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Sex Steroid-like Substances in the Ovaries, Hepatopancreases, and Body Fluid of Female *Mictyris brevidactylus*

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Jin-Taur Shih (1997) Sex steroid-like substances in the ovaries, hepatopancreases, and body fluid of female *Mictyris brevidactylus*. *Zoological Studies* **36**(2): 136-145. This study was designed to determine if certain tissues of the soldier crab *Mictyris brevidactylus* contain sex steroid-like hormones. Ethanol extracts were prepared from ovaries, hepatopancreases, and body fluid of female crabs. A progesterone-like substance was detected by HPLC and RIA in the steroid residues of all 3 tissues. High levels of the progesterone-like substance were found in the prereproductive season (September-December) and low levels in the reproductive (egg-carrying, January-April) and nonreproductive seasons (May-August). An estradiol-like substance was detected by RIA only in ovaries, hepatopancreases, and body fluid of the prereproductive season. Ovaries had high levels of progesterone-like and estradiol-like substances in November, or December, while these 2 hormones reached their peak phases in the hepatopancreases and body fluid at least 1 to 2 months before in the ovary. The possible role of sex steroids in this crab is discussed.

Key words: Crab, Progesterone-like substance, Estradiol-like substance, Reproductive cycle.

The existance of steroid hormone biosynthesis in crustaceans remains untested. However, biochemical studies have indicated that sex steroid-like hormones, such as estradiol, estriol, estrone, progesterone, and 17-alpha-hydroxyprogesterone are detectable in tissues of crab and shrimp (Lisk 1961, Kanazawa and Teshima 1971, Teshima and Kanazawa 1971, Rateau and Zerbib 1978, Sandor 1981, Couch and Hagino 1983, Skinner 1985, Couch et al. 1987, Fingerman 1987, Sasser and Singhas 1988 1992, Shih 1992 1993, Shih and Wang 1993). Physiological studies have shown that ecdysteroids enhance protein synthesis in the adipose tissue of amphipods (Zerbib 1976). The hemolymphic level of ecdysteroid increases during the reproductive season (Blanchet et at. 1976). Progesterone and 17-alpha-hydroxyprogesterone were found to increase the biosynthesis of yolk protein in the ovaries of isopodes and penaeid shrimp (Kulkarni et al. 1979, Quackenbush and Keeley 1987 1988). The maturation of the penaeid shrimp ovary is enhanced by progesterone and 17-alpha-progesterone (Sarojini et al. 1985 1986, Yano 1987, Tsukimura and Kamemoto 1988). Quackenbush (1992) found that progesterone stimulates ovarian protein synthesis by 2.5 fold in penaeid shrimp ovary fragments. Estradiol had less, while testosterone and ecdysteroid had no effect on protein synthesis. Recently, Quackenbush (1994) stated that steroid hormones produced in the lobster ovary may regulate yolk production.

Shih (1992 1993) and Shih and Wang (1993) reported that the hemolymphic progesterone-like substance levels of *Uca arcuata* and *Mictyris brevidactylus* reach the peak phase 2 to 3 months before their egg-carrying periods started. At the same time, the gonadosomatic index (GSI) also reaches its peak phase. It suggests, but does not yet prove, that this progesterone-like substance is an endogenous product of the crab. This hormone may also have an effect on crustacean reproduction, for example, ovarian maturation as suggested by Quackenbush (1989a,b 1992).

There is still a lack of either qualitative or

quantitative evidence showing the presence of sex steroids in tissues other than body fluid of *M. brevidactylus* (Shih 1993). The location of sex steroid biosynthesis in this crab is unknown. Therefore, it is important to know whether sex steroids exist in various tissues. This paper reports the detection of sex steroid-like substances by high-performance liquid chromatography (HPLC) and radioimmunoassay (RIA) in the steroid residues of *M. brevidactylus* ovaries, hepatopancreases, and body fluid. A progesterone-like substance was detected in these 3 tissues and at high levels in samples from the prereproductive season. An estradiol-like substance was only detected in samples from the prereproductive season.

MATERIALS AND METHODS

Chemicals

Organic solvents used were LC grade from Alps Chemical Company, Taiwan. Authentic steroids were from Sigma Co.,(St. Louis, MO, USA.)

Extraction of steroids

Mictyris brevidactylus females (carapace width > 0.60 cm) were collected monthly in the Tanshui mangrove swamp of northern Taiwan (Shih et al. 1991). Crabs collected on the same date were weighed together in groups of 40-50. After chilling in a refrigerator for 10-15 min, 10 crabs were gently pressed in a small juice maker and the body fluids were pooled and filtered through 4 layers of cheesecloth. This body fluid was centrifuged at 1 000 × g at 4 °C for 10 min. The supernatant was designated as body fluid which was believed to consist of hemolymph and probably other contamination as well. Other crabs (26-38 crabs) were dissected to remove ovaries and hepatopancreases. The pooled ovaries and hepatopancreases were weighed and placed in absolute ethanol.

