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The Defensive Alkaloids of *Cryptolaemus montrouzieri* (Coleoptera: Coccinellidae)

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Abstract

The chemical defensive system of the ladybird *Cryptolaemus montrouzieri* consists mainly of three related alkaloids. Two of these have been identified as *cis*-1-(6-methyl-2-piperidyl)propan-2-one (3) and 1-methyl-9-azabicyclo[3,3,1]nonan-3-one (2); the third is very unstable and readily isomerizes to (3).

Defensive alkaloids are widespread in the beetle family Coccinellidae[1] although the structures of only a few (from species of the tribes Hippodamini and Coccinellini) have been determined. Precoccinelline (1) and its *N*-oxide coccinelline, and related alkaloids contain a characteristic tricyclic system and have been shown[1] to be effective in repelling ants and birds. Tests with whole insects of the species *Adalia bipunctata*, which contain the alkaloid adaline[2] (2; R = C_5H_{11}) likewise demonstrated repellent properties. In this paper we report our findings on related alkaloids from the Australian mealybug ladybird, *Cryptolaemus montrouzieri* Mulsant (tribe Scymnini).

Gas chromatography of the *Cryptolaemus* alkaloids on column 1 (see Experimental) showed the presence of three major components (A, B and D) and one minor component (C) but on Carbowax columns component B was completely isomerized to A. The largest component (D, 60–65%) gave a mass spectrum with a molecular ion at *m/z* 153 (45%), which suggests an empirical formula of $C_9H_{15}NO$. When deuterium oxide was introduced into the source, partial exchange (*c.* 20%) was evident in the molecular ion and in several major fragments, demonstrating the presence of an active hydrogen atom. Component D was readily acetylated to give a product significantly less volatile and more polar (as evidenced by g.l.c. retention data on columns 1 and 3) than D itself. Accurate mass spectral measurements on this acetyl derivative indicated an empirical formula $C_{11}H_{17}NO_2$ and confirmed that tentatively assigned to component D.

D was resistant to catalytic reduction with Adams catalyst in ethanol but was reduced by lithium aluminium hydride in ether to give a product of longer retention

[1] Pasteels, J. M., Deroe, C., Tursch, B., Braekman, J. C., Daloze, D., and Hootele, C., *J. Insect Physiol.*, 1973, **19**, 1771.

[2] Tursch, B., Braekman, J. C., Daloze, D., Hootele, C., Losman, D., Karlsson, R., and Pasteels, J. M., *Tetrahedron Lett.*, 1973, 201.

time (column 3) and with molecular weight 155. The presence of a carbonyl group was further demonstrated when component D reacted with 1,1-dimethylhydrazine to give a dimethylhydrazone.

The empirical formula indicated three degrees of unsaturation, one of which was accounted for by the carbonyl group, and as no double bonds appeared to be present D was probably bicyclic. These findings and the general resemblance of the mass spectrum of D to that of the known ladybird alkaloid adaline[2] (particularly the fragments at M−29, M−42, M−43 and M−57) suggested that D was a C_4 lower homologue of adaline. This conclusion was supported by the Kovats indices on column 1 and 3 which were consistent with a chain length difference of four carbon atoms.

Such a lower homologue of adaline [1-methyl-9-azabicyclo[3,3,1]nonan-3-one (2; R = Me)] was already known and characterized from *Euphorbia atoto*.[3] Component D was found to be identical with this plant material by the coincidence of mass spectra and of retention times on columns 1 and 3.

Component A (9–15%) gave a mass spectrum with a molecular ion at *m/z* 155, which suggests an empirical formula of $C_9H_{17}NO$. Like D component A readily formed an acetyl derivative and accurate mass spectral measurements on this compound confirmed the tentative empirical formula for A.

Component A was slowly reduced by hydrogen and Adams catalyst in ethanol and readily by lithium aluminium hydride in ether to give the same product, of longer retention time and molecular weight 157. Thus A, like D, appeared to have a carbonyl group, and an >NH group but no unsaturation and so was presumably monocyclic.

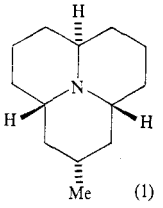
These findings and the mass spectrum of A were suggestive of formula (3; R = Me) or possibly (4); the intense fragments at M−43 (loss of C_2H_3O) and 43 were consistent with a methyl ketone and the base peak at M−57 could arise from loss of the complete side chain. Such a structure as (3; R = Me) would be biosynthetically related to component D and structure (3; R = Me) was considered more likely than (4) as it is derivable from a polyketide chain. Thus component A would be a homologue of the well known alkaloid pelletierine (3; R = H) and skeletally similar to pinidine[4] (5). The latter relationship was confirmed when component A was subjected to a Wolff–Kishner reduction and gave a product identical with dihydropinidine in mass spectrum and retention times on columns 1 and 5.

