When 70 µM N-ethylmalenimide (NEM), which completely inhibited the formation of ATP, due to the inhibition of Pi-translocation across the mitochondrial membrane, was present in the reaction medium, the respiratory rates induced by these 3 peptide antibiotics in the presence of Pi were the same as those induced in the absence of Pi. The results with hypelcin-A are shown in figure 3. Thus the translocation of Pi is concluded to be very important for exhibiting the uncoupling of hypelcins and alamethicin.

Furthermore, to know whether the effects of these antibiotics are directly related to the ionophoric action in mitochondria as observed with valinomycin[11], the effects of $Na^+$ and $K^+$ on the activity of the antibiotics were examined using $Na^+$ and $K^+$-depleted medium where all the acids were neutralized with Tris·Cl. It was found that these cations have no effect on the action of hypelcins and alamethicin.

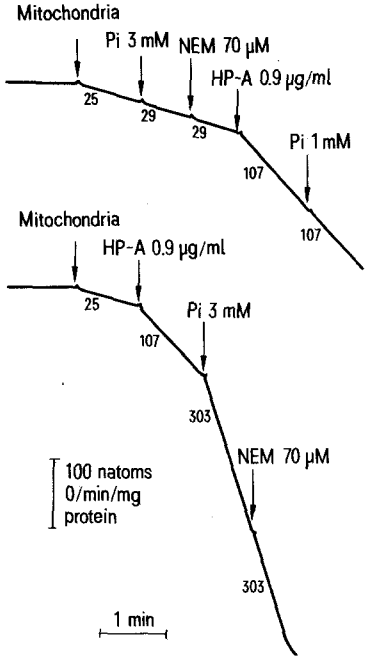



Fig. 3. Effect of N-ethylmalenimide (NEM) on the uncoupling of hypelcin-A(HP-A) in the presence of inorganic phosphate (Pi). The Tricine-medium containing 10 mM succinate with 3 µg rotenone was used as reaction medium in a total volume of 7.0 ml. Numbers adjacent to the traces are respiratory rate in natoms O/min/mg protein. Mitochondria: 0.7 mg/ml.

These results indicate that hypelcins and alamethicin are unique uncouplers of oxidative phosphorylation in mitochondria; they exhibit uncoupling action effectively in the presence of inorganic phosphate. The inorganic phosphate-requiring uncoupling action has sometimes been observed with cationic uncouplers, such as the cyanine dye NK-19[12], and peptide ionophors, such as valinomycin[11], though the role of inorganic phosphate is not clear. It is of interest that hydrophobic peptides, such as hypelcins, alamethicin, and valinomycin act on mitochondria with a common requirement of inorganic phosphate, like that of cationic uncouplers. It should be noted that a neutral antibiotic, hypelcin-A, exhibited about the same activity as the acidic antibiotic alamethicin did, while the peptide hypelcin-B, which is more alamethicin-like with regard to chemical composition, exhibited less activity than alamethicin. The presence of an acidic residue (glutamic acid) in the peptide chain seems not to be directly related to the uncoupling effect of these peptide antibiotics, although the presence of a dissociable proton is generally regarded as essential for exhibiting the action of weakly acidic uncouplers[13,14]. The activity of these antibiotics may be related to the membrane modifying action found with alamethicin[15].

1 T. Fujita, Y. Takaishi and T. Shiromoto, J. chem. Soc. chem. Commun. *1979*, 413.
2 R.C. Pandey, J.C. Cook, Jr, and K.L. Rinehart, Jr, J. Am. chem. Soc. *99*, 8469 (1977), and references therein.
3 P. Muller and D.O. Rudin, Nature *217*, 713 (1968).
4 L.G.M. Cordon and D.A. Hydon, Biochim. biophys. Acta *255*, 1014 (1972).
5 L.R. Jones, H.R. Besch, Jr, J.W. Fleming and A.M. Watanabe, J. biol. Chem. *252*, 3315 (1977).
6 H.R. Besch, Jr, L.R Jones, J.W. Fleming and A.M. Watanabe, J. biol. Chem. *252*, 7905 (1977).
7 G.H. Hogeboom, Meth. Enzymol. *1*, 16 (1955).
8 D.K. Myers and E.C. Slater, Biochem. J. *67*, 558 (1957).
9 S. Muraoka and H. Terada, Biochim. biophys. Acta *275*, 271 (1972).
10 R.B. Beechey, Biochem. J. *98*, 284 (1966).
11 C. Moore and Pressman, Biochem. biophys. Res. Commun. *15*, 562 (1964).
12 H. Terada, in: Cation Flux across Biomembranes, p. 365. Ed. Y. Mukohata and L. Packer. Academic Press, New York 1979.
13 H. Terada, M. Uda, F. Kametani and S. Kubota, Biochim. biophys. Acta *504*, 237 (1978).
14 H. Terada and S. Kubota, FEBS Lett. *100*, 37 (1979).
15 J.C. Bonnafous, J. Dornand and J.C. Mani, Biochem. biophys. Res. Commun. *86*, 536 (1979).

