# ECDYSTEROID METABOLISM IN A CRAB : CARCINUS MAENAS L.

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## ABSTRACT

Ponasterone A (25-deoxy-20-hydroxyecdysone) and 20-hydroxyecdysone were the major ecdysteroids detected in crab hemolymph, although some ecdysone was also present.

The metabolism of ponasterone A was examined in intermolt and premolt crabs either by injecting the radiolabeled hormone or by incubating tissues in its presence. Metabolites were extracted from the surrounding seawater and from tissues and separated by high-performance liquid chromatography.

Ponasterone A metabolism proceeds through (1) C-25 and C-26 hydroxylation, followed by formation of inactivation products via oxidation of the terminal alcoholic group to a carboxylic residue, (2) conjugation, (3) "binding" to very polar compounds and (4) side-chain scission.

The conversion of ponasterone A into 20-hydroxyecdysone, inokosterone (25-deoxy-20,26-dihydroxyecdysone), 20,26-dihydroxyecdysone and ecdysonoic acids, as well as the formation of conjugates and of very polar compounds, occurs in various tissues.

These metabolites were excreted by both intermolt and premolt crabs.

## INTRODUCTION

Prior to 1979, the chemical identity of the predominant molting hormone of crustacea was assumed to be 20-hydroxyecdysone (1).

Recently, ponasterone A (25-deoxy-20-hydroxyecdysone) was identified in three species of crab, Callinectes sapidus (2), Carcinus maenas (3, 4) and Gecarcinus lateralis (2, 5), by means of thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), radioimmunoassay (RIA) and mass spectrometry. This hormone was estimated to be at least as abundant as 20-hydroxyecdysone in the premolt hemolymph (5), in the ovaries (3) and in the eggs (2, 4, 6) of

crabs. The concentration of both 20-hydroxyecdysone and ponasterone A was shown to increase in the hemolymph of premolt Gecarcinus (5) and in the ovaries of Carcinus during their maturation (3). In crab eggs, ponasterone A is the principal ecdysteroid, particularly at the end of embryonic development (4, 6).

We have established that the major metabolite of ecdysone in the crab Carcinus maenas was 20-hydroxyecdysone (7) and demonstrated that various tissues convert ecdysone into 20-hydroxyecdysone (8). In view of the significant amount of ponasterone A evidenced in that crab species, it was of interest to analyze its metabolism during the molting cycle and to elucidate whether this conversion proceeds through $C_{25}$ and $C_{26}$ hydroxylation as in the insect Bombyx mori (9).

## MATERIALS AND METHODS

Animals. The shore crabs Carcinus maenas (L.) were obtained from Roscoff Marine Station and were fed twice weekly. The intermolt and premolt stages were defined by Drach and Tchernigovtzeff's method (10) : intermolt crabs were in stages $C_4$-$D_0$ and premolt crabs in stages $D_1$-$D_2$.

Ecdysteroids. Reference ponasterone A (25-deoxy-20-hydroxyecdysone) was a gift of Dr. D. Horn (Melbourne, Australia). Inokosterone (25-deoxy-20,26-dihydroxyecdysone) was from Rhoto Pharmaceutical Chemistry (Osaka, Japan), 20-hydroxyecdysone was from SIMES (Milan Italy). Authentic 25-deoxy-20-hydroxyecdysonoic acid was the gift of Dr. J. Koolman (Marburg, West Germany). [$^3$H]ponasterone A (specific activity 180 Ci/mmol), which was a gift of Dr. J.A. Hoffmann, (Strasbourg, France) and Dr. J. Koolman, was purified by two consecutive high-performance liquid chromatography (HPLC) steps prior to use (11). [$^3$H]-20-hydroxyecdysone was prepared enzymatically by incubating tritiated ecdysone with insect fat body (12).

Incubation. Various tissues of crabs (testis, hindgut, antennal gland, anterior caeca, posterior caecum, epidermis of the branchiostegite region, pieces of midgut gland, of ovary and of spermatheca of premolt females) were removed and dissected from adhering tissues. They were then rinsed twice and placed in 2.5 mL of sterile seawater containing 16 mg/100 mL of penicillin G, 0.1 g/100 mL of glucose, 1.5 mg/100 mL of phenylthiourea, 0.12 g/100 mL of TRIS and 5 microcuries of

labeled ponasterone A. This volume was sufficient to just cover the tissues. Incubations were performed in darkness at 20°C in the air.

