Isolation and Purification of the hormone Ecdysone

Author: Dr.Mrs.Tasneem Jahan,Lecturer Department of Zoology,St.Ann's college for Women,Mehdipatnam,Hyderabad.

ABSTRACT

*A hormone regulating growth and moulting of decapod crustacean is the ecdysone ,secreted by Y-organs. The crustacean moult controlling system consists of eyestalk neurosecretory cells (X-organs) with enlarged axonal endings in the neurohaemal sinus gland (SG) and paired peripheral steroidogenic Y-organs , which produced ecdysone .The characterization of ecdysone was first carried out in insects . The insect moulting gland homologue in the crustaceans Y-organ resulted in the characterization of ecdysone as the primary secretory product .The present study was carried out in crustacean,**Barytelphusa guerini** in which secreted ecdysone is rapidly hydroxylated to 20 – hydroxyecdysone,crustaceans produces 20- E as a predominant ecdysteroid which is extracted and subsequently characterized by Hplc .*

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KeyWords: ***Barytelphusa guerini ,Isolation,Hplc,Ecdysone,***

INTRODUCTION

Steroids are widespread molecules derived from cholesterol mainly controlling moulting , reproduction , development and homeostasis .The steroid arthropod moulting hormone was first isolated from insects and it was called ecdysone (Butenandt and Karlson , 1954). After a decade the structure of the more active and most predominant form of the hormone was subsequently determined to be 20-hydroxyecdysone (Huber and Hope,1965) .

The structure of moulting hormone was isolated and determined in the crustacean , *Jasus lalender*.The crustacean hormone was originally called crustecdysone or ecdysterone. The chemical structure was similar to insect moulting hormone (20-hydroxyecdysone) . 20 – hydroxylase transforms ecdysone (E) into physiologically more active ecdysteroid 20-ecdysone (20-E) (Horn *et al*.,1966).

The Y-organs are paired glands in crustaceans that secrete a class of steroid hormones i.e, ecdysteroids.The glandular secretions are identified as ecdysone (E),25-deoxyecdysone (25 dE),3-dehydroecdysone (3-DE) and 3-dehydro- 25-deoxyecdysone (3D25dE) (Horn *et al*.,1966) .

The moulting gland , or Y-organ , is the primary site for ecdysteroid synthesis in decapods crustaceans . The ecdysteroids are released into the haemolymph and subsequently hydroxylated by target tissue into 20-hydroxyecdysone (20 E), (Chang O Connor, 1978).

Ecdysteroid biosynthesis is divided into two stages :-

(1) In the first reaction cholesterol is converted to 7 –dehydrocholesterol by 7,8-dehydrogenase.

(2) The stage two reactions generate four major products , depending on species , ecdysone , 3-dehydroecdysone (3 DE) , 25 – deoxyecdysone and 3-dehydro 25-deoxyecdysone (3D25dE) . Peripheral tissues convert these compounds to the active hormone 20 – hydroxyecdysone

(20 E) and 25- deoxy-20 hydroxy - ecdysone (25d 20E). The concentrations of ecdysteroids vary over the moult cycle and are determine by the combined effects , biosynthesis , metabolism and excretion (Donald , 2011).

Ecdysteroid hormone also helps in the control of embryogenesis .The synthesized ecdysteroids from the Y-organs transported through the haemolymph into the ovaries .The ovaries accumulate significant amounts of haemolymph ecdysteroid which are then passed into eggs , where they are involved in the control of embryogenesis (Subramoniam ,2000) .
Disruption of the ecdysteroid signaling pathways in crustaceans has been associated with aberrations in moulting , growth , metamorphosis , reproductive maturation , sex determination , and sex differentiation (Le Blanc, 2007) .
In the present investigation the ecdysteroids from the Y-organ were isolated and purified .

MATERIAL AND METHOD

Isolation and purification

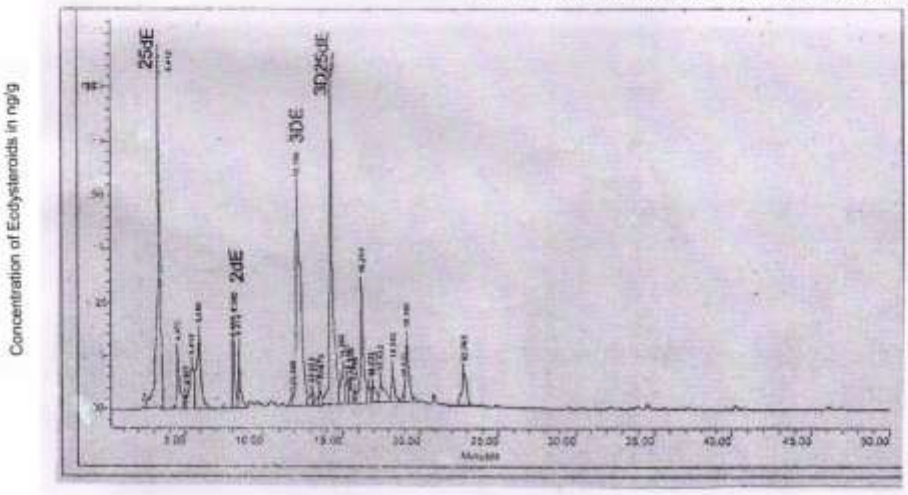
The Y-organs were excised out and homogenized in 90 % methanol (5mg/ml). About 25µg /ml samples were passed through Sep Pak C-18 chromatographic cartridges (Waters ; Milford, MA) to isolate ecdysteroids (Watson and Spaziani, 1982).

The Sep Pak eluent volume was reduced to dryness with rotatory evaporation under vaccum and dissolved in 35% methanol for Hplc analysis .Ecdysone was isolated with Nova- pak (Waters) C-18 reverse phase Hplc column using 35-70% methonal gradient (Rudolph and Spaziani , 1992) and measured by integration of peaks.

