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Original article

Comparison of the dietary and tissue sterols of the greater wax moth, *Galleria mellonella* (L)

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Summary — The neutral sterols of the greater wax moth *Galleria mellonella* were determined and compared to the sterols isolated from the used brood comb upon which the insects were reared. Analysis by gas-liquid chromatography and mass spectrometry revealed that used brood comb contained primarily 28- and 29-carbon sterols, with cholesterol accounting for less than 1% of the total sterols detected. This differed considerably from the insect, where cholesterol comprised over 85% of the tissue sterols. These results indicate the wax moth is able to convert dietary 24-alkylsterols to cholesterol. The potential for using inhibitors of sterol metabolism to control *G mellonella* is discussed.

Galleria mellonella* / neutral sterols / cholesterol / chemical control / parasite / *Apis mellifera

INTRODUCTION

Insects are unable to synthesize the steroid nucleus de novo and therefore require a dietary source of sterol for membrane integrity, normal growth, development and reproduction (Hobson, 1935; Clark and Bloch, 1959). Though plants contain little or no cholesterol, cholesterol is often the predominant tissue sterol detected in many phytophagous insects, a result of their ability to convert the 24-alkylsterols commonly found in their diet to cholesterol. In these insects, cholesterol is then used to synthesize molting hormones, like ecdysone and 20-hydroxyecdysone (Rees, 1985; Svoboda and Thompson, 1985; Grieneisen, 1994). Other insects, notably the honey bee *Apis mellifera* L, do not possess the ability to convert their dietary sterols to cholesterol, and the tissue sterols of honey bees contain little, if any, cholesterol (Svoboda et al, 1981; 1983a, b). Furthermore, honey bees apparently utilize 24-alkylecdysteroids, like makisterone A, as their molting hormone (Feldlaufer et al, 1985; Rachinsky et al, 1990), which has

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been shown to be synthesized from the plant sterol campesterol (Feldlaufer et al, 1986a).

Several vertebrate hypocholesterolemic agents have been shown to inhibit the conversion of 24-alkylsterols to cholesterol in insects, by interfering with the Δ^{24}-sterol reductase enzyme system involved in this conversion (Svoboda and Robbins, 1967). These, along with other related compounds were shown in several insect species to be potent inhibitors of metamorphosis, disrupting development at the time of molting (Svoboda et al, 1972; Robbins et al, 1975; Svoboda and Thompson, 1985). Interestingly though, when three of these inhibitors, two azasteroids and an alkyl amine, were included in honey bee diet, none of the chemicals had a harmful effect on honey bee brood development to the adult (Svoboda et al, 1987). Presumably, honey bee development was not affected because the bees did not obtain sterol from the metabolic pathway these compounds inhibit. These reports open the possibility that Δ^{24}-sterol reductase inhibitors may have potential use in control programs aimed at honey bee pests that rely upon converting dietary sterols to cholesterol, particularly members of the Lepidoptera, upon which many of the initial inhibition studies were conducted (Svoboda and Robbins, 1971; Chippendale and Reddy, 1973; Al-Azzi and Hopkins, 1982). As an initial step in a program to determine the potential of these compounds to control the greater wax moth *Galleria mellonella*, we have analyzed the tissue sterols of pupal moths and compared them to the neutral sterols of brood comb upon which they were reared. The results of this study are the subject of this communication.

MATERIALS AND METHODS

Biological material

Prepupae of *Galleria mellonella* were obtained from a culture at the Bee Research Laboratory that is maintained at 30 °C in the dark. Used brood comb, on which the insects were reared, was obtained from our bee yard.

Isolation of sterols

Used brood comb and *G mellonella* prepupae were saponified under reflux using 5% potassium hydroxide in a solution of ethanol/benzene/water (10:1:1, by vol). After 5 h, the solution was allowed to cool and 2 vol of water was added. The solution was then acidified with 6N hydrochloric acid and extracted with hexane (3x) and diethyl ether (1x). After drying the pooled organic phases over sodium sulfate, brood comb and insect samples were taken to total dryness under vacuum. Residues were fractionated on a Florisil® column (Fisher Sci, Fair Lawn NJ) in a diethyl ether system as previously described (Chitwood et al, 1987). Fractions containing sterols from the brood comb sample were subsequently fractionated on an aluminum oxide column (neutral grade II; Merck, Darmstadt, Germany) eluted with the following solvents: hexane (40 mL), 5% ether in hexane (30 mL), 40% ether in hexane (25 mL), and ether (100 mL). All column fractions were monitored by thin-layer chromatography (TLC) and capillary gas-liquid chromatography (GLC). All analyses were performed in triplicate.

