0014-4754/94/010072-03$1.50 + 0.20

Variability of sterol utilization in stored-products insects[1]

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Received 18 August 1993; accepted 20 September 1993

Abstract. A comparison of sterol utilization by 3 stored-products insects revealed very different capabilities. The flour beetle, *Tribolium castaneum*, dealkylates and converts dietary sitosterol to about equal amounts of cholesterol (43.7%) and 7-dehydrocholesterol (39.8%), whereas another flour beetle, *Tenebrio molitor*, produces considerably less 7-dehydrocholesterol (16.8%) and relatively more cholesterol (66.7%) from sitosterol. The lepidopteran, *Plodia interpunctella*, utilized dietary sterol very similar to plant-feeding Lepidoptera, producing primarily cholesterol (86.5%) from sitosterol.
Key words. Stored-products insects; sterol metabolism; *Tribolium castaneum*; *Tenebrio molitor*; *Plodia interpunctella*; C_{28} and C_{29} phytosterols; 7-dehydrocholesterol; cholesterol.

Insects are unable to biosynthesize sterols, and therefore are dependent on dietary sterols for normal growth, development, and reproduction. Considerable diversity in sterol utilization and metabolism exists, however, between various insect groups and species[2]. Many of these differences undoubtedly reflect the fact that insects, during the course of evolution, have adapted to virtually every type of environment. While nearly all insects are able to utilize cholesterol as the sole dietary source of sterol, many phytophagous species obtain little or no cholesterol from their diets. Most phytophagous and omnivorous species, however, are able to dealkylate and convert the common C_{28} and C_{29} phytosterols (e.g. campesterol, sitosterol, and stigmasterol) to cholesterol, which is their predominant sterol, while certain species metabolize phytosterols differently[3,4]. Sterol metabolism studies with the confused flour beetle, *Tribolium confusum*, of the family Tenebrionidae, revealed the first unusual aspect of sterol utilization in a phytophagous insect. Using a well-defined diet, we found *Tri. confusum* capable of dealkylating radiolabeled campesterol, stigmasterol and sitosterol, but nearly equivalent amounts of cholesterol and 7-dehydrocholesterol ($\Delta^{5,7}$,-cholestadien-3β-ol), together totalling more than 90% of the insect's sterols, were found in the insect, regardless of the dietary sterol[5]. Similar results were obtained when either cholesterol, 7-dehydrocholesterol, or desmosterol was added as the dietary supplement, indicating that an equilibrium exists between cholesterol and 7-dehydrocholesterol in this species. Earlier studies that relied upon UV spectrophotometric analysis had indicated that 7-dehydrocholesterol was the major sterol of *Tri. confusum*[6]. However, in another major stored-products pest, the khapra beetle, *Trogoderma granarium*, we discovered a phytophagous species that is unable to dealkylate and convert C_{28} and C_{29} phytosterols to cholesterol[7]. Although the khapra beetle is capable of selective uptake of cholesterol from minute levels (0.5%) in the diet, cholesterol only accounts for 1.2% of the total sterols in the insect. Unlike the confused flour beetle, no 7-dehydrocholesterol could be identified in the sterols of *Tro. granarium*. Thus, two species of beetles, which ordinarily occupy fairly similar ecological niches, utilize dietary sterols very differently. With regard to sterol utilization, the khapra beetle is more similar to other members of the family Dermestidae such as the hide beetle, *Dermestes maculatus*[8], which usually feeds on animals or animal products that contain adequate amounts of cholesterol. In light of the differences in sterol utilization found between *Tri. confusum* and *Tro. granarium*, we compared the sterol metabolism capabilities in three other stored-products species. These include two other members of the family Tenebrionidae, the red flour beetle, *Tribolium castaneum*, and the yellow mealworm, *Tenebrio molitor*. Also, for comparison with a species not belonging to the order Coleoptera, the Indian meal moth, *Plodia interpunctella* (family Pyralidae, order Lepidoptera), was included in this study.