## Lycorine – a resistance factor in the plants of subfamily Amaryllidoideae (Amaryllidaceae) against desert locust, *Schistocerca gregaria* F.

R. P. Singh[1] and N. C. Pant[2]

*Division of Entomology, Indian Agricultural Research Institute, New Delhi (India), 27 August 1979*

*Summary.* Lycorine, an alkaloid was isolated from the bulbs of *Hymenocallis littoralis* Salisb. The percentage of lycorine was 0.00083. When sprayed on cabbage leaves this alkaloid inhibited the feeding of the desert locust, *Schistocerca gregaria* at 0.05% concentration.

The plants of the subfamily Amaryllidoideae, popularly known as lilies, are famous for their beautiful fragrant flowers. They are also of pharmacological interest[3,4]. A few plants are reported to contain a toxic principle[4,5]. *Crinum asiaticum* Linn. is reputed to be repellent to noxious insects[3]. Because of the above characteristics of the plants, it was thought desirable to screen the plants of this subfamily.

When pieces of leaves from 5 plant species, viz. *Crinum asiaticum* Linn., *C. bulbispermum* Milne-Redhead and Schweikerdt, *Hymenocallis littoralis* Salisb., *Zephyranthes grandiflora* Lindl. and *Hippeastrum hybridum* Herb.

belonging to this subfamily, were offered to 24-h starved, sexually immature desert locusts, they were completely rejected. A few locusts, when caged on the leaves of *Hymenocallis littoralis*, preferred death due to starvation rather than feeding on this plant. Detailed investigation led to the isolation of the above alkaloid from the bulbs of this plant, and it proved to be a powerful feeding deterrent.

*Materials and methods.* Isolation of the total alkaloid and its further separation were carried out by the method described by Wildman and Kaufman[6] and later followed by Highel[7].

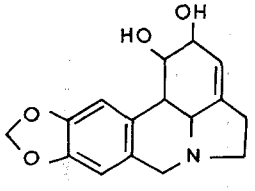
The bulbs of *H. littoralis* were collected from the campus of this institute during May and June, 1975. 3 kg of bulbs yielded about 560 mg alkaloid (0.018% based on the wet weight of the bulbs). Lycorine and tazettine consituted about 0.00083% and 0.016% respectively of the wet weight of the bulbs.

Sexually immature adult locusts reared on cabbage leaves were starved for 24 h at 30 °C before they were released on treated cabbage leaf pieces (5 cm × 5 cm) kept in glass jars (15 cm × 10 cm). Leaf pieces of the above size were cut from a cabbage leaf and weighed on a chemical balance. Pieces having a weight of 3 g ± 50 mg were selected for the experiments. Different concentrations of total alkaloid, lycorine and tazettine were prepared by dissolving them in ethyl alcohol and then diluting with distilled water. The alcohol level in the final concentration was maintained at 20%. 2 ml of the solution was sprayed (1 ml on each side of the leaf piece) from a Potter's tower at 24 cm mercury pressure. Petri dishes containing leaf pieces were dried under a fan before they were offered to locusts in glass jars. These jars were covered with muslin cloth and kept at 30 °C. A set of controls was treated with 20% alcohol. Side by side, a set of the same number of replicates without treatment was also kept to provide information about the shrinkage of cabbage leaves at the above temperature.

After 24 h, the uneaten area left in each jar was measured by putting it on graph paper (5 cm × 5 cm) divided into 100 squares. The percentage feeding and the percentage protection was worked out using the following 2 formulae:

$$\text{Percentage feeding} = \frac{\text{Area given for feeding} - \text{Corrected area left}}{\text{Area given for feeding}} \times 100$$

$$\text{Percentage protection due to treatment} = \frac{\text{Percentage protection in treated samples} - \text{Percentage protection in control}}{100 - \text{Percentage protection in control}} \times 100$$




Lycorine.

