

DEFENSIVE SECRETIONS OF TIGER BEETLES: CYANOGENETIC BASIS

MURRAY S. BLUM,¹ TAPPEY H. JONES,¹ GARFIELD J. HOUSE¹
and WÄLTER R. TSCHINKEL²

¹Department of Entomology, University of Georgia, Athens,
Georgia 30602, U.S.A. and ²Department of Biological Science,
Florida State University, Tallahassee, FL 32306, U.S.A

(Received 28 November 1980)

Abstract—1. The defensive secretion of the tiger beetle *Megacephala virginica* contains benzaldehyde, HCN, and mandelonitrile.

2. The significance of convergent biochemical evolution in the Arthropoda for the production of benzaldehyde and HCN by cyanogenesis is discussed.

INTRODUCTION

Among the insects, carabid beetles have proven to be an especially rich source of natural products that are utilized in defensive contexts. The pygidial glands of the Carabidae synthesize a wide variety of defensive allomones that include aliphatic acids, hydrocarbons, esters, ketones, cresols, and quinones (Moore & Wallbank, 1968; Schildknecht *et al.*, 1968; Moore, 1980). The tiger beetles, members of the subfamily Cicindelinae, are particularly distinctive in producing benzaldehyde (Moore & Brown, 1971; Moore, 1980), a compound that has not been detected as a glandular product of species in any of the other subfamilies analyzed. In the present paper we report that in tiger beetles benzaldehyde is derived from the same apparent cyanogenic precursor that has been independently evolved by arthropods in a variety of disparate taxa.

MATERIALS AND METHODS

Adult beetles, *Megacephala virginica* and *M. carolina*, were collected in Athens, GA and after freezing, the pygidial gland reservoirs were dissected and placed directly in methylene chloride. Extracts were analyzed by gas chromatography-mass spectrometry on a LKB 2091 instrument utilizing a 2.0 m column of 3% OV-1 programmed from 60–220°C. The presence of HCN was qualitatively determined by holding beetles over moist filter papers impregnated with copper acetate-benzidine acetate (Feigl, 1966).

RESULT

The pygidial gland reservoirs of both species are well developed and rather typical of these structures as described for other carabid species (Forsyth, 1972).

Two compounds were detected by gas chromatographic analysis of glandular extracts of *M. virginica*, the earlier eluting of which accounted for more than 90% of the observed volatiles. A molecular ion at m/z 106 and characteristic fragments at m/z 105, 77, and 51 established the identity of this compound as benzaldehyde. In GC analyses of glandular extracts of *M. carolina* this was the only compound detected.

The minor constituent present in *M. virginica* extracts possessed a molecular ion at m/z 133 and a base peak at m/z 77. Strong fragments were also present at m/z 106 and 105, and good signals were detected at m/z 132, 78, 51 and 50. The mass spectrum of this compound was completely congruent with that of mandelonitrile. This compound, which is very unstable, could only be detected in initial analyses of the extracts of *M. virginica*.

The presence of HCN in the secretions of both species was demonstrated by the immediate appearance of a strong blue color in copper acetate-benzidine acetate treated papers placed under beetles that were actively discharging their pygidial gland products.

DISCUSSION

Benzaldehyde appears to be a characteristic defensive product of carabid beetles in the genus *Megacephala*, having been previously identified in three Australian species (Moore & Brown, 1971; Moore, 1980), in addition to the two reported here. Significantly, this aromatic aldehyde has only been identified in the secretions of species in the subfamily Cicindelinae, notwithstanding the fact that over 200 species representing 60% of the carabid subfamilies have been examined (Moore, 1980). The rarity of benzaldehyde as a carabid defensive compound is probably correlated with the lack of a cyanogenetic pathway for producing this compound in members of other subfamilies.