1-(6-Methyl-2-piperidyl)propan-2-one (3; R = Me) was synthesized from 2,6-lutidine via lutidyllithium and 1-(6-methyl-2-pyridyl)propan-2-one, and was found to be identical with component A in mass spectrum and retention times on columns 1, 3 and 5. Similarly 1-(6-methyl-2-piperidyl)propan-2-ol obtained from the lithium aluminium hydride reduction of the synthetic ketone (3; R = Me) was identical with the alcohol derived from component A.

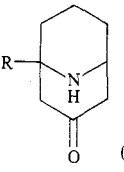
The results of the Wolff–Kishner reduction indicated that component A was a *cis*-stereoisomer of 1-(6-methyl-2-piperidyl)propan-2-one since Tallent and Horning[5] and Hill *et al.*[6] concluded that pinidine (and therefore, presumably, dihydropinidine)

[3] Hart, N. K., Johns, S. R., and Lamberton, J. A., *Aust. J. Chem.*, 1967, **20**, 561.
[4] Tallent, W. H., Stromberg, V. L., and Horning, E. C., *J. Am. Chem. Soc.*, 1955, **77**, 6361.
[5] Tallent, W. H., and Horning, E. C., *J. Am. Chem. Soc.*, 1956, **78**, 4467.
[6] Hill, R. K., Chan, T. H., and Joule, J. A., *Tetrahedron*, 1965, **21**, 147.

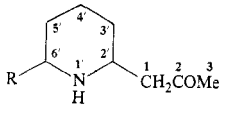
had a *cis* configuration. However in view of the ready conversion of component B into A further evidence was sought. The catalytic reduction of 1-(6-methyl-2-pyridyl)-propan-2-one yielded component A but no other isomer, supporting the *cis* configuration for A as the reaction would be expected to give *cis* products.[5] That the reduction was actually giving *cis* products was demonstrated when 2% of *cis*-2-methyl-6-propylpiperidine (dihydropinidine), but no candidate peak for the *trans* isomer, was detected (g.l.c., mass spectrum) in the reaction mixture. 1-(6-Methyl-2-piperidyl)propan-2-ol was present also as only one component.



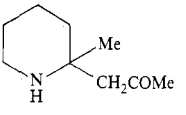
(1)



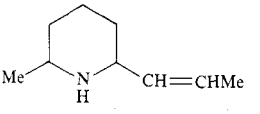
(2)



(3)



(4)



(5)

The carbon-13 magnetic resonance spectrum (^{13}C n.m.r.) of component A in comparison with that of 2,6-dimethylpiperidine[7] showed that the chemical shifts of C4′, C2′, C5′ and C6′ were only slightly affected (−0·76 ppm or less); that of C3′ shifted rather more (−2·53 ppm). A molecular model of *cis*-1-(6-methyl-2-piperidyl)propan-2-one showed that in the equatorial position the carbonyl group was remote from all the ring carbon atoms except C3′. However, in the *trans* isomer one or other of the ring substituents would be axial and in much closer proximity to most of the ring carbon atoms. Therefore the ^{13}C n.m.r. spectrum was consistent with a diequatorial constitution and confirmed the *cis* configuration of component A.

Component B (20–27%) gave a mass spectrum with a molecular ion at *m/z* 155. This spectrum was qualitatively similar to that of component A but ions at 97, 69 and 43 were relatively more intense. On columns 3 and 5 component B apparently underwent complete conversion into A and the sharpness and symmetry of the resulting peak showed that this change occurred very rapidly after injection. With column 1, however, provided that it had been deactivated recently by silylation, there was little evidence of degradation occurring during the analysis.

That component B was indeed isomerizing to A was proved when successive collections of B from columns 1 and 3 gave a material shown to be entirely component A when analysed on column 1. Component B, therefore, is possibly *trans*-1-(6-methyl-2-piperidyl)propan-2-one and isomerizes to the thermodynamically more stable component A (with its diequatorial configuration) when chromatographed on all but chemically very inert columns. However in view of its instability and the small quantities available, the problem of its structure could not be settled conclusively.

