	Reflected light from drops of mucilage; movement of various degree of rapidity
Sarracenia sp.	Odour; attractive colour; brightness
Utricularia sp.	Movement of various degree of rapidity

In the insectivorous plant Heliamphora sp., the insect proteins are made available for metabolism also by bacterial digestion, and the nitrogenous end products are absorbed by the plant body. In another insectivorous plant Sarracenia sp., diastase, protease, invertase, lipase, maltase, urease and esterase may be present (39-42). In the bladders of Darlingtonia californica, stimulation by the introduction of meat-bits, milk or beef-extracts, increases the amount of internal fluid; also diastase activity has been noted (43). As in human stomach, the fluids return to neutrality, whatever the nature of the introduced reagent. In the pitcher plant Nepenthes sp., the pitchers secrete a quantity of fluid before they open. This limpid, colourless, odorous, tastey and non-volatile acid-containing fluid has been found to become more acidic as flies enter into the pitchers (44). A pepsin-like enzyme, secreted from the inner walls of the pitchers, digests the insect proteins (45-49). These facts seem to bring the whole phenomenon of protein digestion by insectivorous plants into line with that in higher animals. Nepenthin (50), a protease, exists as zymogen in the tissues of the pitchers of the pitcher plant, and is essentially tryptic in character; among its products of digestion, peptones are present (51-56). Otherwise, this enzyme is a true pepsin (57, 58).

Stern and Stern (59) have found that there are two enzymes present in the pitcher fluid, - a catheptic present in sterile pitcher fluids and a tryptic occurring only in opened pitchers to which bacteria have access (60). In vitro,

the fluid from the pitchers of Cephalotus follicularis, in presence of added acid, digests fibrin, and contains a digestive ferment which will break up proteins into peptone-like bodies (61). Sessile Drosophyllum leaves secrete enzymes only being stimulated by the addition of proteins (62, 63). In Pinguicula there is a milk clotting tryptase active at pH 9.0, and a weak pepsidase active at pH 8.0 (55, 64). Dernby (65) has obtained a glycerine extract of an enzyme from the leaves of Drosera (cf. to pepsin), active at pH 5.0. Working with acetone extract of Drosera leaves, Okahara (66) has noted the pepsin-like activity of the enzyme at pH 1.5. This plant may have two enzymes, at least one of which is a proteinase (67) active at pH 3.2, non-identical to pepsin, and rather takes a middle position between pepsin and papain (32), regarding biochemical properties.

In order to exhibit maximum proteolytic activity toward substrates (synthetic or natural), intracellular proteinases like papain, ficin, and bromelin, require activation by one of the variety of chemicals like glutathione, cysteine, H_2S, HCN, etc. Crude preparations of these enzymes are always accompanied by sufficient natural activator like glutathione, to permit at least some proteolytic activity. Further addition of any one of the substances like cysteine, H_2S, HCN or even glutathione, may increase the rate of enzymic action. These chemicals bind inhibitory metal ions like Cu^{2+}, Hg^{2+} etc., and react directly with inactive enzyme proteins like papain. Probably, the

activators serve as reducing agents for the conversion of disulphide linkages in the inactive enzyme protein to sulfhydryl groups essential for enzymic activity (68). Activation of

Papain : R-S-S-R + 2 cysteine $\longrightarrow$ 2 R-S-H + cystine.
Inactive papain Active papain

Alternatively, when volatile activators like HCN, H_2S, etc., are removed from an active enzyme preparation under anaerobic conditions, the activity of the enzyme may be lost, indicating a reversible addition of the activator to the enzyme protein (69-71). Activation of papain by volatile substances :

Papain + H_2S $\longrightarrow$ Papain - H_2S
Inactive Active

In order to effect maximum activation of an extensively dialyzed preparation of papain with HCN, traces of cysteine are required. The combination of HCN or of another activator like cysteine, H_2S, etc., with the inactive proenzyme must be preceded by a reduction of the disulphide groups in the inactive enzyme protein. Carboxyl reagents like hydroxylamine, phenyl hydrazine, etc., inhibit papain. So, the reversible addition of an activator may involve a reaction with a carbonyl group of the enzyme protein to form a hemimercaptol with cysteine or H_2S, or to form a cyanohydrin with HCN. Of course, an increase (above 4×10^{-5} M) in the concentration of the activator, counteracts the inhibition. The intra-cellular proteinases of animal tissues like cathepsin B, carboxypeptidase, etc., also require activation by sulfhydryl compounds.

The proteinases, which require activation by cysteine, glutathione, HCN, etc., are completely inhibited by small concentrations (even 1 x 10^{-4} M) of iodoacetate. Generally, the inhibition involves a combination of the reagent with essential sulfhydryl groups on the enzyme protein (72). Combination of iodoacetate with sulfhydryl group of enzyme protein :

$$\text{Protein-SH} + ICH_2COO^- \longrightarrow \text{Protein-S-}CH_2COO^- + HI$$

The general properties of purified plant proteases (72, 73) have been represented in Table V.

