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ANALYSIS OF ECDYSTERONE IN *BRADINOPYGA GEMINATA* (RAMBUR) LARVAE BY REVERSE PHASE – HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, RP-HPLC (ANISOPTERA: LIBELLULIDAE)

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Ecdysterone or 20-hydroxyecdysone (20E) is a polyhydroxylated ecdysone that plays a major role in insect growth and metamorphosis. The 20E level was analyzed in 2 larval instars of the dragonfly using RP-HPLC. The presence of 20E was demonstrated for the first time in dragonflies, with the higher levels occurring in the older larval instar (larger larvae), while in the younger instar (smaller larvae) low or negligible levels were recorded. This has implications for extending the use of odon. larvae as biocontrol agents in aquatic ecosystems.

INTRODUCTION

The moulting and metamorphosis of insects are controlled by a system of hormones (MANI, 1994). These moulting hormones (ecdysteroids) regulate moulting in immature stages of insects (KOOLMAN, 1989). When insects reach the appropriate size known as the critical weight, prothoracicotropic hormone (PTTH) is released from the brain and stimulates the secretion of ecdysone (E) from the prothoracic gland, which triggers moulting (NIJHOUT, 1994). Ecdysteroids elicit moulting and metamorphosis as a result of their stage specific effects on target tissues (RIDDIFORD, 1980). Ecdysterone or 20-hydroxyecdysone (20E) is polyhydroxylated ecdysone that plays a major role not only in moulting but also in insect growth and metamorphosis. Apart from insects, the presence of ecdysteroids has been detected and isolated in some trematodes (FOSTER et al., 1992) and nematodes (MENDIS et al., 1983). The hormone's role as a human

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lymphocyte and neutrophil modulator has also been evaluated (TRENIN & VOLODIN, 1999). Hence, ecdysteroids are an important group of steroid hormones. With a better understanding of their biology, the potential of odonates as biocontrol agents and bioindicators both as larvae and adults can be utilized more efficiently in the control of insect vectors such as mosquitoes, blackflies and gnats.

MATERIAL AND METHODS

Bradinopyga geminata is a common dragonfly in Tambaram near Chennai, India where the study was conducted. Live larvae were collected from the lake of Madras Christian College, Tambaram and were segregated into two groups viz., Stage I and Stage II, based on their body length of 1.1 cm and 1.4 cm, respectively.

Binary gradient HPLC (Shimadzu 10 Avp, Japan) with Shim pack CLC-ODS Column (C_{18} Column) and Photodiode array detector (190-800nm) were used to analyze the levels of 20E in the two larval stages. The standard Ecdysterone (20-hydroxyecdysone, $C_{27}H_{44}O_7$) was purchased from Sigma-Aldrich Chemicals Private Ltd.

For the preparation of steroid extract, the larvae were weighed (0.5g) after removing their legs and wing buds and homogenized using Insect Ringer's solution. Ethylacetate and Cyclohexane (1:1) were added and stirred well, followed by centrifugation for 10 minutes at 8000 rpm. The supernatant was transferred to clean Eppendox vials and evaporated to obtain the steroids. The extracts of free steroids were injected into the column, and an elution rate of 1ml/min with acetonitrile in water (40:60) in the HPLC.

The hormone was detected by the HPLC-UV detector set at 254nm (TAKASHI et al., 1981, ISAAC & REES, 1984).

RESULTS AND DISCUSSION

The Standard 20E showed maximum UV absorbance at 246 nm with a retention time (RT) of 5.443 minutes (Fig. 1a). Of the two stages analyzed based on their body size, the larger of the two recorded a peak closely correlating with Standard 20E, confirming the presence of 20E with RT of 5.763 minutes (Fig. 1c) and maximum absorbance at 246nm. None of the peaks of the smaller stage coincided with the Standard, showing that 20E is negligible in this stage (Fig. 1b). A peak (RT: 4.7 min.) almost correlating with that of Standard 11-ketotestosterone (RT: 4.897 min.) was recorded in the younger larvae but is not confirmed (Fig. 1b). The other peaks recorded with RT viz. 1.857, 2.277 and 3.817 minutes respectively, are presumed unknown and present further opportunities for study on these steroids. The quantitative measurement of 20E in the Stage II larvae was calculated using the peak area of absorbance of chromatograms (Fig. 2) and it showed as 15.6 µg/g of tissue.

20E is a steroid found in insects and some arthropods that plays a major role in the growth and metamorphosis of those organisms. TAKASHI et al. (1981) detected the presence of ecdysteroids in the eggs of *Bombyx mori* at 254 nm. The results of the present study on *B. geminata* larvae support the occurrence of 20E

at 254nm. WARREN et al. (2006) used RP-HPLC and differential RIA analysis to determine whole body titers of 20E during the last larval instar of *Drosophila melanogaster*. GELMAN et al. (2005) subjected the whole body extracts of mature 4th instar and newly formed pharate adults of *Bemisia tabaci* (biotype B) and *Trialeurodes vaporarium* to RP-HPLC, confirming the identity of E and 20E as the whitefly moulting hormones by Normal Phase-HPLC. Here, RP-HPLC

