

## Prostaglandin Synthetase Inhibitors in Insect Defensive Secretions

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Prostaglandins (PG) are ubiquitous metabolites of arachidonic and related fatty acids that have been isolated from nearly every type of mammalian tissue [1], from a variety of non-mammalian vertebrates [2], from insects [3] and from several marine invertebrates [4]. In mammals, prostaglandins have been implicated in nearly every major physiological function [5] and in invertebrates, prostaglandins have been shown to be involved in ion regulation [6], oviposition behavior [7] and the normal emergence and flight capabilities of a mosquito [8]. Future studies will undoubtedly show that prostaglandins regulate as wide a variety of physiological functions in invertebrates as they do in mammals.

Given that prostaglandins do play such

a vital role in the normal physiology of animals, it seemed odd to us that no animals have been reported to secrete chemicals which inhibit the biosynthesis of prostaglandins in their competitors, predators and/or parasites. We reasoned that a potential source for such inhibitors would be the defensive secretions of insects, which are noted for their quantitative abundance and qualitative complexity [9]. These secretions are frequently composed of three distinct groups of chemicals: immediate-acting irritants or toxicants, such as quinones, terpenes, and alkaloids; penetrants, surfactants, or "bio-solvents" such as alkanes, alkenes and medium-chain-length fatty acids; and a heterogeneous group of compounds for which the mode of action is usually not known.

We recently reported that the defensive secretion of the red flour beetle, *Tribolium castaneum* (Herbst), contains 2'-hydroxy-4'-methoxyacetophenone and 2'-hydroxy-4'-methoxypropiofenone and demonstrated that these  $\beta$ -hydroxy ketones were potent prostaglandin synthetase inhibitors (PSI) of both mammalian and insect-derived prostaglandin synthetases [10].

This beetle, however, is primarily a pest of human grain and flour storage facilities, and it is conceivable that its PSIs represent a unique adaptation to this specialized niche. We now report, however, that insects from at least three taxonomic orders, representing a diversity of ecological niches, also contain potent prostaglandin synthetase inhibitors in their defensive secretions. We

Table 1. Inhibition of PGE<sub>2</sub> synthesis in bovine seminal vesicle microsomes by insect defensive compounds and acetylsalicylic acid (Aspirin)

Inhibitor level [mM]	PGE <sub>2</sub> synthesis [nmol/h] $\pm$ SD (n)						
	Aspirin	Methyl anthranilate	Amino acetophenone	Methyl salicylate	Salicylaldehyde	2,5-Dihydroxy phenylacetic acid $\gamma$ -lactone*	1-Heptadecene
0	2.9 $\pm$ 0.6 (8)	2.9 $\pm$ 0.6 (8)	2.9 $\pm$ 0.6 (8)	2.9 $\pm$ 0.6 (8)	2.9 $\pm$ 0.6 (8)	2.9 $\pm$ 0.6 (8)	2.9 $\pm$ 0.6 (8)
0.1	2.1 $\pm$ 0.3 (3)	2.1 $\pm$ 0.3 (2)	2.3 $\pm$ 0.3 (3)	2.2 $\pm$ 0.1 (2)	2.2 $\pm$ 0.3 (3)	2.6 $\pm$ 0.3 (2)	2.8 $\pm$ 0.4 (3)
0.5	1.9 $\pm$ 0.3 (3)	1.9 $\pm$ 0.1 (2)	2.7 $\pm$ 0.2 (3)	1.5 $\pm$ 0.3 (2)	2.2 $\pm$ 0.3 (3)	2.0 $\pm$ 0.2 (3)	2.9 $\pm$ 0.1 (3)
2.5	1.6 $\pm$ 0.2 (3)	0.6 $\pm$ 0.1 (2)	1.9 $\pm$ 0.1 (3)	0.1 $\pm$ 0 (2)	1.4 $\pm$ 0.4 (3)	0.6 $\pm$ 0.2 (3)	2.8 $\pm$ 0.4 (3)

\* Occurs in the insect as the methyl ester rather than as the commercially available  $\gamma$ -lactone

Table 2. Inhibition of PGE<sub>2</sub> synthesis in *Periplaneta americana* fat body microsomes by insect defensive compounds and acetylsalicylic acid (Aspirin)

Inhibitor level [mM]	PGE <sub>2</sub> synthesis [pmol/h] ± SD (n)			
	Aspirin	Methyl anthranilate	Methyl salicylate	2,5-Dihydroxy phenylacetic acid $\gamma$ -lactone <sup>a</sup>
0	556.2 ± 85.8 (3)	360.4 ± 41.1 (2)	360.4 ± 41.1 (2)	360.4 ± 41.4 (2)
0.1	455.6 ± 72.5 (3)	403.3 ± 23.7 (3)	238.5 ± 3.5 (3)	380.3 ± 47.9 (3)
0.5	398.2 ± 52.9 (3)	314.6 ± 22.4 (3)	193.6 ± 47.1 (3)	262.5 ± 34.3 (3)
2.5	170.8 ± 1.5 (3)	152.5 ± 26.7 (3)	127.0 ± 20.1 (3)	27.4 ± 15.9 (3)

<sup>a</sup> Occurs in the insect as the methyl ester rather than as the commercially available  $\gamma$ -lactone

also report that these compounds are present in sufficient quantity to deliver a potentially pharmacologically significant dosage to their predators, parasites or competitors.

