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Pyrrolizidine alkaloids of probable host-plant origin in the pronotal and elytral secretion of the leaf beetle *Oreina cacaliae*

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Abstract

Oreina cacaliae (Coleoptera, Chrysomelidae) produces in its elytral and pronotal defensive secretion seneciphylline N-oxide together with small amounts of another pyrrolizidine alkaloid tentatively identified as senecionine N-oxide. This is a strong departure from the chemical composition of the defensive secretions in related species, characterized by complex mixtures of cardenolides, synthesized by the beetles from cholesterol. It is suggested that *O. cacaliae* sequesters the alkaloids from its host-plant, *Adenostyles leucophylla.* Other specimens of *O. cacaliae* from far distant populations feeding on *Senecio nemorensis, Petasites paradoxus* or *P. album* also produced pyrrolizidine alkaloids, but not *O. speciosissima* feeding on the same food plants and producing cardenolides. In addition to pyrrolizidine alkaloids, *O. cacaliae* secretes ethanolamine, which is also found in all the cardenolide-producing species.

Introduction

Numerous leaf beetles (Chrysomelidae) are well known for their chemical defences. Most of the chemically defended species studied so far biosynthesize toxins *de novo* (review in Pasteels *et al.,* 1988). However there have been several reports recently on the utilization of food plant precursors or, of direct sequestration of, plant toxins by oligophagous leaf beetles to produce toxins (Pasteels *et al.,* in press). For example, the larvae of the genus *Chrysomela* and of *Phratora vitellinae* derive the salicylaldehyde, secreted by their serial defensive glands, from salicin, a phenolglucoside present in their food plant (*Salix* and *Populus* (Rowell-Rahier & Pasteels, 1982; Pasteels *et al.,* 1983; Rowell-Rahier & Pasteels, 1986)). Similarly, the juglone produced by larvae of *Gastrolina depressa* (Matsuda & Sukawara, 1980) most probably derives from a glucoside present in the *Juglans* leaves on which they feed. Another well documented example is the sequestration and accumulation of the cucumber cucurbitacins by all life stages of several *Diabrotica* species and *Acalymna vittatum* (Ferguson & Metcalf, 1985). Bowers (in press) found iridoid glucosides in the alticine *Dibolia borealis* feeding on Scrophulariaceae, although further quantitative work is necessary to determine if these compounds are accumulated in quantities large enough for defensive purpose. There has been a report of sequestration of hypericin by the specialized *Hypericum*-feeder *Chrysolina brunsvicensis* (Rees, 1969), but Duffey & Pasteels (in preparation) have not been able to confirm it.

We present here results suggesting a new example of chemical influence of the food plant on a leaf bee-

tle defensive secretion.

Oreina spp. are large and very brightly coloured leaf beetles. They are particularly abundant and rich in species in the alpine region of central Europe, and their distribution ranges from 600 to 2500 m in elevation. In this genus, the adults but not the larvae have defensive glands. Like many other leaf beetles of the closely related genus *Chrysolina,* they secrete cardenolides (Pasteels *et al.,* 1984; Van Oycke *et al.,* 1988), which they biosynthesise from cholesterol (Van Oycke *et al.,* 1987). We have now found one species, *Oreina cacaliae* which stands out as an exception: the major component of the secretion is the pyrrolizidine alkaloid seneciphylline N-oxide. This species is a specialist on Asteraceae known to contain pyrrolizidine alkaloids. Until now, there was no known example of sequestration of plant natural products into the elytral and pronotal glands of adult leaf beetles, although plant derived defensive toxins were found either in the larval exocrine secretions or in the adult body fluids.

Material and methods

The beetles were collected near Vallouise (Briançon, France) at elevations between 1800 and 2300 m. In that locality and altitude, all the *O. cacaliae* belong to a blue colour morph and feed exclusively on *Adenostyles leucophylla* (Asteraceae). The beetles were manipulated with fine forceps in order to cause emission of secretion which was collected on filter papers and immediately placed in methanol for storage until further use. The beetles were kept in laboratory cultures on their food plants and 'milked' at weekly intervals.

Thin layer chromatographic analyses were done on silica gel F254 plates (Macherey-Nagel); eluent: dichloromethane-methanol 8:2; visualisation by UV light at 254 nm, or sprayed with ceric sulfate, Dragendorff reagent, Kedde reagent or ninhydrin.

Seneciphylline N-oxide was isolated by flash silica gel column chromatography (eluent:dichloromethane-methanol 8:2).

The NMR spectra were recorded on a Bruker WM 250 spectrometer in CD_3OD with TMS as internal standard. Mass spectra were run on a VG 70 S apparatus. Rotation measurements were made on a Perkin-Elmer 141 polarimeter.

Reduction of seneciphylline N-oxide

Seneciphylline N-oxide (8 mg) was treated (Christie *et al.,* 1949) for 2 h at room temperature with an excess of zinc and $CuSO_4$ in 2N H_2SO_4 (4 ml). The reaction mixture was basified with NH_4OH, extracted three times with CH_2Cl_2 and the combined organic phases were evaporated to dryness. The residue was purified by silica gel column chromatography (eluent:dichloromethane-methanol 95:5), yielding 6 mg of seneciphylline, identified on the basis of its physical properties (see Results).

