In vitro metabolism of the trichothecene 4-monoacetoxyscirpenol by fungus- and non-fungus-feeding insects

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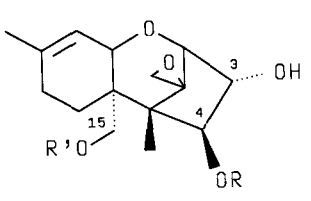
Received 5 July 1988; accepted 8 December 1988

Summary. The metabolism of the trichothecene 4-monoacetoxyscirpenol by intact gut tissue was determined in the fungus-feeding Nitidulid, *Carpophilus hemipterus* (L.) and the non-fungus-feeding caterpillars, the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and the corn earworm, *Heliothis zea* (Boddie). The primary metabolite was the hydrolysis product scirpentriol. The amount of metabolism by the *C. hemipterus* larvae was ca 10 times that of the caterpillars on a per mg protein basis, suggesting metabolic adaptation for feeding on fungi that may contain mycotoxins.
Key words. Mycotoxin; detoxification; *Carpophilus hemipterus; Spodoptera frugiperda; Heliothis zea.*

Secondary metabolites are known to serve a defensive function in both higher plants and fungi. However, in many cases, insects are still able to exploit these resources as a food source, and so, must in some way be able to detoxify these chemicals if they are to survive. While the metabolism of phytotoxins by insects that successfully feed on them has been widely studied (see Brattsten[5] and Dowd[6] for recent reviews), this aspect has not been investigated in regard to mycotoxins and insects which are successfully able to feed on substrates containing them. The ability of insects that specialize on plants that contain phytotoxins to metabolize them has been found to be greater than that of insects which are not specifically adapted[7]. Once again, this parameter has not been investigated in insects that feed on fungi or substrates infested by fungi.

Trichothecenes, such as diacetoxyscirpenol, **1**, and 4-monoacetoxyscirpenol, **2**, represent a class of mycotoxins which are toxic to both mammals[8] and insects[9]. These toxins are produced by a variety of fungi, including those that invade crops, such as *Fusarium* spp. While these fungal natural products are apparently capable of protecting infected kernels of maize from caterpillars due to their inherent toxicity (and are in fact toxic at 25 ppm in diet)[10], sap beetles apparently feed freely on material infected by these fungi, and often act as vectors of the inoculum[11,12]. For example, both larvae and adults of the sap beetle *Carpophilus hemipterus* appear unaffected by naturally occurring levels of trichothecenes (25 ppm in diets) (Dowd, unpublished data). This information suggests that the *Carpophilus* spp. beetles are more successful in dealing with the trichothecenes than are the caterpillars, perhaps by more efficiently detoxifying them. Therefore, in vitro metabolism of a representative trichothecene, 4-monoacetoxyscirpenol (MAS) by intact gut tissue was investigated in the dried fruit beetle, *Carpophilus hemipterus* (L.), and two caterpillars: the corn earworm, *Heliothis zea* (Boddie) and the fall armyworm, *Spodoptera frugiperda* (J. E. Smith).



1 R=Ac; R'=Ac

2 R=Ac; R'=H

3 R=H; R'=H

Materials and methods. Insects. All insects were reared at 27 ± 1 °C, 14:10 light:dark photoperiod, and 40 ± 10% relative humidity on pinto bean-based diet[13]. Third instar larvae of *S. frugiperda* and *H. zea* (weighing ca 35 mg) and last instar larvae of *C. hemipterus* (weighing ca 2.5 mg) were used for the assays.

Chemicals. Diacetoxyscirpenol and scirpentriol, **3**, were obtained from Sigma Chemical Co. Pyridinium chlorochromate and acetic acid were obtained from Aldrich Chemical Co. The NaB^3H_4 was obtained from New England Nuclear. All other chemicals were of reagent grade. The [15-^{3}H]-4-MAS was prepared from diacetoxyscirpenol[14] by formation of the 3-tetrahydropyranyl ethers[15] (90% yield), selective microbial hydrolysis of the 15-acetate with *Streptomyces grisius* (similar to Claridge and Schmitz[16]) (45% yield), oxidation to the 15-aldehyde with pyridinium chlorochromate (85% yield), reduction with NaB^3H_4 (85% yield), and hydrolysis of the tetrahydropyranyl ether with acetic acid (85% yield). The synthetic MAS exhibited spectral and chromatographic properties which were in complete agreement with reported values[16–18]. Thus, the MAS was tritium-labelled regio-specifically in the C-15 position, and had a specific activity of 3 mCi/mmole. Unlabelled 4-MAS was prepared and identified in the same manner.