[7] Jones, A. J., and Hassan, M. M. A., *J. Org. Chem.*, 1972, **37**, 2332.

Reduction of a *Cryptolaemus* extract with lithium aluminium hydride gave, in addition to a product from component D, three alcohols with extremely similar mass spectra and molecular ion at *m/z* 157. One of these products was the alcohol derived from component A and the other two, which were not completely resolved, were considered to be the *threo* and *erythro* forms of the product derived from B. The ratios of the products were 1 (from A):2:0·9 and these reflected the ratio of A to B in the extract. Component A, like B, might be expected to give two isomeric alcohols on reduction but only one peak was seen. This could be due either to lack of resolution on g.l.c. or to stereoselective reduction favouring one of the possible isomers. Even with component B one of the alcohols was clearly more abundant than the other.

The facile conversion of component B into A suggested that the latter might be an artefact; however, dead beetles that had been stored at 4° for several weeks (and which presumably had suffered some enzymic change) showed only a small peak due to component A but a large one due to B when analysed on column 1. Thus very little if any of B isomerized to A under these conditions of analysis. Moreover, reduction of the total extract with lithium aluminium hydride produced only little of the alcohol derived from component A but substantial amounts of those from B (column 3).

A few fresh beetles were crushed in methanol and the extract analysed directly on column 1. This revealed essentially the same ratio of components as was detected after workup by the method given in the Experimental section. Thus the composition had not been affected by exposure to acidic and basic conditions during the latter procedure and component A was evidently a genuine constituent of the insects.

Component C (2–3%) gave a mass spectrum with a molecular ion at *m/z* 153 (4%). This minor component may be an isomer of component D but lack of material prevented its further investigation.

The relative amount of the major components varied little from batch to batch of insects and the Canberra population was little different from its Sydney countetpart in this regard. However, the Canberra insects showed none of the minor component C but instead a similar proportion (2–3%) of another isomer with a mass spectrum almost identical with that of component C but differing in g.l.c. retention time on column 3.

Analysis of a batch of larvae showed the presence of the same alkaloid components but with relatively more of the monocyclic materials; components A and B combined now represented 62% of the mixture.

Tursch *et al.*[2] suggested a biosynthetic relationship between adaline and the coccinelline type alkaloids via a hypothetical intermediate of structure (3; $R=C_5H_{11}$) and this was extended by Ayer and Browne[8] who adduced evidence for the polyketide origins of these alkaloids from feeding experiments with C1- or C2-labelled [^{14}C]-acetate. The monocyclic compound (3; $R = C_5H_{11}$) was postulated as a key intermediate which, by alternative ring closures, could afford either adaline or precoccinelline. The presence in *Cryptolaemus* of a compound of this molecular type, together with the corresponding adaline homologue, lends considerable support to this biosynthetic scheme.

[8] Ayer, W. A., and Browne, L. M., *Heterocycles*, 1977, **7**, 685.

Experimental

General Procedures

Melting points are uncorrected. Mass spectra were determined on a VG-Micromass 70–70 instrument coupled to a Varian gas chromatograph. High-resolution mass spectra were measured on an AEI MS 902 mass spectrometer by peak matching; the samples were introduced on a ceramic probe. ^{1}H n.m.r. spectra were recorded in $CDCl_3$ on a Varian CFT-20 instrument operating at 80 MHz. Carbon-13 spectra were determined on a Bruker HFX-270 instrument operating at 67·89 MHz.

Analytical and small-scale preparative gas chromatography were conducted on a Varian 2100 instrument with a Hewlett–Packard 3370A digital integrator or a Varian CDS-111 data processor, and with a nitrogen flow of 25 ml/min and the following glass columns:

Column 1: 2 m by 3 mm of 5% OV-101 on Gas-chrom Q.

Column 2: 2 m by 2 mm of 5% OV-101 on Gas-chrom Q.

Column 3: 4 m by 3 mm of 6% Carbowax-20M/2% KOH on Gas-chrom Z.

Column 4: 2 m by 2 mm of 6% Carbowax-20M/2% KOH on Gas-chrom Z.

Column 5: 4 m by 3 mm of 5% Carbowax-20M on Gas-chrom Z.

The g.l.c./m.s. system used helium as the carrier gas with a flow of 20 ml/min. Larger-scale preparative g.l.c. was conducted on a Loenco Prep-matic machine, with thermal conductivity detectors and 2 m by 10 mm stainless steel columns packed with 20% OV-1 or 20% Carbowax-20M on Gas-chrom Z. Helium was the carrier gas.

