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Variability of Metabolism and Function of Sterols in Insects

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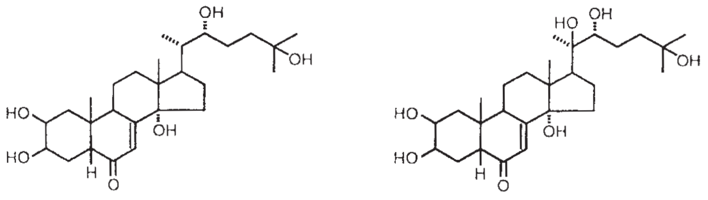
CONTENTS

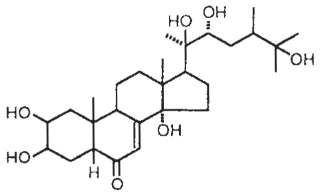
I. INTRODUCTION

It is generally accepted that insects are unable to biosynthesize the tetracyclic steroid nucleus and depend on an exogenous or dietary source of sterol to support normal development and reproduction.[1] In a few known exceptions, microbial symbionts provide sufficient essential sterol for the insect.[2] As in many organisms, sterols serve a dual role both as components of cell membranes and as precursors to steroid hormones which, in insects, are the molting hormones (ecdysteroids). Of these ecdysteroids, the 27-carbon steroids, ecdysone and 20-hydroxyecdysone, are the most prevalent (Figure 1). Perhaps other as yet unknown physiological needs for sterols also exist.



Ecdysone

20-Hydroxyecdysone



Makisterone A

Figure 1 Structures of C_{27} ecdysteroids ecdysone and 20-hydroxyecdysone, and the C_{28} ecdysteroid makisterone A.

Cholesterol will satisfy the dietary need for sterol in insects in all but two known cases, in which a Δ^7-dietary sterol was shown to be required by *Drosophila pachea*[3] and *Xyleborus ferrugineus*.[4] Although most plant material contains little or no cholesterol, most phytophagous insects are capable of obtaining adequate cholesterol by converting C_{28} and C_{29} phytosterols to cholesterol via dealkylation of the C-24 alkyl group (Figure 2).[5] Even the primitive firebrat, *Thermobia domestica*, was shown by means of thorough biochemical studies to be capable of dealkylating.[6] For many years, it was believed that, in general, phytophagous and omnivorous insects were able to dealkylate phytosterols, whereas zoophagous species were not; but, as more species were examined, a number of exceptions to this rule were discovered. Although sterol metabolism has been investigated in a small number of insect species, as an increasing number of species is examined, it becomes increasingly clear that it is very difficult to generalize about sterol metabolism in insects. A number of variations in sterol utilization and metabolism will be discussed in this chapter. These include differences between members of the same order and between insects that occupy similar ecological niches and utilize similar diets. Discussion in this chapter will concentrate on neutral sterol metabolism and touch only briefly on ecdysteroid biosynthesis. For detailed discussion of ecdysteroid metabolism the reader is referred to recent detailed accounts by Rees,[7] Thompson et al.,[8] and Grieneisen.[9]