Results

1200 Milkings of *O. cacaliae* afforded 66 mg of crude secretion after the evaporation of the solvent.

No cardenolides were detected on the tlc plates (Kedde reagent). However, a single major component was visualised by UV light, ceric sulfate and Dragendorff reagent. An additional more polar constituent was revealed by spraying the plates with ninhydrin. Flash chromatography afforded 28 mg of the major constituent identified as seneciphylline N-oxide on the following grounds: amorphous solid; $[\alpha]_{579}^{20} = -68°$ (CH_3OH, c = 0.65); FAB/MS, positive mode: $(M + H)^+$ at m/z 350; negative mode: M^- at m/z 349; DCI/MS: $(M + H)^+$: m/z 350; $(M + H - O)^+$: m/z 334; IR: 3500–2900, 1730 and 1715 cm^{-1}; UV: λ_{max} 210 nm (ϵ9.900); ^1H and ^{13}C NMR spectra nearly identical with those of seneciphylline N-oxide (Molyneux *et al.,* 1982; Segall & Dallas, 1983).

This identification was confirmed by zinc dust reduction of the natural constituent (see Methods) into seneciphylline, identified on the basis of its physical properties: m.p. 206–208°; $[\alpha]_{579}^{20}$: −132° ($CHCl_3$, c = 0.23) ((literature: m.p. 208–209°; $[\alpha]_D^{20} = -129°$ ($CHCl_3$, c = 1.86), Orekhov & Tidebel, 1935)); ^1H NMR nearly identical with that reported for seneciphylline (Segall & Dallas, 1983).

The ^1H NMR spectrum of the sample shows, be-

sides the signals of seneciphylline N-oxide, those of a minor component (about 15%) which is tentatively identified as senecionine N-oxide.

The ninhydrin positive constituent was identified as ethanolamine, by comparison with an authentic sample on tcl (eluent:n-BuOH-AcOH-:H_2O, 8:2:2).

Discussion

Comparative studies of chrysomelid beetles have demonstrated a good correlation between current classification and the chemical nature of the secretions (Pasteels *et al.,* 1984, 1988). In most species of *Oreina* and *Chrysomela* (Chrysolinina, Chrysomelini, Chrysomelinae), the defensive secretion are characterized by complex mixtures of cardenolides accompanied by ethanolamine (Pasteels *et al.,* 1988; Van Oycke *et al.,* 1988). Contrary to well known examples of the use of cardenolides for defence in other insects (e.g. von Euw *et al.,* 1967; Brower & Glazier, 1975; Scudder *et al.,* 1986), these cardenolides are not sequestered directly from the plants, but the beetles synthesize them from cholesterol (Van Oycke *et al.,* 1987). The presence of seneciphylline N-oxide in the defensive secretion of *O. cacaliae* is thus a striking departure from the usual pattern and should be considered as a derived condition. Additionally, the host plants of *O. cacaliae* are known to contain pyrrolizidine alkaloids in their N-oxide form in their leaves (Robbins, 1981). Thus it is probable that the alkaloids of *O. cacaliae* are derived from its food plant. The sequestration of pyrrolizidines for defense or as pheromone precursors is well known in danaid butterflies and arctiid moths as well as in a few other insects (review in Boppré, 1986).

Although the population of *O. cacaliae* studied was found only on *Adenostyles leucophylla* in the field, the beetles also feed on other Asteraceae in the laboratory, for example *Senecio nemorensis, Petasites paradoxus* and *P. album*. These plants are hosts in the field in other, geographically separated populations of *O. cacaliae.* Moreover, preliminary results indicate that in at least some of these other populations (e.g. French Vosges or Swiss Apenzell) pyrrolizidine alkaloids are also present in the secretions. These secretions are somewhat different quantitatively and qualitatively to that of the Vallouise population. We do not know at this point if the observed differences are the result of differences in the alkaloid patterns in the different host plants and/or to differences between the beetle populations in the way they sequester and metabolize the alkaloids. A closely related species, *O. speciosissima* (in fact so difficult to separate from *O. cacaliae* that it was confused with it in previous publications, Pasteels & Daloze, 1977; Pasteels *et al.,* 1984, 1988) feeds on exactly the same range of asteraceous food plants, but does not sequester pyrrolizidine alkaloids. Like the adults of other *Oreina* species feeding on Apiaceae or on *Centaurea,* those of *O. speciosissima* secrete cardenolides. A similar evolution from *de novo* synthesis of toxins to the use of plant-derived compounds is known in some chrysomelid larvae (i.e. *Chrysomela* spp., *Phratora vitellinae* and *Gastrolina depressa,* see Introduction), but not in the adults. The situation of *O. cacaliae* and *O. speciosissima* parallels that observed in the larvae of *Phratora vitellinae* and *Ph. laticollis* or *Ph. tibialis.* All three *Phratora* species feed on *Salix* or *Populus,* their spectra of plant foods partially overlap, but only one species, *Ph. vitellinae,* utilizes a food-plant precursor (salicin) for its defensive secretion (salicylaldehyde), the others biosynthesize *de novo* iridoid monoterpenes. In both the *Oreina* adults and the *Phratora* larvae the biosynthesized or the sequestered defensive compounds are stored in and released from the same organs. Thus *Oreina* adults and *Phratora* larvae provide excellent models to study how the utilization or sequestration of host plant toxins has evolved in specialized insects, and how preexisting tissues and structures are modified for this novel function from production of *de novo* defenses. We are engaged in further investigation of these topics.

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