Supplies of beetles were collected in the field. Two populations, from Sydney and Canberra respectively, were sampled. Pinidine was obtained from the needles of *Pinus sabiniana* growing in Canberra, by the method of Tallent *et al.*[4] Adaline was obtained from *Adalia bipunctata*[2] collected in Britain. (+)-1-Methyl-9-azabicyclo[3,3,1]nonan-3-one from *Euphobia atoto* was supplied by Dr J. A. Lamberton.

Degradation Techniques

(A) Hydrogenations were carried out on a micro-scale in septum-sealed Reacti-vials with hydrogen and a trace of Adams platinic oxide catalyst. Ethanol served as solvent and the products in solution were removed with syringe and applied directly to the gas chromatograph.

(B) Lithium aluminium hydride reductions (small scale) were carried out in ether solution followed by quenching with water. The reaction mixture was extracted with ether (3×) and the ethereal solutions concentrated prior to g.l.c. analysis.

(C) Acetylations were carried out in Reacti-vials with a small quantity of 1:1 acetic anhydride/pyridine at 100° for 5 min. The resulting mixture was used directly for g.l.c. Samples of acetyl derivatives for high-resolution mass spectroscopy were collected from column 1 at 140° by trapping in capillary tubes cooled by carbon dioxide snow.

(D) The Wolff–Kishner reduction was carried out in the following manner. Component A was collected from column 5 and was then treated with 10% hydrazine hydrate in ethanol (20 μl) with a trace of formic acid for 30 min. The solvent was then removed under reduced pressure (20 mmHg) and 10% potassium hydroxide in triethylene glycol (20 μl) was added. The mixture was heated in a sealed tube at 200° for 30 min. The product was diluted with water and extracted with chloroform (2×), the chloroform extract was concentrated prior to analysis by g.l.c.

(E) The dimethylhydrazone was prepared, in a Reacti-vial, by reaction (70°, 10 min) with 10% 1,1-dimethylhydrazine and 1% formic acid in ethanol. The product was analysed directly by g.l.c./m.s. on column 2 at 110° programmed at 2°/min.

Extraction, Gas Chromatography and Mass Spectroscopy of Alkaloids

The ladybirds (Sydney population) were immersed in 1% sulfuric acid in methanol, crushed and left for 16 h at 4°. The liquid was then removed and filtered, the residue was washed with methanol (2×) and the washings filtered and combined with the original extract. The mixture was diluted with water and concentrated under a stream of nitrogen to remove most of the methanol and the aqueous residue extracted with ether (3×) to remove neutral materials. The aqueous solution was

then made alkaline with 2 N sodium hydroxide and extracted twice with chloroform. The chloroform solution of alkaloids when concentrated was ready for g.l.c. analysis. The beetles were estimated, by g.l.c. responses, to contain *c.* 100–200 μg of combined alkaloids each.

On column 1 at 110° four components were detected and designated A, B, C and D; these had retention times of 4·26, 5·30, 6·10 and 8·07 min respectively, and relative abundances of 9–15, 20–27, 2–3 and 60–65%. Temperature programming to 200° produced no further peaks. However, on columns 3 and 5 at 150° only peaks attributable to A, C and D were seen. G.l.c./m.s. showed that the first peak, which now had increased to 30–35%, was due entirely to A and not a mixture of A and B.

Component D gave Kovats indices of 1301 (column 1 at 150°) and 2010 (column 3 at 170°). Mass spectrum (g.l.c./m.s. column 4): *m/z* 153 (M, 45%), 11 (32), 110 (100), 96 (78), 83 (28), 82 (37), 43 (26), 42 (45) and 41 (26). The acetyl derivative of D gave mass spectrum *m/z* 195·1254 (M, 46%; Calc. for $C_{11}H_{17}NO_2$ 195·1259), 153 (34), 152 (100, $C_9H_{14}NO$), 111 (47, C_6H_9NO), 110 (98, C_6H_8NO), 96 (69, $C_6H_{10}N$), 95 (34, C_6H_9N) and 43 (78).

