

## CHEMISTRY OF DEFENSIVE SECRETIONS OF BOMBARDIER BEETLES (BRACHININI, METRIINI, OZAENINI, PAUSSINI)\*

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(Received 25 May 1977)

**Abstract**—Data are given on the chemical composition of the defensive secretions of species of carabids of the genera *Brachinus*, *Stenaptinus*, *Metrius*, *Goniotropis*, *Pachyteles*, *Ozaena*, and *Homopterus*. All produce quinonoid secretions, containing from one to three of four different benzoquinones (1,4-benzoquinone; 2-methyl-1,4-benzoquinone; 2,3-dimethyl-1,4-benzoquinone; 2-methyl-3-methoxy-1,4-benzoquinone). In most species the secretions also contain *n*-alkanes (tridecane, tetradecane, pentadecane).

### INTRODUCTION

ONE OF the most remarkable defense mechanisms of arthropods is that of the so-called bombardier beetles (Carabidae, Brachinini). These insects respond to disturbance by ejecting a quinonoid secretion from a pair of glands that open at the tip of the abdomen (SCHILDKNECHT, 1957). They aim their discharge by revolving the abdominal tip and direct their spray with accuracy toward any region of the body subjected to assault (EISNER, 1958). The quinones in the secretion are generated explosively by oxidation of hydroquinones, which are stored together with hydrogen peroxide in the major chamber of the glands and are mixed at the moment of discharge with catalases and peroxidases from an accessory chamber (SCHILDKNECHT and HOLOUBEK, 1961). The formation of quinones is accompanied by liberation of heat, with the result that the spray is ejected at the astonishingly high temperature of 100°C (ANESHANSLEY *et al.*, 1969). An audible detonation accompanies the discharge. The spray is an effective deterrent to predators (EISNER, 1958; EISNER and DEAN, 1976).

It has been known for some time that certain Carabidae other than Brachinini, of the subfamily Pausinae, also crepitate when disturbed, and that the secretion they emit tans human skin (PÉRINGUEY, 1838; KIRBY and SPENCE, 1956), as benzoquinones generally do. The Pausinae are an interesting subfamily, comprising free-living forms (Ozaenini) and myrmecophilous species (Protopaussini, Paussini). That they do

indeed produce benzoquinones has been confirmed (MOORE and WALLBANK, 1968; ANESHANSLEY *et al.*, 1969; SCHILDKNECHT and KOOB, 1969).





We have recently found that a beetle of yet another carabid subfamily—*Metrius contractus*, the only American species of the Metriinae—also discharges quinone audibly and hence qualifies as a bombardier. This finding, plus the fact that we have had the good fortune of being able to secure live for studies in our laboratories not only *Metrius* but also several species of the Brachinini and Ozaenini and one of the Paussini, prompted us to undertake a comparative study of the defense mechanism of bombardiers. Our approach was multifold, and involved a study of the behavior of the beetles, the chemistry and thermal properties of the secretions, the biochemistry and acoustical concomitants of the discharge mechanisms, and the morphology and mode of operation of the glands. In this first of several papers on this comparative study, we present our findings on the chemistry of the secretions.

### MATERIALS AND METHODS

The beetles studied are listed by subfamilial and tribal assignment (in accord with BALL, 1968) in Table 1. The table also gives the geographical source of the beetles and the numbers of specimens that were "milked" to provide secretion. *Metrius contractus* was identified by Dr. John Doyen (University of California, Berkeley). The other species were identified by Dr. Terry L. Erwin (National Museum of Natural History, Smithsonian Institution, Washington, D.C.). One species, *Pachyteles* n. sp. proved to be undescribed. It corresponds to species No. 24 of Dr. Erwin's

\* Paper No. 57 of the series *Defense Mechanisms of Arthropods*. Paper No. 56 is Meinwald J. *et al.*, *Proc. nat. Acad. Sci. U.S.A.* (in press).