We have selected five aromatic compounds, which have been previously reported as major components of unknown function in insect defensive secretions, and assayed them for their ability to inhibit mammalian and insect prostaglandin synthetases. These compounds are methyl anthranilate from male ants (Hymenoptera) [11], o-aminoacetophenone from male seed bugs (Hemiptera) (Harrington and Howard, unpub.) and from male ants [11] and, methyl salicylate, 2,5-dihydroxyphenylacetic acid  $\gamma$ -lactone, and salicylaldehyde, which occur in a number of beetles (Coleoptera) [12]. In addition we assayed 1-heptadecene (a major defensive secretion component of *T. castaneum*) as a "biosolvent" control and acetylsalicylic acid (Aspirin) as a standard prostaglandin synthetase inhibitor [13]. All compounds were purchased from commercial suppliers and were at least 98% pure.

We assayed prostaglandin synthetase activity in vitro using microsomal enzyme preparations from bovine seminal vesicles and from the fat body of last instar or adult males of the American cockroach (*Periplaneta americana* L.). The details of the assay were reported earlier [10]. Briefly, lyophilized bovine microsomes were obtained from Miles Laboratories, Inc., Kankakee, Illinois, U.S.A. and 2 mg were used per assay. Cockroach fat body microsomes were prepared as described earlier [10] and 2 mg of protein was used in each assay. Protein concentrations were determined using the method in [14] with

bovine serum albumin as the standard. [5,6,8,9,11,12,14,15-<sup>3</sup>H]Arachidonic acid (1  $\mu$ Ci) (83.8 Ci/mM, New England Nuclear) was used to monitor the conversion of arachidonic acid to prostaglandin E<sub>2</sub> (PGE<sub>2</sub>). Incubations were carried out at 30 °C for 1 h. Extraction and separation of PGE<sub>2</sub> using silicic acid chromatography followed the procedure in [15].

A comparison of the ability of the six insect-derived chemicals and acetylsalicylic acid to inhibit PGE<sub>2</sub> synthesis from bovine seminal vesicles is shown in Table 1. The most potent inhibitor was methyl salicylate followed by methyl anthranilate, 2,5-dihydroxyphenylacetic acid  $\gamma$ -lactone, and salicylaldehyde. All of these compounds were as effective or more effective than acetylsalicylic acid in inhibiting the bovine PG synthetase, but were about an order of magnitude less effective than the *Tribolium* ketones [10]. The o-aminoacetophenone was a little less effective than acetylsalicylic acid, and as expected, 1-heptadecene showed no inhibition of the bovine seminal vesicle PG synthetase.

The three most active chemicals from the bovine assay were then tested against the cockroach PG synthetase (Table 2). The  $\gamma$ -lactone was a more potent inhibitor than acetylsalicylic acid, and the methyl anthranilate and methyl salicylate were comparable in potency to acetylsalicylic acid.

Since insects are relatively small organisms one must ask if the quantities of PG synthetase inhibitors in their defensive secretions are great enough to be of pharmacological importance against their competitors, predators and parasites. Individual components in insect defensive secretions typically occur in

the range of 0.1 to 100  $\mu$ g per insect. The invertebrate predators, parasites and competitors of insects are also usually relatively small, with typical body volumes of ca. 0.01 to 0.5 ml, so that exposure to microgram levels of these chemicals by either contact or ingestion could produce in them PG inhibitor concentrations of ca. 0.01 to 100 mM. If the chemicals are sequestered in particular tissues, the resulting concentration would be even higher. Since the I<sub>50</sub> values for all of the defensive components that we have assayed previously [10] and in this study ranged between ca. 0.1 and 2 mM, the quantities of PSI in insect defensive secretions would thus appear to be sufficient to interfere with those physiological functions of the target organism that involve prostaglandins. What such an interference means in ecological terms is not yet clear, however.

We have examined only a small proportion of the types of compounds known to occur in insect defensive secretions. We chose the five aromatic hydroxy or amino carbonyl compounds for this study on the basis of their resemblance to the two *Tribolium* compounds and to acetylsalicylic acid. The consistent effectiveness of the seven compounds that we have examined in specifically inhibiting both mammalian and insect-derived prostaglandin synthetases suggests that other similar aromatic hydroxy- or amino-substituted aldehydes, ketones, esters, and acids are also likely to be potent prostaglandin synthetase inhibitors, either directly or after metabolic activation [16]. We recognize of course that these compounds can serve other functions as well, including that of being semiochemicals. We note that a wide variety of other chemical structures related to natural products have been shown by pharmacologists to be excellent PG synthetase inhibitors [17], suggesting that other novel inhibitors remain to be discovered in the vast repertoire of insect exocrine secretions. Studies are now underway in our laboratories to identify such chemicals, to elucidate their ecological roles and to further clarify the physiological functions of prostaglandins in insects.

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