The extraction of steroids from ovaries and hepatopancreases was carried out using a modified procedure described by Shih (1992) and Shih and Wang (1993). Ovaries or hepatopancreases were homogenized twice in cold absolute ethanol in a mortar. The pooled homogenate was filtered. The filtrate was extracted twice in 10 volumes of methanol and chloroform (1:2, v/v). The organic phase was air dried. This extract was dissolved in 70 ml of methanol and mixed with 30 ml of $CaCl_2$ (1.0 M). This mixture was refrigerated overnight. The precipitate was removed by filtering. The above procedure was repeated once. The steroids in the aqueous phase were extracted by dichloromethane. Into 40 ml of dichloromethane extract, 8 ml each of water, 0.1 N NaOH, and 0.1 N acetic acid were added and mixed in a separatory funnel. After removing the aqueous phase, the residue in the dichloromethane was evaporated to dryness. The resultant residue was designated as the steroid residue used for this study. Because the body fluid contained water, it was necessary to separate the aqueous phase by adding diethyl ether to the body fluid. The ether phase containing steroids was collected; after the ether was evaporated, the residue was dissolved in absolute ethanol and subjected to the same procedure as for the ovaries. In order to check the efficiency of the extraction procedure, for each batch of samples, 1 sample was added with tritium-labeled progesterone. The efficiencies found in this study were between 52.2% and 69.50%.

High-performance liquid chromatography

The HPLC system used for this study was composed of a Knauer HPLC pump (Type 364, Germany), a sample injector, a Spectral photometer, (N_f/No_o. 731 879), a Chromatocorder 11 (SIC, Japan) and a column of LiChroCART 250-4, RP-C18 (4.0 mm 1 244 mm, Merck, Germany). The elution procedure was carried out according to Huang et al. (1983). The elution was first performed by an isocratic elution with solvent A (water: methanol: acetonitrile: isopropanol, 55:32:6.5:7.5, v/v) for 15 min, followed by a linear gradient elution built up to 80% of solvent B (water: methanol: n-butanol, 40:40:20, v/v) within 35 min.

If not otherwise stated, UV-absorbing steroids were detected in all chromatograms at 254 nm; attenuation was set at 32 a.u./cm. A sample of 20 μl was injected for each run. Steroid residues were dissolved in 1.0 ml of methanol, filtered through a millipore membrane (0.45 μm), and prepared for HPLC. Authentic steroid standards were: progesterone (pregn-4-one-3,20-dione), estradiol (17-beta-estradiol), 17-alpha-hydroxyprogesterone (4-pregnen-17-alpha-ol-3,20-dione), and aldosterone (4-pregnen-18-alpha,11 beta,21-diol-3,20-dione). For identity examination, the eluates which had OD_{254} peaks including the estradiol-like or progesterone-like substance were collected. The eluates without OD peaks were also collected at 1 to 2-min intervals. All eluates were air dried in preparation for radioimmunoassays.

Preparation of samples for radioimmunoassay (RIA)

Steroid residues obtained from chemical extraction and HPLC elution were dissolved in 1.0 ml of 0.1 M phosphate buffer saline (PBS, pH 7.4) (Shih and Wang 1993) containing 0.1% gelatin (PBSG). This mixture was incubated at 50 °C in a waterbath for 1 h and used for RIA. For each batch of samples, 1 sample was measured by RIA at dilutions of 1/2, 1/4, and 1/8 to check for contamination. All samples were then tested for progesterone using an Amerlex-M progesterone RIA kit (Johnson and Johnson Clinical Diagnostics Ltd., Amersham, UK). The progesterone antibody was prepared from sheep. The working RIA range for progesterone were 0.03 to 40 ng/ml of PBSG. In this study, the observed concentration of progesterone-like substance was 0.03-40 ng/ml of PBSG. Samples were tested for 17-beta-estradiol using a Coat-A-Count Estradiol kit (TKE 21 Diagnostic Products Corporation, CA, USA). The working RIA range for estradiol was 2.0 to 1 000 pg/ml of PBSG. The observed concentrations of estradiol-like substance were 8.0 to 313 pg/ml of PBSG. Any assay with a concentration below the minimum limit was rejected.

