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Alkaloids and isoprenoids as defensive and signalling agents among insects

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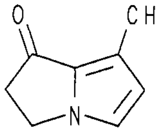
ABSTRACT - While extensive effort has been devoted to the study of female insectan pheromones, male pheromones have been much less studied. Our research with Professor T. Eisner has established that the metabolites of acquired pyrrolizidine alkaloids serve some male lepidoptera as aphrodisiac pheromones. Quantitative studies reveal that the alkaloids themselves may be transferred from males to females during mating, and then from females to eggs, which are rendered unpalatable. As a consequence of these transfers, insects can use compounds which probably evolved as plant defensive agents to protect not only themselves, but also their offspring. The male's "aphrodisiac" can be understood to be a sympol of its "chemical fitness." We have now found a simpler example of this type of strategy in which certain male beetles seek out and ingest the well-known isoprenoid cantharidin, which is then transmitted to females and subsequently to eggs. In this case, the cantharidin itself also serves as an aphrodisiac. Based on these experimental studies, unanticipated insights into the relationship between chemical defense and chemical communication have been gained.

Since the seminal study of the chemistry of the sex attractant emitted by the virgin female silkworm moth, *Bombyx mori*, was carried out by A. Butenandt and his collaborators three decades ago (ref. 1), hundreds of female lepidopteran pheromones have been characterized (ref. 2). Not only the structures, but also in many cases the biosyntheses of these molecular messengers have been elucidated in considerable detail (ref. 3). It is clear that these fatty acid derived pheromones are often produced as mixtures of closely related components whose ratios play an important role in recognition and response. An additional dimension was added to our understanding of pheromone communication by the finding that in the arctiid moth *Utetheisa ornatrix*, female pheromones are emitted in short pulses, giving rise to a temporally modulated electrical signal (*ca.* 1.5 Hz) in the receiving male's antennae, when the male is at close range (ref. 4). The implications of temporal patterning of this sort are still being studied.

While much less attention has been devoted to research on pheromones emitted by male insects, this area has also proven itself to be of some interest. In our earliest collaborative investigation, involving a Trinidad species of danaid butterfly, *Lycorea ceres*, males were shown to accumulate a bicyclic, heterocyclic hetone, *danaidone*(1), on a pair of readily everted organs known as hairpencils (ref. 5). While it proved impractical to study the behavioral role of 1 in *L. ceres*, we later found the identical compound in a related species, the Florida queen butterfly (*Danaus gilippus berenice*). In this case, both electrophysiological and behavioral studies established that 1 serves as an authetic pheromone, contributing significantly to the success of a courting male (refs. 6, 7, 8). The puzzling observation that the queen butterfly fails to produce 1 when raised on its normal food (milkweed) in a greenhouse remained a mystery until we showed that in a related East African species, *Danaus chrysippus*, adult males have to extract the pyrrolizidine alkaloid lycopsamine (2) from certain senescent plants (*Heliotropium steudneri*) in order to carry out the biosynthesis of their courtship pheromone (ref. 9). This bizarre dependence of an insect on a plant alkaloid as the biosynthetic precursor of an important signalling molecule was not easy to understand from an evolutionary viewpoint. How and why might this curious plant/insect relationship have come about?



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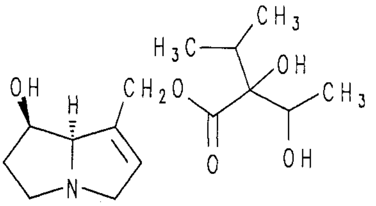

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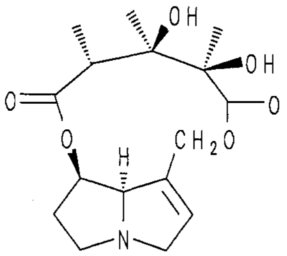

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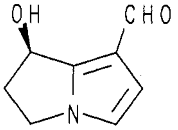

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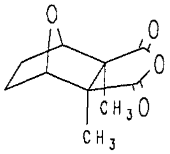

