THE BUPRESTINS: BITTER PRINCIPLES OF JEWEL BEETLES (COLEOPTERA: BUPRESTIDAE)

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Abstract

The occurrence of a series of bitter principles is reported from a wide range of buprestid beetles. These substances, which are named buprestins, are β-D-glucose-1,2,6-triesters in which the main esterifying moiety is pyrrole-2-carboxylic acid. Sugar solutions, when mixed with the most abundant buprestin A, became unpalatable to meat ants (*Iridomyrmex purpureus* Smith). The buprestins are considered to be repellents that have been evolved exclusively in Buprestidae and their bearing on biology and mimicry in these beetles is discussed.

Introduction

The beetle family Buprestidae is remarkable for its high proportion of brilliantly coloured species, a situation that is reflected in the popular name "jewel beetles". However, some buprestids show more restrained colours, sometimes in apparently cryptic patterns, and a few, such as the fire beetle, *Merimna atrata* (Hope), are entirely black. The last mentioned is also exceptional within the family in being nocturnal.

The biological roles served by the brilliant colours have not been adequately investigated but the widespread occurrence of certain generalised patterns, notably that of 2 or 3 transverse orange or yellow bands on a dark metallic ground, suggests mimetic convergence within the family. Mimicry of other groups of aposematic insects is also apparent but there is no published evidence to indicate that buprestids are themselves chemically protected against predation.

Our interest in this possibility arose from our studies of the mimetic complex centred upon the aposematic lycid, *Metriorrhynchus rhipidius* (Macleay). We had earlier shown (Moore and Brown 1981) this model to be highly protected by warning odours, bitter principles and antifeedants, and its associated mimetic complex was already known (Nicholson 1927) to include a number of buprestids belonging to the large and dominant Australian genus *Stigmodera* Eschscholtz. Chemical examination of some of these buprestid mimics has now led to the discovery of a group of bitter principles of a novel general structure (Fig. 1), which we here name "buprestins" and which are evidently widespread in both mimetic and non-mimetic species of this important family of beetles.

Materials and methods

Live buprestids were collected mostly in the Canberra district but supplies of the large *Stigmodera (Stigmodera) macularia* (Donovan), used as a major source of the buprestins, were obtained from blossom in the sandstone country near Buxton, New South Wales, and a specimen of *S. (Themognatha) heros* Géhin was provided by Dr S. Barker from South Australia. Museum specimens were drawn from the Australian National Insect Collection and the B.P. Moore collection.

Gas chromatography (GC) was conducted on a Varian-2100 instrument, with a Varian CDS-111 data processor, a 2 m $\times$ 3 mm column of 6% Carbowax-20M/2% KOH on Gas-Chrom Z, and a nitrogen flow of 25 mL/min. A flame ionisation detector was normally used but when required, a Hall electrolytic conductivity detector was operated in the reducing mode for specific detection of nitrogen.

Thin-layer chromatography (TLC) was performed on silica gel with 1% of a zinc sulphide fluor, developed with 15% methanol in dichloromethane and irradiated under short-wave UV light, when the buprestins appeared as a dark bank at R_f 0.55.

The test for biological activity was conducted on 22 Feb. 1984 at "Calosoma" via Gundaroo, New South Wales. Buprestin A (0.1 g) was dissolved in aqueous brown sugar solution (10% w/v 20 mL). This solution (2mL) was exposed in each of 2 watch-glasses, in alternation with 2 similar controls containing the sugar solution only, around the periphery of an occupied mound of meat ants (*Iridomyrmex purpureus* Smith), sheltered from the north and east by eucalypt woodland. The tests were placed in position at dawn, before the ants were foraging and were sited away from any obvious trunk trails. The tests were monitored at hourly intervals from 0900 to 1600 h (Eastern summer time) and the numbers of ants at each dish were recorded. Consumption of the controls necessitated their replenishment at noon and evaporation losses, which became important after 1300 h, when the tests were in full sun, were made good at 1400 h for all 4 stations. The tests were terminated at 1600 h, when the ants had ceased foraging and the ambient temperature was 25 C. The results are given in Table 1.

