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Sterol composition of three species of sawflies (Hymenoptera: Symphyta) and their dietary plant material

J. A. Svoboda, N. M. Schiff[a] and M. F. Feldlaufer

Insect Neurobiology and Hormone Laboratory and [a]Bee Research Laboratory, Agricultural Research Service, Beltsville, (Maryland 20705, USA)
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Abstract. Three species of sawflies, along with their particular host plants, were examined for neutral sterols. Cholesterol was the predominant sterol in the sawflies *Dolerus nitens* (56.6%) and *Aneugmenus flavipes* (73.0%), with lesser amounts of sitosterol, campesterol and desmosterol. *Xiphydria maculata*, however, contained unusually high levels (74.9%) of 7-dehydrocholesterol. Based on the sterol composition of their individual host plants, which contained primarily 28- and 29-carbon sterols, it is likely that all three sawfly species are capable of dealkylation of phytosterols.
Key words. Sawflies; sterols; 7-dehydrocholesterol; cholesterol; dealkylation.

Since insects are unable to synthesize sterols from smaller molecules, they require a dietary source of sterol for normal growth, development and reproduction. While many plant-feeding insects are capable of converting dietary C_{28} and C_{29} plant sterols to cholesterol, which serves as the precursor for their molting hormones, other insect species are not capable of this conversion[1]. The relationship between dietary sterols and tissue sterols in Hymenoptera is interesting and diverse. Wasps, hornets and other predacious or omnivorous hymenopterans contain from 45–81% cholesterol, presumably because cholesterol is a significant dietary sterol[2]. Other species, though, that are secondarily phytophagous, like honey bees, solitary bees, and phytophagous ants, contain little or no cholesterol, indicative of an inability to convert dietary plant sterols to cholesterol[3–8]. However, the only primarily phytophagous hymenopteran species examined to date, the Virginia pine sawfly, *Neodiprion pratti* Dyar, was shown to be capable of dealkylating and converting plant sterols to cholesterol, which is their predominant tissue sterol[9]. In this study we compare the tissue sterols of three additional species of sawflies with the sterols found in their particular host plant.

Materials and methods

Adult female *Dolerus nitens* Zaddach (13.32 g) were collected near Boyce, VA and preserved in methanol at 4 °C. Samples of Kentucky fescue (*Festuca arundinacea*), a known host plant in this region were also collected and stored in methanol. Larvae of *Xiphydria maculata* Say were collected in infested sugar maple (*Acer saccharum*) logs around Beltsville in February, and the adults (2.07 g) were reared out in the spring. Xiphydriids were preserved in methanol at 4 °C approximately 48 h after adult emergence, and samples of maple wood shavings were also stored in methanol. *Aneugmenus flavipes* (Norton) were collected by sweeping adults (1.71 g) from Bracken fern (*Pteridium aquilinum*) around Beltsville. Insect and plant samples were individually homogenized in chloroform: methanol (2:1) and filtered. The filtrates were partitioned against distilled water, and the aqueous-methanol phase was back-extracted twice with fresh chloroform. Crude lipids were recovered by evaporating the solvent from the combined chloroform phases. After saponification of the crude lipids, sterols were isolated and purified by column chromatography on acid-grade and neutral-grade alumina (Woelm, ICN Pharmaceuticals) in ether:hexane systems previously described[2]. Sterol fractions from all samples were qualitatively and quantitatively analyzed by a Varian 3700 gas chromatograph equipped with a J&W DB-1 fused silica capillary column (15 m × 0.25 m; 0.25 μm film) at 235 °C. Sterol identifications were made by comparing retention times relative to cholestane as an internal standard. All identifications were confirmed by GLC-MS using a Finnigan Model 4510 under conditions previously described[5]. When necessary, certain sterols were fractionated by HPLC (see below).

For ecdysteroid analysis, the aqueous-methanol phase from the above partition was dried, and the residue partitioned between countersaturated butanol and water. The dried butanolic residue was fractionated on a silica SEP-PAK® with 5 ml portions of varying concentrations of ethanol/chloroform[5]. The appropriate fractions were then eluted from a C_{18} SEP-PAK® as previously described[10].

Table. Relative percentages of major neutral sterols of sawflies and their host plants as determined by GLC-Mass spectrometry.

	D. nitens	Fescue	*A. flavipes*	Bracken fern	*X. maculata*	Maple wood
C_{27} Sterols						
Cholesterol	56.6	1.1	73.0	2.0	5.7	1.2
Desmosterol	13.6	-	6.0	-	-	-
Lathosterol	2.4	-	-	-	0.4	-
7-Dehydrocholesterol*	-	-	-	-	74.9	-
Cholestanol	-	-	-	-	7.9	-
C_{28} and C_{29} Sterols						
Campesterol	7.6	21.3	3.6	9.0	-	1.9
Campestanol	-	-	-	-	-	7.9
Sitosterol	17.6	62.0	16.9	68.7	-	26.8
Isofucosterol	-	4.4	-	16.6	-	-
Stigmasterol	-	3.5	-	-	-	32.9
Ergosterol	-	-	-	-	2.3	-
$\Delta^{7,22}$-Ergostadienol	-	-	-	-	-	1.5
Stigmastanol	-	-	-	-	-	12.9
Δ^{22}-Stigmastenol	-	-	-	-	-	7.8
Δ^{7}-Stigmastenol	-	-	-	-	-	5.8
$\Delta^{7,22}$-Stigmastadienol	-	-	-	-	-	1.1
Other	2.2	7.7	0.5	3.7	8.8	0.2

*The $\Delta^{5,7}$-diene system was verified by HPLC in conjunction with UV Spectrophotometry.