RESULTS

Qualitative analysis of steroid-like substances of crab tissues

As shown in Fig. 1, retention times for the authentic steroids, aldosterone (A), 17-beta-estradiol (E_2), 17-alpha-hydroxyprogesterone (P), and progesterone (P_4, 100 ng each except E_2 at 500 ng) are 7.74, 23.60, 24.55, and 31.60 min, respectively. The ovary steroid residue from female *Mictyris brevidactylus* (October 1994) was analyzed by HPLC; results are shown in Fig. 2a. Some peaks with optical densities (OD) were seen during the isocratic elution, a peak (P') with a retention time of 31.87 min was seen in the gradient elution. In order to assure the identity of P' of Fig. 2a, a chromatogram was taken of the ovary steroid residue with 4 authentic steroid standards. As shown in Fig. 2b, P' of ovary residue contained progesterone (P_4) and had a retention time (31.2 min) which is similar to authetic progesterone. Therefore, P' of Fig. 2a was identified as a progesterone-like substance. Though there were some small OD peaks a few minutes after the gradient elution started, no distinguishable OD peak at the retention time of estradiol (23.0 to 24.0 min) was identified.

An HPLC chromatogram of steroid residues of *M. brevidactylus* hepatopancreas and body fluid (October 1994) is shown in Fig. 3. P' of Fig. 3a and 3b had retention times of 31.91 and 31.01 min, respectively. Similarly, P' of the hepatopancreas and body fluid residues was identified as a progesterone-like substance. Again, no distinguishable OD peak corresponding to estradiol was seen.

Quantitative analysis of steroid-like substances of crab tissues

After the authentic 17-beta-estradiol (500 ng) and progesterone (100 ng) passed through HPLC, the eluates of these 2 hormones were assayed

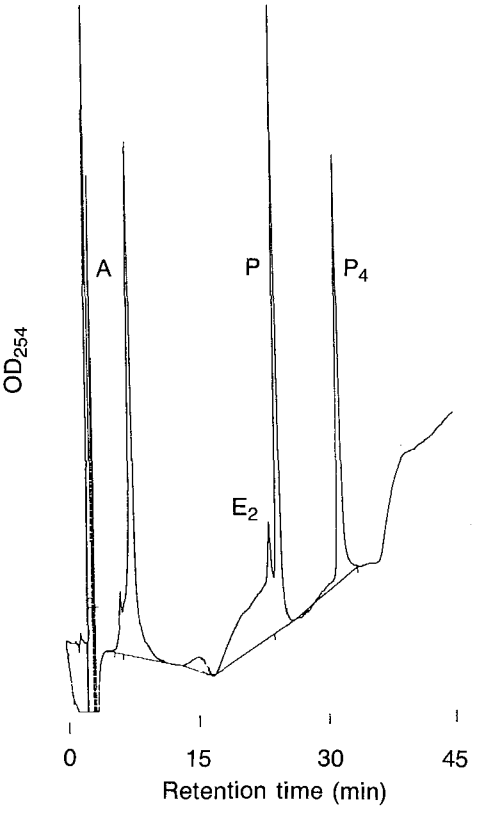

Fig. 1. HPLC chromatograms of authentic steroids: aldosterone (A), 17-beta-estradiol (E_2), 17-alpha-hydroxyprogesterone (P), and progesterone (P_4, at 100 ng each except for E_2 at 500 ng). The elution was first performed by an isocratic elution with solvent A (water: methanol: acetonitrile: isopropanol, 55:32:6.5:7.5, v/v) for 15 min, followed by a linear gradient elution built up to 80% of solvent B (water: methanol: n-butanol, 40:40:20,v/v) within 35 min. UV-absorbing steroids were detected at 254 nm and the attenuation was set at 32 a.u./cm.

by RIA. The eluates showed a positive reaction by RIA and had 75.8% and 78.2% recovery efficiencies for 17-beta-estradiol and progesterone, respectively. When the hepatopancreas steroid residue (without steroid standards; at 20 μl containing 935 pg of RIA-detected estradiol-like substance and 2.66 ng of progesterone-like substance) of the November 1994 sample passed through HPLC, the eluates which came out along the whole profile of the chromatogram were collected. The eluates were assayed by RIA, and results are shown in Table 1. A total of 78% (730.6 pg) of the estradiol-like substance applied to the column was recovered after chromatography. About 69.0% of the chromatographed as estradiol-like substance emerged at a retention time (27.0 min) corresponding to authentic estradiol. Fifteen per cent of the estradiol-like substance eluted at retention times close to that of authentic estradiol could be considered as contained in the shoulders of the OD peak of that hormone. Therefore, less than 16.0% of the chromatographed estradiol-like substance was identifiable in the isocratic and gradient elutions.