Table 1. Effect of buprestin A on aggregation of *Iridomyrmex purpureus* at sugar solutions

Time of day (h)	Ambient temperature (°C)	Numbers of ants at bait			
		C1	C2	B1	B2
		10% sugar only		10% sugar + 0.5% buprestin A	
0900	15	2	2	1	2
1000	19	20	10	1	1
1100	21	13	10	0	1
1200	22	6	7	1	0
1300	22	11	4	2	1
1400	24	2	3	0	0
1500	26	4	1	0	0
1600	25	0	0	0	0
Totals*		58a	37a	5b	5b

* Figures with different post-scripts differed significantly ($P < 0.01$) in the "U"-test.

Results

Preliminary GC analyses of acetone/2N sulphuric acid extracts of various mimetic and non-mimetic *Stigmodera* species showed a common, broad and tailing peak which, from its configuration, appeared to be due to on-column degradation of some comparatively involatile component. Since this peak was visible with the nitrogen-selective Hall detector, it was evidently due to a nitrogenous substance, which was soon identified as pyrrole from mass spectral data. When the beetles were treated with methanolic sulphuric acid, methyl pyrrole-2-carboxylate was readily identified in the extract, by similar means. Thus it was apparent that the original broad peak attributable to pyrrole had resulted from thermal decarboxylation of free pyrrole-2-carboxylic acid. However, since this acid showed a strong peak of absorption in the UV at 262 nm (in methanol), whereas the extract of the beetles peaked at 267 nm (in methanol), it seemed likely that both the free acid and its methyl ester were artefacts and that in the natural state, the pyrrole moiety existed in combination with another substrate. This was supported by thin-layer chromatographic analyses, when the dark band from the beetles ran at an Rf different from those of pyrrole-2-carboxylic acid and its methyl ester.

In a larger-scale experiment (Brown *et al.* 1985), the acetone extract from 6 *S. macularia* (combined weight: 10 g) was subjected to high performance liquid chromatography (HPLC) in both normal and reversed-phase systems. This led to isolation of 2 components with the characteristic chromophore, namely buprestin A and buprestin B, in proportions of about 9:1 (total yield: 92 mg). The full structures of buprestins A and B were determined by mass spectral and nuclear magnetic spectral studies and were shown to be triesters of β-D-glucose, represented by the general formula given in Fig. 1, where $R_1 = R_2$ = pyrrole-2-carbonyl, and R_3 = pyrrole-2-carbonyl (buprestin A) or 4-hydroxybenzoyl (buprestin B). Further minor buprestins, showing more extended 4-hydroxycinnamoyl and 3,4-(methoxy, hydroxy) cinnamoyl chromophores have since been detected in buprestids of the genera *Chalcotaenia* and *Curis* (Table 2). These moieties were identified by study of mass spectra and by GC/MS of the fully methylated derivatives obtained by hydrolysis of the buprestins, followed by treatment of the hydrolysates with diazomethane.

Since the major buprestins normally amounted to *ca* 1% of the body weight in species where they occurred, they proved readily detectable in crude acetone extracts in such cases, through their characteristic UV spectra (in methanol) and their performance in HPLC and TLC. Thus a survey of species that were available as fresh specimens was a relatively simple matter. Buprestin A was also shown to have survived, for nearly 60 years in dried museum specimens. However, the presence of naphthalene and of degradation products in such specimens obscured the UV spectra obtained

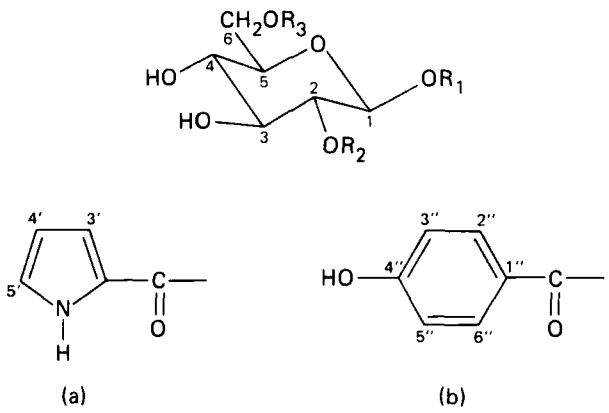

FIG. 1—General formula for the buprestins, where R_1 = pyrrole-2-carbonyl (a). Buprestin A: R_2 = R_3 = (a); buprestin B: R_2 = (a), R_3 = 4-hydroxybenzoyl (b).

