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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peared in all the seedlings of the control group. In case of the benzophenone-treated group, healthy seedlings emerged and no disease symptoms appeared, also thereafter.

The effect of the benzophenone on the fungal hyphae was examined. The fungus was grown (48 h) in sterilized Richard's medium (150 ml) to which the benzophenone was added in 3 different concentrations. The extent of the mycelial growth in the control and benzophenone-treated groups was quantitated as follows. The mixtures were incubated at 21 °C for 7 days. Subsequently, the dry weight of the mat grown in the control and the benzophenone-treated groups was recorded. Lysis of the hyphal cells was observed within 72 h of the benzophenone treatment, the mycelium became black, and protoplasts were disintegrated. Shrinkage of the cell well was followed by the dissolution of the cell wall in some of the hyphae. The major portions of the mycelium was not stained when lactophenol cotton blue was applied. At the lowest concentration (1×10^{-5} M), however, the benzophenone appeared to promote growth of the mycelium, while at the highest concentration (1×10^{-3} M), it significantly retarded the mycelial growth. The mycelial growth (in g $\pm$ SEM) in the control and the benzophenone-treated (1×10^{-3} M) groups were 0.422 ± 0.0023 and 0.118 ± 0.002, respectively ($p < 0.01$).

The potentiality of the benzophenone as a foliar fungicide was examined. The benzophenone was sprayed on the cotyledon leaves of 2-day-old seedlings showing typical symptoms of the infection. Remarkable recovery from the affliction was observed in the treated group. The first leaves of the treated seedlings were normal in size and shape, while those in the control group showed typical abnormality associated with the infection.

The ability of translocation of the benzophenone from leaves to roots of safflower was demonstrated. 10-day-old seedlings of safflower, grown on sterilized sand, were sprayed 4 times on the *leaves* with the benzophenone at 12-h-intervals. After 24 h from the last spray, the seedlings were uprooted, surface-sterilized, and sections of the leaves, stems, and roots were cut. These were placed on PDA plates which were seeded with the pathogen (spore suspension ca. 1,000,000/ml). The plates were incubated at 21 °C for 48 h. Around the sections of the leaves, stems and roots of the treated seedlings, a clear inhibition zone of the fungus was observed. The maximum inhibition zone was observed, as expected, around the sections of the treated leaves on which the benzophenone was sprayed. Each of the root sections also showed appreciable inhibition zone.

The above results suggest that 2,2'4-trihydroxybenzophenone could be used as a protective and a curative agent against the safflower wilt. Additionally, it possesses the unique property of translocation from leaves to roots of safflower. It has thus the potentiality for use as a foliar fungicide against this pathogen. Further studies about the practical significance of these results are currently underway.

Aflatoxin metabolism and absence of cytochrome P-450 in rat colon tissue during vitamin A malnutrition[1]

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Summary. Homogenized mucosal linings prepared from vitamin A adequate and deficient male rats were used in metabolic studies of aflatoxin B_1 (AFB_1). Cytochrome P-420 was identified in both groups which metabolized AFB_1 to 4 metabolic products in vitro. The implications of this observation are discussed in relation to colon carcinoma.

Aflatoxin B_1 is a metabolite of the toxigenic fungus *Aspergillus flavus* (Link ex Fries, U.I.81), cultures of palmsap[3] and several agricultural commodities[4] which, like most lipophilic organic compounds, are known to be metabolized by the cytochrome P-450 dependent monoxygenase system present in liver microsomes[5]. Recently, it was reported[6] that dietary vitamin A caused a decline of drug metabolism in rat liver and a lowering of cytochrome P-450. Under marginal vitamin A, aflatoxin was reported[7] to induce colon carcinoma. Since the condition may occur in man, this report could be of further biochemical and nutritional interest. Thus, it seemed to us necessary to investigate the mechanistic pattern of AFB_1 metabolism in the colon under this condition.

Materials and methods. For producing vitamin A deficiency in the experimental animals, male weanling Sprague-Dawley-derived rats (50–55 g) were fed for 45 days on a corn-based diet without the vitamin. Control animals received a diet supplemented with vitamin A (5 mg vitamin A palmitate per kg diet) for the same period, both groups receiving water ad libitum. The rats were then decapitated and their colons were dissected out and washed free of fecal materials with 0.9% saline. The mucosal linings of the colon were scraped, pooled for each group and stored at 0 °C. The pooled samples were minced in 0.2 M phosphate buffer, pH 7.4, and homogenized in the buffer in a motor-driven Potter-Elvehjem Teflon-glass homogenizer (in an iced bath) at 600 rev/min. Protein and vitamin A were determined by technique of Lowry et al.[8] and Neeld and Pearson[9] respectively. While we were attempting to characterize cytochrome P-450 from this preparation by the method of Omura and Sato[10], we encountered a pig-

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