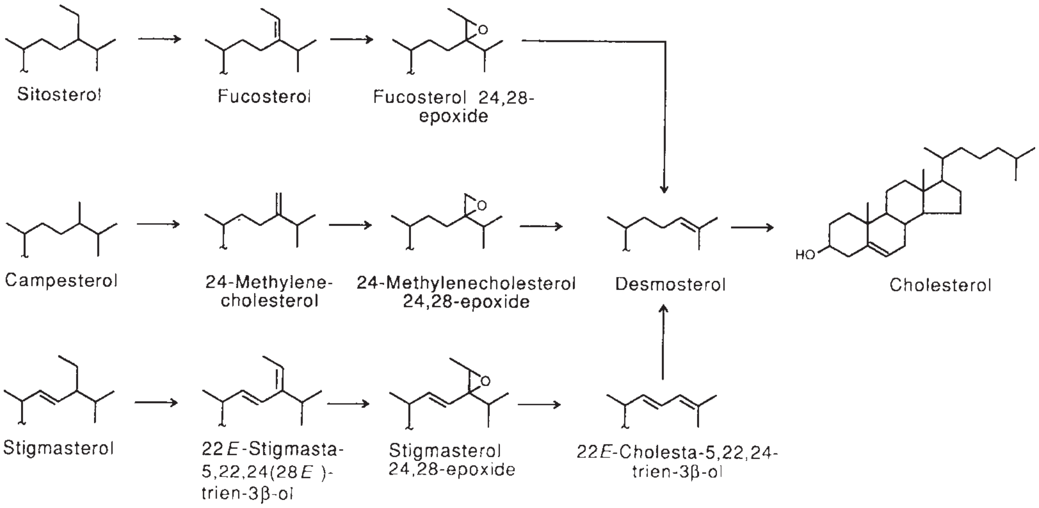


Figure 2 Pathways of dealkylation and conversion of major C_{28} and C_{29} phytosterols to cholesterol functional in most phytophagous and omnivorous insect species.

II. MOST COMMON PATHWAYS OF DEALKYLATION

In the first demonstration of C-24 dealkylation in an insect, ergosterol was shown to be metabolized to 22-dehydrocholesterol in the German cockroach.[10] This insect is unable to reduce the Δ^{22}-bond, but was subsequently shown to be able to convert sitosterol to cholesterol.[11]

A generalized scheme for the metabolic pathways involved in the dealkylation and conversion of some of the most common C_{28} and C_{29} phytosterols to cholesterol in insects is shown in Figure 2. The dealkylation mechanism includes an initial oxidation to produce a $\Delta^{24(28)}$-bond with a subsequent epoxidation prior to the actual dealkylation to form a Δ^{24}-bond. Desmosterol is the common terminal intermediate in the conversion of a number of phytosterols to cholesterol. Most of the intermediates shown in Figure 2 were first determined in studies with *Manduca sexta*[2] and *Bombyx mori*.[12] However, 22-*E*-stigmasta-5,22,24(28*E*)-trien-3ß-ol and stigmasterol-24,28-epoxide were first determined to be intermediates in the conversion of stigmasterol to cholesterol in studies with *Spodoptera littoralis*.[13] In addition to sitosterol, campesterol, and stigmasterol, other 24-alkyl sterols including brassicasterol, 22,23-dihydrobrassicasterol, 24-methylenecholesterol, and fucosterol were shown in related studies to be converted to cholesterol by *M. sexta*.[14]

Using radiolabeled sitosterol as the dietary sterol, desmosterol was the first intermediate identified in the dealkylation and conversion of a phytosterol to cholesterol in *M. sexta*.[15] Subsequently, fucosterol, 24-methylenecholesterol, and 22*E*-cholesta-5,22,24-trien-3ß-ol were determined to be intermediates in the conversion of sitosterol, campesterol, and 24-methylenecholesterol to cholesterol in *M. sexta*.[16] Fucosterol 24,28-epoxide was first implicated as an intermediate in the conversion of sitosterol to cholesterol in *B. mori*.[17] Likewise, 24-methylenecholesterol 24,28-epoxide was isolated as an intermediate in the conversion of campesterol to cholesterol in *B. mori*.[12]

The pathways in Figure 2 have been shown to exist in the vast majority of phytophagous and omnivorous insect species that have been examined in detail.[2,18] For some time, it was accepted that all phytophagous (and omnivorous) insects utilized dietary phytosterols in a similar fashion, but as a wider variety of insects was examined, it became obvious that there are a number of exceptions.