When necessary, sterols were fractionated by reversed-phase HPLC on a YMC C_{18} column (250 mm × 4.6 mm; 5 µm particle size) with 100% methanol. The eluate was monitored by UV spectrophotometry (190–400 nm) using a Waters 990 Photodiode array detector. For ecdysteroid analyses, reversed-phase HPLC was performed on an IBM octyl column (150 mm × 4.6 mm; 5 µm particle size) eluted with 35% aqueous methanol at one ml/min. After analysis of one-ml fractions by RIA, immunoreactive areas were fractionated on a Waters NOVA-PAK column (150 mm × 4.6 mm; 5 µm particle size) eluted with methylene chloride:2-propanol:water (125:25:2) at one ml/min, and one-ml fractions were analyzed by RIA. Column temperature for all separations was held at 33 °C. The 'H-22' antiserum used in this study was a gift of L. I. Gilbert (Chapel Hill, North Carolina) and was raised in rabbits injected with a hemisuccinate derivative of ecdysone, conjugated at C-22[11]. [23, 24-^{3}H] ecdysone (spec. act. 83 Ci/mmol; Dupont) was used as the labeled ligand. All assays were performed in triplicate as previously described[12].

Results and discussion

The major sterols isolated from the three species of sawflies and their particular host plants are given in the table. Cholesterol was the predominant sterol in both *D. nitens* (56.6%), which fed on grasses, and *A. flavipes* (73.0%), which fed on Bracken fern. Desmosterol was also detected in these two species, in lesser amounts (13.6% and 6.0%, respectively). While cholesterol accounted for only 5.7% of the sterols in *X. maculata*, other C_{27} sterols like 7-dehydrocholesterol (74.9%) and cholestanol (7.9%), accounted for the majority of the sterols in this sawfly species. All host plants contained low levels (2.0% or less) of cholesterol, having instead an abundance of 28- and 29-carbon sterols including sitosterol, stigmasterol, isofucosterol and campesterol. Maple wood, in particular, had a high percentage of stigmasterol (32.9%) along with related 24-ethylsterols. Given the unusually high percentage of 7-dehydrocholesterol and very low levels of cholesterol in *X. maculata*, we attempted to determine the chemical nature of the ecdysteroids in this species. Both reversed-phase and silica separations yielded immunoreactive fractions that corresponded to the elution volumes of the C_{27} ecdysteroids 20-hydroxyecdysone and ecdysone. There was insufficient material for confirmation by mass spectrometry. There was no immunological evidence of any 24-alkyl ecdysteroids, such as makisterone A. Like the Virginia pine sawfly, *N. pratti*, all three species of sawflies examined in this study contained primarily C_{27} sterols. This is indicative of the ability to dealkylate, given the small amount of cholesterol in their respective diets. The ability of sawflies to convert dietary plant sterols to cholesterol sets them apart from all other phytophagous hymenopterans like ants, honey bees, and solitary bees, that have been examined to date. The sterol composition of *X. maculata* differed from both *D. nitens* and *A. flavipes* in that the predominant sterol in *X. maculata* was 7-dehydrocholesterol, which accounted for almost 75% of the total sterol in this species. While 7-dehydrocholesterol was detected in the Virginia pine sawfly by Schaefer et al.[9], it accounted for only 6% of the total sterol. Only in certain beetles of the family Tenebrionidae have the levels of 7-dehydrocholesterol

been shown to approach those found in *X. maculata*. Based upon UV analysis, Beck and Kapadia[13] reported that 7-dehydrocholesterol comprised over half of the sterol found in the confused flour beetle, *Tribolium confusum*. Svoboda et al.[14] used radiolabeled sterols in combination with a defined diet to demonstrate that *T. confusum* was capable of dealkylation, and that the predominant sterols consisted of nearly equal amounts of cholesterol and 7-dehydrocholesterol. More recently, in a study involving several species of stored products insects, Svoboda and Lusby[15] found that the red flour beetle, *T. castaneum*, converted sitosterol to about equal amounts of cholesterol (43.7%) and 7-dehydrocholesterol (39.8%), while another flour beetle, *Tenebrio molitor*, contained 16.8% 7-dehydrocholesterol.

While 7-dehydrocholesterol has been implicated in the early stages of ecdysone biosynthesis[16–19], the purpose of such high levels of this $\Delta^{5,7}$-sterol in *X. maculata* is unknown. A species of ambrosia beetle, *Xyleborus ferrugineus*, requires a Δ^{7}-sterol for pupation, and lives in close association with fungi that have high levels of the $\Delta^{5,7,22}$-sterol, ergosterol[20–22]. Whether Xiphydriid sawflies also have a requirement for Δ^{7}-sterols is not known. There is some evidence, however, that a related species, *X. prolongata*, lives in association with certain fungi[23,24]. The possibility then exists that *X. maculata* may also obtain their dietary sterols from associated fungi and not directly from the wood of its host tree.

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