Assays. Entire guts were dissected from each organism in pH 7.4, 0.1 M phosphate buffer, and emptied of their contents by slitting the gut and coaxing the material out

with a probe. Due to the intimate association of the fat body of *C. hemipterus* with the gut, it was left attached. The tissues were added to 100 µl of buffer, 3.09×10^{-10} moles of ^{3}H-MAS (ca 1000 cpm) was added in 1 µl of ethanol, and the tissues were incubated at 35 °C for 1 h. After incubation, the buffer was removed and immediately spotted on the absorbent area of LK5DF plates (Whatman). The plates were developed in toluene: acetone (50:50); and authentic MAS and scirpentriol (the hydrolysis product) were used as standards. The position of the standards was detected by absorption of I_2 and by spraying the TLC plates with 3% $Ce(SO_4)_2$ in 2 N H_2SO_4 and heating at 110 °C for ca 10 min. To extract compounds potentially remaining in the tissues, the tissues were extracted three times each with 500 µl of ethyl acetate, followed by methanol. The extracts were concentrated under nitrogen to ca 50 µl, and then characterized chromatographically in the same manner as described for the buffer solution. The entire plates were scraped in 1-cm sections, and the radioactivity was quantitated by liquid scintillation counting. The remaining gut tissue was homogenized in 1 ml of distilled water, and quantitated for protein content with the Bio-Rad protein assay kit [19].

Results. The MAS was metabolized primarily to scirpentriol in all three insect species (table 1). A second area of metabolite(s) was located at the origin. The remaining radioactivity was randomly distributed on the plates. The *C. hemipterus* produced a greater proportion of scirpentriol, while the *H. zea* produced a higher proportion of the metabolite(s) that remained at the origin compared to the other insects. No radioactivity was detected either in the methanol or the ethyl acetate extracts of the tissues. Recovery of the radiolabel was at least 80% in all cases (table 2).

The *C. hemipterus* and *S. frugiperda* tissues metabolized the MAS at approximately the same rate on a per tissue basis (table 2). However, on a per mg protein basis, the tissues of *C. hemipterus* were ca 8–10 times more effective in metabolizing MAS than those of *H. zea* or *S. frugiperda*.

Discussion. The presence of scirpentriol in controls may be due to spontaneous hydrolysis. The metabolism of MAS by other organisms has not been reported except as a part of 4,15-diacetoxyscirpenol (DAS) metabolism studies. The 15-MAS was the only metabolite of DAS reported for metabolism studies with rat and rabbit liver [20] and bovine rumen microorganisms [21]. Both 15-MAS and scirpentriol were reported as metabolites of DAS by pig blood serum [22]. Sakamoto et al. [23] also reported two additional metabolites by rats in vivo: 12,13-diene analogs of MAS and scirpentriol. The scirpentriol and its deepoxy-analog cochromatographed in the TLC solvent systems used [23]. Thus, it is possible that the scirpentriol reported in the present study may be a combination of these two metabolites. However, this is unlikely, since no metabolic reactions where an epoxide is converted to an olefin have ever been reported in insects. The identity of the metabolite(s) at the origin in the present study is unknown. Their position indicates they are more polar than the scirpentriol. It is likely that the area is represented by conjugates, since this type of reaction occurs widely in insects when they detoxify xenobiotics [24]. Although no conjugates of MAS have been reported, metabolic conjugates of the trichothecene T-2 [25, 26] and DAS have been described [26, 27]. Since epoxide hydrolases are also involved in detoxification in insects [28], the radiolabel at the origin may include some form of a 12,13-diol as well. However, this epoxide is chemically very resistant to hydrolysis, and to date no metabolites of this sort have been reported for any trichothecenes.

The production of scirpentriol from the MAS by the insects in the present study can be considered to be a detoxification reaction, since scirpentriol is 30 times less toxic to HEp2 cells than MAS [29]. The compound(s) present at the origin, whether conjugates or hydrolyzed epoxides, are also likely to be less toxic than MAS. Insect esterases frequently hydrolyze phytotoxins that contain carboxylic esters, so the high production of scirpentriol as a MAS metabolite by all three insect species examined is not unexpected.