Injections. Purified [$^3$H] ponasterone A was diluted in 50 % ethanol/seawater. Ten microliters of that solution, corresponding to 5 microcuries, was injected at the base of the crab chela. After the injection, each crab was maintained in 100 mL of artificial seawater at 17°C.

Extractions of ecdysteroids. Tissue extracts: after a 48 hour incubation, tissues and medium were homogenized and centrifuged. The pellets were rinsed twice with distilled water and the radioactivity of the combined supernatants was measured. The extracts were partially purified by partitioning with 1/2 the volume of chloroform. The ecdysteroids in the aqueous phase were extracted by chromatography on Sep-Pak $C_{18}$ cartridges (13, 14). The Sep-Paks were eluted with 5 mL of methanol. After evaporation of the methanol under reduced pressure, the extracts were redissolved with the appropriate solvent before HPLC injection.
Seawater extracts: The ecdysteroids were adsorbed from seawater on a Sep-Pak $C_{18}$ cartridge and eluted with 100 % methanol. The eluates were dried down and then taken up in the appropriate solvent for HPLC. At each step of purification, the radioactivity of the liquid fractions was measured.

Radioimmunoassay of blood extracts. Owing to clotting, the hemolymph of crabs cannot be injected through the Sep-Pak. For that reason, the procedure for extracting ecdysteroids from crab hemolymph was the same as for crab tissues. After HPLC on a reverse phase column (RP) each fraction was dried and suspended in a known volume of 0.1 M citrate buffer at pH 6.2. The aqueous phases were assayed for ecdysteroids using the radioimmunoassay (RIA) developed by De Reggi et al. (15).

Chromatographic separation of ecdysteroids. Ecdysteroid extracts from blood, tissues or seawater were analyzed by high-performance liquid chromatography (HPLC). Reversed phase (RP) HPLC was carried out with a Zorbax ODS (DuPont) or an Ultrasphere ODS (Beckman) column. After injection, radiolabeled products were eluted with a linear gradient (8 to 40 %) of acetonitrile in 20 mM TRIS $HCLO_4$ buffer pH 7.5 for 60 minutes (flow rate 1 mL/min.). Normal phase (NP) HPLC analyses were carried out with a Zorbax-Sil column (DuPont) eluted with a methylene chloride/propan-2-ol/water mixture. The actual conditions are indicated as necessary. Ecdysteroids from Carcinus blood were extracted and analyzed by RP HPLC with methanol/water as the solvent. After RP HPLC the ponasterone A, ecdysone and 20-hydroxyecdysone zones were injected on a NP column and the three peaks corresponding to these hormones were collected and tested by RIA.

Treatment with Helix pomatia enzymatic mixture. After evaporation, the eluted polar products were treated by Helix pomatia digestive juice (I.B.F., Gennevilliers, France) overnight at 30°C in 50 mM acetate buffer, pH 5.3, or in seawater adjusted to pH 5.3.

## RESULTS

### Identification of the major ecdysteroids of Carcinus hemolymph.

Total blood ecdysteroid titers during the molting cycle of Carcinus were previously estimated to be $1.6 \times 10^{-7}$M in intermolt crabs and $9.8 \times 10^{-7}$M in premolt crabs (7). In the study reported here, our aim was to identify the major ecdysteroids in Carcinus blood by two successive HPLC analyses followed by RIA.

In a series of ten intermolt crabs ($C_4$-$D_0$), the average titers of ecdysone and 20-hydroxyecdysone were respectively 10 pmol/mL ($10^{-8}$M) and 23 pmol/mL ($2.3 \times 10^{-8}$M). No ponasterone A was detected in these animals.

In a series of ten premolt crabs ($D_1$-$D_2$) the concentration of the ecdysteroids was estimated to be 350 pmol/mL ($3.5 \times 10^{-7}$M) for ponasterone A, 250 pmol/mL ($2.5 \times 10^{-7}$M) for 20-hydroxyecdysone and 25 pmol/mL ($2.5 \times 10^{-8}$M) for ecdysone.