Table 1. Relative percentages of 1,4-benzoquinones and *n*-alkanes in defensive secretions of bombardier beetles. Means and ranges are given by height of rectangles and vertical bars respectively. Concentrations below 10% are given by transverse bars with means indicated numerically. The number (*N*) of beetles analyzed per species is given in the corresponding genus-species box. Over 96% of total secretion is accounted for by the sum of relative percentages given, except in *Metrius* where the total is 84% (see text)

Subfamily	CARABINAE		METRIINAE		PAUSSINAE			Relative %
	Tribe Brachinini		Tribe Metriini		Tribe Ozaenini		Tribe Paussini	
Genus-species	A	A	B	C	D	D	D	
	<i>Brachinus quadrigemus</i>	<i>Brachinus subbevis</i>	<i>Stenopellus insignis</i>	<i>Metrius confusus</i>	<i>Gonolopsis nigroaeneus</i>	<i>Pachyletes</i> n. sp.	<i>Ozaena magna</i>	<i>Hormoterus arrowi</i>
	N = 3	N = 2	N = 9	N = 5	N = 9	N = 2	N = 1	N = 1
						5.5		
								
	0.8	0.9			3.7	1.4	9.2	
								
$\text{CH}_3(\text{CH}_2)_{13}\text{CH}_3$		0.3					7.4	
$\text{CH}_3(\text{CH}_2)_{12}\text{CH}_3$				1.2	0.5	0.6		
$\text{CH}_3(\text{CH}_2)_{11}\text{CH}_3$				0.3	0.02			

Geographical source of beetles is given by letters in genus-species box, as follows: A: Lake Placid, Highlands County, Florida; B: Nyeri, Nyeri District, Kenya; C: Blodgett Experimental Forest, Eldorado County, California; D: Barro Colorado Island, Canal Zone.

Central American series in the National Museum collection.

All beetles were shipped live to our Cornell laboratories. The animals were maintained singly or in groups within small cages provided with moist sand and strips of paper toweling. They were fed daily on pieces of freshly killed mealworms (larvae of *Tenebrio molitor*) or cockroaches (*Nauphoeta cinerea*). Except for the single paussine, which was moribund on arrival, all beetles took well to captivity, surviving for months in most instances and for over two years in some.

The animals were individually milked of secretion by seizing their bodies in forceps and pinching their legs with a second pair of forceps until they discharged. Pieces of filter paper held against the gland openings at the rear of the beetles served to trap the secretion. The papers were promptly transferred to

chilled vials ( $-78^\circ\text{C}$ ) for brief storage until extracted with ether for analysis.

No discharge could be prompted from the moribund paussine. Because previous anatomical work on paussines (EISNER and ALSOP, D. W., unpublished) had shown that the glands of these beetles are 2-chambered devices comparable to those of Metriini and Ozaenini, in which hydroquinones and hydrogen peroxide are stored in one chamber and appropriate enzymes for hydroquinone-quinone conversion in the other (essentially as in Brachinini) (EISNER and JONES, unpublished), it was assumed that explosive secretory production might be artificially elicited from the paussine glands simply by mixing the contents of their two chambers. This proved feasible. The glands were excised from the paussine, transferred to a porcelain depression dish, and teased open. As soon as the contents of the two gland chambers came into contact,

there was an intense bubbling (hydrogen peroxide decomposition) and a darkening of the fluid (quinone production). The fluid was soaked up in filter paper and immediately extracted (ether) and analyzed.

Gas chromatographic analyses were carried out with a Varian 2100 gas chromatograph equipped with a flame ionization detector using a 2.4 m × 2 mm glass column packed with 5% OV-1 on Gaschrom Q. Mass spectra were obtained using the same column in a Finnigan model 3300 gas chromatograph/mass spectrometer coupled with a System Industries model 150 computer.

Relative amounts of components were obtained using a Spectra-Physics Autolab Minigrator in conjunction with the Varian 2100, except in the case of the paussine in which the relative amounts of components were estimated from the reconstructed gas chromatogram from the Systems Industries 150 computer.