As shown in Table 1, 80.8% (2.15 ng) of the progesterone-like substance applied to the column was recovered from chromatography. There was 73.9% of the chromatographed progesterone-like substance detected in the eluate, with a retention time like that of authentic progesterone (32.8 min). Less than 16.0% of the chromatographed progesterone-like substance was found in other fractions.

When the ovarian steroid residue (803.0 pg of estradiol-like substance and 2.04 ng of progesterone-like substance) of the December 1994 sample passed through HPLC, as described above for hepatopancreas residue, the recovery efficiencies

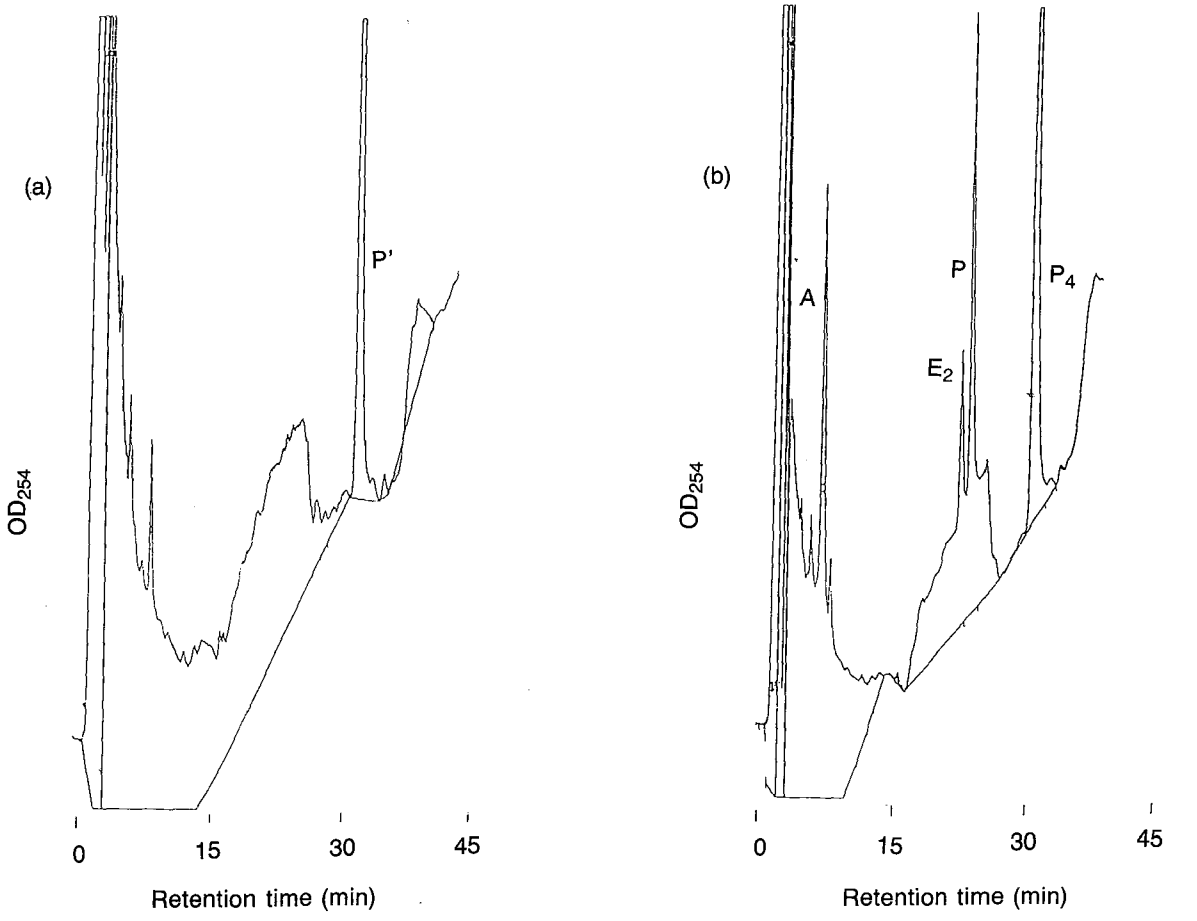

Fig. 2. HPLC chromatograms of steroid residue from ovaries of female *Mictyris brevidactylus* (October 1994). (a) Steroid residue of ovaries run on HPLC without authentic steroid standards. P' represents a progesterone-like substance. (b) Sample of (a) run on HPLC with authentic steroid standards. A, E_2, P, and P_4 represent the authentic steroids, aldosterone, 17-beta-estradiol, 17-alpha-hydroxyprogesterone, and progesterone, respectively. The elution procedure was as described in Fig. 1.

for estradiol-like and progesterone-like substances were 68.0% and 67.6%, respectively. Results are shown in Table 2. Seventy-eight per cent of the chromatographed estradiol-like substance was detected in the eluate with a retention time of authentic estradiol (27.5 min). Again, less than 21.0% of the eluted estradiol was detected in other fractions. In the case of the progesterone-like substance, 65.2% of the chromatographed material was detected in the eluate with a retention time of the authentic hormone. About 28.0% of the chromatographed progesterone-like substance was found in the isocratic elution phase.