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Some insight into this question was provided by an investigation of the courtship of *Utetheisa ornatrix*, a moth whose larval food is a pyrrolizidine alkaloid producing plant, such as *Crotalaria spectabilis*. *C. spectabilis* produces the macrocyclic alkaloid monocrotaline (3). *U. ornatrix* males possess a pair of courtship organs, the coremata, which we found to carry hydroxydanaidal (4). Once more, electrophysiological and behavioral evidence demonstrates that 4 is a true courtship pheromone. Without a dietary source of monocrotaline (or some similar pyrrolizidine alkaloid), no hydroxydanaidal is produced, and success in courtship is reduced (ref. 10). In this case, the alkaloid itself was found to be sequestered by larvae of both sexes, with the result that the moths are rendered unpalatable to predators such as spiders. Most exciting was the discovery that even the eggs of *U. ornatrix* normally contain *ca.* 0.5% of monocrotaline, which acts as a potent anti-feedant for predators such as coccinellid beetles. In experiments where only the female contained 3, the male having been raised on an artifical diet, the eggs were, not surprisingly, still chemically protected. In a complementary experiment, however, females raised on an alkaloid-free diet were allowed to mate with normal males. The eggs from these matings were also largely rejected by coccinellid beetles, and were found to contain about 30% of the normal alkaloid content. From these and similar experiments, we conclude that the pyrrolizidine alkaloid ingested by larvae can be used subsequently to protect the most vulnerable stage in this moth's life cycle, the egg, and that this parental investment involves both maternal and paternal contributions (ref. 11).

Since there can be serious competition among larvae in the field for a good supply of alkaloid, the alkaloid content of individuals varies over a wide range in nature. From the point of view of a female, there would be a great advantage (chiefly to the success of her offspring) if the alkaloid titer of a courting male could be estimated, since the size of his alkaloidal "nuptial gift" is proportional to his alkaloid content. As it turns out, the hydroxydanaidal on a male's coremata provides unambiguous evidence of his success in sequestering alkaloid.

Based on these studies, a reinvestagation of the Florida queen showed that in this case, males will ingest monocrotaline (as its N-oxide) avidly. They can store roughly 60% of what they ingest. Over 60% of this alkaloid is to be found in the male's accessory reproductive glands, from which it is transferred to the female during copulation. Females, in turn, place over 90% of the received alkaloid into the eggs, with the overall result that fully one third of the monocrotaline *ingested* by a male can end up in the next generation! Since in this case females do not normally sequester alkaloid, they are dependent on the males for the protection of their eggs. A male's ability to do this is signalled by his courtship pheromone, an alkaloid metabolite. Once more, the male pheromone 1 can be viewed as a symbol of "chemical fitness" (ref. 12).

Is this finding of a chemical basis for mate selection, based on an acquired defensive compound, restricted to the exploitation of pyrrolizidine alkaloids by certain lepidoptera? It is not. Certain male pyrochroid beetles (*Neopyrochroa flabellata*) are attracted to the highly vesicant terpenoid acid anhydride, cantharidin (5). Once more, Prof. Eisner suspected that males would be found to ingest this cantharidin and that ingested cantharidin would be transferred to females during mating for incorporation into their eggs. Countless analyses showed this to be the case. Particularly striking was the observation that females sample the contents of a cephalic gland in the male forehead before mating. A male fed on cantharidin is readily accepted, while one who was not given access to cantharidin is vigorously evaded. Analysis of the secretion of the cephalic gland contents from the successful males reveals the presence of cantharidin. No cantharidin is found in males who were not fed cantharidin previously. Thus, cantharidin can be seen to act as an "aphrodisiac" pheromone for these insects.

This example of a male pheromone which is simply an acquired defensive compound is particularly interesting. It suggests that the strategy of female mate choice based on male chemical fitness must have arisen several times independently. Thus, we have seen examples involving butterflies, moths, and a beetle, with the unpalatable, defensive compound coming either from a plant or an animal source. The pheromones themselves can be as different as alkaloid metabolites and terpenes. These explorations into the chemical ecology of natural products have yielded unanticipated insights into the roles played by secondary metabolites in nature, and have raised some intriguing questions about the evolutionary relationships among plant defensive substances, insect defensive chemistry, and insect pheromones.

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