### Ecdysteroid metabolites excreted into seawater.

After injection of ponasterone A, we analyzed the radiolabeled products eliminated by the crabs into the surrounding seawater. The first extraction steps (using $C_{18}$ Sep-Pak cartridges) revealed radiolabeled material both adsorbed and not adsorbed by the Sep-Pak cartridge. We therefore analyzed separately the metabolites adsorbed on the Sep-Pak and eluted with methanol and the very polar products which are not adsorbed by the Sep-Pak.

Excreted ecdysteroid metabolites adsorbed on a Sep-Pak cartridge.

The methanolic extracts were chromatographed on an RP column and a total of seven significant peaks of radioactivity were present (Figure 1).

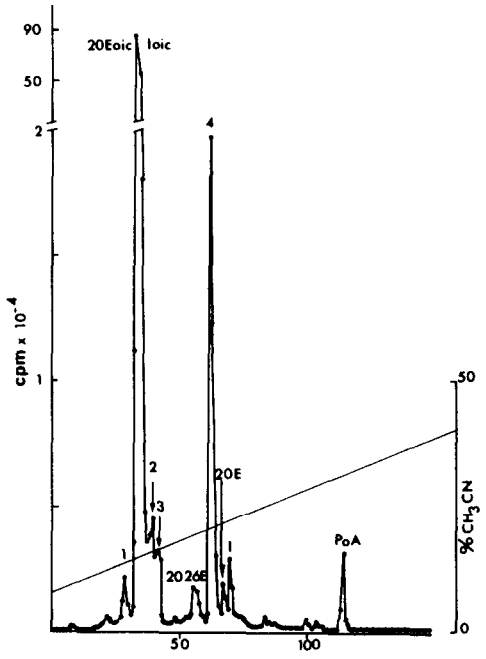


Figure 1 : HPLC separation of the various metabolites of [$^3$H]-ponasterone A excreted by intermolt crabs: Analysis of metabolites adsorbed on a $C_{18}$ Sep-Pak cartridge. Operating conditions : column ultrasphere ODS, 4.6 mm i.d., 250 mm length ; flow rate : 1 mL/min ; linear gradient 8 % to 40 % $CH_3CN$ in 20 mM TRIS $HCLO_4$ buffer, pH 7.5 in 60 min. Fractions of 0.4 mL were collected and counted with 1.2 mL scintillation cocktail. 1 : conjugate of 20,26-dihydroxyecdysone ; 20Eoic : 20-hydroxyecdysonoic acid ; Ioic : 25-deoxy-20-hydroxyecdysonoic acid ;
2-3 : conjugates of 20-hydroxyecdysone and inokosterone ; 20-26E : 20,26-dihydroxyecdysone; 4 : conjugate of ponasterone A ; 20E : 20-hydroxyecdysone ; I : inokosterone ; PoA : ponasterone A.

Three of these products were chromatographed with authentic 20,26-dihydroxyecdysone, 20-hydroxyecdysone and inokosterone. After RP HPLC in a linear gradient of 8% to 40% $CH_3CN$ in 60 minutes (Figure 1) the zones corresponding to these two last metabolites were analyzed separately by RP HPLC in a linear gradient of 16% to 20% $CH_3CN$ in 30 minutes. All the radioactivity was eluted with the retention time characteristic of 20-hydroxyecdysone and inokosterone respectively. Inokosterone was eluted as two isomers, the more polar isomer representing 60% of the radioactivity. NP HPLC of the presumed 20,26-dihydroxyecdysone gave one peak of radiolabeled product which comigrated with authentic 20,26-dihydroxyecdysone in a methylene chloride/propan-2-ol/water (125/50/2) solvent (retention volume: 18 mL).

Two of the three other metabolites were polar products corresponding to 20-hydroxyecdysonoic acid and 25-deoxy-20-hydroxyecdysonoic acid, respectively. These polar components were characterized in two successive HPLC runs by comigration with the authentic products (16). The more polar isomer represented 80% of the total radioactivity for 25-deoxy-20-hydroxyecdysonoic acid.

After hydrolysis of the products 1, 2 , 3 and 4 (Figure 1) with Helix pomatia enzyme mixture, the ecdysteroid extract yielded respectively 20,26-dihydroxyecdysone, 20-hydroxyecdysone, inokosterone and ponasterone A. These peaks disappeared after enzymatic hydrolysis of the whole methanol extract ; they were therefore conjugates of these four ecdysteroids.