Standard - The Hplc chromatogram of the Y-organ of *Carcinus maenas* is taken as a standard reference .The standard profile shows four major products i.e, 25dE,2De,3DE ,3D25de and other polar metabolites (Donald ,2011) .

FIG-1

X Axis - Retention in Minutes
Y Axis - Concentration of Ecdysteroids in ng/g



Rp – HPLC profile of the standard Y-organ ecdysteroids of the crustacean *Carcinus maenas* showing 25dE (with retention time of 2-4min) , 2dE (10 - 11 min) , 3DE (12- 13 min), 3D25dE and other polar metabolites .

Column - C_{18} ODS , Solvent Acetonitrile , Flow rate 1ml .min^{-1}

Source – Donald (2011)

Result:

LEGEND FOR FIGURE- 2

Single step purification of the crustacean ecdysone from the Y-organ of the crab *Barytelphusa guerini* on 1st day of the moult cycle.

Column : C_{18} ODS column .

Solvent A: 30% Methanol.

Solvent B: 60% aqueous Acetonitrile.

Column temperature : 20^0 C.

Flow rate : 1ml/min.

Elution profile of the crude extract : 25µg/ml.

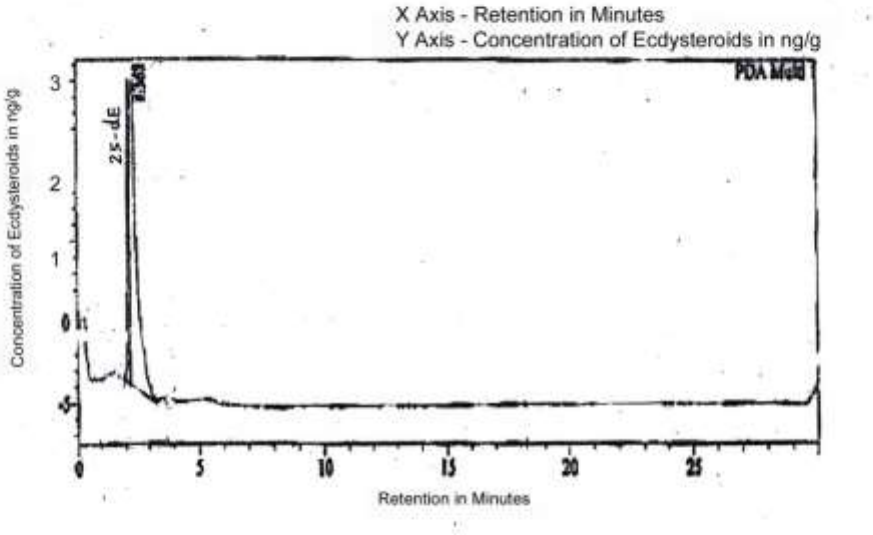
A gradient elution from 30% - 80% B concentration was run for 1hour at room temperature .

The absorbance of the elute was monitored at 214nm.

One major peaks of 25dE were measured between a rentention time of 2-3 minutes and compared with the standard reference .

The amount of ecdysone (25dE) isolated from the major peak is 2.369 ng/g .

FIG-2


X Axis - Retention in Minutes
Y Axis - Concentration of Ecdysteroids in ng/g
Concentration of Ecdysteroids in ng/g
Retention in Minutes
PDA Multi
2.5 - dE
0
5
10
15
20
25

Discussion

The Y-organ is the primary site for ecdysteroid synthesis in decapod crustaceans. Biosynthesis of ecdysteroids is divided into two stages . The first stage involves the conversion of cholesterol to diketol and the second stage reactions generate four major products depending on species i.e, ecdysone, 3dehydroecdysone (3 -DE), 25 – deoxyecdysone (25 dE) and 3dehydro- 25 deoxyecdysone (3D25d E).

In *Cancer antennarius* (Spaziani *et al* ., 1989) and *Penaeus vannamei* the major ecdysteroid secreted by the Y-organ is 3-dehydroecdysone. In cray fish , *Orconectes limosus* , the major secretory product of the Y-organ are ecdysone and 3-dehydroecdysone (Bocking *et al* ., 1993) .

The ecdysteroid levels during the moult cycle have been determined in several species of crustaceans,like *Carcinus maenas* (Adelung ,1967,1969), *Pachygraspus grassipes* (Chang and O'Connor , 1976),*Orconectus limosus* (Spindler *et al* .,1980), , *Callinectus sapidus* (Soumoff and Skinner ,1983) *Orchestia cavimana* (Graf and Delbecque,1987) .

In larval lobsters,manipulation of the appearance of the ecdysteroids peaks can be achieved either through eyestalk ablation (by the removal of MIH). Both juvenile and adult lobsters display a characteristic pattern of low concentrations of ecdysteroids during post moult and inter moult starting at early premoult it declines prior to ecdysis .

The larval crustaceans also shed their confining exoskeleton as a prerequisite to moulting , growth and development . The ecdysteroids levels rise and fall during each larval statge (Chang and Bruce, 1981).

Ecdysteroids may serve as morphogens or promote protective membranes during embryonic development and functions as moulting hormones from larval to adult life.In adults they may function as gonadotropins (Subramoniam,2000).

The products are released from the Y-organ into the haemolymph. Peripheral tissues convert these compounds to the active hormones 20- hydroxyecdysone (20 E) and pona- sterone A (25 deoxy-20hydroxyecdysone or 25 d20E).The concentrations of ecdysteroids vary during the moult cycle (Donald,2011).

In the present investigation the major ecdysteroid secreted by the Y-organ was found to be 25-deoxyecdysone (25 dE) .

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