III. UNUSUAL DEALKYLATION PATHWAYS

The first unusual aspect of phytosterol dealkylation was encountered in sterol metabolism studies with the confused flour beetle, *Tribolium confusum* (Figure 3). We discovered that, regardless of whether the dietary sterol was radiolabeled cholesterol, desmosterol, sitosterol, campesterol, 7-dehydrocholesterol acetate, or stigmasterol, 7-dehydrocholesterol constituted over half of the sterols isolated from the insect.[19] Cholesterol was the next most abundant sterol. More recent studies revealed that a hymenopteran, the phytophagous sawfly, *Xiphydria maculata*, had unusually high levels of 7-dehydrocholesterol (74.9% of total).[20]

Another quite unique aspect of sterol metabolism was discovered in the Mexican bean beetle, *Epilachna varivestis*, when it was found that saturated sterols (stanols) were the major products of phytosterol metabolism (Figure 4). Although this insect does dealkylate C_{28} and C_{29} phytosterols, cholestanol, rather than cholesterol, is the major sterol produced in all stages of the insect.[21] In addition, a significant amount (>10% of total) of lathosterol (Δ^{7}-cholestenol) is produced. Studies with radiolabeled dietary sterols indicated that the Δ^{5}-bond is saturated prior to C-24 dealkylation, and that even $[^{14}C]$cholesterol is reduced to $[^{14}C]$cholestanol and, to a lesser extent, $[^{14}C]$lathosterol. Most of the members of the family Coccinellidae are predacious, but the subfamily Epilachninae includes phytophagous species such as the Mexican bean beetle. In a related study with the insectivorous *Coccinella septempunctata*, cholesterol was the major sterol and the total sterols reflected those of the phytophagous insect species that *C. septempunctata* fed upon.[22] It appears that phytophagy arose secondarily in the phytophagous members of the Epilachninae and that they evolved from predacious ancestors. Thus, it seems that phytophagous coccinellids have developed unique biochemical modifications in order to adapt to different diet regimens.

IV. DIFFERENCES IN STEROL METABOLISM WITHIN ORDERS

A. HYMENOPTERA

The Virginia pine sawfly, *Neodiprion pratti*, a hymenopteran, was one of the first insects shown to be capable of phytosterol dealkylation using adequate biochemical studies.[23] Recently, several other sawfly species, including the previously mentioned *Xiphydria maculata*, were shown apparently to be capable of dealkylation based on comparison of sterols of the sawflies with those of their host plant sterols.[20] However, as extensive studies with the honey bee, *Apis mellifera*, have shown, not all phytophagous Hymenoptera are capable of dealkylating and converting phytosterols to cholesterol.[24] The honey bee was found to be capable of maintaining 24-methylenecholesterol as its major sterol in all stages of development regardless of the dietary sterols fed in the brood food.[25] It appears that the solitary bee, *Megachile rotundata*, also lacks the ability to dealkylate and utilizes dietary sterols similarly to the honey bee.[26]

Also, the honey bee differs from most phytophagous insects in that the C_{28} ecdysteroid makisterone A (Figure 1) is the major molting hormone in all postembryonic stages.[27 29] Use of radiolabeled precursors revealed that this insect produces makisterone A from campesterol.[30] Another solitary bee, the cactus bee, *Diadasia rinconis*, apparently utilizes dietary sterols similarly to the honey bee, but in this case, 20-hydroxyecdysone is the major ecdysteroid rather than makisterone A.[31]

Investigations with two species of leaf-cutting ants have provided very unusual information on sterol metabolism. In exacting studies with *Atta cephalotes isthmicola*, which cultivates fungi for food, no cholesterol could be found, even in the sterols of the brain or nervous tissue.[32] $\Delta^{5,7}$-24-Methylsterols comprised the major sterols of the workers and soldiers, reflecting the sterols of the fungal diet. The sterols of pupae of another leaf-cutting ant, *Acromyrmex octospinosus*, contained mostly $\Delta^{5,7}$-24-methylsterols,

