

PROSTAGLANDIN SYNTHETASE INHIBITORS IN THE DEFENSIVE SECRETION OF THE RED FLOUR BEETLE *TRIBOLIUM CASTANEUM* (HERBST) (COLEOPTERA:TENEBRIONIDAE)

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Abstract—The defensive secretion of the red flour beetle, *Tribolium castaneum* (Herbst) contains two aromatic hydroxy ketones which inhibit the conversion of arachidonic acid into prostaglandin E_2 by prostaglandin synthetases of both mammalian and insect origin. Gas chromatographic analyses of secretions from individual beetles indicated that the major ketone component, 2'-hydroxy-4'-methoxypropiofenone occurs at 1–10 μg per beetle levels, and that the minor ketone component, 2'-hydroxy-4'-methoxyacetophenone (paeonol) occurs at 0.1–1 μg per beetle levels. To our knowledge this is the first report of exogenously released prostaglandin synthetase inhibitors derived from animal sources.

Key Word Index: Prostaglandins, mass spectrometry, prostaglandin synthetase inhibitors, defense secretion, chemical ecology

INTRODUCTION

Insect communities are characterized by populations that compete in space and time. A prominent feature of such competition is the utilization of chemical defenses against parasites, predators and microbial pathogens (Blum, 1981). A relatively unexplored facet of chemical ecology, however, is the possible involvement of defensive secretions in the regulation of community dynamics by intra- or interspecific interference with critical hormonal or other physiological processes. One such group of physiologically important biochemicals is prostaglandins. They have been reported from a number of insect species (Brady, 1983), and as in mammals (Samuelsson *et al.*, 1978) they are proving to be important mediators of a wide variety of physiological processes that govern growth, development and reproduction. We now report that the defensive secretion of *T. castaneum* contains two aromatic ketones, 2'-hydroxy-4'-methoxyacetophenone (paeonol) (1) and a previously reported homologue, 2'-hydroxy-4'-methoxypropiofenone (2) (Suzuki *et al.*, 1975b), which inhibit the conversion of arachidonic acid into prostaglandin E_2 (PGE_2) by prostaglandin synthetases of both mammalian and insect origin. To our knowledge this is the first report of exogenously released prostaglandin synthetase inhibitors (PGI) derived from animal sources.

MATERIALS AND METHODS

Insects

Beetles (*T. castaneum*, Sooty strain) were originally ob-

tained from the *Tribolium* Stock Center, Department of Biology, University of California, San Bernardino, California, U.S.A., and have been in culture at Manhattan, Kansas, U.S.A., for about 50 generations. Cockroaches, *Periplaneta americana* L., were reared in 120 l metal containers held at room temperature and were fed dog-chow and water *ad libitum*.

Isolation and structure elucidation

Beetles were chilled to 0°C causing them to discharge their defense secretions. Secretions from individual beetles were dissolved in hexane and analysed by capillary gas chromatography (GC)-electron impact mass spectrometry (EI-MS) on a Hewlett-Packard 5790A GC containing a bonded phase 30 m non-polar DB-1 capillary column, and interfaced to a Hewlett-Packard 5790 mass selective detector*. Infra-red spectra were obtained as thin films on KBr plates in a Perkin-Elmer Model 467 Grating Infra-red Spectrophotometer.

Enzyme assays

Prostaglandin synthetase activity in the presence or absence of 1, 2, or aspirin (acetylsalicylic acid) was assayed *in vitro* using microsomal enzyme preparations from bovine seminal vesicles and from the fat body of the American cockroach. The assay is a modification of Yoshimoto *et al.* (1970).

Incubations were performed in 1.8 ml of 50 mM KH_2PO_4 , pH 8.0, containing 2.4 mM reduced glutathione, 0.25 mM hydroquinone and 25 $\mu\text{g}/\text{ml}$ hemoglobin. Arachidonic acid was added at 5.5 μM and 1 μCi of [5,6,8,9,11,12,14,15-³H]arachidonic acid (83.8 Ci/mmol, New England Nuclear) was added to monitor the conversion of arachidonic acid to PGE_2 . Inhibitors dissolved in 100% ethanol were added at the indicated concentrations. Lyophilized microsomes of bovine seminal vesicles were purchased from Miles Laboratories Inc., Kankakee, Illinois, U.S.A., and 2 mg were used per assay. Prostaglandin synthetase of cockroach fat body was prepared by dissecting fat bodies from adult males and homogenizing in ice-cold phosphate buffer (50 mM potassium phosphate with 1 mM EDTA, pH 8.0). The homogenate was centrifuged at 200 g for 10 min and the supernatant

*Mention of a proprietary product in this paper does not imply its approval by the USDA or the exclusion of other products that may also be suitable.