Component A gave mass spectrum (g.l.c./m.s. column 4): *m/z* 155 (M, 12%), 140 (22), 112 (23), 98 (100), 82 (34), 70 (21) and 43 (42). The acetyl derivative of A gave mass spectrum: *m/z* 197 (M, 8%), 154·1232 (70; Calc. for $C_9H_{16}NO$ 154·1232), 140 (31, $C_8H_{14}NO$), 112 (36, $C_7H_{14}N$), 98 (100, $C_6H_{12}N$), 82 (25, C_5H_8N) and 43 (54). The alcohol from the reduction of component A gave mass spectrum (g.l.c./m.s. column 4): *m/z* 157 (M, 1%), 156 (1), 142 (9), 124 (3), 112 (3), *99* (*9*), *98* (*100*), *96* (*3*), *84* (*3*), *82* (*6*), *81* (*6*), *70* (*8*) and *55* (*12*).

Component B gave mass spectrum (g.l.c./m.s. column 4): 155 (M, 9%), 140 (20), 112 (16), 98 (94), 97 (72), 82 (64), 69 (100) and 43 (100).

Component C gave mass spectrum (g.l.c./m.s. column 4): *m/z* 153 (M, 4%), 110 (100), 96 (13), 83 (15), 82 (15) and 43 (68).

Adaline gave Kovats indices of 1701 (column 1 at 150°) and 2400 (column 3 at 170°). Mass spectrum: *m/z* 209 (M, 49%), 180 (15), 167 (30), 166 (100), 153 (87), 152 (29), 138 (19), 110 (55), 96 (49), 55 (17), 43 (20) and 41 (24).

1-(6-Methyl-2-pyridyl)propan-2-one

This substance was prepared by the method used by Wibaut *et al.*[9] for (2-pyridyl)propan-2-one. In the present case 2,6-lutidine (17 ml) was the starting material and the reaction proceeded via lutidyllithium. The product was worked up as described and fractionally distilled. The fraction of b.p. 80–130°/2 mmHg (5 g) was shown by g.l.c. (column 1 at 120°) to be *c.* 75% of 1-(6-methyl-2-pyridyl)propan-2-one. Purification was achieved by preparative-scale g.l.c. (20%OV-1 at 170°) and gave a sample better than 98% pure. This product gave a picrate with m.p. 138° (ethanol) (lit.[10] 137·7–138·3°).

cis-*1-(6-Methyl-2-piperidyl)propan-2-one*

(6-Methyl-2-pyridyl)propan-2-one (250 mg) was reduced with hydrogen and Adams catalyst (10 mg) in acetic acid (2·5 ml). The reaction was monitored by periodic analysis of aliquots on column 1 at 120° and column 5 at 160°. The reduction was arrested when the desired product *cis*-1-(6-methyl-2-piperidyl)propan-2-one had reached a maximum. The solution was then separated from the catalyst, diluted with water, made alkaline with 10 N sodium hydroxide and extracted several times with ether. Analysis of the ether extract by g.l.c./m.s. (column 4 at 120° programmed at 2°/min) showed that the proportion of *cis*-1-(6-methyl-2-piperidyl)propan-2-one was 31%. Other components were identified as *cis*-2-methyl-6-propylpiperidine (dihydropinidine, 2%), 2-methyl-6-propylpyridine (10%), 1-(6-methyl-2-piperidyl)propan-2-ol (14%), and 1-(6-methyl-2-pyridyl)propan-2-ol (35%) and a little starting material (3·5%). The desired *product* was purified by preparative-scale g.l.c. (20% Carbowax-20M at 190°). This material was shown to be better than 98% pure by g.l.c. analysis on columns 1 and 3 (found: $M^{+\bullet}$, 155·1309. $C_9H_{17}NO$ requires $M^{+\bullet}$, 155·1310). ^1H n.m.r. δ 1·2, d, CH_3CH; 2·05, s, NH, (not fully resolved from the 2·12 peak, removed in contact with D_2O); 2·12, s, CH_3CO; 2·52, d, CH_2CO. The methylene protons (6H) were represented by a band between 1·0 and 2·0 and the methine protons (2H) by a band between 2·5 and 3·2. The ^{13}C n.m.r. spectrum had peaks (relative to $SiMe_4$) at 23·0 ppm, CH_3CH; 24·65, C4′; 30·65, C3; 31·95, C3′; 33·93, C5′; 50·68, C1; 52·09, C2′; 52·4, C6′; 208·45, C2.

[9] Wibaut, J. P., Kloppenburg, C. C., and Beets, M. G. J., *Recl Trav. Chim. Pays-Bas*, 1944, **63**, 134.
[10] Goldberg, N. N., and Levine, R., *J. Am. Chem. Soc.*, 1952, **74**, 5217.

Acknowledgments

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