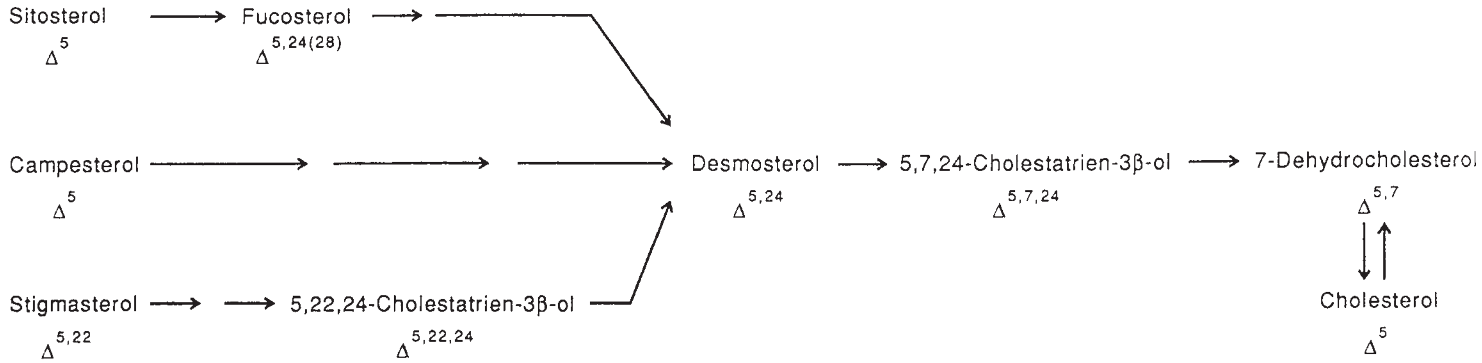


Figure 3 Pathways of conversion of major phytosterols to cholesterol in confused flour beetle, *Tribolium confusum*.

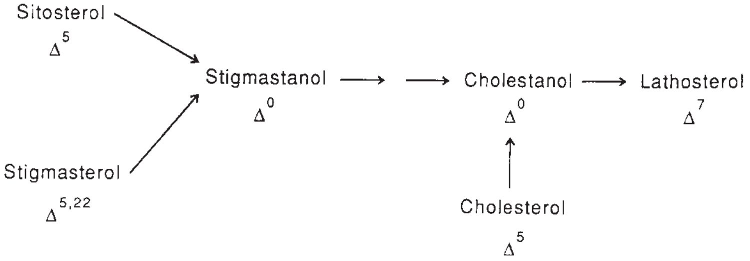


Figure 4 Phytosterol metabolism in Mexican bean beetle, *Epilachna varivestis*.

but a small amount of cholesterol was present in the sterols of this insect.[33] The major ecdysteroids of *A. octospinosus* were C_{28} ecdysteroids; lesser amounts of C_{27} ecdysteroids were also identified. It appears that both these species of leaf-cutting ant are similar to the honey bee in being unable to dealkylate C-24 alkyl phytosterols.[32,34]

B. COLEOPTERA

A number of years ago, the boll weevil, *Anthonomus grandis*, was found to be capable of producing cholesterol from dietary phytosterols via dealkylation,[35] and for some time this was assumed to be generally true for all phytophagous Coleoptera. A unique aspect of sterol metabolism in another coleopteran, *T. confusum*, where there appears to be an equilibrium between cholesterol and 7-dehydrocholesterol, was discussed earlier.[19] Also, a new sterol, 5,7,24-cholestatrien-3ß-ol, was found to be a normal intermediate in the conversion of radiolabeled sitosterol, campesterol, and desmosterol to cholesterol in this insect. A recent report showed that the closely related *Tribolium castaneum* utilizes sterols similarly to *T. confusum*, but the sterols of a more distant member of the same family, *Tenebrio molitor*, contained only about 15% 7-dehydrocholesterol.[36] Although trace amounts of 7-dehydrocholesterol can be found in the sterols of most insects, since it is an intermediate in ecdysone biosynthesis, there is no explanation for the high levels of 7-dehydrocholesterol in these tenebrionid beetles. Unusual aspects of sterol metabolism in the Mexican bean beetle also were previously mentioned;[21] this insect produces mainly Δ^0-sterols along with some Δ^7-cholesterol. In this respect, this coleopteran remains unique among insects in its ability to metabolize dietary phytosterols. The novel requirement for a dietary Δ^7-sterol by the beetle *X. ferrugineus* was also previously cited.[4]