Higher rates of metabolism of xanthotoxin (6–6.5 times faster) by the black swallowtail caterpillar, *Papilio polyxenes*, as opposed to *S. frugiperda* is considered to be an adaptation of *P. polyxenes* to feed specifically on umbelliferous hosts [7]. The present study indicates that *C. hemipterus* also has similarly adapted to feeding on

Table 1. Levels of MAS and metabolites present after incubation with insect guts (% of initial radiolabel)

Compound	Rf	Control	*C. hemipterus*	*S. frugiperda*	*H. zea*
MAS	0.73	93.3 ± 1.2 a	76.0 ± 2.9 b	74.8 ± 2.5 b	83.0 ± 0.8 b
SCTL	0.20	5.8 ± 0.8 a	18.6 ± 2.1 b	16.3 ± 1.7 b	12.1 ± 0.5 b
POL	0.00	0.0 ± 0.0 a	3.9 ± 0.7 b	6.3 ± 0.6 b	7.3 ± 0.9 b
MISC	–	1.0 ± 0.4 a	2.5 ± 1.1 a	2.6 ± 1.6 a	0.0 ± 0.0 a

Values are means ± standard errors of three assays of two replicates each. MAS = monoacetoxyscirpenol, SCTL = scirpentriol, POL = polar metabolites at the origin, MISC = randomly encountered radioactivity remaining. Means in rows followed by the same letter are not significantly different at $p < 0.05$ by linear contrast analysis of variance [30].

Table 2. Rates of total metabolism of MAS by the different insect species relative to controls

Activity type	*C. hemipterus*	*S. frugiperda*	*H. zea*
% of radiolabel recovered	86.4 ± 2.0 a	80.1 ± 2.1 a	89.5 ± 3.2 a
Amount of MAS metabolized (pmole)	55.7 ± 7.6 ab	56.6 ± 4.0 a	36.4 ± 2.4 b
MAS metabolized (pmole/mg gut protein)	11.1 ± 1.8 a	1.2 ± 0.3 b	1.6 ± 0.2 b

Values are means ± standard errors of three assays of two replicates each, for assay run over a 1-h period. Means in rows followed by the same letter are not significantly different at $p < 0.05$ by linear contrast analysis of variance [30].

substrates that are colonized by fungi that produce mycotoxins such as the trichothecenes. Over the 1-h period that metabolism was monitored, *C. hemipterus* detoxified MAS at a rate that was 8–10 times (on a per mg protein basis) that of caterpillars that may also encounter the trichothecenes, but are not adapted to feeding on material containing them. The increased rates of MAS metabolism by *C. hemipterus* relative to *S. frugiperda* and *H. zea* may contribute to the lower toxicity of trichothecenes to *C. hemipterus*. This information, which represents the first study of trichothecene metabolism by insects, indicates that insects are capable of adapting to a range of naturally occurring toxins which also includes mycotoxins through enzymatic detoxification.

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3 Acknowledgment. The authors wish to thank S. Taylor for technical assistance.
4 The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

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0014-4754/89/040393-03$1.50 + 0.20/0

Limitations on visual food-location in the planktivorous antarctic fish *Pagothenia borchgrevinki*

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Received 26 October 1988; accepted 18 November 1988

Summary. The visual threshold for food-location in the antarctic fish *Pagothenia borchgrevinki*, is compared with light conditions and food availability under the ice. Even under the most favourable conditions for vision, *P. borchgrevinki* is operating close to its visual threshold and must often depend on non-visual mechanisms of prey detection.
Key words. Antarctic fish; vision; lateral line; prey detection.

The area immediately beneath the annual sea-ice of polar regions has been termed the cryopelagic habitat[2]. In McMurdo Sound, Antarctica, *P. borchgrevinki* is the only common species of fish occupying this zone. During the austral spring these fish feed on macrozooplankton located in the water column[3]. Observations of fish striking lures indicate that feeding can be visually mediated. However, it has also been shown that the mechanosensory lateral line system of these fish is well suited to the detection of planktonic prey[4, 5]. This system may be particularly important not only during winter, but also in summer when snow and ice cover, and phytoplankton and algal blooms, reduce ambient light levels.

What is the lowest light level at which these fish are still able to locate food visually? To answer this question fish were taken from beneath the sea-ice in McMurdo Sound, Antarctica (water temperature −1.9 °C) and kept in aquaria under controlled lighting conditions. For elec-