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PRECOCCINELLINE AND RELATED ALKALOIDS IN THE AUSTRALIAN SOLDIER BEETLE, *CHAULIOGNATHUS PULCHELLUS* (COLEOPTERA: CANTHARIDAE)

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Abstract—The alkaloids precoccinelline (II), hippodamine (III) and propyleine (IV), previously known only from Coccinellidae, have been identified as components of the defensive secretion of *Chauliognathus pulchellus*. This secretion also contains (Z)-8-dihydromatricaria acid (I), which has already been reported from another species of the genus.

Key Word Index: Defense secretion, *Chauliognathus pulchellus*, (Z)-8-dihydromatricaria acid, precoccinelline, hippodamine, propyleine

INTRODUCTION

THE APOSEMATIC soldier beetle *Chauliognathus pulchellus* (Macleay) is widespread in eastern temperate Australia and is often very abundant, locally. The diurnal adults are active throughout the summer and are often attracted in large numbers to blossom of both native and exotic trees and shrubs. In some years they may so dominate certain nectar sources that honeybees are virtually excluded with, presumably, an adverse effect on honey yields. At night the beetles roost in dense aggregations on grass stems and other low supports beneath the blossom and, when this occurs in closely settled areas, they can become a minor physical nuisance.

C. pulchellus is evidently distasteful to most predators and like others of its genus, it possesses a series of defensive glands in the prothorax and the first to eighth abdominal segments. When roughly handled the beetles exude small drops of a milky secretion from the orifices of these glands, at the lateral margins of the individual segments. A similar mechanism has been described in the American *C. lecontei* Champion by MEINWALD *et al.* (1968), who identified (Z)-8-dihydromatricaria acid (I) as an active component of the secretion. We now report the results of a parallel study of the above Australian species, which indicate that this substance is likewise present but is accompanied by precoccinelline and related bases that have hitherto been confirmed only in beetles of the family Coccinellidae (PASTEELS *et al.*, 1973)*.

MATERIALS AND METHODS

Gas chromatography and mass spectrometry

Gas chromatography (G.C.) was conducted on a Varian 2100 instrument with a Hewlett-Packard 3370A digital integrator, and with flame ionisation detectors and a nitrogen flow of 25 ml/min. Alternatively a Hall electrolytic conductivity detector operated in the reducing mode for nitrogen was used, with a helium flow of 50 ml/min, hydrogen flow of 40 ml/min and a solvent flow (1:1, v/v water:propanol) of 0.5 ml/min; nickel wire was the catalyst and the operating temperature was 900°C. The following glass columns were used: column 1—4 m × 3 mm i.d. of 6% (w/w) Carbowax-20 M/2% (w/w) potassium hydroxide on Gas-Chrom Z, column 2—8 m × 2 mm i.d. of Carbowax—20 M (w/w)/2% potassium hydroxide (w/w) on Gas-Chrom Z, column 3—2 m × 3 mm i.d. of 5% (w/w) phenyldiethanolamine succinate on Gas-Chrom Z, column 4—2 m × 3 mm i.d. of 5% (w/w) OV-101 on Gas Chrom Q.

Samples for mass spectra were collected from the gas chromatograph in capillary tubes cooled by carbon dioxide snow. Mass spectra were determined on an AEI MS 902 mass spectrometer with a cooled probe inlet. The ultra violet spectrum was recorded on a Pye SP 1700 instrument with hexane as solvent.

Insects

Supplies of *C. pulchellus* were obtained from beneath a flowering specimen of *Eucalyptus bicostata* at Black Mountain, A.C.T., or from the blossom of various garden shrubs, according to the season. Samples of the coccinellids needed for reference were collected in the United Kingdom (*Coccinella septempunctata* L., *Propylea quatuordecimpunctata* L.) and California, U.S.A. (*Hippodamia convergens* Guérin) during visits by the authors.

Treatment of C. pulchellus

(a) *Separation of secretion.* Neat secretion was collected by seizing individual beetles with forceps and absorbing the exuded droplets on small wedges of filter paper; the wedges were then extracted briefly with diethyl ether and this solution containing the (Z)-8-dihydromatricaria acid was treated with diazomethane prior to analysis by G.C. Alternatively, to detect the alkaloids, the secretion-containing filter paper was soaked in sodium hydroxide solution which was then extracted with chloroform. The chloroform extract was then submitted to G.C. analysis.

