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Identification and Predictive Bioconversion of Cholesterol to Estran and Androst Hormones from Scalloped Spiny Lobster (*Panulirus homarus*) Gonads

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Abstract: Steroidal hormones process from gonadal maturation of scalloped spiny lobster (*Panulirus homarus*) not established due to undeveloped practice of hatchery technology. Two steroid hormones *i.e.* estran and androst were elucidated previously and involved on gonadal maturation of scalloped spiny lobster. This study aimed to identified and evaluate role of cholesterol as raw material of estran and androst hormones. Eighteen individuals of immature and mature female scalloped spiny lobster gonads were used and analysed with pyrolysis gas chromatography mass spectrometry (py-GCMS). Results found that cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α ($C_{28}H_{46}O$) with molecular weight 368 assumed as raw materials for estran and androst hormones. Scalloped spiny lobster may process the enzymatic systems that able to catalysed the bioconversion of cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α to estran and androst hormones.

Keywords: bioconversion, cholesterol, pyrolysis GC-MS, spiny lobster, steroid hormone

I. INTRODUCTION

Advancing spiny lobster (*Panulirus*) culture not only limited to grow-out system from puerulus to adult spiny lobster but also need support from hatchery that producing seed [1]. Hatchery technology of scalloped spiny lobster not developed massively due to limited practice information and literacy based from research, in particular for broodstock husbandry and spawning and role of hormones that involved [2] and environment supported behaviour [3].

Spiny lobster reproduction likes other crustaceans their controls ruled by mechanism regulated by many organs and hormones [4] [5]. In specific attention to role natural steroidal hormones of crustaceans, [6] it is vital for vitellogenesis activities such as estrogen and progesterone. Others, in captivity of spiny lobster feed with additional of several steroid hormones such as estradiol, testosterone, hydroprogesterone and progesterone may support female egg-berried broodstock, increase fertilised egg and shorter fertilisation period to produce larvae [7] and in short steroid hormones in both ways supported many benefits for spiny lobster reproduction similar founded in teleost [8].

Recently, two steroid hormones founded and have role in gonadal maturation of scalloped spiny lobster (*Panulirus homarus*) *i.e.* estran hormone and androst hormone [9]. Two steroid hormones were found not understandable their origin and role in reproduction process. This study aimed to identified and evaluate role of cholesterol as raw material of estran and androst hormones.

II. MATERIALS AND METHODS

Immature and matured gonads from 18 individuals of female scalloped spiny lobster were collected and analysed with a pyrolysis gas chromatography mass spectrometry. Approximately 1 µl spiny lobster mature gonad of scalloped spiny lobster was weighted with micropipette and pyrolyzed at 400°C. The products of pyrolysis were analysed by GCMS. Gas chromatography separations were carried out with a GCMS-QP2010 (Shimadzu, Tokyo) plus instruments equipped with a rt x 5 ms capillary column (length of 60 cm, diameter of 0.25 mm, and thickness of 0.25 µm). Chromatographic separation was achieved by the following temperature program: 50° for 5 minutes, then it was raised to 280°C for 50

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minutes with pressure 0f 101kPa. Helium was the carrier gas at 0.85 mL/minutes with total flow of 46.5 ml/minutes and flow program mode at a linear velocity of 23.7 cm/seconds, and purge flow of 3.0 ml/minutes. Split injection mode used was the ratio of 1:50. Mass spectra was set with ion source at 200ºC and interface at 280ºC with solvent cut time of 1.5 minutes.

Detector temperature used was 280ºC and cholesterol identification was based on comparison of mass spectra with WILEY7 library database. Compound with the highest similarity (>90%) were identified as secondary metabolites. Library database from WILEY7 was able to identify structural, molecular weight and concentration of cholesterol as a secondary metabolite of scalloped spiny lobster.

III. RESULTS AND DISCUSSION

Complete spiny lobster production need support from hatchery not only enlargement from fishery of puerulus then continuing grow to consumption size in cages or tank for several months [10]. Complicated phase of hatchery technology of scalloped spiny lobster in particular for gonadal maturation need specific attention due to little information about role of hormones and processing that involved. Cholesterol as raw materials of metabolism related to growth, gonadal development and reproduction of crustaceans [11].

Pyrolisis gas chromatography mass spectrometry and Wiley7 library database analysis found cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α within all samples of immature and mature scalloped spiny lobster gonads (Fig.1). Cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α always showed within estran hormone expression in female gonad of scalloped spiny lobster. Androst hormone also detected within female scalloped spiny lobster gonads. In contrast, cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α not available during male gonads of scalloped spiny lobster (data not shown) assumed this cholesterol too little to detect with pyrolysis GCMS even need to clarified with other analysis such as ELISA or gene expression [12].