The leaves of insectivorous plant Drosera, possesss glandular hairs, which function enzymatically in the digestion of entrapped insects (74). These glands secrete a protease of high activity, with a very low pH optima at 3.2. The leaves of Drosera yield an extractable protease which differs from the glandular enzyme in having a pH optima of 4.2 to 4.3,- an optimum range similar to that of the enzymes of the seeds.

Peptidases may be of two types : endopeptidases and exopeptidases. Dipeptidases hydrolyze dipeptides and the nature of their specificity is as follows :

$NH_2CHRCO \overset{\bullet}{\dagger} NHCHRCOOH$ and/or $NH_2CHRCO \overset{\bullet}{\dagger} NH.....$

$\bullet$ Point of attack

Aminopolypeptidase, tryptic carboxypolypeptidase, catheptic carboxypolypeptidase, etc., hydrolyze polypeptides, and the nature of their specificity is as follows :

$..... CO \overset{\bullet}{\dagger} NHCHRCOOH$

Point of attack

TABLE V

General Properties of Purified Plant Proteases

Enzymes	Source	Synthetic substrates	pH optima	Activation by reduction
Papain	Latex of fruit of Carica papaya	Hippurylamide	7 to 7.5	+
Chymopapain	"	Proteins and certain peptides; amides and amino acid esters	7.0	+
Ficin	Latex of Ficus sp.	Proteins and certain peptides and amides. Also catalyzes transpeptidations	7.0	None
Bromelin	Ananas sativus fruit	CBZ.GGGA*	6.7	+
Pinguinain	Bromelia pinguin fruit	----	3.0	----
Asclepain	Latex of Asclepias sp.	Proteins	7 to 7.5	+
Mexicanain	Leaves and fruits of Pileus mexicanus	----	----	----
Tabernamontain	Latex of fruit of Tabernamontana grandiflora	----	5.0 to 6.0	+
Euphorbain	Latex of Euphorbia sp.	----	6.0	+
Solanain	Fruit of Solanum elaeagnifolium	----	8.5	None
Hurain	Latex of Hura crepitans	----	8.0	None
Arachain	Seeds of Arachis hypogaea	BAA**	6.5 to 7.5	None

* Carbobenzoxyglycylglutamylglycylamide; ** Benzoylarginynamide;
"----" indicates complete data not available; + indicates activation by reduction.

The general properties of plant peptidases are listed in Table VI.

TABLE VI

General Properties of Plant Peptidases

Enzymes	Occurrence	Substrate requirement	Typical substrate	Metal required for activation
Dipeptidase	Leaves and seedlings	Free amino acid carboxyl groups adjacent to peptide bond	Glycylglycine	Co^{2+}
Leucylpeptidase	Leaves	Leucyl residue in peptide linkage	Leucylamide	Mn^{2+} or Mg^{2+}
Prolinase	Yeast	Proline with carboxyl group combined in peptide linkage	Prolylglycylglycine	----
Aminopolypeptidase	Fungi	Peptides with free amino group and three or more residues	Cystinylglycylglycine	----
Yeast polypeptidase	Yeast	Peptide bond adjacent to a free amino group and at least two residues removed from a free carboxyl group	Leucylglycylglycine	----

"----" indicates complete data not available.

Proteinases, peptidases and amidases exhibit hydrolysis of the protein molecule by condensation or replacement :

$$RCO - NHR' + H_2O \rightleftharpoons RCOOH + R'NH_2$$

$$RCO - NHR' + R''NH_2 \rightleftharpoons RCO - NHR'' + R'NH_2$$

The specificity of the peptidases and proteinases are interesting. Crystalline pancreatic carboxy peptidase hydrolyzes CO - NH bonds, in substrates of the general formula $RCO \overset{\downarrow}{-} NHCH(R)COOH$. A structural requisite of substrates of this enzyme is the presence of a free α-carboxyl group adjacent to the peptide bond capable of being hydrolyzed. Carboxypeptidase will not hydrolyze the peptide bond of glycylglycinamide ($NH_2CH_2CO-NHCH_2CO-NH_2$), but this substance will serve as a substrate for a different type of peptidase (aminopeptidase), which requires in its substrate a free α-amino group adjacent to the sensitive peptide bond. For the action of certain of the proteinases, the sensitive peptide bond must be involved in the participation of particular amino acid residues, e.g., crystalline trypsin acts on the CO-NH linkages that are involved in the carbonyl group of a lysine or arginine residue. Substitution of either of these by another amino acid prevents enzymic action. Similarly, crystalline chymotrypsin acts at linkages in which the carboxyl group of tyrosine, phenylalanine, tryptophan, or, to a lesser extent, methionine is involved. Thus, trypsin will not act on the same peptide bonds as chymotrypsin, nor will chymotrypsin attack the linkages broken by trypsin. Neither

trypsin nor chymotrypsin requires the presence of free α-amino or α-carboxyl groups in its substrates (1).