Table 2. Buprestid species tested for presence of buprestins

Genus	Species	Fresh or Museum Material	Origin	Buprestins
Acmaeodera	*cylindrica* (F.)	M	Spain	+
Agrilus	*australasiae* Laporte and Gory	F	Australia	(+)†
Chalcotaenia	*laeta** Waterhouse	F	Australia	+
Chrysodema	*simplex* Waterhouse	M	Christmas Is. (Indian Ocean)	+
Cisseis	*marmorata** (F)	F	Australia	+
Curis	*caloptera** (Boisduval)	F	Australia	+
Cyria	*imperialis* (F)	M	Australia	+
Habroloma	*socialis* (Lea)	M	Australia	—
Julodimorpha	*bakewelli* (White)	M	Australia	+
Julodis	*variolaris* (Pallas)	M	U.S.S.R.	+
Merimna	*atrata* (Hope)	M	Australia	+
Nascio	*vetusta* (Boisduval)	M	Australia	+
Prospheres	*aurantiopictus* (Laporte and Gory)	M	Australia	+
Stigmodera s.str.	*macularia** (Donovan)	F	Australia	+
Stigmodera (*Themognatha*)	*heros** Gehin	F	Australia	+
Stigmodera (*Castiarina*)	*australasiae* Laporte and Gory	F	Australia	+
,,	*bella** Saunders	F	Australia	+
,,	*bifasciata** (Hope)	F	Australia	+
,,	*crenata* (Donovan)	F	Australia	+
,,	*erythroptera* (Boisduval)	F	Australia	+
,,	*hilaris* Hope	F	Australia	+
,,	*kirbyi** (Guerin)	F	Australia	+
,,	*nasuta* Saunders	F	Australia	+
,,	*octospilota** Laporte and Gory	F	Australia	+
,,	*rufipennis** (Kirby)	F	Australia	+
,,	*scalaris* (Boisduval)	F	Australia	+
,,	*sexplagiata** Gory	F	Australia	+
Torresita	*cuprifera* (Kirby)	M	Australia	+

* Species were surveyed by HPLC; others were by TLC.
† This response was weak.

directly from crude extracts and a preliminary purification by TLC, followed by excision and extraction of the appropriate band, was necessary before the characteristic spectrum of the buprestin could be detected. Buprestin B is less stable than buprestin A and could not be detected in museum specimens.

A total of 14 species of *Stigmodera*, embracing all 3 recognised subgenera, have been surveyed for buprestins from fresh material and all gave strongly positive results. Eight of these species were examined by HPLC and 7 showed similar proportions (9:1) of buprestin A and buprestin B, with no evident differences between the sexes, but *S. heros* contained a higher proportion of buprestin B (ratio = 4:1). Four species belonging to 4 other genera (Table 2) were also collected for this purpose and 3 were strongly positive, but *Agrilus australasiae* Laporte and Gory gave only a weak response. The aposematic (red and black) elaterid *Ophidius elegans* Candéze, included for comparison, was completely negative.

Tests with museum material confirmed the presence of buprestin A in species of *Acmaeodera*, *Chrysodema*, *Cyria*, *Julodimorpha*, *Julodis*, *Merimna*, *Nascio*, *Prospheres* and *Torresita*, but the minute *Habroloma socialis* (Lea) and the cryptic elaterid *Paracalais gibboni* (Newman) gave negative results.

A fresh specimen of *Stigmodera (Castiarina) bifasciata* (Hope) was dismembered and the discrete sections were extracted separately with acetone. Examination of the individual extracts, diluted with methanol, by UV spectroscopy, revealed distributions of total buprestins as follows: elytra 17%, combined legs 7%, prothorax 27%, hindbody 49%.