After applying the correction factors, contents of estradiol-like substance in ovaries, hepatopancreases, and body fluid during the study period were tabulated in Table 3. The contents of the progesterone-like substance of these tissues are shown in Table 4. Since ovaries were small from January to June, residues were not prepared from them. Due to the absence or low level of estradiol in tissues, it was not detected by HPLC using the system in this study. However, an estradiol-like substance was detected by RIA in HPLC eluates and chemically extracted steroid residues. The ovarian residue of the July 1994 sample had a low level of estradiol-like substance (8.8 pg/g of tissue). Levels began increasing in August and reached a peak phase in December (70.0 pg/g of tissue). The estradiol-like substance was not detected in the hepatopancreas residues from January to June except in April (6.4 pg/g of tissue). The peak phase was in October with 122.9 pg/g of tissue. The estradiol-like substance was not detected in the body fluid residue during April to July. Steroid residues of body fluid from August to December had detectable estradiol-like substance, but levels were low.

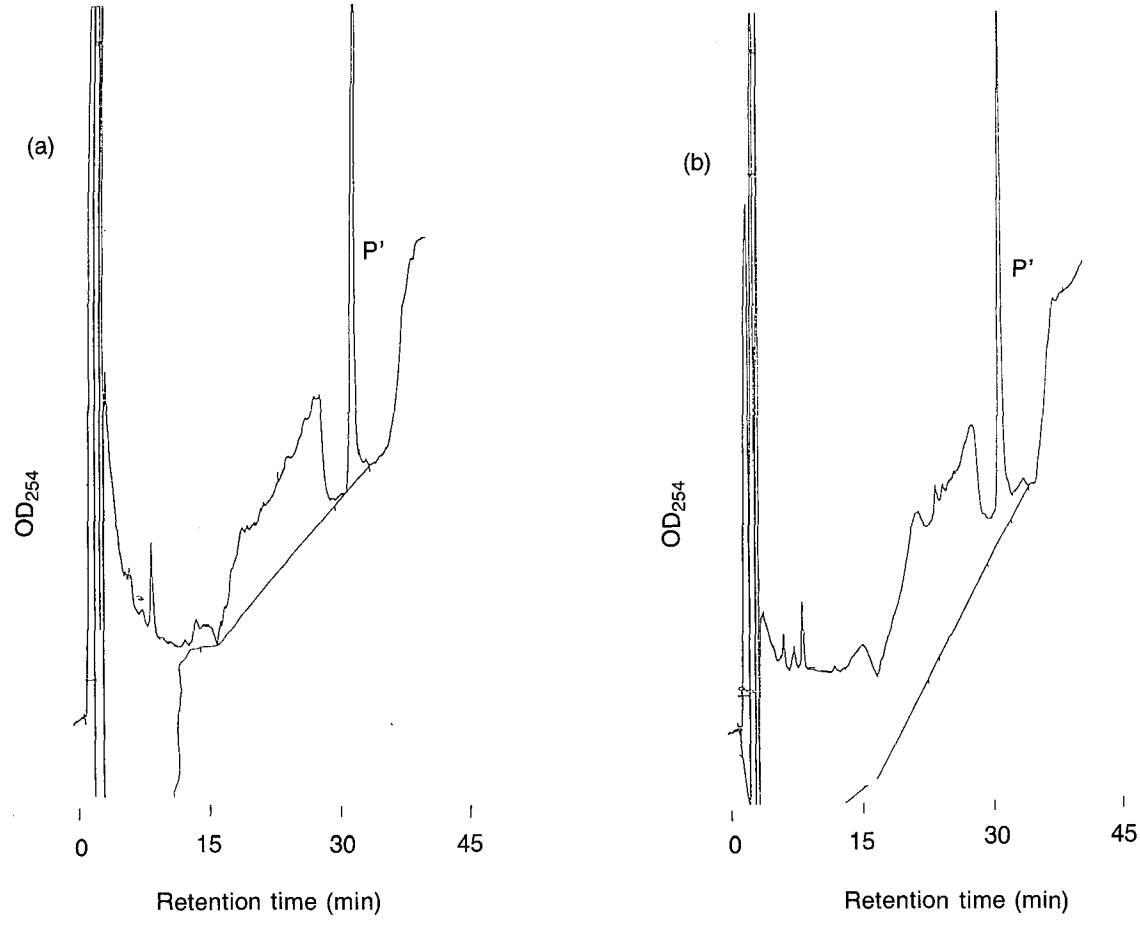

Fig. 3. HPLC chromatograms of steroid residues from hepatopancreases (a) and body fluid (b) of female *Mictyris brevidactylus* (October 1994). P' in (a) and (b) which had similar retention times as authentic progesterone (P_4 of Fig. 1) represents the progesterone-like substance of steroid residues. The elution procedure was as described in Fig. 1.

Concentrations of the progesterone-like substance of ovaries were 22.69 to 34.00 ng/g of tissue from July to October. However, levels increased to the peak phase in November and December with 408.52 to 1 791.27 ng/g of tissue, respectively. Ovaries from January 1994 had a relatively low level of progesterone-like substance (16.12 ng/g of tissue). Concentrations of the progesterone-like substance of hepatopancreas were 3.54 to 27.16 ng/g of tissue from February to August. These levels increased from September (155.07 ± 6.85 ng/g of tissue) and reached the peak phase (6 668.29 ng/g of tissue) in November; in January 1994 the concentration was 111.22 ± 22.23 ng/g of tissue. The steroid residues of body fluid from April to August 1994 had low levels of progesterone-like substance (below 0.28 ng/g of bw). This reached the peak phase (1.19 ng/g of bw) in September and returned to low levels again during October to December.