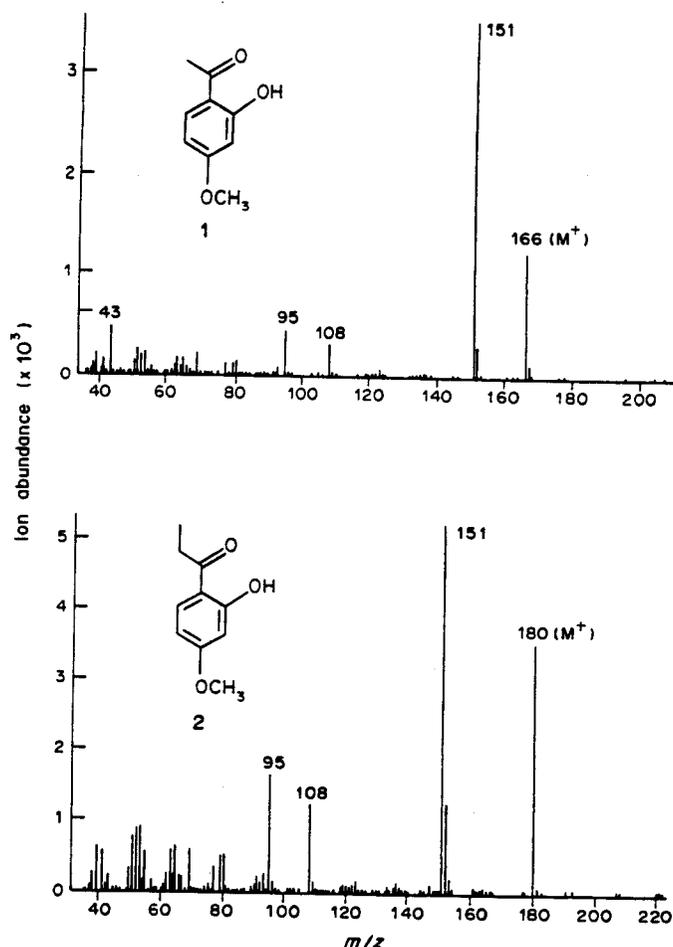


Fig. 1. EI-MS of paeonol (1) and 2'-hydroxy-4'-methoxypropiophenone (2).

was recentrifuged at 11,400 *g* for 15 min. The 11,400 *g* pellet was discarded and 10 mg of supernatant protein was used in the assay. Protein concentrations were determined using the Lowry method (Lowry *et al.*, 1951) with bovine serum albumin as the standard. Incubations were carried out at 30°C and after 1 hr the reaction was stopped by addition of 1 ml 0.1 N HCl and extracted three times with 3 ml ethyl acetate. The ethyl acetate extract was concentrated under a stream of nitrogen and prostaglandins separated using silicic acid chromatography by a modification of the procedure of Caldwell *et al.* (1971). The amount of radioactivity in the PGE₂ fraction was measured by liquid scintillation counting. The rate of production of PGE₂ was determined using the percent conversion of [³H]arachidonic acid into [³H]PGE₂. A boiled protein control was included with all experiments to determine the amount of auto-oxidation of arachidonic acid and the non-enzymatic conversion of 20:4 into PGE₂. This control figure was subtracted from the results of each assay. Fifty percent inhibition values were estimated using linear regression techniques.

RESULTS

Identification of ketones

In addition to 2-methylbenzoquinone, 2-ethylbenzoquinone, and the 15- and 17-carbon olefins (Happ, 1968; Suzuki *et al.*, 1975a), the defense secretion of *T. castaneum* contains aromatic ketones 1 and 2 at approx. 0.1–1 and 1–10 μg levels, respectively.

Mass spectral data indicated a molecular formula of C₉H₁₀O₃ for 1 and C₁₀H₁₂O₃ for 2. Both had prominent molecular ions and their base peaks were at M-15 and M-29 respectively (Fig. 1). The similarity of their mass spectra at *m/z* below the base peak suggested that the two compounds differed only in the side chain. Infra-red spectral data for 2 contained diagnostic absorbance bands at 2850 cm⁻¹ (aromatic OCH₃), 1630 cm⁻¹ (ketone strongly chelated with —OH) and at 1585, 1510, 840 and 805 cm⁻¹ (1,2,4-trisubstituted phenyl). Both 1 and 2 readily reacted with 1,1-dimethylhydrazine (McDaniel and Howard, 1985) to yield the corresponding hydrazones, which were characterized by EI-MS. The structure of paeonol (1) was confirmed by direct capillary GC-MS comparison to a commercially available standard (Aldrich Chemical Co., Milwaukee, Wisconsin, U.S.A.). Compound 2 was synthesized from 2-hydroxy-4-methoxybenzaldehyde via a Grignard reaction with ethyl magnesium bromide, and subsequent Jones oxidation with CrO₃ of the resulting secondary alcohol. Synthetic 2 had identical chromatographic and spectral properties as 2 from the insects and agreed closely with the properties reported by Suzuki *et al.* (1975b). We also examined 2'-hydroxy-5'-methoxyacetophenone and 2'-hydroxy-5'-methoxypropiophenone, the other possible