Perhaps as interesting as any of these differences in sterol metabolism among Coleoptera is the fact that the khapra beetle, *Trogoderma granarium*, is completely unable to dealkylate 28-and 29-carbon phytosterols.[37] This was one of the first phytophagous insects found to be unable to make this conversion. This worldwide pest feeds on stored grain, utilizes the dietary sterols essentially unchanged, but is capable of selective uptake of trace (<0.5%) amounts of cholesterol in the dietary sterols. The khapra beetle is a member of the family Dermestidae, and most of the members of this family feed on animal material which contains adequate cholesterol. Even though this dermestid has adapted to a very different diet regimen than that available to most other members of this family, it lacks the ability to dealkylate and utilize dietary sterols in the same way as other stored product pests such as the tenebrionid flour beetles that produce large quantities of 7-dehydrocholesterol and cholesterol via dealkylation. Since the order Coleoptera includes more species than any other insect order, occupying a wide variety of ecological niches, one might expect to see a significant number of variations from the norm with respect to sterol metabolism and utilization.

C. DIPTERA

Members of the order Diptera, which includes flies and mosquitoes, have also been found to display considerable variability in the utilization of dietary sterols. Detailed biochemical studies with the housefly, *Musca domestica*, established that it was unable to convert C_{28} and C_{29} phytosterols to cholesterol.[38,39] For nearly 20 years, it was assumed that Diptera in general were unable to convert phytosterols to cholesterol. However, the yellow fever mosquito, *Aedes aegypti*, was the first dipteran shown to have the capability to dealkylate C-24 alkyl groups by means of definitive studies utilizing radiolabeled dietary sitosterol, campesterol, and desmosterol and feeding a 24-reductase inhibitor with sitosterol.[40] Subsequent studies with *Drosophila melanogaster*, a higher dipteran more closely related phylogenetically to the housefly, showed that it is clearly unable to dealkylate phytosterols.[41] Related research with several cactophilic *Drosophila* species was in agreement with our findings for *D. melanogaster*.[42,43] However, the requirement for a dietary Δ^7-sterol by *D. pachea*, mentioned earlier, was a significant discovery.[3] When *M. domestica* and *D. melanogaster* were examined for ecdysteroid biosynthesis capabilities, both species produced mainly C_{27} ecdysteroids from trace amounts of endogenous cholesterol obtained from selective uptake of dietary sterols, although both species also produced lesser amounts of the C_{28} ecdysteroid makisterone A.[44,45]

D. HEMIPTERA

Sterol utilization in a number of species of Hemiptera (the true bugs) has been investigated in depth, and all hemipterans so far studied are unable to dealkylate C_{28} and C_{29} phytosterols to produce cholesterol. Although for many years it was assumed that phytophagous insects in general could dealkylate, the very first phytophagous insect found to be incapable of this conversion was the milkweed bug, *Oncopeltus* *fasciatus*.[46] Also, the first isolation of makisterone A from insect material resulted from studies with *O. fasciatus*, when this C_{28} ecdysteroid was found to be the major molting hormone in the eggs of this insect.[47] Several other phytophagous Hemiptera were subsequently found to lack the capability to dealkylate dietary sterols and have adapted to producing makisterone A as their major ecdysteroid. These include *Nezara viridula, Dysdercus cingulatus*,[48,49] *Dysdercus fasciatus*,[50] and *Magalotomus quinquespinosus*.[51] Recently, a C_{29} ecdysteroid, makisterone C, was found to be the major ecdysteroid in the embryonic stage of *D. fasciatus*.[52] This was the first evidence that a C_{29} steroid (24-ethyl) could serve as a molting hormone. It is noteworthy that a predacious hemipteran, *Podisus maculiventris*, produces makisterone A as its major molting hormone although there is adequate cholesterol in the diet of this species and 90% of its total sterols is cholesterol.[48,49] Since this secondarily predacious species evolved from phytophagous ancestors, it appears that the mechanism for biosynthesis of makisterone A is strongly conserved. In contrast, several blood-feeding Hemiptera, including *Rhodnius prolixus, Arilus cristatus*, and *Cimex lectularius* produce only the C_{27} ecdysteroids ecdysone and 20-hydroxyecdysone,[49] which reflects the fact that cholesterol historically has been the predominant sterol in their diet.