Materials and methods

Tri. castaneum was reared on a diet consisting of white flour/whole wheat flour/extracted brewer's yeast (44:44:12) supplemented with 0.1% sitosterol. A similar experiment included 500 ppm 25-azacoprostane (a potent 24-sterol reductase inhibitor) in the diet. The *Te. molitor* diet was commercial bran meal with no additional sterol added. No sitosterol supplement was added to the *Te. molitor* diet since sufficient sitosterol plus isofucosterol was present in the bran meal (see table) and these are both metabolized to cholesterol in this insect. *P. interpunctella* was fed a diet including white corn meal/whole wheat flour/glycerol/honey/extracted brewer's yeast/rolled oats/wheat germ

(28:26:17:16:6:4:3) plus 0.05% sitosterol. Another test included 2 ppm 25-azacoprostane in the diet. Insects were held at 30 °C and 55% RH and were reared to mature larvae or prepupae. Insects were weighed and stored in methanol at 4 °C until extracted. The fresh weights of insect samples were: *Tri. castaneum*, 0.45 g without inhibitor and 0.25 g with inhibitor; *Te. molitor*, 1.10; and *P. interpunctella*, 1.39 g without inhibitor and 1.19 g with inhibitor. Insect samples and portions of the diets (10 g each) were individually homogenized and extracted in $CHCl_3$-MeOH (2:1), prior to saponification of the crude lipids. Sterols were isolated and purified by column chromatography on alumina (Woelm, ICN Pharmaceuticals, Cleveland, Ohio, USA) and column fractions were monitored by TLC[9]. Care was taken to protect all samples from light and exposure to air to reduce the deterioration of labile sterols such as 7-dehydrocholesterol. Sterol fractions from both insect and diet samples were quantitatively and qualitatively analyzed by GLC on J & W DB-1 fused silica capillary column, 15 m × 0.25 mm i.d. (0.25 mm film) at 235°C, helium carrier gas at 25 cm/s linear velocity, 23:1 split ratio, in a Varian model 3700 gas chromatograph interfaced with a Shimadzu C-R1B chromatopac data processor. GLC identifications were made by comparing retention times relative to cholestane as an internal standard. Insect and diet sterols were also analyzed by gas chromatography-mass spectrometry with a Finnigan model 4510 equipped with a J & W DB-1 column.

Results and discussion

The relative percentages of major 4-desmethyl sterols from insect and diet samples are listed in the table. The sterols from *Tri. castaneum* larvae were predominantly cholesterol (43.7%) and 7-dehydrocholesterol (39.8%). Since sitosterol comprised 90.2% of the dietary sterols yet only 16.0% of the insect sterols, and the diet was cholesterol-free, the red flour beetle must be capable of dealkylating sitosterol. The fact that the diet contained 6.5% campesterol and the insect sterols included only 0.6% campesterol provides further evidence of dealkylation. In addition, the incorporation of 25-azacoprostane in the diet greatly reduced cholesterol production and caused accumulation of 5,7,24-cholestatrienol and desmosterol, as has been previously found with *Tri. confusum*. These results indicate that *Tri. castaneum* and *Tri. confusum* metabolize phytosterols similarly, including the equilibrium between cholesterol and 7-dehydrocholesterol as previously described[5]. The yellow mealworm, although a member of the same family, Tenebrionidae, metabolized ingested C_{28} and C_{29} phytosterols somewhat differently from *Tri. confusum* or *Tri. castaneum*. Although 7-dehydrocholesterol is present at much higher levels (16.8%) than is usually found in insects, cholesterol at 66.7% is by far the major sterol of *Te. molitor*, whereas none was identified in the diet sterols. Any metabolic equilibrium between cholesterol and 7-dehydrocholesterol is skewed in the direction of cholesterol. Sitosterol and campesterol levels in the insect sterols are considerably less than in the dietary sterols. This species apparently efficiently utilizes isofucosterol, since none was identified in the insect sterols, whereas 36.7% of the dietary sterol was isofucosterol. The conversion of 28-isofucosterol to cholesterol was previously reported in studies on the mechanism of dealkylation in *Te. molitor*[10]. Other mechanistic studies with this species using radiolabeled sterols demonstrated the conversion of 24-methylenecholesterol[11], fucosterol and isofucosterol epoxides[12] to cholesterol. However, none of these studies included analyses of the total insect sterols or mention of 7-dehydrocholesterol. This labile sterol may not have been obvious to these investigators since the conjugated $\Delta^{5,7}$-diene is destroyed in a very short time unless care is taken to protect the samples from exposure to light and oxygen. Although a major portion of the literature reporting C_{28} and C_{29} phytosterol dealkylation has been

Relative percentages of major 4-desmethyl sterols of stored-product insects and their diets

