A ribonuclease from *Nepenthes* spp

Insectivorus plants have a variety of specialised mechanisms for capturing insects and, presumably, for using the products of digestion or autolysis of the insect bodies. Some 400 species of insectivorus plants have been recorded, but little attention appears to have been paid to the enzymes which may be present in the secretions which they produce. About two years ago we examined the secretions from several species of *Nepenthes* and found they contained substantial ribonuclease activity, with a specificity differing from pancreatic ribonuclease. Since the mode of action of the *Nepenthes* ribonuclease appears to be similar to others recently described from pea leaves[1,2], tobacco leaves[3,4] and ryegrass[5], a condensed account of our results is given here to provide information on an enzyme that is relatively simple to prepare for use.

We have tested four sources of the secretion: from *Nepenthes gracilis*, *N. ampullaria* and *N. rafflesiana* collected in a forest near Singapore, and from an unidentified, large-pitchered species growing in the Auckland Botanic Gardens. The liquid from the pitcher is filtered to remove debris, mainly remains of insect bodies. It may then contain 0.1–1.0 mg/ml of total solids and has a pH of about 3. RNA from *Bacillus cereus* or from TMV was used as substrate and the methods for determination of products were largely those developed by MARKHAM AND SMITH and summarised by MARKHAM[6]. Ribonuclease activity was maintained over the pH range tested (2–9) but was greatest at pH 5. Tests were routinely made with secretion brought to pH 5 with acetate buffer (0.1 *M* final concentration). After a few-hours' incubation at pH 5 and 37° the secretion degrades TMV RNA almost quantitatively to the four nucleoside 2',3'-cyclic phosphates. The pyrimidine compounds are resistant to further action, but the purine cyclic phosphates are cleaved at a slower rate to the 3'-monophosphates. Adenosine 2',3'-cyclic phosphate is attacked at abouttwice the rate of the guanine compound. In the first sample of the secretion we tested (from the Auckland source) phosphatase activity was not detected. If present, it had less than 0.1 % the rate of the nuclease activity. In all the other samples tested (including material from plants grown from cuttings from the first source) variable amounts of phosphatase activity were found.

Ribonuclease activity is slowly lost over a period of months on storage of the secretion at 4°, and more slowly when stored frozen. There is no detectable loss of activity on heating at pH 5 to 70° for 5 min but over 95 % activity is lost on heating to 80° for 5 min. The activity for the conversion of adenosine 2',3'-cyclic phosphate to the 3'-phosphate shows a parallel sensitivity to heat.

The mode of action of *Nepenthes* ribonuclease appears to be closest to that described from tobacco leaves[1,5].

The function of the nucleases found in leaves is unknown. In view of its occurrence in the pitcher secretion the *Nepenthes* enzyme very probably has a strictly degradative function, making constituents of the RNA from insect bodies available to the plant. Incubation of *Nepenthes* secretion under a range of conditions with the various 2',3'-cyclic phosphates gave no indication that the enzyme has any ability to cause polymerization of the nucleotides, but it would be preferable to make such tests with a purified preparation of the enzyme.

Abbreviations: RNA, ribonucleic acid; TMV, tobacco mosaic virus.

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The reactivation of the succinate-cytochrome *c* reductase system by a hydrocarbon residue

There are many reports[1–12] on the reactivation of isooctane-extracted cytochrome preparations by fat-soluble vitamins and a variety of lipids. From the present communication it should be recognized that following the evaporation or redistillation of practical or spectral-grade isooctane (2,2,4-trimethylpentane, No. P2396 and No. S2396, Eastman Organic Chemicals, Distillation Products Industries and Phillips Petroleum Company) a lipid-like residue has been found which is fully able to reactivate isooctane-extracted succinate-cytochrome *c* reductase preparations. Column- and paper-chromatographic analysis, chemical tests and spectral analysis demonstrate that this active residue is a non-polar, branched hydrocarbon.

In the course of the determination of the succinate-cytochrome *c* reductase reactivating ability of various lipid fractions obtained by silicic acid column chromatography[13] of the isooctane-extracted lipids from particulate cytochrome preparations, it was found that the most marked reactivating activity occurred in a lipid fraction (Fraction C, Table I) which was eluted by petroleum ether and which constituted less than 1 % of the total lipid. This lipid fraction was observed on paper chromatograms (carried out on paper impregnated with silicic acid, using as solvent *n*-heptane–diisobutyl ketone (96 : 6)[14]) of the isooctane-extracted lipids of the cytochrome preparations as a rapid-moving spot with an R_F value of 0.85. The spot appeared yellow when viewed under u.v. light following staining with rhodamine-G. However, this lipid fraction was not present when the particulate cytochrome preparations were extracted by the conventional chloroform–methanol method[15] rather than with isooctane. An examination of the active lipid material obtained by isooctane extraction of the cytochrome preparations showed that this material was initially present in the practical-grade isooctane which was used to extract the enzyme system and that it was also present to a lesser extent in spectral grade and redistilled practical grade isooctane.

A sample of the material present in the isooctane was obtained by the distillation of 4000 g of practical-grade isooctane. The resulting yellow oily residue (41 mg) was