Crabs injected with [$^3$H]-20-hydroxyecdysone converted this hormone into 20,26-dihydroxyecdysone, 20-hydroxyecdysonoic acid,

conjugates of 20,26-dihydroxyecdysone (1), and conjugates of 20-hydroxyecdysone (2) (Figure 2). Unchanged 20-hydroxyecdysone and these compounds were excreted into the surrounding seawater.

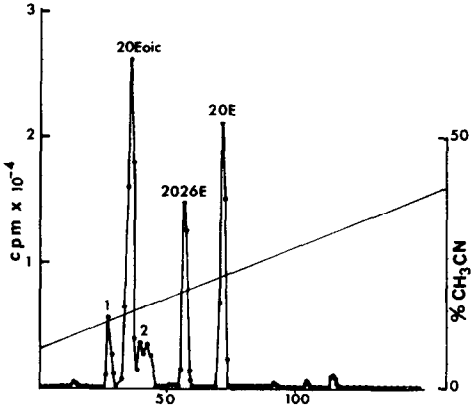


Figure 2: HPLC separation of the metabolites of [$^3$H]-20-hydroxy-ecdysone excreted by intermolt crabs : Analysis of metabolites adsorbed on a $C_{18}$ Sep-Pak cartridge. Same conditions as in Figure 1.

Excreted ecdysteroid metabolites not adsorbed by a Sep-Pak $C_{18}$ cartridge.

The very polar metabolites which were not adsorbed by a Sep-Pak $C_{18}$ cartridge were subjected to enzymatic hydrolysis without success. However, after rotary evaporation of the seawater at 40°C, the radioactivity was distributed between the evaporated water and the dry residue. The properties of the volatile fraction were compared with those of radiolabeled 4-hydroxy-4-methylpentanoic acid formed by chemical side-chain cleavage of 20-hydoxyecdysone (17). Neither was adsorbed on a $C_{18}$ Sep-Pak cartridge and both were volatile ; in

addition, both became soluble in ether when the water was acidified with hydrochloric acid. The non-volatile radiolabeled products of the dry residue were taken up in water and, again, they were not adsorbed on a $C_{18}$ Sep-Pak cartridge. However, if they were first taken up in methanol, dried down and then dissolved in water they were adsorbed on such a cartridge. Two major ecdysteroids were released by that treatment : ponasterone A and 20-hydroxyecdysone. Neither 20-hydroxyecdysonoic acid nor 25-deoxy-20-hydroxyecdysonoic acid, nor 20,26-dihydroxyecdysone nor conjugates were found in that mixture. Crabs injected with [$^3$H]-20-hydroxyecdysone converted this hormone into volatile and non-volatile very polar products; only 20-hydroxyecdysone was found in the non-volatile very polar fraction.

Metabolism of ecdysteroids during the molting cycle of Carcinus maenas.

In both the intermolt and the premolt stage [$^3$H]-ponasterone A was metabolized and excreted by crab tissues. Within 24 hours after injection of radiolabeled ponasterone A, one of the principal peaks revealed by HPLC of the surrounding seawater was ponasterone A. It is possible, however, that this corresponded to the loss of a part of the injected ponasterone A from the needle wound, since the radioactivity eliminated in the following periods contained only little non-metabolized ponasterone A.

In intermolt crabs (vitellogenic females, females with white ovaries, and males), 25-deoxy-20-hydroxyecdysonoic acid is the predominant metabolite. Within 48 hours after injection of radiolabeled 20-hydroxyecdysone into intermolt crabs, the major

metabolites were conjugates and 20-hydroxyecdysonoic acid. Between 48 hours and 15 days after injection, very polar products which were not adsorbed by a $C_{18}$ Sep-Pak cartridge, became more and more abundant until they were the only metabolites excreted by the crab.

In premolt crabs, within 24 hours after injection of [$^3$H]-ponasterone A, the major metabolites were conjugates. Between 24 hours and 15 days after injection, exclusively 25-deoxy-20-hydroxyecdysonoic acid and very polar products were found. Very polar products (not adsorbed on a $C_{18}$ Sep-Pak cartridge) constituted 30% to 90% of the excreted metabolites. We observed that in experiments conducted in autumn or in winter, the volatile products constituted 30 % of the very polar products, whereas in crabs injected during spring time they constituted 90% of these very polar compounds.

