Effects of i.p. injections of desipramine on amino acid contents of mouse brain

Amino acid (μmole/g, wet wt)	Control injection (0.9% NaCl)	Desipramine-HCl (mg/kg) 10	25	50	100
Phosphoserine	0.29 ± 0.06 (4)	0.30 ± 0.04 (3)	0.22 ± 0.02 (4)	0.21 ± 0.02 (4)	0.12 ± 0.007 (8) [a]
Phosphoethanolamine	1.71 ± 0.19 (4)	2.11 ± 0.14 (3)	1.78 ± 0.14 (4)	1.60 ± 0.21 (4)	0.92 ± 0.03 (8) [b]
Taurine	9.71 ± 0.66 (4)	11.03 ± 0.75 (3)	9.70 ± 0.43 (4)	8.97 ± 0.48 (4)	6.44 ± 0.17 (8) [c]
Aspartate	3.04 ± 0.14 (4)	3.02 ± 0.14 (4)	2.87 ± 0.21 (4)	2.56 ± 0.09 (4) [a]	2.39 ± 0.05 (8) [b]
Threonine	0.31 ± 0.04 (4)	0.37 ± 0.08 (3)	0.37 ± 0.03 (4)	0.29 ± 0.01 (4)	0.37 ± 0.02 (6)
Serine, glutamine + asparagine	5.29 ± 0.14 (4)	5.23 ± 0.38 (3)	5.18 ± 0.20 (4)	4.65 ± 0.31 (4)	3.98 ± 0.06 (8) [c]
Glutamate	9.17 ± 0.45 (4)	9.99 ± 0.58 (3)	9.13 ± 0.19 (4)	8.66 ± 0.31 (4)	8.34 ± 0.22 (8)
Glycine	0.88 ± 0.05 (4)	0.99 ± 0.04 (3)	0.89 ± 0.03 (4)	0.79 ± 0.04 (4)	0.78 ± 0.03 (8)
α-Alanine	0.48 ± 0.03 (4)	0.49 ± 0.05 (3)	0.40 ± 0.03 (4) [a]	0.35 ± 0.03 (4) [a]	0.30 ± 0.02 (8) [c]

Mice were killed 1 h after injection of the drug; means ± SEM, numbers of animals in parentheses; [a, b] and [c] represent, respectively, $p < 0.05$, $p < 0.01$ and $p < 0.001$, when these values were compared with those for control animals.

Results and discussion. Results shown in the table revealed that the cerebral contents of some amino acids (or derivatives) were decreased 1 h after desipramine injection, especially at the 100 mg/kg dose. However, glutamate and glycine levels showed no changes. The most significant changes; i.e., the decreases in α-alanine and aspartate, were dose-dependent. Hypothermia was evident in all mice which received 25–100 mg/kg, but this effect was not experimentally determined. This study has revealed that the cerebral levels of several amino acids, especially α-alanine and aspartate, which are linked directly with oxidative metabolism, are decreased by the tricyclic antidepressant, desipramine. These effects, as well as the increase in GABA levels produced by this drug[8], are likely to be linked to its hypothermic action. The extent to which these changes in cerebral amino acids are involved in the antidepressant action of desipramine in humans is not known, but it is suggested that such changes should be considered, along with the effects that this agent produces on cerebral noradrenergic and cholinergic mechanisms, in explaining this action.

Polyoxin fungicides: Demonstration of insecticidal activity due to inhibition of chitin synthesis

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Summary. Polyoxin A, a selective inhibitor of chitin synthetase, was found to be insecticidal when injected into the abdomen of grasshoppers nymphs, with an LD_{50} of 1.26 ± 0.20 μg per insect. Symptomatology and the absence of any toxicity toward adult insects indicate that toxicity is due to interference with cuticle deposition.

The polyoxin complex is an antifungal mixture of nucleoside peptide antibiotics elaborated by *Streptomyces cacaoi* var. *asoensis*. The structures of major and minor components of this mixture have been described[2–4]. The polyoxins have been used as practical fungicides in Japan. They act through their ability to be powerful inhibitors of chitin synthesis in filamentous fungi[5]. The structural similarity of the polyoxins to uridine disphospho-N-acetyl glucosamine (UDPNAG), the natural substrate of chitin synthetase, accounts for the competitive nature of this inhibition[6]. The polyoxins have been shown to inhibit in vitro chitin synthesis in insects, both in an organ culture system[7] and in excised abdominal integument incubated under appropriate conditions[8]. To the best of our knowledge there have not been any reports on the in vivo toxicity of polyoxins toward insects. Interest in the possibility of insect control through interference with cuticle deposition, has been recently heightened by the discovery of a new group of insecticides that actually display such a mode of action[9,10]. These are substituted urea compounds and there is no similarity of structure between them and the polyoxin or any natural precursor of chitin. The work to be reported in this note was an adjunct to the development of a simple in vitro assay system for measuring chitin synthesis in insects[8]. It was found that polyoxin A was a powerful inhibitor of chitin synthesis in the system when either glucose, glucosamine, or UDPNAG were used as precursors[8]. It was therefore of interest to see whether or not this compound could affect the process of chitin deposition in vivo.