DISCUSSION

Results of this study on *Mictyris brevidactylus* are: 1) a progesterone-like substance was detected in the steroid residues of ovaries, hepatopancreases, and body fluid, 2) a progesterone-like substance was at low levels in the reproductive and nonreproductive seasons, but at high levels

Table 1. Radioimmunoassays of eluates from HPLC of *Mictyris brevidactylus* hepatopancreas steroid residue of the November 1994 sample[a]

Fraction no.	OD_{254}	Retention time (min)	RIA detected	
			E_2-like substance (pg)	P_4-like substance (ng)
1[b]	> 0.500	2.5	3.1	0.09
2	0.025	5.7	5.0	0.08
3	0.000	8.0	0.0	0.00
4	0.010	9.0	5.2	0.00
5	0.000	11.0	0.0	0.00
6	0.000	13.0	0.0	0.00
7	0.000	15.0	13.8	0.00
8	0.000	17.0	2.9	0.00
9	0.000	19.5	0.0	0.00
10	0.000	22.0	10.7	0.00
11	0.006	25.0	7.5	0.04
12	0.007	26.0	81.7	0.03
13	0.008	27.0	504.7	0.03
14	0.009	28.0	32.0	0.03
15	0.010	29.0	12.8	0.05
16	0.010	30.0	7.0	0.11
17	0.013	31.2	4.5	0.04
18	0.012	32.0	2.7	0.00
19	0.058	32.8	22.8	1.59
20	0.010	34.0	11.9	0.06
Total			730.6[c]	2.15

[a]A sample of 20 μl of hepatopancreas steroid residue (in methanol, without steroid standards) containing RIA-detected 935 pg of estradiol-like substance and 2.66 ng of progesterone-like substance was applied onto an HPLC column. Elution procedure is described in Materials and Methods.
[b]Fractions 1-10 emerged in the isocratic elution. Fractions 11-20 emerged in the gradient elution. Fractions 13 and 19 had retention times corresponding to authentic estradiol (26.0-27.0 min) and progesterone (32.0-33.0 min), respectively.
[c]Total recovered amount from chromatography.

Table 2. Radioimmunoassays of eluates from HPLC of *Mictyris brevidactylus* ovary steroid residue of the December 1994 sample[a]

Fraction no.	OD_{254}	Retention time (min)	RIA detected	
			E_2-like substance (pg)	P_4-like substance (ng)
1[b]	> 0.500	2.5	2.2	0.00
2	0.001	5.2	3.1	0.02
3	0.002	8.0	7.8	0.00
4	0.008	9.2	4.3	0.04
5	0.001	11.0	0.0	0.05
6	0.001	12.5	0.0	0.00
7	0.000	13.5	0.0	0.11
8	0.000	14.5	0.0	0.08
9	0.000	15.5	0.0	0.06
10	0.000	18.5	0.0	0.04
11	0.005	25.5	4.0	0.00
12	0.008	26.4	0.0	0.00
13	0.009	27.5	427.5	0.06
14	0.009	28.7	14.6	0.00
15	0.010	29.2	11.1	0.00
16	0.015	31.0	10.9	0.00
17	0.013	31.6	6.1	0.00
18	0.048	32.6	6.3	0.90
19	0.017	34.1	8.6	0.02
20	0.013	35.4	17.4	0.00
Total			546.0[c]	1.38