(b) *Total extraction. C. pulchellus* beetles (14 g) in chloroform were blended and the chloroform extract separated by filtration, the residue was re-extracted with chloroform and filtered again. The chloroform solutions were combined and concentrated then extracted with 1 M sulphuric acid. The aqueous layer was washed (three times) with chloroform to remove any non-basic materials, basified with aqueous sodium hydroxide and extracted with chloroform. The extract was dried over sodium sulphate and concentrated for G.C. analysis; it contained approx 150–200 μg of alkaloids.

*An alkaloid of this type was reported by HEDIN *et al.* (1974) as a constituent of the boll weevil but its identity was not precisely established.

The chloroform solution remaining after extraction with sulphuric acid was extracted twice with potassium carbonate solution. The aqueous layer was extracted (twice) with diethyl ether to remove any remaining neutral materials acidified with hydrochloric acid and extracted with ether. The ether extract was washed twice with water, dried over sodium sulphate and treated with ethereal diazomethane. The resulting solution was concentrated for G.C. analysis; it contained approx. 1.5 mg of methyl esters.

Reaction with stannous chloride

A chloroform solution of *C. pulchellus* alkaloids, with an added appropriate quantity of N-methylaniline, was analysed by G.C. on column 1 at 150°C. The solution was then allowed to evaporate and the residue treated with stannous chloride in 2 M hydrochloric acid. The mixture was then basified with aqueous sodium hydroxide until all the tin hydroxides formed had dissolved, and extracted with chloroform. The chloroform extract was then concentrated and analysed by G.C. as described above.

Reference compounds

Precoccinelline was obtained from *Coccinella septempunctata* (TURSCH *et al.*, 1971), hippodamine from *Hippodamia convergens* (TURSCH *et al.*, 1972a) and propyleine from *Propylea quatuordecimpunctata* (TURSCH *et al.*, 1972b). Myrrhine was prepared from precoccelline by the method of TURSCH *et al.* (1975).

RESULTS

Preliminary G.C. analysis of acetone extracts of *C. pulchellus* beetles on column 1 at 125°C with the Hall detector indicated the presence of volatile nitrogenous bases. Therefore batches of beetles were worked up in a manner appropriate for such compounds, as described above, and the resulting chloroform extracts analysed by G.C. on column 1 at 145°C. Three major components were indicated with retention times of 13.0 min (A), 13.6 min (B) and 15.1 min (C); the relative amounts of these varied, especially the proportion of C, but B was always the largest component.

The mass spectra of components A and B were very similar; both showed molecular ions at m/e 193 and significant fragmentation peaks at 192 (base peak), 178, 164, 151, 150, 137, 136 and 122. C showed a molecular ion at m/e 191 (base peak) and significant fragment ions at 176, 162, 149 and 148.

The mass spectra of components A and B were almost identical with those reported for the coccinellid alkaloids precoccinelline, hippodamine (PASTEELS, 1975, personal communication) and myrrhine (TURSCH *et al.*, 1975). The mass spectrum of component C was very similar to that reported (PASTEELS, 1975, personal communication) for the unsaturated alkaloid propyleine. As the mass spectra of precoccinelline and its two possible stereochemical isomers hippodamine and myrrhine (TURSCH *et al.*, 1975) are so similar it was not possible to identify A and B on that basis. However the G.C. retention times of all three isomers on column 2 at 160°C proved to be quite distinct and led to the identification of A and B as precoccinelline and hippodamine respectively; A also matched precoccinelline on column 3. Component C was identified as propyleine when its G.C. retention time on column 1 was shown to match that of authentic propyleine. This finding was confirmed when, after treatment with zinc dust and 1 M sulphuric acid, C gave a mixture of mainly precoccinelline with some hippodamine.

2-Phenylethylamine was identified on retention times and mass spectrum, from one batch of *C. pulchellus*, but this material was also present in the blossom upon which the beetles had been feeding and they had presumably derived it from that source.