Benzaldehyde has a haphazard distribution in the defensive secretions of arthropods and in every case in which these exudates have been carefully analyzed, it is accompanied by HCN. In arthropods, the defensive duet of benzaldehyde and HCN has been demonstrated to have its ultimate origin from cyanogenetic precursors, principally mandelonitrile. In polydesmoid millipedes this cyanogen is derived from aromatic amino acids (Duffey *et al.*, 1974), and it will not prove surprising if this is the case for arthropods in other taxa as well. Cyanogenesis, resulting in the secretion of benzaldehyde-HCN, has been detected in geophilid centipedes (Jones *et al.*, 1976), larvae of

chrysomelid beetles (Moore, 1967), and a large variety of polydesmoid millipedes (Conner *et al.*, 1977; Duffey *et al.*, 1977). In the cases of the centipedes, millipedes, and the carabid beetles described in the present paper, the presence of mandelonitrile in the defensive exudates is consistent with the conclusion that the arthropods in these three orders have independently evolved a common cyanogen from which their major defensive products are derived. This does not necessarily imply that the absolute configurations of the mandelonitrile produced by the species in these three arthropod taxa are identical.

The spotty distribution of benzaldehyde in the defensive secretions of arthropods may indicate that this compound is not easily produced by these invertebrates. Since cyanogenesis constitutes its only known means of derivation in the defensive secretions of arthropods, in the absence of other demonstrable biosynthetic evidence, it may well be that benzaldehyde cannot be readily produced by arthropods unless they have evolved a pathway for generating a precursory cyanogen. The instability of mandelonitrile militates against its ready detection and, since HCN can also be easily overlooked utilizing gas chromatographic detection methods, the presence of benzaldehyde in a defensive exudate in the apparent absence of these compounds may not necessarily indicate that they were not present. If benzaldehyde is subsequently detected as a glandular product of arthropods in other taxa, it would seem highly desirable to check for the presence of HCN and a cyanogen in order to further establish the distributional limits of cyanogenesis.

Acknowledgements—We thank Dr H. M. Fales, Laboratory of Chemistry, National Heart, Lung, and Blood Institute for providing GC-MS facilities.

REFERENCES

- CONNER W. E., JONES T. H., EISNER T. & MEINWALD J. (1977) Benzoyl cyanide in the defensive secretion of polydesmoid millipedes. *Experientia* **33**, 206–207.
- DUFFEY S. S., UNDERHILL E. W. & TOWERS G. H. N. (1974) Intermediates in the biosynthesis of HCN and benzaldehyde by a polydesmoid millipede, *Harpaphe haydeniana* (Wood). *Comp. Biochem. Physiol.* **43B**, 753–776.
- DUFFEY S. S., BLUM M. S., FALES H. M., EVANS S. L., RONCADORI R. W., TIEMANN D. L. & NAKAGAWA Y. (1977) Benzoyl cyanide and mandelonitrile benzoate in the defensive secretions of millipedes. *J. chem. Ecol.* **3**, 101–113.
- FEIGL F. (1966) *Spot Tests in Organic Analysis*, pp. 546–547. Elsevier, New York.
- FORSYTH D. J. (1972) The structure of the pygidial glands of Carabidae. *Trans. zool. Soc. Lond.* **32**, 249–309.
- JONES T. H., MEINWALD J., HICKS K. & EISNER T. (1977) Benzoyl cyanide and mandelonitrile in the cyanogenetic secretion of a centipede. *J. chem. Ecol.* **2**, 421–429.
- MOORE B. P. (1967) Hydrogen cyanide in the defensive secretions of larval *Paropsini* (Coleoptera:Chrysomelidae). *J. Aust. ent. Soc.* **6**, 36–38.
- MOORE B. P. (1980) Chemical defense in carabids and its bearing on phylogeny. In *Carabid Beetles, Their Evolution, Natural History and Classification* (Edited by ERWIN T. L., BALL G. E., WHITEHEAD D. R. & HALPERN A. L.), pp. 193–203. Proc. 1st Intern. Symp. Carabidology, The Hague.
- MOORE B. P. & BROWN W. V. (1971) Benzaldehyde in the defensive secretion of a tiger beetle (Coleoptera:Carabidae). *J. Aust. ent. Soc.* **10**, 142–143.
- MOORE B. P. & WALLBANK B. E. (1968) Chemical composition of the defensive secretion in carabid beetles and its importance as a taxonomic character. *Proc. R. ent. Soc. Lond. (b)* **37**, 62–72.
- SCHILDKNECHT H., WINKLER H. & MASCHWITZ U. (1968) Vergleichend chemische Untersuchungen der Inhaltsstoffe der Pygidialwehrrblasen von Carabiden. *Z. Naturf.* **23**, 637–644.