Both intermolt and premolt crabs were shown to eliminate also free 20,26-dihydroxyecdysone, 20-hydroxyecdysone and inokosterone. The first two compounds occurred at very low levels (1 to 5% of the total radioactivity recovered) whereas the last was from 2 to 10 times more abundant than 20-hydroxyecdysone.

Metabolism of ecdysteroids by incubated tissues.

In tissues incubated for 24-48 hours, 1 to 50 % of the recovered radioactivity was present as ponasterone A metabolites (Table 1). All ponasterone A metabolites found in the *in vivo* experiments were also detected in the *in vitro* experiments (Figure 3). These different metabolites were characterized by the same methods used for excreted metabolites.

The predominant ecdysteroid recorded was mostly

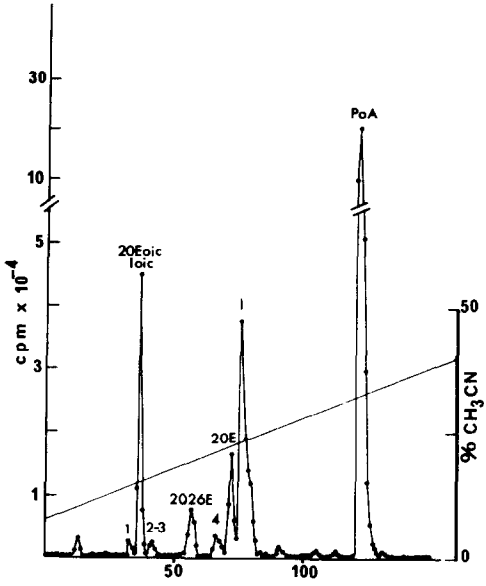


Figure 3: HPLC separation of the various metabolites of [$^3$H]-ponasterone A after incubation of premolt crab epidermis in the presence of the hormone : Analysis of metabolites adsorbed on Sep-Pak cartridge. Same conditions as in Figure 1.

20-hydroxyecdysone (Table 1). The major fact to emerge from this series of experiments is that the 20-hydroxyecdysone levels within the tissues generally exceeded those of inokosterone, in contrast to the results obtained in the in vivo experiments (see above).

Large amounts of 25-deoxy-20-hydroxyecdysonoic acid (30 to 60% of the total radiolabeled metabolites) were formed in epidermis of premolt crabs and in hindgut of both premolt and intermolt animals. Conversion of ponasterone A to 20,26-dihydroxyecdysone and 20-hydroxyecdysonoic acid occurred to a very limited extent.

Table 1 : Metabolism of ($^{3}$H)-ponasterone A by incubated tissues. (I):intermolt crabs; (P):premolt crabs; 20Eoic:20-hydroxyecdysonoic acid; Ioic:25-deoxy-20-hydroxyecdysonoic acid; 2026E:20-26-dihydroxyecdysone; C:conjugates; 20E:20-hydroxyecdysone; I:inokosterone; VPP:very polar products.

| Crab tissues | Testis (I and P) | Vitellogenic ovary (I) | Non-vitellogenic ovary (P) | Hindgut (I and P) | Anterior caeca (I and P) | Posterior caecum (I and P) | Spermatheca (P) | Midgut gland (I and P) | Epidermis (I) | Epidermis (P) | Antennal gland (I and P) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Metabolites of ($^{3}$H)-PoA | | | | | | | | | | | |
| 20Eoic | - | - | + | + | + | traces | - | + | traces | + | - |
| Ioic | traces | - | + | +++ | + | + | + | + | traces | +++ | traces |
| 2026E | - | - | + | + | - | traces | - | + | + | + | traces |
| C | - | - | + | ++ | - | traces | - | - | traces | + | traces |
| 20E | +++ | traces | ++ | ++ | +++ | + | ++ | +++ | +++ | ++ | + |
| I | ++ | traces | ++ | ++ | + | + | ++ | ++ | +++ | +++ | +++ |
| VPP | - | - | - | - | - | - | - | - | +++ | +++ | + |
| non-metabolized ponasterone A | 90% | 99% | 85% | 80% | 80% | 90% | 90% | 70% | 50% | 60% | 85% |

Enzymatically hydrolysable conjugates of ponasterone A were also present in incubated hindgut, epidermis and white ovaries at low percentage levels.