Pure buprestin A, when tasted by both of us, produced a long-lasting and intensely bitter sensation and the results of the biological test (Table 1) indicated that it renders brown sugar solution unacceptable to meat ants.

Discussion

The discovery of the buprestins provides the first firm indication that buprestid beetles are chemically protected against predation. Although the present study has been based mainly upon Australian species, its coverage of some 9 tribes, ranging from the primitive Prospheresini (Levey 1978; Cobos 1980) and Julodini to the advanced Trachyini (Cobos 1980), and 15 genera (Table 2), is sufficient to show that these bitter principles are widespread, if not universal, within the family, only the position of the Trachyini being uncertain. The results also confirm that such chemical protection is not restricted to conspicuous and brightly coloured species. On the other hand, the negative tests with 2 species of Elateridae (1 aposematic and 1 cryptic) in the present work, and our failure to detect pyrrole or other evidence of buprestins in any other family, during our studies of chemical defence in Coleoptera, suggest that buprestins will prove to be confined to the Buprestidae.

The low ($< 10\%$ of average) concentrations of buprestins in living *A. australasiae* seem hardly likely to serve effectively in a defensive function and it would be interesting to determine whether such a situation is general in this large and worldwide genus of small, slender species. Likewise, the negative findings with the minute, compact *H. socialis*, which was available only as 3 museum specimens, will need to be confirmed on fresh material and tested against a wider range of related species. The 2 relevant tribes, Agrilini and Trachyini respectively, although formerly regarded as primitive (Théry 1929) are now placed among the most advanced Buprestidae, on account of their adult and larval adaptions to specialised stem-boring or leaf-mining habits (Cobos 1980). One may therefore speculate that the buprestins evolved autapotypically in the family but are being secondarily lost in Agrilini and Trachyini and perhaps in certain other advanced forms, where marked reduction in body size concomitant with specialised feeding habits has rendered tham redundant.

At first consideration, it is somewhat surprising that a group of predominantly wood-feeding insects should resort to nitrogenous substances in the large amounts needed for defence purposes. However, although there is currently no information concerning the biosynthesis of the buprestins, it seems likely that the pyrrolecarboxylic moiety of these substances is derived from its tetrahydro-derivative proline, which is an

amino acid that is present as an osmoregulator in wood and other plant tissues, but one that is not an essential requirement for most insects (Gilmour 1961). The phenolic moieties that partly replace the pyrrolecarboxylic residue in the minor buprestins are also readily available from plant material.

From their general structure and ready hydrolysis in acidic media, the buprestins are unlikely to be toxic to potential predators, but their intensely bitter taste to humans and their rendering of sugar unacceptable to meat ants leave no doubt as to their repellent properties. However, protection is not absolute, for buprestids are attacked occasionally (though perhaps not consumed) by meat ants (Gwynne and Rentz 1983) and are also eaten by birds (Silberglied and Eisner 1969). In view of the solubility of the compounds in water and their wide distribution within the bodies of the beetles, they may be components of the haemolymph. However, as buprestids have a hard and rigid cuticle and show no reflex bleeding, they would be unlikely to survive sampling by naive vertebrate predators. The education of the latter would therefore involve some sacrifice but this would be minimised, at least in the case of avian predators, by the convergence in colour patterns which occurs in many buprestid species.

Within *Stigmodera*, subgenus *Castiarina*, the buprestins are probably universal and their concentrations are similar in lycid-mimetic (*erythroptera*, *nasuta* and *rufipennis* of Table 2) and non-mimetic species. In view of this and because the lycid mimics are not very closely related among themselves but rather, are drawn from several distinct species groups (S. Barker pers. comm.), it is evident that lycid mimicry in this subgenus has evolved independently and on several occasions as a secondary process. Moreover, since these mimics are now known to be chemically protected and although they lack the pyrazine-based warning odours of their lycid models, their place is obviously nearer to the Müllerian than to the Batesian side of the mimetic spectrum.

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