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CHEMICAL DEFENSE OF INSECT - *PHEROPSOPHUS HILARIS* (COLEOPTERA: CARABIDAE)

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ABSTRACT

Insect make up the largest and most diverse group of organisms on earth, contributing to as much as 80 – 90 % of the world's biodiversity. Approximately 950,000 species of insects have been described, some estimate there are 4,000 000 species in total. Over 70 % of drugs on the market are derived from natural compounds, however, insect are one of the least explored groups in drug discovery. A large portion of animals on earth, particularly insects, utilize chemicals as their primary tools of warfare and defense. Various strategies such as chemical defense or mimicry have evolved that protect insects from predators. Most defensive chemical in beetles are odoriferous and repugnant to humans and other animals. This *pheropsophus hilaris* is called a 'bombardier' because it ejects a hot, highly noxious spray of aqueous benzoquinones, oxygen and steam as a defense mechanisms against would be predators.

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INTRODUCTION

Among invertebrates, especially in arthropods, insects have received much attention in the study of morphology. The high biotic potential of insects makes the reproductive process a subject of importance in applied entomology. In recent years, the functional aspects of insect have attracted more attention (Adiyodid and Adiyodi, 1974). The great complexity of morphology and physiology of insects renders them an interesting group of investigation. Individually, each insect exhibits an unique way of reproduction. Physiology of insects is a complicated phenomenon and it deals with the structure and functions of various tissue components of the system. Insect behavior is considered under three more or less closely related subjects; tropisms, instincts, and intelligence. Insects, like other organisms, are continually subjected to various environmental influences to which they directly or indirectly respond. These stimuli are of chemical and physical nature and fall in various categories. The reactions to these stimuli are known as tropisms (*tropotaxes*). Since tropisms may be positively or negatively tropic. Tropisms seldom operate individually. The response to touch, Thigmotropism may involve Rheotropism the response to water currents, Anemotropism the response to air currents, or geotropism, the response to gravitation.

This study is further complicated by the fact that the reactions of insects may result from hidden or internal stimuli as well as the more obvious external stimuli. The vast majority of animals on earth are insects. Beetle's alone make up the group (Order Coleoptera) with the largest number of described species of any other group of animals on earth (Hammond 1992). A large portion of animals on earth, particularly insects, utilize chemicals as their primary tools of warfare and defense. The plethora of chemical compounds they produce for defense is similarly as vast and diverse (Blum, 1981; Dettner, 1987; Eisner, 2003; Eisner et al., 2005). Aldehydes and ketones are common functional groups used by insects for chemical defense. Aldehydes are a portion of a molecule consisting of a carbon atom double bonded to an oxygen atom and single bonded to a hydrogen atom and to another carbon atom. Aldehyde containing compounds tend to cause a burning sensation. Ketones are portions of a molecule consisting of a carbon atom double bonded to an oxygen atom and single bonded to two other carbon atoms. Carbon atoms almost always have exactly four chemical bonds attached to them. Beetles, as well as many other insects and even plants, also tend to produce monoterpenes as active components of their chemical weapons. For example, chrysomelidial produced by leaf beetles(Family Chrysomelidae) is an isomer of the double bond (Meinwald et al., 1977). Dolichodial, anisomorphal, and peruphasmal are diastereomers (compounds with the same chemical formula, but different configurations at

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one or more of the functional groups, resulting in compounds that are not mirror images) and are the active components of several walking stick insect defensive sprays (Dossey *et al.*, 2006, 2008; Eisner 1965; Meinwald *et al.* 1962), as well as some ants (Cavill *et al.*, 1976; Cavill and Whitfield, 1964; Pagnoni *et al.*, 1976). Probably the best known beetle defensive substance is cantharidin, a compound from the blister beetles (Family Meloidae) that has a long history of medicinal use. Blister beetles have a very interesting way of deploying this chemical weapon. They can spontaneously bleed through the joints in their legs a blood concoction enriched in cantharidin (Eisner, 2003; Eisner *et al.*, 2005). In some types of beetles, such as “fire-colored beetles” (Family Neophyochroa), cantharidin is actually passed from males to females during mating (Eisner *et al.*, 2005). One of the most famous chemical defense mechanisms used by a beetle is that of the Bombardier beetle, characterized by the work of Eisner and Meinwald (Eisner, 2003; Eisner *et al.*, 2005). The rather spectacular spray given off by bombardier beetles when attacked is the result of a violent chemical reaction, which occurs upon the mixture of reactants and enzymes in the animal’s defensive apparatus. Specifically, hydroquinone and hydrogen peroxide, stored ready and waiting in the beetle’s defensive reservoirs, are brought together by enzymes in a high-energy oxidation/reduction reaction to form benzoquinone water, and heat. The reaction creates temperatures up to 100°C (212°F). During the reaction, pressure builds up in the defense gland reservoir until the substance can no longer be contained (Bedford, 1975). At that point a rapid-fire series of pulsed sprays of hot toxic chemicals are deployed directly at the offending stimulus. Thus, it is the boiling of the mixture of these components and water which builds pressure and causes the explosion of toxins in the face or mouth of a predator unlucky enough to select a bombardier beetle as their next meal—or simply an unfortunate passerby who gets too close for the beetle’s comfort.