It appears that premolt and intermolt crab epidermis yielded very polar products which were not adsorbed by the Sep-Pak cartridge (Table 1).

It should be noted that no metabolites were formed by vitellogenic ovaries and that testis converted [$^3$H]-ponasterone A essentially into 20-hydroxyecdysone.

Under the same incubation conditions, [$^3$H]-20-hydroxyecdysone was not significantly metabolized by intermolt crab tissues except by epidermis. In this tissue, 20-hydroxyecdysone was converted into 20,26-dihydroxyecdysone, 20-hydroxyecdysonoic acid and conjugates.

The profile of catabolism of labeled ponasterone A was not modified by the addition of physiological concentrations of non-labeled ponasterone A ($10^{-6}$ M and $10^{-8}$M) to the culture medium.

Some degradation of [$^3$H]-20-hydroxyecdysone and [$^3$H]-ponasterone A may be expected to occur during incubation and/or sample processing. The radioactivity found eluting between inokosterone and ponasterone A might be accounted for in this way, in as much as similar peaks were obtained by incubating [$^3$H]-ponasterone A and [$^3$H]-20-hydroxyecdysone in culture medium devoid of tissue.

## DISCUSSION

In agreement with previous reports (3,4), our experiments have shown that 20-hydroxyecdysone, ecdysone and ponasterone A are the

major ecdysteroids in the crab Carcinus maenas. It was therefore interesting to elucidate the changes observed in the biochemical pathway of ponasterone A metabolism during the molting cycle of a crab.

Hydroxylation of ecdysone to 20-hydroxyecdysone has been classically demonstrated in crustacea (5, 7, 8, 18-24). The present data show that crabs possess another source of 20-hydroxyecdysone by 25-hydroxylation of ponasterone A. This hormone can also be 26-hydroxylated to yield inokosterone which is a crustacean ecdysteroid too (25). Accordingly, in Carcinus maenas, ponasterone A is a potential precursor of both 20-hydroxyecdysone and inokosterone. In that connection, it is worth recalling that both 25-hydroxylation and 26-hydroxylation were proved to occur in the insect Bombyx mori when ponasterone A, normally absent, was injected in radiolabeled form (9). This conversion is accomplished by various tissues: testis, ovaries, midgut gland, anterior caeca, hindgut, posterior caecum, antennal glands and epidermis.

Inokosterone and 20-hydroxyecdysone, in addition to ponasterone A and 20,26-dihydroxyecdysone, are eliminated into the seawater as free molecules. However, like insects, crabs eliminate ecdysteroids via the formation of highly polar products (conjugates and ecdysteroids with an acidic side chain) (26). Free ecdysteroids (ponasterone A, 20-hydroxyecdysone, inokosterone and 20,26-dihydroxy-ecdysone) are conjugated by crabs and excreted into the seawater. These products are formed by non-vitellogenic ovary, hindgut and epidermis. Some conjugates were detected in crab ovaries, and particularly in eggs, during embryogenesis of Carcinus maenas (3,4).

Carcinus maenas seems to possess also another type of excretion

product corresponding to very polar compounds not adsorbed by $C_{18}$ Sep-Pak cartridges. These metabolites consist of two classes of polar products: volatile and non-volatile ones. The volatile compounds resemble 4-hydroxy-4-methylpentanoic acid ; however, side-chain scission could affect all compounds bearing a free 20,22 diol e.g. 20-hydroxyecdysone, ponasterone A, 20,26-dihydroxyecdysone, inokosterone and ecdysonoic acids, yielding poststerone and different side-chain cleavage products. In our experiments, the nature of the volatile products was not definitively established. We plan to characterize these compounds by preparing chemical side-chain scission products of ponasterone A and those of its metabolites which possess a free 20,22 diol. The non-volatile compounds are extremely polar, even more polar than sulfo or phospho conjugates which are adsorbed on the $C_{18}$ Sep-Pak cartridge. Their cleavage by methanol suggests that these products are very fragile. They were not, however, hydrolyzed by Helix pomatia enzyme mixture. Therefore, they are more likely to be "non-covalent complexes". This "binding" of ecdysteroids to a polar molecule (polyoside or polypeptide) seems restricted to ponasterone A and to 20-hydroxyecdysone. In view of the evidence that they are synthesized by incubated epidermis in vitro only, we are more inclined to consider them as a specific system rather than a trivial artifact. The question then arises as to the nature and the biological significance of such compounds. Experiments are now proceeding to characterize them.