Analyses and instrumentation

All solvents for extraction and purification were reagent grade, redistilled. TLC was done on high performance silica gel 60 F_{254} plates (Merck) developed in hexane/diethyl ether/acetic acid (60:40:1, by vol). Capillary GLC was performed at 245 °C on a Shimadzu GC-9A gas chromatograph (Columbia MD) equipped with a J&W DB-1 fused silica column (15 m × 0.25 mm; J&W Scientific, Folsom CA) and a Shimadzu CR-3A integrator. Mass spectra were obtained on a Finnigan 4500 gas chromatograph/mass spectrometer under conditions previously described (Lusby et al, 1993).

RESULTS

The sterols isolated from used brood comb and from *G mellonella* prepupae are given in

table I. The predominant sterol isolated from used brood comb was 24-methylenecholesterol, which accounted for almost 52% of the total sterol detected. Other C_{28} and C_{29} sterols, including isofucosterol (21.0%), sitosterol (14.2%), campesterol (8.0%) and stigmasterol (4.3%), comprised the bulk of the remaining sterols. The 27-carbon sterol cholesterol, accounted for less than 1% of the sterol isolated from used brood comb.

In contrast, cholesterol accounted for over 85% of the tissue sterols of *G mellonella* prepupae, the remainder consisting of sitosterol (10.7%) and campesterol (2.5%), with lesser amounts of isofucosterol (0.8%) and 24-methylenecholesterol (0.8%). No stigmasterol was detected in the samples.

DISCUSSION

The predominant neutral sterol isolated from used brood comb was 24-methylenecholesterol, with lesser amounts of other C_{28} and C_{29} sterols, like sitosterol and isofucosterol. 24-methylenecholesterol has been shown to be a major component of many pollens (Barbier et al, 1960; Standifer et al, 1968; Svoboda et al, 1983a; Lusby et al, 1993), the dietary source of sterols for honey bees. In addition, the overall sterol composition of used comb is similar to the sterols isolated from several honey bee stages and tissues (Barbier and Schindler, 1959; Svoboda et al, 1983a, 1986; Feldlaufer et al, 1986b). Since fresh beeswax contains primarily hydrocarbons, esters, acids and alcohols (Schmidt and Buchmann, 1992; Tulloch, 1980), it is reasonable to assume that brood comb sterols originate from a combination of pollen residues and bee by-products, like larval feces and cast larval and/or pupal skins.

Table I. Relative percentage of brood comb sterols and tissue sterols isolated from *Galleria mellonella* prepupae.

Sterol [a]	*Brood comb* %	Galleria mellonella %
Cholesterol	0.6	85.2
24-methylenecholesterol	51.9	0.8
Campesterol	8.0	2.5
Stigmasterol	4.3	-
Sitosterol	14.2	10.7
Isofucosterol	21.0	0.8

[a] Sterols were identified by capillary gas chromatography-mass spectrometry, and relative percentages were determined by either peak area (*Galleria*) or total ion chromatogram (comb). Percentages represent the average of three determinations.

While cholesterol accounted for less than 1% of the comb sterols, a finding also consistent with the low levels of cholesterol associated with both pollen and bee tissue, it constituted over 85% of the sterols isolated from *G mellonella*. This is consistent with the reports that every developmental stage of the wax moth examined, contained a mixture of 27-carbon ecdysteroids (Bollenbacher et al, 1978; Hsiao and Hsiao, 1979; Smith and Bollenbacher, 1985), which are all synthesized from cholesterol. The high levels of cholesterol in wax moth prepupae are also indicative of this insect's ability to dealkylate dietary sterols to cholesterol, and open the possibility of using chemical inhibitors of the dealkylation pathway in a wax moth control program. These compounds have previously been shown to disrupt development in other species of moths (Svoboda and Robbins, 1971; Chippendale and Reddy, 1973; Al-Azzi and Hopkins, 1982), and in another study, shown not to harm honey bees (Svoboda et al, 1987). In addition, one of the compounds, N, N-dimethyldodecanamine (designated IPL-12) was previously shown to have promise as an antifungal agent directed at chalkbrood (Herbert et al, 1985, 1987). While suitable methods of delivery must be formulated for this or any related compound to realize their potential, inhibitors of sterol metabolism may offer an effective and useful alterna-

tive to fumigation in management of the greater wax moth.