E. HOMOPTERA

Over the course of a number of years, several reports suggested that sterol biosynthesis by certain aphid symbiotes provided essential sterols to their hosts.[2] Aphids belong to the order Homoptera, which used to be included in the Hemiptera. However, contrary to earlier reports, in a very thorough study, the aphid *Schizaphis graminum* was found to be able to convert [^{14}C]sitosterol to [^{14}C]cholesterol, and no sterol biosynthesis could be demonstrated in this aphid or its symbiotes.[53] It is of interest to note that phytosterol dealkylation occurs in this homopteran, but has not been demonstrated in any of the previously discussed phylogenetically more advanced Hemiptera that have been examined.

V. VARIABILITY BETWEEN SPECIES FROM A SIMILAR ENVIRONMENT

A recent study comparing sterol utilization by three species of stored-products insects indicates that various species occupying similar ecological niches can utilize dietary sterols very differently.[36] 7-Dehydrocholesterol comprised nearly 40% of the sterols isolated from the red flour beetle, *Tribolium castaneum*, a proportion fairly comparable to what was found in the case of the previously mentioned *T. confusum*.[19] Another flour beetle, the yellow mealworm, *Tenebrio molitor*, produced only about 17% 7-dehydrocholesterol but about 67% cholesterol. However, the sterols of the lepidopteran Indian meal moth, *Plodia interpunctella*, contained nearly 87% cholesterol, indicating that this species metabolizes dietary C_{28} and C_{29} phytosterols similarly to plant-feeding Lepidoptera. Thus, the similar environments of these stored-products insects do not seem to have induced similarity in sterol metabolism. Phylogeny appears to influence sterol metabolism more than environmental pressures. Previously, a similar relationship was mentioned comparing the khapra beetle, which is a stored-products pest that is unable to dealkylate, with other dermestids (hide beetles) that feed on animals or animal products.[37]

VI. CONCLUSIONS

From the foregoing discussion, it is clear that sterol metabolism in insects is a fascinating area of steroid biochemistry. The amount of variability in sterol metabolism discovered so far is quite striking when one considers the relatively small percentage of the nearly one million identified insect species that have been investigated with respect to sterol metabolism. However, insects do occupy practically every conceivable ecological niche, and, in the process of adapting to various environmental conditions and diets, they have apparently often altered their sterol metabolism capabilities. We know now that phytophagy does not necessarily indicate that an insect can convert C_{28} and C_{29} phytosterols to cholesterol by dealkylating the sterol side chain at the C-24 position. It is also difficult to predict the sterol metabolizing capabilities of insects based on phylogenetic relationships.[54] Very primitive insects such as the firebrat are able to dealkylate.[6] Also, the next most primitive group studied, the hemimetabolous Orthoptera (cockroaches, locusts) can convert phytosterols to cholesterol.[2] In certain orders, the more primitive species are able to dealkylate, whereas more advanced species are unable to make this conversion, e.g., in the Diptera and Hymenoptera. However, this relationship does not always hold true as we found among the Coleoptera, where members of the more primitive dermestid family cannot dealkylate, but members of the more advanced tenebrionids can.

The most prevalent molting hormones of insects, ecdysone and 20-hydroxyecdysone, both C_{27} ecdysteroids, are derived from cholesterol. However, makisterone A, the C_{28} ecdysteroid, is the major molting hormone of many insects that are unable to convert phytosterols to cholesterol. This is true even in certain insects that ordinarily have adequate cholesterol in their diets, such as *P. maculiventris* (Hemiptera) and in the higher Diptera, indicating a well-conserved biochemical mechanism.

The variability in sterol metabolism capabilities could be exploited in development of selective insect control technology. For example, certain inhibitors that disrupt sterol metabolism in those insects that can convert phytosterols to cholesterol (e.g., Lepidoptera) and thereby affect growth and development, have no effect on the beneficial honey bee, which cannot dealkylate.[55] Any suitable means of limiting availability of utilizable sterol in a pest insect could be exploited for new control technology.[56] Undoubtedly, as a greater variety of insects is examined with respect to sterol metabolism capabilities, even more unusual and exciting discoveries will be made.

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