In the crab Gecarcinus lateralis, the metabolism of ecdysone proceeds through hydroxylation at positions $C_{20}$ and $C_{26}$ to form 20-hydroxyecdysone and 20,26-dihydroxyecdysone and via conjugation of

the three free ecdysteroids. The presence of "extremely polar material" which is not hydrolyzable by an enzymatic mixture has also been reported (22,23), and this polar material could be the same as what we call "very polar products" in our work.

Like crabs, insects metabolize [$^3$H]-20-hydroxyecdysone to 20-hydroxyecdysonoic acid (via 20,26-dihydroxyecdysone) and [$^3$H]-ponasterone A to 25-deoxy-20-hydroxyecdysonoic acid (via inokosterone) (16). Formation of ecdysonoic acids (especially 25-deoxy-20-hydroxyecdysonoic acid) and very polar products seem to be the principal hormone inactivation pathways in Carcinus maenas (Figure 4).

Crayfishes, injected with [$^3$H]-ecdysone, formed essentially 20-hydroxyecdysone and an apolar substance which has not been identified in Carcinus (21,27,28).

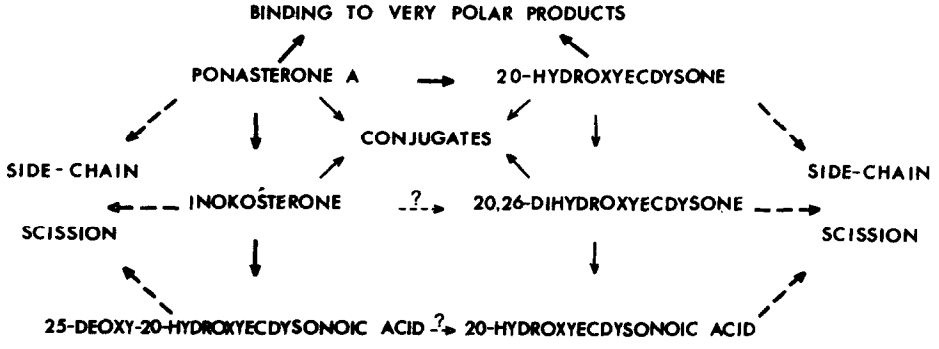


Figure 4: Summary of [$^3$H]-ponasterone A metabolic pathway in Carcinus maenas. The heavy arrows indicate the most active reactions.

The major insights provided by this study on ecdysteroid metabolism in Carcinus are the concomitant occurrence of two 20-hydroxyecdysone precursors: ponasterone A and ecdysone, and the equally high levels of ponasterone A and 20-hydroxyecdysone during ovarian development, embryogenesis and molting cycle.

This would suggest a mutual regulation of these two hormones in the same way that ponasterone A inhibits 20-hydroxylation of ecdysone in the crab Pachygrapsus crassipes (20).

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## TRIVIAL AND IUPAC EQUIVALENT NAMES

20-Hydroxyecdysone: 2β, 3β, 14α, 20R, 22R, 25-hexahydroxy-5β-cholest-7-en-6-one

Ponasterone A: 2β, 3β, 14α, 20R, 22R-pentahydroxy-5β-cholest-7-en-6-one

Inokosterone: 2β, 3β, 14α, 20R, 22R, 26-hexahydroxy-5β-cholest-7-en-6-one

20,26-Dihydroxyecdysone: 2β, 3β, 14α, 20R, 22R, 25,26-heptahydroxy-5β-cholest-7-en-6-one

Poststerone: 2β, 3β, 14α-trihydroxy-5β-pregn-7-en-6,20-dione

20-Hydroxyecdysonoic acid: 2β, 3β, 14α, 20R, 22R, 25-hexahydroxy-6-oxo-cholest-7-en-26-oic acid

25-Deoxy-20-hydroxyecdysonoic acid: 2β, 3β, 14α, 20R, 22R-pentahydroxy-6-oxo-cholest-7-en-26-oic acid