The N-oxides coccinelline and convergine, which correspond with precoccinelline and hippodamine and occur concomittantly with them in various coccinellids, were not detected in the basic extract from *C. pulchellus*. Treatment of this extract with stannous chloride, after addition of N-methylaniline to serve as an internal standard, led to no significant increase in total volatile alkaloid (the ratio total alkaloid : standard moved from 0.99–1.05). The propyleine, however, declined and was presumably reduced mainly to precoccinelline. Under similar treatment, the total bases from *Coccinella septempunctata*, which are known to contain much coccinelline, showed a substantial increase in precoccinelline (ratio moving from 1.10–2.40).

The ethereal solution of acids from *C. pulchellus*, after treatment with diazomethane, was analysed on column 4 at 130°C but only one major peak, with a retention time of 9.15 min, was observed. A programmed rise of temperature to 180°C revealed no further peak. The mass spectrum of the component eluting at 130°C showed a molecular ion at m/e 176 (base peak) and major fragments at 161, 145, 133, 115, 105, 103, 91, 77, 63, 51 and 39. The u.v. spectrum was also distinctive, with sharp maxima at 228, 239, 252, 266 and 282 nm. Both spectra showed very good agreement with those reported by MEINWALD *et al.* (1968) for methyl (Z)-8-dihydromatricariate, derived from the North American *C. lecontei*.

Subsequent G.C. analysis of extract from the neat defensive secretion, before and after treatment with diazomethane, confirmed the presence of precoccinelline, hippodamine, propyleine and (Z)-8-dihydromatricaria acid.

DISCUSSION

The detection, in *Chauliognathus*, of alkaloids which were previously known only from Ladybird beetles of the family Coccinellidae raises interesting questions of comparative ethology and evolution.

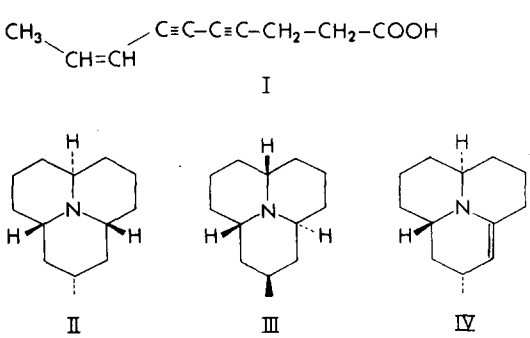

Fig. 1. Defensive components of *Chauliognathus pulchellus*. I, (Z)-8-dihydromatricaria acid; II, precoccinelline; III, hippodamine; IV, propyleine.

That such sophisticated repellents should occur in two widely disparate groups of aposematic beetles is certainly noteworthy but on both taxonomic and behavioural grounds, it appears necessary to attribute the concurrence to convergent evolution. Thus, the two families involved show no morphological resemblence and are currently placed in separate superfamilies, the Cantharoidea and Cucujoidea. Moreover, the manner of storing and presenting the alkaloids differs in the two groups: in the cantharid these substances are components of a specifically defensive secretion that is stored in separate reservoirs and discharged through discrete orifices; in the coccinellids they are components of the haemolymph and are released by reflex bleeding through weak areas of the intersegmental membranes. Much of the alkaloid content of coccinellid haemolymph is in the form of N-oxides, whereas the unoxygenated bases are predominant (and perhaps, exclusive) in the cantharid secretion. N-oxides are of course, much more soluble in water than are their parent bases and their accommodation, at suitably high concentrations, in haemolymph, would pose fewer problems. In the cantharid secretion, the difficulty of restricted water solubility does not arise because of the presence of considerably larger quantities of the dihydromatricaria acid, a substance that would not, presumably, be permissible as a haemolymph component.

From what is known concerning the biology of coccinellids and cantharids, and despite the demonstrated similarities in defensive chemistry, it would appear likely that the repellent qualities of the two groups of beetles are directed against different ranges of potential predators. Thus the coccinellids in question, which feed upon aphids, scales and other sap-sucking Hemiptera, would be in constant competition with ants, against which coccinelline and convergine in particular are very effective repellents (PASTEELS *et al.*, 1973). On the other hand, the blossom-feeding and more free-flying cantharids would be expected to come into contact more with larger predators, such as birds and other vertebrates, with greater learning powers and against which a specifically defensive secretion might be expected to be more effective.

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