Metabolic Fate of Injected and Ingested Ecdysteroids in the Larvae of the Silkworm, *Bombyx mori*, and the Tobacco Budworm, *Heliothis virescens*

by

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Abstract

Ecdysteroids, as endogenous molting hormones, are active at low concentrations regulating insect metamorphosis. However, bioactivity of exogenous ecdysteroids introduced to some insects by feeding was low. Some factors, especially the absorption, distribution and metabolic fate of injected and ingested ecdysone, were studied in the larvae of the silkworm, *Bombyx mori*, and the tobacco budworm, *Heliothis virescens*, in order to understand the mechanisms involved in reducing the bioactivities of ingested ecdysteroids in these insects.

Some ecdysteroids used for the metabolic studies were isolated from the root bark of *Vitex strickeri* (Verbenaceae). Among them, 11α-hydroxyecdysone and ajugasterone C-20,22-monoacetonide were new natural products.

Metabolic pathways of ingested ecdysteroids were found to be different from those of injected ecdysteroids in these two insects. Ecdysone hydroxylation followed by oxidation to 20-hydroxyecdysonoic acid was the major pathway of injected ecdysone metabolism for both species. An additional pathway, epimerization followed by sulfate conjugate formation of 3-epiecdysone, was found in *B. mori* larvae fed with ecdysone.

Ecdysteroid-22-acylesters were the major metabolites of the ingested ecdysteroids in *H. virescens* larvae.

Selected characteristics of ecdysteroid-22-*O*-acyltransferase in *H. virescens* were studied. The apparent K_m and V_{max} values were $1.2 \pm 0.4 \times 10^{-2}$ mM and $8.5 \pm 0.9 \times 10^{-5}$ µmol of ecdysone-22-oleate formed per min per mg of protein, respectively. Fatty acyl-CoA is required for this enzyme activity. The apparent optimum pH and temperature range were 6.9 and 10 - 20 °C, respectively. The enzyme is sensitive to heat at 60 °C and inhibited by divalent cations but not affected by K^+ and EDTA.

Most ecdysteroid-22-*O*-acyltransferase activity was found in the membrane fractions. Analyses localized the major activity of this enzyme on gut epithelial cell membrane. It was active only during feeding stages. The activity decreased as the larvae became committed to pupation, and it was not enhanced by feeding ecdysteroids to the fifth instar larvae. Preliminary results showed that the esterification was very specific to 22-OH in ecdysteroids.

In vitro assays showed that the permeability of gut epithelial cell membrane to ecdysteroids was very low. No evidence for carrier mediated ecdysteroid absorption in the gut tissues was observed in these two insects.

These studies suggest that the low permeability of gut epithelial cell membrane to ecdysteroids, additional ecdysteroid metabolic pathway in the gut tissue, and high rate of ecdysteroid metabolite excretion were the major factors that reduced the bioactivity of the ingested ecdysteroids in these insects. High resistance of *H. virescens* to ingested ecdysteroids is due to the high rate of ecdysteroid-22-*O*-acylester formation and excretion.

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