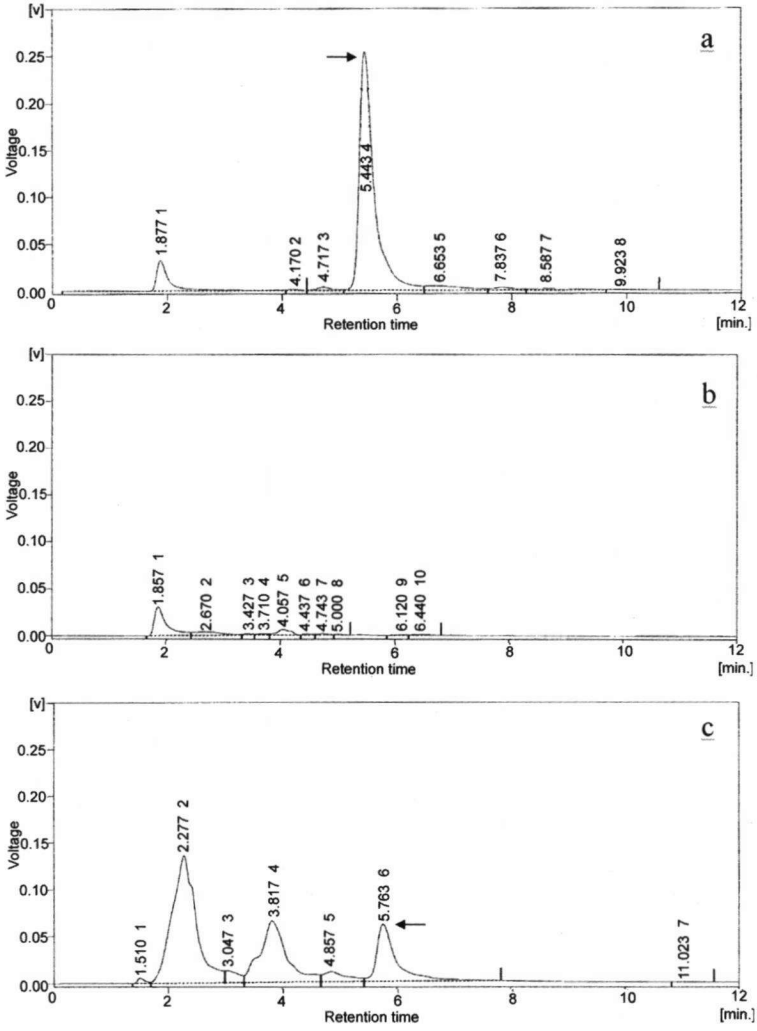

Fig. 1. RP-HPLC separation of steroids from the larvae of B. geminata: (a) Standard Ecdysone at RT 5.4 min; – (b) stage I larvae showing absence of 20E; – (c) stage II larvae showing presence of 20E at RT 5.7 min.

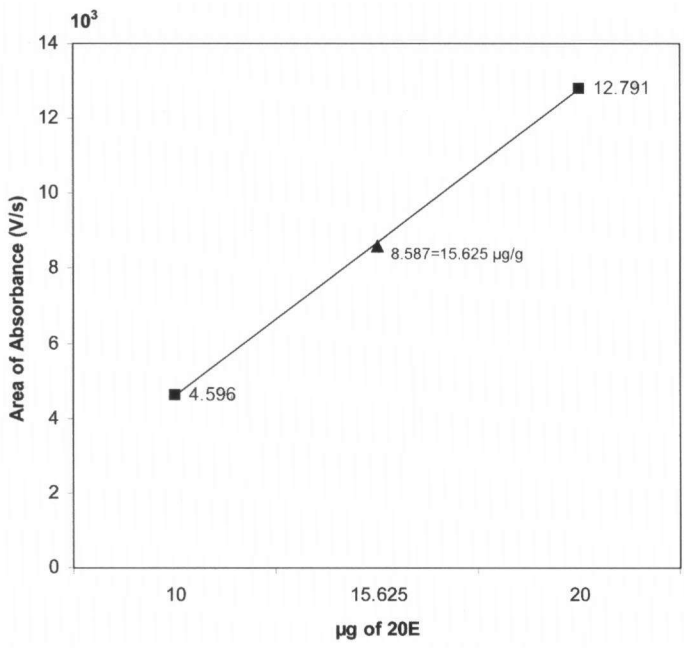

Fig. 2. Graph showing the 20E quantitative measurements of the standards (10µg and 20µg ■) and the stage II larvae (▲)

analysis was made on the whole body titers of two stages of *B. geminata* larvae to determine the levels of 20E. CHEN et al. (1996) detected and analyzed using HPLC-RIA, the in vitro secretion of ecdysteroids from the prothoracic glands of the last instar larvae of *Spodoptera littoralis*. In general, larval moults display a single massive peak prior to moulting, and in hemimetabolous insects the larva-adult moult likewise displays a single peak (GERSTENLAUER & HOFFMAN, 1995). From the results obtained, it is concluded that the older of the larvae studied were nearing their next larva-larva moult and that the younger were not since they recorded a negligible presence of 20E.

In understanding the factors controlling the 20E secretion, the duration of the odonate larval stages may be increased in order to better exploit the role of dragonfly larvae as biocontrol agents in the aquatic ecosystems.

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