Transformation of cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α to steroid hormones that involved in gonadal maturation of scalloped spiny lobster similar found in vertebrate of 5-hydroytrypatime [13] and can accomplished within specific pathways such as *in vivo* conversion [14] or bioconversion [15] due to limited metabolism capacity of spiny lobster. Role of several enzymes *i.e.* steroidogenic factor 1, StAR-related lipid transfer protein, estradiol receptor and progesterone-like protein were found in freshwater prawn may supported future research [16].

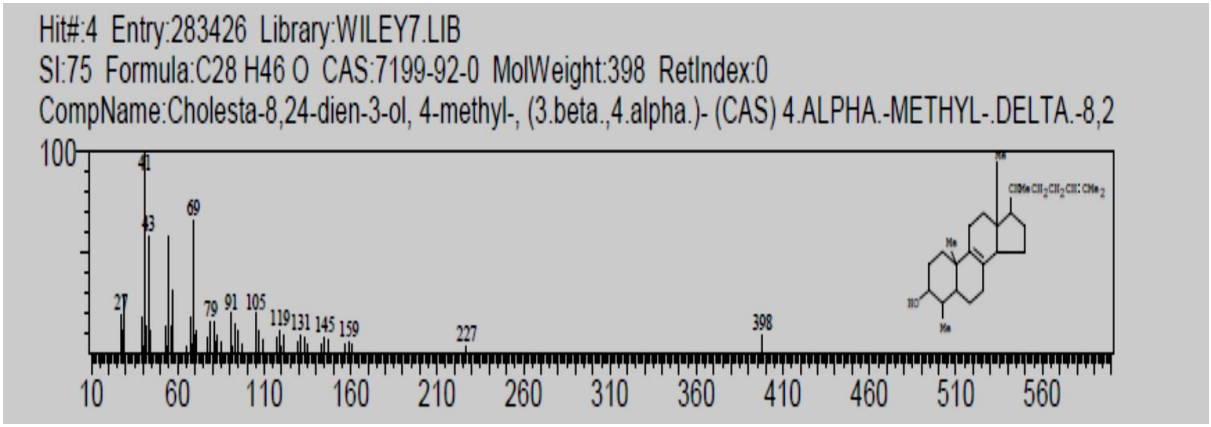

Fig. 1 Identification of cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α with GC-MS pyrolysis. Cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α as raw materials for estran and androst, two steroids hormones were involved in scalloped spiny lobster (*Panulirus homarus*) gonadal maturation process.

This cholesterol consisted with 28 atoms of Carbon, 46 atoms of hydrogen, one atom of oxygen and molecular weight of 398 (Fig.2). Not similar with other cholesterol molecule and molecular weight, cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α is large and may useful not only as raw materials of estran and androst hormones but for metabolism in particular for membrane development. Otherwise, cholesterol of cholesta-8,24-dien-3-ol, 4-methyl-3ß-4α may also supported others specific system such as reproduction that found similar in other crustaceans and lobster came from nutritional source that consumed [17].

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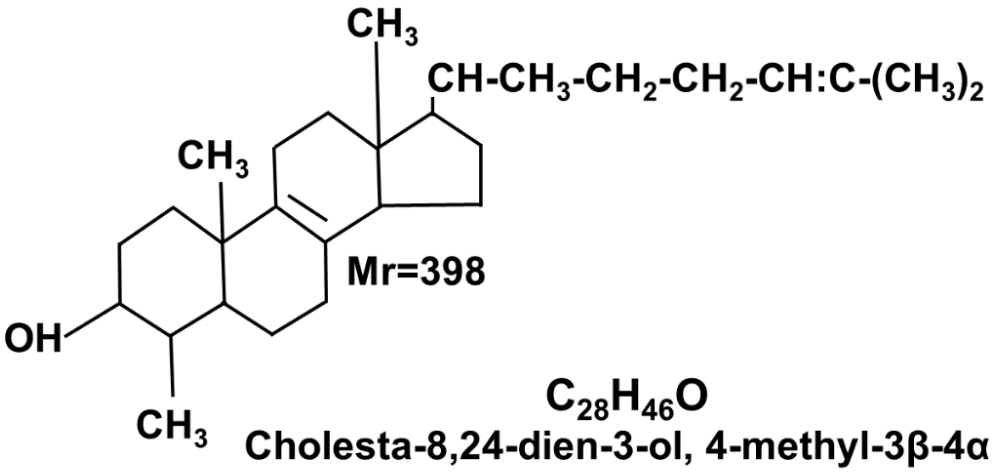

Fig. 2 Structure and molecular weight of cholesta-8,24-dien-3-ol, 4-methyl-3β-4α.

IV. CONCLUSION

Bioconversion of cholesta-8,24-dien-3-ol, 4-methyl-3β-4α to estran and androst hormones that involved on gonadal maturation of scalloped spiny lobster.

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