2,3-Dimethyl-1,4-benzoquinone, 2-methoxy-3-methyl-1,4-benzoquinone, *n*-tridecane, *n*-tetradecane, and *n*-pentadecane were identified on the basis of direct gas chromatographic and mass spectral comparisons with authentic samples. 1,4-Benzoquinone and 2-methyl-1,4-benzoquinone were identified by their mass spectra, which were indistinguishable from those published (STENHAGEN *et al.*, 1974).

## RESULTS AND DISCUSSION

As is clear from the results (Table 1), the secretions produced by the various bombardiers are remarkably similar from a chemical viewpoint. All produce 1,4-benzoquinones, accompanied in all cases except two (*Stenaptinus*, *Homopterus*) by *n*-alkanes. Given the anomalous procedure by which the *Homopterus* secretion was collected, some question remains whether the absence of *n*-alkanes in this species is real (the filter paper soaked with the aqueous secretory contents of the excised glands may not have picked up the hydrophobic hydrocarbons). In the case of *Metrius*, two minor components were not characterized: one of these seemed to be a heptadecadiene from its mass spectra (EI and CI), but the amount available was insufficient for complete characterization.

Our findings are generally in line with work previously done by others on bombardiers. Members of the Brachinini, including species of *Brachinus* and *Pheropsophus*, were previously shown to produce quinones (SCHILDKNECHT and HOLOUBEK, 1961; MOORE and WALLBANK, 1968), although neither 2,3-dimethyl-1,4-benzoquinone nor *n*-alkanes were detected in these species. 1,4-Benzoquinones have also been reported from a European paussine (SCHILDKNECHT and KOOB, 1969), as well as from an Australian protopaussine and an ozaenine (MOORE and WALLBANK, 1968), but *n*-alkanes were undetected, and the quinones did not include 2,3-dimethyl-1,4-benzoquinone or 2-methyl-3-methoxy-1,4-benzoquinone. No metriines had previously been studied chemically.

On the basis of chemical evidence alone, it is clearly impossible to make assertions regarding the possible phyletic relationships of the various bombardiers. No one would question the intrinsic affinities of species within any one group. Thus, although *Stenaptinus* and *Brachinus* have somewhat different secretions, there can be no doubt that their inclusion in the same tribe is taxonomically justified (ERWIN, 1970). Even the chemical differences between *Homopterus* and the Ozaenini cannot be taken as indicative of a major phyletic gap, since the close relationship of Paussini and Ozaenini, implicit in their inclusion in the same subfamily, is well established (DARLINGTON, 1950). The problem arises when one attempts to relate the Brachinini and the subfamilies Metriinae and Paussinae to one another. Although it has been argued (MOORE and WALLBANK, 1968), on the basis of Jeanne's (1940-41) taxonomic criteria, that the Paussinae and Brachinini must have evolved their 2-chambered bombarding apparatuses independently, our own morphological evidence (EISNER and ALSOP, D. W., unpublished) suggests that this may not be so. In fact, it appears certain that the glandular apparatuses of Paussinae and Metriinae are homologous (EISNER and ALSOP, D. W., unpublished), and possible also that they were acquired evolutionarily from the same ancestor that also gave rise (*via* the Crepidogastini or a crepidogastrine-like stage) to the Brachinini (EISNER, ERWIN, T. L., and ALSOP, D. W., unpublished). Evidence in support of this view will be presented in our subsequent papers on the comparative biology of bombardier beetles.

*Acknowledgements*—Study supported in part by the National Institutes of Health (Grant Nos. AI-02908 and AI-12020, and Fellowship No. 1-F32-CA05139 to T. H. J.), the National Science Foundation (Grant No. PCM-15084), and Hatch project NY(C) 191405. We thank Dr. JOHN DOYEN and Dr. GLENN PRESTWICH for providing, respectively, the live *Metrius* and the *Stenaptinus*. The *Brachinus* were collected on the grounds of the Archbold Biological Station, Lake Placid, Florida. We thank the personnel of the Station for help and hospitality.

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