Résumé — Comparaison des stérols alimentaires et des stérols des tissus chez *Galleria mellonella* (L). Les insectes doivent tirer les stérols de leurs aliments et de nombreux insectes phytophages sont capables de convertir les stérols à 28 et 29 carbones, couramment présents dans les plantes, en cholestérol. D'autres insectes, tels que l'abeille mellifère, sont incapables de faire cette conversion et renferment peu, si ce n'est aucun, cholestérol. Nous avons voulu comparer les stérols des tissus de la grande teigne de la ruche, *Galleria mellonella*, avec les stérols des rayons de couvain usagés que l'insecte parasite. A l'aide d'une séparation solvant:solvant suivie d'une série de chromatographies sur colonne, nous avons pu isoler et purifier les stérols des prénymphes de *G mellonella* et des rayons sur lesquels elles étaient élevées. Les analyses par chromatographie gazeuse sur capillaire et par chromatographie gazeuse-spectrométrie de masse ont montré que le rayon de couvain comportait principalement du 24-méthylène-cholestérol et d'autres 24-alkylstérols et renfermait moins d'1 % de cholestérol (tableau I). Nous avons supposé que ces stérols provenaient des résidus de pollen et/ou des exuvies d'abeilles. Pourtant, d'après l'analyse des prénymphes de *G mellonella*, le cholestérol représentait plus de 85 % des stérols totaux des tissus. Nous avons attribué ce résultat à la capacité de *G mellonella* de déalkyler les stérols alimentaires en cholestérol. Ces résultats montrent que les composés qui inhibent le système enzymatique impliqué dans la conversion des 24-alkylstérols en cholestérol pourraient être utilisés à l'avenir dans les programmes de lutte contre la grande teigne de la ruche.

Galleria mellonella* / stérol neutre / cholestérol / lutte chimique / parasite / *Apis mellifera

Zusammenfassung — Vergleich von Sterolen aus der Nahrung und dem Körpergewebe der großen Wachsmotte, *Galleria mellonella* (L). Insekten müssen Sterole aus Nahrungsqellen aufnehmen. Viele pflanzenfressende Insekten können die allgemein in Pflanzen vorkommenden C 28- und C 29-Sterole in Cholesterol umwandeln. Andere Insekten, wie die Honigbiene, sind nicht in der Lage diesen biochemischen Umbau durchzuführen. Sie enthalten nur wenig oder wenn überhaupt nur Spuren von Cholesterol. Wir interessierten uns für den Vergleich von Gewebesterolen der großen Wachsmotte, *Galleria mellonella,* mit Sterolen der Brutwaben, die diesem Schädling als Nahrung dienen.

Nach einer Vorreinigung mit verschiedenen Lösungsmittelphasen und durch chromatische Auftrennungen mit einer Serie von Säulen gelang es uns, sowohl die Sterole von den Vorpuppen als auch die ihrer Waben, in denen sie sich entwickelt hatten, zu isolieren und aufzureinigen. Eine Analyse mit Kapillar- Gas Chromatographie und der kombinierten Gas-Chromatographie / Massen-Spektrometrie zeigte, daß die Sterole der Brutwabe vor allem aus 24-Methylencholesterol und weiteren 24-Alkylsterolen bestand. Das Vorkommen von Cholesterol lag unter 1% (Tabelle I). Wir vermuten, daß diese Sterole von Pollenresten und/oder Resten von Bienenhaut stammen. Die Analyse von *G melonella* Vorpuppen zeigte jedoch, daß bei ihnen Cholesterol über 85% der Gesamtmenge von Gewebesterolen ausmacht. Wir schreiben diese Befunde der Fähigkeit von *G mellonella* zu, Nahrungssterole zu Cholesterol zu dealkylieren. Diese Ergebnisse zeigen, daß Verbindungen, die das Enzymsystem zur Umwandlung von 24-Alkylsterolen in Cholesterol hemmen, in Zukunft eine Rolle bei der Bekämpfung der großen Wachsmotte spielen könnten.

Wachsmotte / *Galleria mellonella* / neutrale Sterole / Cholesterol

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