[a]A sample of 20 μl of ovary steroid residue (in methanol, without steroid standards) containing RIA-detected 803 pg of estradiol-like substance and 2.04 ng of progesterone-like substance was applied onto an HPLC column. Elution procedure is described in the Materials and Methods.
[b]Fractions 1-10 emerged in the isocratic elution. Fractions 11-20 emerged in the gradient elution. Fractions 13 and 18 had retention times corresponding to authentic estradiol (26.0-27.0 min) and progesterone (32.0-33.0 min), respectively.
[c]Total recovered amount from chromatography.

in the prereproductive season; and 3) an estradiol-like substance was only detected in steroid residues during the prereproductive season.

A progesterone-like substance was separated by the HPLC system used in this study. Results (Figs. 2, 3) only show the chromatograms of steroid residues from October 1994 which represent the prereproductive season. Residues of the nonreproductive and egg-carrying seasons (January, April, June, and December 1994) were also assayed by HPLC. A peak which had a retention time similar to that of authentic progesterone was detected in all samples (data not shown). This result is consistent with those of Shih (1992) and Shih and

Table 3. Estradiol-like substance contents of steroid residues from ovaries, hepatopancreases, and body fluid of *Mictyris brevidactylus* in 1994

Date of collection	Ovaries (pg/g of tissue)[a]	Hepatopancreas (pg/g of tissue)	Body fluid (pg/g of bw)
January	—[b]	—[c]	
February	—	0	
March	—	0 (2)[d]	
April	—	6.4	0
May	—	0	0
June	—	0.6	0
July	8.8	24.4 ± 2.7 (2)	0
August	10.4 ± 0.9 (2)	5.8	0.4
September	18.5	15.3	0.3
October	23.6	122.9	0.6
November	43.6	9.2	0.4
December	70.0	20.3 ± 10.6 (2)	0.2

[a]The RIA-detected estradiol-like substance is expressed as pg/g of wet weight of tissue for ovary and hepatopancreas, or body weight (bw) for body fluid.
[b]Ovaries were small during the reproductive and nonreproductive seasons and were not examined.
[c]Not determined.
[d]The numbers in parentheses represent the number of extractions of steroid residues. The contents of the steroid are expressed as the mean and standard errors.

Table 4. Progesterone-like substance contents of steroid residues from ovaries, hepatopancreases, and body fluid of *Mictyris brevidactylus* in 1994

Date of collection	Ovaries (ng/g of tissue)[a]	Hepatopancreas (ng/g of tissue)	Body fluid (ng/g of bw)
January	16.12	111.22 ± 22.34 (2)[b]	
February	—[c]	20.02	
March	—	27.16	
April	—	9.40	0.07
May	—	4.55	0.28
June	—	3.54	0.19
July	34.00	9.93	0.22
August	29.30	21.54	0.19
September	23.70 ± 1.60 (2)	155.07 ± 6.85 (2)	1.19
October	22.69 ± 4.93 (2)	244.66 ± 18.37 (2)	0.32
November	408.52	6 668.29	0.25 ± 0.06 (2)
December	1 791.27	263.85 ± 9.77 (2)	0.11 ± 0.05 (2)

[a]The RIA-detected progesterone-like substance is expressed as ng/g of wet weight of tissues for ovary and hepatopancreas, or body weight (bw) for body fluid.
[b]The numbers in parentheses represent the number of extractions of steroid residues. The contents of the steroid are expressed as the mean and standard errors.
[c]Ovaries were small during the reproductive and nonreproductive seasons and were not examined.

Wang (1993) who reported that a progesterone-like substance was detected by HPLC and RIA in tissues of *Uca arcuata*.

For identification of the eluates from HPLC of the crab tissue, all eluates were tested by RIA's for steroid-like substances. Though some fractions showed immunoreaction for steroids in either isocratic and gradient elutions. Most (> 70%) of the loaded steroid-like substances emerged at retention times corresponding to those of authentic estradiol or progesterone. For ovarian steroid residue (December 1994), about 28% of the immunoreaction were progesterone in the isocratic elution. According to Huang et al. (1983) only slightly water soluble steroids were eluated in isocratic phase (containing 55% water). Therefore, these RIA reactive materials could be either some steroid-like substances, but certainly not estradiol-like or progesterone-like substances, or could represent interference of the testing procedure. Separate HPLC analysis of ovary and hepatopancreas steroid residues of the November 1994 sample were carried out. Results (data not shown) are similar to those of Tables 1 and 2.