In the present investigation, the insectivorous plant Utricularia aurea Lour. (75, 76) (Fig. 1), of the family Lentibulariaceae (77-79), has been used as a source of proteolytic enzymes. This plant is distributed in tropical and temperate regions and are sciophytes. About 20 sps. are found in ponds and ditches of India. For experimental purpose of the present investigation, these plants have been collected from South 24-Parganas district of West Bengal.

This plant is a rootless, aquatic, perennial herb. The leaves are rosette, or when submerged, bear upto 7 mm. x 3.5 mm. globular pinkish bladder (Fig. 2) or insect - trap as it is called, with a truncate mouth (Fig. 3). The mouth is provided with an one-way valve (Fig. 4) opening inwardly, and remains sealed after entrapping an insect (80-82).

There are three types of hairs present in the bladders : (a) Antennae and the hairs forming a fringe round the ridge of the mouth, - these afford protection to the bladders;

(b) Irritable hairs situated at the apex of the dome formed by the valve. These hairs receive and transmit stimulus caused by contact with any animalcule and finally bring about the opening of the valve and sucking in of the prey (Figs. 5,6), and

(c) The inner wall of the bladder is studded with 3 to 5 pronged hair like glands (Fig.21). These hairs have secretory functions and are called bifids and quadrifids (83).

The bladders of Utricularia, which are promising source of proteases, serve as the traps for the animalcules, when the latter is digested possibly by the enzymic action (84-86). Luetzelberg (87) has found slight enzyme activity in an acid and an alkaline media with bladder homogenate and he has further noticed that the expressed juices can liquify gelatine in four days. Presence of Benzoic acid has been discovered in the trap fluid. Luetzelberg has unspecifically concluded that a trypsin like enzyme may be present in the content of the bladder.

Adowa (88, 89) has made saline and hydrochloric acid extracts of the whole of U. vulgaris plant. He has tested the efficacy of the extracts in digesting gelatine, fibrin, milk casein and egg albumin. The extracts contain two proteoclastic ferments : α - and β -proteases; - the latter being active in acid medium. In neutral medium α -protease may have rendered a little more activity with the addition of $CaCl_2$. Adowa then has made extracts of (a) stems, (b) green or young traps and (c) coloured or matured traps, and has tested their activities separately. In neutral gelatine, the hydrolytic effects of these three extracts are reported in the ratios of 18.5 for green traps, 6.5 for coloured traps, and 3.5 for stems. In alkaline gelatine the ratios are 22.0, 23.5 and 6.0 respectively. In acid gelatine, the effects are rapid at first but quick, while in the alkaline and neutral media the actions have been found to be continuous. Thus the trap extracts have been found to contain

more α -protease than that of the stems. The extracts of green traps have been found to hydrolyze alkaline gelatine over a long period of 24 days to the same extent as that of coloured traps. The extract of green traps have been reported to act more energetically on neutral gelatine than that of the coloured traps. The protease content of branches are very insignificant. Alakline gelatine has been reported to be the best medium for digestion by undiluted extracts, and neutral gelatine for diluted (upto 50%) extracts. β -protease, both from the branches and from the traps has exhibited a weaker activity than α -protease. Extracts diluted 8 to 16 times, have been reported to have acted in neutral but not in alkaline medium. Hada (90) has found that the captured aquatic animals are decomposed by the enzymes secreted by the plant, as also by the bacteria, which increase rapidly on the remnants of the body, inside the bladders.

In the present investigation a systematic biochemical study has been made to characterize the proteolytic enzyme obtained from Utricularia aurea, an aquatic insectivorous plant. An attempt has been made to purify the enzyme partially, and the effects of variation of assay conditions have been studied with the partially purified enzyme preparations. Thermal sensitivity as well as the effects of different activators and inhibitors have also been studied with the partially purified enzyme preparations. Also an attempt has been made to throw some light on the specificity of the enzyme preparations.

The lines of work of the present investigation are :

1) Extraction of proteolytic enzyme from the bladders of Utricularia aurea Lour., an aquatic insectivorous plant, and fractionation of the extract by ammonium sulphate,

2) Determination of proteolytic activities of the crude homogenate and of the ammonium sulphate precipitable fractions,

3) Effects of variation of incubation conditions like pH, temp. and time on the proteolytic activities of the different fractions,

4) Effects of preincubation temperature treatments of the prepared fractions on the proteolytic activity,

5) Effects of activators and inhibitors on the proteolytic activities of the prepared fractions,

6) Proteolytic activities of the prepared fractions towards different synthetic substrates and Co-substrates, measured by colorimetric and photometric methods, and identification of the end products by descending paper chromatography.

7) Further purification of ammonium sulphate precipitable fractions by ECTEOLA-cellulose column chromatography and estimation of activity of the effluent enzyme protein, and

8) Histochemical localization of proteolytic activity in the plant tissue.