SUMMARY

1. Previous work on plant cysteine proteases has been reviewed.

2. Isolation, purification and crystallization of two cysteine proteases, Fraction D-1 and Fraction D-2, from the latex of Calotropis gigantea have been described. Purification has been done by ion-exchange chromatography on CM-cellulose and SP-sephadex C-50. Purity has been checked by disc electrophoresis at pH 4.5 and SDS-polyacrylamide gel electrophoresis at pH 7.2.

3. At pH 4.5, the electrophoretic mobility of Fraction D-2 has been found to be higher than that of Fraction D-1.

4. In SDS-polyacrylamide gel electrophoresis, both the Fractions D-1 and D-2 have been found to contain each single polypeptide chain and Fraction D-1 has got the higher relative mobility than that of Fraction D-2.

5. The rate of hydrolysis of azoalbumin and haemoglobin at pH 7.5 have been found to be proportional to the concentration of Fractions D up to 25 μg. The rate of hydrolysis of casein was not proportional to the concentration of Fraction D.

6. The pH optimum for both fractions has been found to lie between 7.5 and 8.0 at 37°.
7. A temperature optimum of 55° has been found for both Fractions D-1 and D-2 using azoalbumin as substrate in 0.1M phosphate buffer containing 0.001M EDTA and 0.05M 2-mercaptoethanol, pH 7.5.

8. Effect of activators, like cystein, 2-mercaptoethanol, dithioerythritol and glutathiones, on the rate of proteolysis of Fractions D-1 and D-2 in the concentration range of 2.5 mM-25 mM, has been studied. Cystein and 2-mercaptoethanol have been found to activate both the enzymes almost completely. With dithioerythritol, the activity of both Fractions D-1 and D-2 has been found to increase upto the concentration of 7.5 mM of DTE and then decreased with the increasing concentration of the activator. In both cases, glutathione appeared as a weaker activating agent.

9. The substrate saturation curves of Fractions D-1 and D-2 have been obtained in hyperbolic forms with azoalbumin as substrate. The Michaelis-Menten constants for Fraction D-1 and 0-2, calculated from Lineweaver-Burk plot, have been found to be 2.0 x 10³ mg/litre and 2.5 x 10³ mg/litre respectively.

10. The specific activities of Fractions D-1 and D-2 on azoalbumin have been found to be 6.4 units and 6.3 units per mg of enzyme respectively.

11. Nitrogen content of both the fractions as determined by micro-Kjeldahl method has been found to be 16.3%.
12. Extinction coefficients of both the fractions found to be 19.5 at 280 nm in the protein concentration 1 g/100 ml.

13. Both the Fractions D-1 and D-2 sedimented as single symmetrical peaks at different protein concentrations in 0.1M NaH2PO4-0.1M Na2HPO4-0.001M EDTA-0.001M Na2SO4, pH 6.66. The s20,w values for both Fractions D-1 and D-2 have been found to be independent of protein concentration in the range 0.4-1.2 g/100 ml and the average values have been found to be 2.71 x 10^-13 sec for Fraction D-1 and 2.65 x 10^-13 sec for Fraction D-2.

14. Diffusion coefficients of Fractions D-1 and D-2 as determined from boundary spreading in the ultracentrifuge have been found to be 9.80 x 10^-7 cm^2 sec^-1 and 9.56 x 10^-7 cm^2 sec^-1 respectively.

15. The molecular weights of Fractions D-1 and D-2 as calculated from sedimentation and diffusion data have been found to be 24,280 and 24,339 respectively.

16. The ratios of frictional resistance of hydrated molecule to that of anhydrous sphere, calculated from sedimentation and diffusion data have been observed to be 1.14 for Fraction D-1 and 1.17 for Fraction D-2.

17. From the results of Archibald experiments in 0.1M NaH2PO4-0.1M Na2HPO4-0.001M EDTA-0.001M Na2SO4, pH 6.66, molecular weights of Fractions D-1 and D-2 have been calculated as 24,702 and 24,685 respectively.
18. Molecular weights were also determined for both fractions in SDS-polyacrylamide gel electrophoresis and the values were obtained as 22,650 and 23,440 for Fractions D-1 and D-2 respectively.

19. Tyrosine and tryptophan contents of Fractions D-1 and D-2 were determined spectrophotometrically. The average values of tyrosine and tryptophan were obtained as 10.84 moles and 3.64 moles respectively per mole of Fraction D-1 and 13.07 moles and 4.62 moles respectively per mole of Fraction D-2.

20. The content of free sulphhydril group of Fractions D-1 and D-2, papain and ficin was determined by the reaction of active enzyme with Ellman's reagent and each contained one mole of sulphhydril group per mole of enzyme.

21. Amino acid compositions of both the Fractions D-1 and D-2 have been determined and molecular weights from such results were calculated as 23,427 for Fraction D-1 and 24,163 for Fraction D-2.

22. Partial specific volumes as calculated from their respective amino acid compositions have been obtained as 0.724 ml/g for both the fractions.

23. Peptide mapping has been done on the tryptic digests of carboxymethylated Fractions D-1 and D-2 by high voltage paper electrophoresis followed by chromatography in the second dimension.
The results have yielded a high proportion of peptides in common.

The results have been discussed and the properties of different plant cysteine proteases have been compared.