M. brevidactylus of Taiwan has an annual reproductive cycle (Shih 1993 1995). It is important to study the annual pattern of certain hormones, to learn whether levels of endogenous progesterone fluctuate during the reproductive cycle. In this study, seasonal variation of progesterone-like substance levels in the body fluid, ovaries, and hepatopancreases was: peak values of body fluid occurred between September and October; that of ovaries was in December; those of hepatopancreases were between October and November. It should be noted that a high gonadosomatic index and high hemolymphic progesterone-like substance levels were also found during October to December (Shih 1993). During the egg-carrying period, levels of both steroid-like substances decreased significantly. This phenomenon was also seen in *U. arcuata* whose hemolymphic progesterone-like substance levels dropped to the yearly low right after egg-carrying (Shih 1992).

An estradiol-like substance was not detected in the steroid residues from the sample of January to June 1994. It is possible that tissues did not contain this hormone, or that levels were too low to be detected. However, a low level of an estradiol-like substance was detected in body fluid and hepatopancreas residues during August to December and reached peak values in October. On the other hand, peak values of the estradiol-like substance in the ovary residue occurred in December. Again, high levels of an estradiol-like substance were found in body fluid and hepatopancreas at least 1 to 2 months before in the ovary.

This author reported similar results for *U. arcuata* (Shih 1992) in which both progesterone-like and estradiol-like substances reached peak levels 1 to 2 months before the egg-carrying stage. It therefore is reasonable to suggest that sex steroids may serve a physiological function in crustacean reproduction; they may enhance the growth of ovaries or oocytes, or promote the timely maturation of crustacean oocytes (Quackenbush 1986 1989a, Fingerman 1987).

Though progesterone-like and estradiol-like substances were detected in tissue of *M. brevidactylus*, there are still 2 questions. 1) Are these 2 steroid hormones synthesized in the same tissues of this crab? 2) Do these hormones have a physiological role in reproduction? In a recent review, Quackenbush (1994) stated that steroid hormones produced in the ovary of American lobster may regulate yolk production. In this study, steroid residues of ovaries and hepatopancreases from the sample of November 1994, 1995, and December 1995 were tested by RIA for 17-alpha-hydroxyprogesterone, aldosterone, and cortisol. Results showed a positive immunoreaction to 17-alpha-hydroxyprogesterone, but negative to aldosterone and cortisol. In vitro experiments were conducted in this laboratory in which tritium-labeled cholesterol was added to the culture medium of ovaries or hepatopancreases of *M. brevidactylus*. After purification of tissues and media for steroids, HPLC analyses showed that labeled progesterone was detected. However, more quantitative data are needed to support the possibility that crab can synthesize sex steroids endogenously.

Quackenbush (1992) treated the penaeid shrimp ovary fragments with progesterone and found that the synthesis of ovarian protein was enhanced by 2.5 fold. Progesterone stimulated specific yolk protein synthesis by 50% compared to the control, while testosterone, estrogen, and ecdysterone had no effect. Quackenbush (1992) suggested that progesterone may have a normal biological role in shrimp ovarian maturation. Similar experiments have not been carried out on ovary and hepatopancreas of *M. brevidactylus*. The effect of progesterone or estradiol in reproduction of this crab needs further study.

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短趾和尚蟹 (*Mictyris brevidactylus*) 體液、卵巢和肝胰臟中之類固醇性激素

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本報告的目的是測試雌性短趾和尚蟹體內是否有類固醇性激素。雌性短趾和尚蟹的體液、卵巢及肝胰臟經純化，並以 HPLC 及 RIA 分析後，發現三種組織中均可測得類助孕酮。由全年的型態得知類助孕酮於生殖前期 (9-12 月) 在三種組織中的含量最高，生殖期 (抱卵，1-4 月) 與非生殖期 (5-8 月) 的含量則較低。另外經 RIA 測試，類雌二醇僅發現於生殖前期的三種組織中。在三種組織中，卵巢內的類助孕酮和類雌二醇的含量是於 11-12 月達到高峰，而肝胰臟和體液中的含量則較卵巢早一至兩個月達到全年的高峰，文中對類固醇性激素與短趾和尚蟹生殖的關係有所討論。

關鍵詞：螃蟹, 類助孕酮, 類雌二醇, 生殖周期。

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