Sterols	Red flour beetle (*Tri. castaneum*)			Yellow mealworm (*Te. molitor*)		Indian meal moth (*P. interpunctella*)		
	Larvae		Diet	Larvae	Diet	Prepupae		Diet
	N	I				N	I	
Cholesterol	43.7	4.9	-	66.7	-	86.5	14.0	-
Desmosterol	-		-	-	-	1.2	54.2	-
7-Dehydrocholesterol	39.8	52.3*	-	16.8	-	-	-	-
5,7,24-Cholestatrienol	-	15.1	-	-	-	-	-	-
Campesterol	0.6	0.9	6.5	3.8	11.8	1.5	6.1	9.3
Stigmasterol	-	-	0.5	1.5	4.3	-	-	1.4
Sitosterol	16.0	25.9	90.2	11.2	47.2	10.8	25.1	78.8
Isofucosterol	-	-	2.6	-	36.7	-	-	10.4

N, normal diet; I, diet includes 25-azacoprostane inhibitor.
*Desmosterol and 7-dehydrocholesterol combined.

obtained from Lepidoptera[3,4], primarily the tobacco hornworm, *Manduca sexta*, and the silkworm, *Bombyx mori*, no information is available concerning sterol metabolism in a stored-products lepidopteran. Our examination of sterol utilization in the Indian meal moth indicates that *P. interpunctella* metabolizes phytosterols very similarly to the leaf-feeding Lepidoptera previously studied[3,4]. Sitosterol, isofucosterol, and campesterol comprised 78.8, 10.4, and 9.3%, respectively, of the dietary sterols and no cholesterol was identified. The predominance of cholesterol (84.0%) and the much reduced levels of phytosterols in the prepupal sterols provide good evidence that the Indian meal moth is as capable of deaklylating C_{28} and C_{29} phytosterols as *M. sexta* and *B. mori*. Thus, in its ability to metabolize phytosterols, this stored-products pest is more similar to other Lepidoptera, even though they inhabit very different environments, than it is to either species of stored products Coleoptera (*Tri. castaneum* and *Te. molitor*) included in this study. We also found that the 24-reductase inhibitor, 25-azacoprostane, greatly reduces cholesterol production and causes an accumulation of desmosterol and unmetabolized sitosterol when fed with sitosterol, just as had been found with other Lepidoptera[13]. The other stored-products Coleoptera previously examined, the confused flour beetle[5] and the khapra beetle[7], also are very different from the Indian meal moth in their sterol metabolism capabilities.

The ability of various stored-products insects to survive in such similar environments and yet to differ so markedly with respect to sterol metabolism is striking. Not surprisingly, since they are members of the same genus, the red flour beetle metabolizes sterols similarly to the confused flour beetle, producing about equivalent amounts of cholesterol and 7-dehydrocholesterol. The yellow mealworm, although a member of the same family as the two *Tribolium* species, also has considerably more 7-dehydrocholesterol than is usually found in the sterols from an insect, but appreciably less than the *Tribolium* species. The reason for the occurrence of such relatively large amounts of 7-dehydrocholesterol in these flour beetles is unknown. This sterol is an early intermediate in the conversion of cholesterol to ecdysone[14], but only a minute portion of the 7-dehydrocholesterol present would be required as an ecdysone precursor. One might argue that this may be related to the very dry environment of these insects, but this is not true of all stored-products insects we examined. Even though the khapra beetle, *Tro. granarium*, occupies a similar ecological niche, is of a similar size to the *Tribolium* species, and resembles them in gross morphology, it is unable to dealkylate, and no more than trace amounts of 7-dehydrocholesterol are found. It obtains adequate cholesterol for physiological needs through selective uptake from the diet[7]. Also, the lepidopteran Indian meal moth dealkylates, but produces mainly cholesterol from dietary C_{28} and C_{29} phytosterols, as do leaf-feeding Lepidoptera, thus differing significantly from all the coleopteran species examined. Consequently, even though all these stored-products species occupy similar ecological niches, their sterol metabolism capabilities seem to be more phylogenetically than environmentally influenced, and these results emphasize the diversity of sterol utilization and metabolism among insect species.

1 The authors thank O. J. Duncan III and D. Harrison for technical assistance. Mention of a company name or proprietary product does not imply endorsement by the US Department of Agriculture.

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