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Materials and methods. Because of its hydrophilic nature, polyoxin A was unlikely to penetrate insect cuticle; we therefore tested for toxicity by injecting into the abdominal cavity. The insects used were 5th instar nymphs of the grasshopper *Melanoplus sanguinipes*. Polyoxins (samples of Polyoxin A and D were gifts from Dr K. Isono) were injected in 2–10 µl of water using a micrometer syringe. Control groups of insects received injections of water only. Mortality counts were made on the seventh day after injection and results subjected to computerized probit analysis with appropriate correction for natural mortality.

Results and discussion. From toxicity trials with polyoxin A we obtained an LD_{50} of 1.26 ± 0.20 µg per insect. A limited number of trials with polyoxin D indicated that the latter compound was somewhat less toxic. Most of the deaths occurred during the molting process. It seemed that the newly formed exoskeleton did not possess the strength requirement to withstand the stresses involved in ecdysis. Invariably, the exoskeleton split with loss of haemolymph and desiccation of the insect. Injection of large doses of polyoxin A (up to 50 times LD_{50}) was totally without effect if applied to adult insects, a finding strengthening the contention that toxicity was strictly due to inhibition of chitin synthesis. Polyoxin A was not very toxic when applied topically to last instar nymphs. However it was possible to get localized effects from topical applications. A high percentage of nymphs treated topically by depositing a 5-µl drop of a solution of polyoxin in water (100 µg per insect) under the wing pads, developed into adults with wing aberrations. A limited number of toxicity trials were done with polyoxin A (injected) and the migratory locust as the experimental animal. Toxicity and symptoms of death were similar to what is described above for the grasshopper. In conclusion, the results of this study showed that polyoxins can be very effective insecticides if immature rather than adults insects are treated, and if the compounds are injected into the insect rather than applied topically. The symptoms obtained agree with a mode of action that involves a disruption of the process of chitin synthesis. Perhaps some practical insecticides can be based on the polyoxin structure if it is modified so that the compounds become lipophilic enough to efficiently penetrate insect integument.

Toxic substances produced by *Fusarium* VI. Anti-*F. oxysporum* f. sp. *carthami* effect of 2,2′,4-trihydroxybenzophenone

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Summary. Anti-*Fusarium oxysporum* f. sp. *carthami* activity of 2,2′,4-tri-hydroxybenzophenone was evaluated. Pretreatment with the benzophenone offered complete protection to safflower seeds and seedlings, and recovery of the latter from the fungal infection.

Phenolic substances have been reported to be responsible for the general resistance which higher plants show towards parasitic bacteria and fungi[2]. On the basis of these reports, we had investigated and recently reported[3] the antifungal activity of mangiferin, a naturally occurring xanthone-C-glucoside from *Canscora decussata* Schult (Gentianaceae), against *Fusarium oxysporum* Schl. f. *carthami* Klisiewicz and Houston. The fungus is the causal agent of wilt of safflower[4]. Hydroxybenzophenones, which are regarded as intermediates to polyoxygenated xanthones (e.g. mangiferin), were expected to produce stronger interactions with pathogenic fungi because of the flexibility of their 2 aryl rings. This possibility was tested with a number of synthetic hydroxybenzophenones against *F. oxysporum* f. sp. *carthami*. The present communication describes the antifungal activity of the most potent among these compounds, viz., 2,2′,4-trihydroxybenzophenone. The compound was synthesized as previously described[5].

Aqueous sodium carbonate solution (1%) of 2,2′,4-trihydroxybenzophenone, in 3 different concentrations (1×10^{-5}, 1×10^{-4} and 1×10^{-3} M), was used for determining the antifungal activity. Unless stated otherwise, the data given indicate the effect of the benzophenone at a concentration of 1×10^{-4} M. In all seed treatment experiments, 100 seeds (10 seeds in each batch) were used for the control and the benzophenone-treated groups, the former receiving only the vehicle (1% aqueous sodium carbonate solution).

The antifungal activity of the benzophenone against 3 strains of the pathogen (IMI-186539, IMI-186543, and IMI-186544) was evaluated. In the interaction of the 3 strains of the fungus and the benzophenone, no qualitative difference was observed. The results reported here were those obtained by using the most virulent strain (IMI-186539).

The effect of the benzophenone against the fungal invasion of the seeds and seedlings of safflower was determined. Surface-sterilized seeds were soaked (12 h) in the vehicle or in the benzophenone solution. The solution was wiped from the outer surface of the seeds and these were placed on the fungal mat grown on a potato dextrose agar (PDA) medium. After 24 h, the seeds were picked up, washed successively with aqueous mercuric chloride (0.1%) and sterile distilled water, and again placed on PDA plates for incubation at 21 °C. Within 96 h, the fungus appeared on the surface of all the control seeds while the benzophenone-treated seeds remained completely unaffected. In another experiment, safflower seeds soaked in the benzophenone solution were sown in infested potting soil (5 g of the inoculum, grown in Richard's medium, was added to 1 kg garden soil). Typical disease symptoms ap-

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