Table 1. Percentage protection of cabbage leaves against the desert locust due to the antifeeding property of total alkaloid

| S. No. | Concentration (%) | Mean feeding | Mean protection % | Protection due to treatment % |
|---|---|---|---|---|
| 1 | 0.4 | 0.0 | 100.0 | 100.0 |
| 2 | 0.3 | 0.2 | 99.8 | 99.8 |
| 3 | 0.2 | 17.4 | 82.6 | 81.8 |
| 4 | 0.1 | 63.0 | 37.0 | 34.0 |
| 5 | 0.05 | 85.0 | 15.0 | 10.9 |
| 6 | 0.025 | 92.8 | 7.2 | 2.7 |
| 7 | Control | 95.4 | 4.6 | - |

Table 2. Percentage protection of cabbage leaves against the desert locust due to the antifeeding property of lycorine

| S. No. | Concentration (%) | Mean feeding | Mean protection % | Protection due to treatment % |
|---|---|---|---|---|
| 1 | 0.1 | 0.0 | 100.0 | 100.0 |
| 2 | 0.05 | 0.0 | 100.0 | 100.0 |
| 3 | 0.025 | 9.5 | 90.5 | 89.7 |
| 4 | Control | 92.4 | 7.6 | - |

*Results and discussion.* Alkaloids are known for their biological activity. With this in view, alkaloids were isolated and tested against a highly polyphagous insect, the desert locust. The alkaloids reported from this family occur only in the subfamily Amaryllidoidae. No alkaloids have been reported from the remaining subfamilies, i.e. Agavoideae, Hypoxidoideae and Campynematoideae[8]. Lycorine (figure) and tazettine are the most widespread alkaloids and both are present in all the 5 species offered to the desert locust. Lycorine has been found to occur in all the 26 genera examined without exception[8].

The results of the testing of total alkaloid and lycorine at various concentrations, against the desert locust, are given in tables 1 and 2. It is clear from table 2 that lycorine could inhibit the feeding response completely at 0.05% concentration. The same result was, however, obtained at 0.4% concentration of total alkaloid. Tazettine did not show any activity even at the concentration where alkaloid gave 100% protection. The percentage of lycorine obtained in the present investigation was only 0.00083. This percentage is far less than the concentration at which lycorine inhibited the feeding completely. Testing of different parts of the plant as dust against locusts (not included in this paper), revealed that the leaves possess the least antifeeding property. The death of locusts due to starvation when caged on the plants, therefore, cannot be attributed to lycorine alone. It appears that in addition to lycorine, there are other contributing factors which are responsible for imparting immunity to the plants of this subfamily. Research on this line is in progress and there is an indication that the non-alkaloid fraction also contains a feeding deterrent.

There are various tentative hypotheses regarding the function of alkaloids in plants[9]. The present findings strengthen the view expressed by Cook and Louden[10] that the alkaloid content contributes to the immunity of the plants.

1 Acknowledgements. The authors are grateful to Dr. H.M. Fales and Dr H.A. Lloyed, Department of Health, Education and Welfare, National Institute of Health, Maryland, for sending the sample of lycorine hydrochloride and tazettine.

2 Present address: Commonwealth Institute of Entomology, 56 Queen's Gate, London SW7 (England).

3 A.K. Nandkarni. Indian Materia Medica vol.1, p.389. Dhootapapesnwar Prakashan Ltd, Bombay 1954.
4 K.R. Kritkar and B.D. Basu, Indian Medicinal Plants vol.4, p.2466. L.M. Basu, Allahabad 1935.
5 M.R.S. Sastary, C.K.V. Nayyar, R. Ramaswamy and K. Venkatachalam, 1943. Indian J. Vet. Sci. Anim. Husb. *13*, 65-69 (1943).
6 W.C. Wildman and C.J. Kaufman, J. Am. Chem. Soc. *76*, 5815 (1954).
7 R.J. Highel, J. org. Chem. *26*, 4767 (1961).
8 W.C. Wildman, in: Alkaloid vol.6, p.289. Ed. R.H.F. Manske. Academic Press, New York 1960.
9 A. McKillop, An Introduction to the Chemistry of Alkaloid, Butterworth, London 1969.
10 J.W. Cook and J.D. Loudon, in: Alkaloid vol.2, p.331. Ed. R.H.F. Manske. Academic Press, New York 1952.