Table 1. 2'-Hydroxy-4'-methoxyacetophenone (1) and 2'-hydroxy-4'-methoxypropiofenone (2) and aspirin inhibition of PGE₂ synthesis from arachidonic acid by bovine and American cockroach prostaglandin synthetases

Level of inhibitor	Synthesis of PGE ₂ per hour ± SD (n)		Aspirin level (mM)	Aspirin
	1	2		
μM	Bovine (nmol/hr)			
0	3.2 ± 0.4 (5)	3.2 ± 0.4 (5)	0	3.2 ± 0.4 (5)
20	2.7 ± 0.5 (2)	2.3 ± 0.4 (2)	0.5	2.5 ± 0.2 (2)
60	2.0 ± 0.7 (3)	1.8 ± 0.4 (2)	1.5	1.6 ± 0.4 (2)
150	1.1 ± 0.7 (3)	1.1 ± 0.4 (2)	2.5	1.1 ± 0.2 (2)
mM	Cockroach (pmol/hr)			
0	126.0 ± 29.0 (6)	126.0 ± 29.0 (6)		280.3 ± 13.7 (2)
0.5	75.2 ± 31.3 (2)	59.9 ± 27.9 (4)		Not determined
1.5	70.0 ± 4.3 (3)	42.3 ± 21.2 (4)		71.8 ± 27.9 (3)
2.5	27.5 ± 17.6 (3)	31.7 ± 22.5 (4)		48.8 ± 16.6 (3)
10.0	0.0 (2)	Not determined		15.5 ± 7.0 (3)

1,2,4-trisubstituted phenyl compounds, and showed that they differed significantly in both retention time and mass spectral data from the natural products.

Inhibition of PG synthesis

All three compounds tested inhibited prostaglandin production in an *in vitro* assay using microsomal enzyme preparations from bovine seminal vesicles and from the fat body of the American cockroach. The results (Table 1) show that paeonol and 2 are about 15 times more effective than aspirin in inhibiting PGE₂ production in the mammalian system and are equal in activity to aspirin in the insect system. Under the assay conditions used, aspirin gave 50% inhibition of bovine prostaglandin synthetase at about 1.5 mM whereas both 1 and 2 gave 50% inhibition at about 100 μM . All three compounds inhibited the cockroach prostaglandin synthetase at similar concentrations (50% inhibition at about 1.5 mM).

DISCUSSION

Tribolium castaneum is typical of a wide variety of arthropods that possess large defense glands from which they discharge under appropriate stimuli copious quantities of *p*-benzoquinones admixed with aliphatic hydrocarbons. These quinones and hydrocarbons have been postulated to function as repellants, irritants, toxicants and as anti-microbial and fungistatic agents (Blum, 1981). The finding of prostaglandin synthetase inhibitors in these defensive secretions represents a totally new, and rather novel, function for these secretions.

Paeonol (1) has previously been found in the Chinese medicinal plant *Paeonia suffruticosa* (Miyazawa *et al.*, 1983), and has been reported to be a potent inhibitor of cyclooxygenase and lipoygenase reaction products from rat peritoneal macrophages (Tahara *et al.*, 1983) and human platelets (Hirai *et al.*, 1983), and to enhance cadmium-induced intratesticular hemorrhage in the rat testis (Sajiki *et al.*, 1984). Compound 2 is known as a natural product only from flour beetles (Suzuki *et al.*, 1975b). These workers isolated 2 from a large number of pooled insects rather than from individual beetles, and assigned no biological function to it other than possible repellancy to other flour beetles.

We chose aspirin as a PGI standard of comparison

because its mode of action is relatively well established (Tomlinson *et al.*, 1972; Mizuno *et al.*, 1982) and because it has been utilized as a standard by most other prostaglandin researchers. The range of apparent potency found by us between aspirin and the beetle derived ketones 1 and 2 is entirely consistent with other reports of marked variation in apparent potency of synthetic aspirin analogues between test organisms (Ferreira and Vane, 1974).

The ecological significance of these potent PGIs in the defensive secretion of *T. castaneum* is still unknown. These compounds are released into the insects environment (Howard, unpublished data), and as such have the potential to function so as to alter the population dynamics of either intra- or interspecific competitors. A test of this hypothesis is now in progress.

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