Short Communication

Insect Ecdysis Inhibitors from the East African Medicinal Plant *Ajuga remota* (Labiateae)

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Received May 11, 1981

The bitter tasting leaves and roots of the East African medicinal plant, *Ajuga remota* (Labiateae), are well known to be naturally resistant to insect attack. In fact, four diterpenes, ajugarin-I, -II, -III, and clerodin, have been chemically identified from the ether extract as having antifeedant activity against the major East African pest insect, *Spodoptera exempta*^{1,2)} with leaf disk method.³⁾

This communication reports on ecdysis inhibitors from the methanol extract of the leaves and roots of *A. remota*. Biological activity was monitored with artificial diet feeding assays,⁴⁾ and observations were made with five Lepidopterous larvae. Failure to complete

the molting cycle was induced in three species of tested insects—silkworm, *Bombyx mori*; pink bollworm, *Pectinophora gossypiella*; and fall armyworm, *Spodoptera frugiperda*. The *Heliothis* complex⁵⁾ showed no response to the *A. remota* methanol extract. Further purification of the methanol extract, in conjunction with the biological assay utilizing *B. mori* and *P. gossypiella*, led to the isolation of two active principles. These two ecdysis inhibitors have been identified as cyasterone and ecdysterone by spectroscopic data and by direct comparison with authentic samples.⁶⁾

Table I shows the response of silkworm and pink bollworm when fed cyasterone or ecdysterone. Most prominent among these responses is death, apparently caused by an anatomical inhibition of feeding caused by molting cycle failure. Insect molting cycle is initiated when the cuticular epithelium separates from the overlying cuticle in the process of apolysis.⁷⁾ The molting cycle is terminated, upon the completion of cuticle synthesis, by hydrostatic expansion of the new cuticle during the process of ecdysis. The added phytoecdysones, cyasterone and ecdysterone, apparently upset the temporal patterning of the molting cycle because hardening (sclerotization) of the newly synthesized cuticle occurred before its expansion, so that the

TABLE I. EFFECTS OF CYASTERONE AND ECDYSTERONE ON GROWTH AND DEVELOPMENT OF SILKWORM AND PINK BOLLWORM

Values are based on three or more replicates, each of which consisted of 30 or more insects. ED₅₀ refers to the effective dose for 50% growth inhibition, while LD₉₀ refers to the lethal dose for 90% kill due to ecdysis inhibition.

Species	Compound	Amount in diet (ppm)	Effect	Stage assayed
Silkworm	Cyasterone	10	ED ₅₀	Second instar
		50	LD ₉₀	Second instar
	Ecdysterone	10	ED ₅₀	Second instar
		50	LD ₉₀	Second instar
Pink bollworm	Cyasterone	25	ED ₅₀	Neonate
		40	LD ₉₀	Neonate
	Ecdysterone	35	ED ₅₀	Neonate
		50	LD ₉₀	Neonate

FIG. 1. This Electron Micrograph Depicts a Fall Armyworm, *Spodoptera frugiperda*, after Ingestion of the Crude Methanol Extract of *Ajuga remota* roots.

This insect has three head capsules which mask its functional mouthparts. The insect eventually starved to death. Magnification $\times 42$.

previous cuticle remained adhering to, and thereby masking, the new cuticle. This process repeated itself until individual larvae were with up to three cuticular head capsules, all of approximately equal size and all adhering to one another. Thus, affected larvae were unable to continue to feed because their mouthparts were masked by unshed head capsule(s). In some cases, larvae had impaired locomotory and excretory functions because the whole body remained in the pharate condition due to the retention of the entire cuticular skin, in addition to the heavily sclerotized head capsules.

Without artificially removing the old capsule(s) from the insects, death occurred within 72~96 hours after the onset of the abortive molt. Even when affected larvae were artificially unmasked, only some could continue feeding because of impaired mandibular function due to restricted expansion of the new head capsule. These unmasked larvae which could feed, again experienced masking at the following molt.

Prematurely activated molting fluid phenoloxidase, the enzyme responsible for cuticle hardening,⁸⁾ could sclerotize the soft, new cuticle before cuticular expansion. This would result in the head capsule retention described.

It is noteworthy that some larvae could synthesize a third head capsule even though feeding became impossible after synthesis of the second head capsule. Apparently, the phytoecdysones retain activity inside the insect through two molts.

A lower (than the threshold for ecdysis inhibition) concentration of these phytoecdysones (Table I) causes growth inhibition when fed to silkworm and pink bollworm. The effective dose (ED_{50}) for 50% growth inhibition was comparable for cyasterone and ecdysterone within the two species of insect.

Different responses to these two phytoecdysones were demonstrated by orally injecting them into fourth instar *B. mori*. Oral injection of 10 μ g ecdysterone promoted apolysis to the fifth instar but inhibited ecdysis, while oral injection of 10 μ g cyasterone resulted in only a delay in a normal molt to the fifth instar. Doubling the oral dose to 20 μ g cyasterone induced prothetely in some (20%) of the treated fourth instar insects as they molted directly to pupae. Cyasterone—induced prothetely resulted in pupae and cocoons roughly one-half the size of normal.

The primary purpose of this report is to depict the abnormal retention of cast pink bollworm, fall armyworm, and silkworm head capsules induced by ingestion of the roots and leaves of *A. remota*. The principles causing this activity are two ecdysteroids—cyasterone and ecdysterone. It has long been appreciated that ecdysteroids have marked physiological effects in insects.⁹⁾ This report focuses on a new activity attributed to phytoecdysones—that of ecdysis inhibition through successive molts until death is the final result. The wide range in susceptibility of species to ingested phytoecdysones and the wide range of activities induced in the growth and development of susceptible insect species should renew interests in the possibilities of utilizing phytoecdysones in the control of specific pest species.

Phytoecdysones are widely occurring plant compounds that are nonpolluting, species—specific, and, as illustrated in this report, are capable of inducing some uniquely effective

biological phenomena.

Acknowledgment. Insects were kindly supplied by the agencies of the USDA in Albany, Ca; Phoenix, Az; and Brownsville, Tx. The authors thank A. Chapya for the plant collection. We also thank J. DeBenedictis for his help with electron micrographs. Authentic samples of phytoecdysones were kindly provided by Professor T. Takemoto.

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