

Moulting Hormones of a Crab during Ecdysis

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CRUSTECDYSONE (20-hydroxyecdysone, I) the main moulting hormone of several species of insects¹ also occurs² in low concentration in the marine crayfish *Jasus lalandei* at an intermoult stage. It is thus likely that crustecdysone has a hormonal function in crustaceans, and studies³ of the effect of injected crustecdysone on various crustaceans support this conclusion.

We have examined the extracts of the female marine crab, *Callinectes sapidus* just before and after moulting. Crabs in the three stages, known commercially as "green" (early premoult), "peeler" (late premoult), and "soft-shell" (just after moulting) were collected on Hoopers Island, Maryland. The three batches (each of about 25 kg.) were extracted and processed by methods described earlier.^{1c,4} The amounts of ecdysones isolated from the crabs at the various stages are shown in the Table. The compounds were unequivocally identified by t.l.c., n.m.r., u.v., and mass spectrometry.

In the early premoult stage callinecdysone A is the only

hormone that could be detected. The spectra of callinecdysone A are identical with those of inokosterone (II),

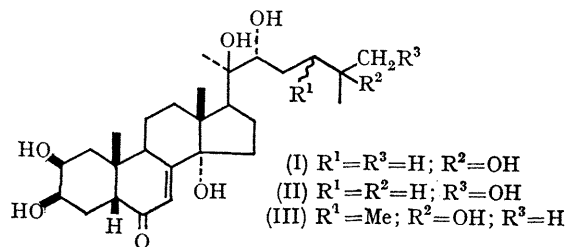
Moulting hormones from Callinectes sapidus at three stages during ecdysis

(Weights in $\mu\text{g.}$ per kg. extracted)

	"Green"	"Peeler"	"Soft-shell"
Callinecdysone A (II) ..	5	20	—
Crustecdysone (I) ..	—	4	280
Callinecdysone B (III) ..	—	—	24

which is a mixture of C-25 stereoisomers.⁶ Callinecdysone A may thus be one of the inokosterone isomers or also a mixture of isomers. At the later premoult stage callinecdysone A is accompanied by a smaller amount of crustecdysone. Finally, after moulting, crustecdysone is the major hormone present together with a smaller amount of callinecdysone B. The spectra of callinecdysone B and

makisterone A (III)⁷ are identical and callinecdysone B is either makisterone A or its C-24 isomer. The latter is more likely since the C₂₈ marine zoosterols and phytosterols, which are likely to be the respective precursors of these hormones are epimeric at C-24.⁸ Inokosterone and makisterone A were originally isolated from plants and this is the first time that ecdysones of this type have been detected in arthropods.



The rising titre of ecdysones during moulting in *Callinectes* provides further compelling support for the view that ecdysones⁹ function as moulting hormones in crustaceans. The high titre of crustecdysone present after moulting is unexpected and suggests that a high titre of hormone is not only associated with cuticle shedding but also with cuticle hardening. Certainly in *Calliphora*, the injection of high doses of crustecdysone into the larvae before pupation leads to precocious cuticle tanning.¹⁰ Events leading up to moulting may thus be sequentially triggered, partly by different ecdysones and partly by a rising hormone titre, with final cuticle hardening taking place only at the highest titre.

We thank Mr. Chan Rippon for collecting the crabs, Professor K. Nakanishi for a sample of Makisterone A, and Professor T. Takemoto for a sample of inokosterone.

(Received, January 8th, 1969; Com. 022.)

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