A wide array of morphological characteristics may act as defensive mechanisms (Edmunds, 1994; Devries, 1987; Evans and Schmidt, 1991; Gross, 1993; Godfray, 1994; Dyer, 1995; Eisner *et al.*, 2007), and these are generally divided into two categories: (A) caterpillar integumental processes, such as spines or hairs; (B) caterpillar coloration, such as brightly colored (aposematic) or visually cryptic. Caterpillar size or developmental rates may also influence predation (Evans, 1983; Gaston *et al.*, 1991; Montllor and Bernays, 1993). In many cases, simply being larger (i.e., later instars) may provide protection through increased effectiveness of their behavioral or physical defenses because they are larger relative to their attacker (Iwao and Wellington, 1970; Schmidt, 1974; Stamp, 1984; Martin *et al.*, 1989). Physiological defenses consist of 3 sublevels and are used primarily to combat pathogens, parasites, and parasitoids (Gillespie *et al.*, 1997; Carton *et al.*, 2008; Strand, 2008; Beckage, 2008). The 3 sublevels include: (1) integument and gut as physical barriers to infection, (2) coordinated action of several subgroups of hemocytes when physical barriers are breached and, (3) induced synthesis of antimicrobial peptides and proteins, mostly by the fat body (Gillespie *et al.*, 1997). This section focuses on the last two sublevels which compose the insect immune response, as these have been the best studied over the past two decades. The immune defense in insects is considered one of the most effective defenses against parasitoids and pathogens (Godfray, 1994; Smilanich *et al.*, 2009).

Defense is undoubtedly one of the most important factors in species life history. To prevent capture by predators, including arthropods (Wilson, 1975) have evolved many defensive behavioral tactics or morphological structures with behavioral impact on potential predators. These defenses include cryptics, mimicry, aposematism, escape tactics, or even retaliation (Alcock, 2001). Biologists have become keenly aware that insects possess a remarkable ability to biosynthesize a large variety of compounds for use as agents of chemical defense against their omnipresent enemies. Many of these compounds are unique products with diverse modes of toxicity against a variety of vertebrate and invertebrate predators. These defense secretions offer originate from unlikely sources that appear to optimize the effectiveness of the chemical defensive systems (Carlberg 1981 and Arbiser *et al.*, 2007). The tremendous abundance of insect constitutes the primary food source of diverse vertebrate and invertebrate predators (Piek, 1986). In insect a variety of orders, blunting the attacks of their omnipresent predators is identified either with the production of defensive compounds in exocrine glands or with the acquisition of these compounds from external sources. It has been generally assumed that the *novo* biosynthesis characterizes the origins of insect defensive compounds. However, recent investigations suggest that novel insect defensive allomones, including the complex amide pederin from staphylinid beetles and unique steroids from dytiscid beetles, are biosynthesized by endosymbiotes (Ajikumar *et al.*, 2008).

RESULTS AND DISCUSSION

The most famous chemical defense mechanism used by a beetle is that of the Bombardier beetle, characterized by the work of Eisner and Meinwald (Eisner, 2003; Eisner *et al.*, 2005). The rather spectacular spray given off by bombardier beetles when attacked is a result of a violent chemical reaction occurs upon the mixture of reactants and enzymes in the animal’s defensive apparatus. Specifically, hydroquinone and hydrogen peroxide, stored ready and waiting in the beetle’s defense reservoirs, are brought together by enzymes in a high-energy oxidation / reduction reaction to form benzoquinone, water and heat (Blum, 1981). The reaction creates temperature up to 100 °C (212 °F). During the reaction, pressure builds up in the defense gland reservoir until the substance can no longer be contained. At the point a rapid-fire series of pulsed sprays of hot toxic chemicals are deployed directly at the offending stimulus (Bedford, 1975). The natural component of the pygidial or defensive secretion is benzoquinones and fatty acids secreted by carabid beetles of *Pheropsophus hilaris* identified by gas chromatography, mass spectrometry and HPLC. Among the more interesting compounds produced by *Pheropsophus hilaris* are 1,4-quinioines and hydroquinones ejected explosively by members of Brachiniini. 1,4-benzoquinone and 2-methyl-1, 4-benzoquinone compound was found to be observed in *Pheropsophus hilaris* (Fig -1). This beetle secreted these substances at body temperature from the reservoir with no sound. In contrast, Brachinidae beetles have a pair of brownish reaction chamber connected with a reservoir.

Two types of fatty acid secreting beetles were found, one secreted formic acid and the other mixed short chain fatty acids. These short chain fatty acids were metabolized from some amino acids, methacrylic acid from valine; angelic acid from isoleucine; senecioic acid from leucine and crotonic acid

from lysine, formic acid from serine and glycine was observed by HPLC. The quantities of isoleucine were found to be more of about 29.9 μ moles/ml (Fig. 2 and Table. 1).

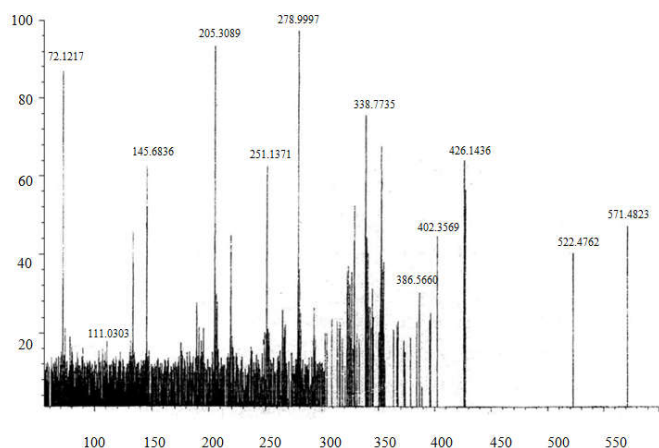


Fig 1. Mass spectra of *Pheropsophus hilaris* pygidial secretion

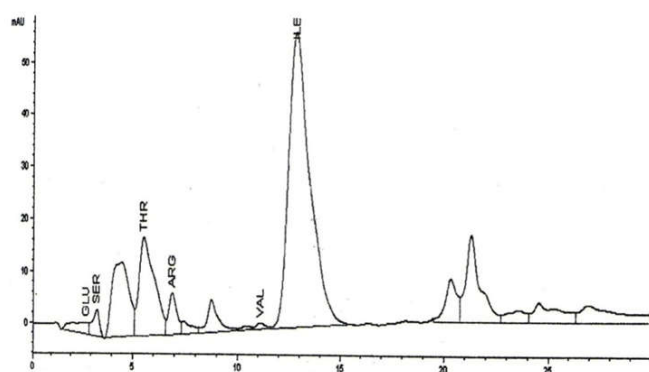


Fig. 2. HPLC spectra of amino acids in *Pheropsophus hilaris*

Table 1. Quantity and quality of amino acids in *Pheropsophus hilaris*

Amino acid level in μ moles/ml	
Amino acids	Pygidial
Aspartic acid	0.7
Glutamic acid	0.5
Serine	1.7
Histidine	0.7
Glycine	1.3
Threonine	13.3
Alanine	0.9
Arginine	3.3
Tyrosine	0.6
Valine	0.4
Methionine	0.8
Phenylalanine	5.4
Isoleucine	27.6
Leucine	7.4
Lysine	0.8

The two reactant chemical compounds, hydroquinones and hydrogen peroxide, are secreted by specialized glands and are stored in separate reservoirs in the rear tip off its abdomen. When threatened the beetle contracts muscles that open the valves of these reservoirs and force the two reactants into a thick-walled mixing chamber lined with cells that produce enzyme including catalases and peroxidases. In the mixing chamber the enzymes rapidly break down the hydrogen peroxide, releasing free oxygen, and hydrogen, and catalyze the oxidation of the hydroquinones into P-quinones (Bouchard *et al.*, 1997). The reaction is very exothermic, and are released

energy raises the temperature of the mixture to near 100°C, vaporizing about a fifth of it. The resultant pressure buildup forces the entrance from the reactant storage chamber to close, thus protecting the beetle's internal organs. The flow of reactants into the reaction chamber and subsequent ejection occur in a series of about 70 pulse, at a rate of about 500 pulses per second. The whole sequence of events takes only a fraction of a second. (Dettner, 1987). Defense chemical in prey are considered to cause some physiological damage to predators, and thus allow prey to escape from attack (Murai *et al.*, 1987). In *pheropsophus hilaris* the secretion are stored in and emitted from glands that have cuticle layers, which are isolated from other organs (Chapman, 1998). Similarly, we suggest that the reservoir and use it during predator attacks, may cause severe injury to predators.

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