Chemoecology 13:55–62 (2003)
0937–7409/03/010055–8

CHEMOECOLOGY

Uptake and metabolism of [^{14}C]rinderine and [^{14}C]retronecine in leaf-beetles of the genus *Platyphora* and alkaloid accumulation in the exocrine defensive secretions

Jacques M. Pasteels[1], Claudine Theuring[2], Donald M. Windsor[3], and Thomas Hartmann[2]

[1]Laboratoire de Biologie Animale et Cellulaire Université Libre de Bruxelles B-1050 Bruxelles, Belgium
[2]Institut für Pharmazeutische Biologie Technischen Universität Braunschweig D-38106 Braunschweig, Germany
[3]Smithonian Tropical Research Institute, Apartado 2072, Balboa-Ancon, Panama

Summary. Sequestration and processing of pyrrolizidine alkaloids (PAs) by leaf beetles of the genus *Platyphora* were investigated. Tracer experiments with labeled alkaloids were performed with *P. eucosma* feeding on *Koanophyllon panamense* (Asteraceae, tribe Eupatorieae). *P. eucosma* catalyzes the same reactions previously demonstrated for *P. boucardi* specialized to *Prestonia portobellensis* (Apocynaceae): (i) epimerization of rinderine to intermedine; (ii) esterification of retronecine yielding insect-specific PAs; (iii) efficient transport of the PAs as free bases into the defensive secretions. *P. bella* feeding on *Tournefortia cuspidata* (Boraginaceae) shows the same sequestration behavior and ability to synthesize the specific retronecine esters. *P. ligata,* a species phylogenetically closely related to the PA adapted species and clustering in the same clade, but feeding on a host plant devoid of PAs, feeds easily on PA treated host-plant leaves, but does not sequester or metabolize PAs. *P. kollari* a species clustering outside the PA clade refused to feed on its food-plant leaves painted with PAs. The results are discussed in relation to host-plant selection of the PA adapted species and the role of PAs in chemical defense.

Key words. Alkaloid sequestration – chemical defense – host-plant selection – *Platyphora* leaf beetles – pyrrolizidine alkaloids

Introduction

Leaf beetles of the related genera *Oreina* and *Platyphora* belonging to the subfamily Chrysomelinae (Coleoptera, Chrysomelidae) feeding on host plants containing pyrrrolizidine alkaloids (PAs) utilize in their exocrine defensive secretions PAs acquired from their host plants in combination with cardenolides or sapononines.

Species of the Palaearctic genus *Oreina* endogenously synthesize cardenolides from general sterol precursors such as cholesterol (van Oycke *et al.* 1987). *Oreina* species feeding on *Adenostyles alliariae* and *Senecio nemorensis* (Asteraceae, tribe Senecioneae) which contain macrocyclic pyrrolizidine alkaloids of the senecionine type (Hartmann & Witte 1995) accumulate these alkaloids in addition to cardenolides in their defensive secretions (Pasteels *et al.* 1988, Rowell-Rahier *et al.* 1991). The host plants contain PAs in the state of their non-toxic *N*-oxides (for details see Hartmann 1999) which are absorbed as such by the beetles and stored in the hemolymph and solid body-tissues. The body compartment functions as a reservoir that replenishes the exocrine glands where the PA *N*-oxides are concentrated and released with the defensive secretions in quantities up to 0.3 mol l^{-1} (Pasteels *et al.* 1995, Hartmann *et al.* 1997). Host-plant PAs entering the beetle's body as pro-toxic tertiary alkaloid are detoxified by glucosylation (Hartmann *et al.* 1999). Among the four *Oreina* species known to sequester PA *N*-oxides there is only one species (*O. cacaliae*) that abandoned the synthesis of cardenolides and totally relies on the host-plant derived defense (Pasteels *et al.* 1995, Dobler *et al.* 1996).

In its defensive chemistry the neotropical genus *Platyphora* shows many parallels to *Oreina* leaf beetles. Instead of cardenolides, *Platyphora* synthesizes pentacyclic triterpene saponins of the oleanan type (Plasman *et al.* 2000 a and b, Plasman *et al.* 2001). The assumed saponin precursor, β-amyrin, is supplied most probably by the host plant; hexane extracts of host-plant leaves were shown to contain α-and β-amyrin (Plasman *et al.* 2001). The known species of the vast genus *Platyphora* are specialized to single host plants out of six plant families (Pasteels *et al.* 2001, Termonia *et al.* 2002). Among these taxa, there are three families (i.e., Asteraceae tribe Eupatorieae, Apocynaceae and Boraginaceae) which share the ability to synthesize unique open-chain monoester PAs of the lycopsamine type (Hartmann & Witte 1995, Hartmann & Ober 2000). All the *Platyphora* species specialized to alkaloid-containing species out of these families are capable of sequestering PAs of the lycopsamine type and accumulating them along with the saponins in their exocrine defensive secretions (Pasteels *et al.* 2001). However, in comparison to the *Oreina* leaf beetles, *Platyphora* beetles developed a completely different biochemical strategy to deal with PAs. Instead of sequestering PAs as non-toxic *N*-oxides, *Platyphora* species reduce PA *N*-oxides in the gut and exclusively absorb the pro-toxic tertiary PAs. In contrast to *Oreina*, absorbed PAs do not accumulate in the body, but are efficiently removed from the

Correspondence to: Thomas Hartmann, e-mail: t.hartmann@tu-bs.de, Jacques M. Pasteels, e-mail: jmpastee@ulb.ac.be

hemolymph and "pumped" into the exocrine glands where they are concentrated and released with the defensive secretions (Hartmann *et al.* 2001). Studies with *P. boucardi* revealed that ingested plant-derived PAs are metabolized in a unique manner: (i) PAs of the lycopsamine type with 7S configuration (e.g., rinderine) are epimerized yielding the respective 7R derivatives (e.g., intermedine); (ii) free necine base (e.g., retronecine) is esterified with 2-hydroxy analogues of aliphatic amino acids yielding unique "beetle PAs" which are not found in the alkaloid patterns of the host plants (Hartmann *et al.* 2001).

A recently established molecular phylogram indicates that all five PA-sequestering *Platyphora* species cluster together in a distinctive PA clade (Termonia *et al.* 2002). The close taxonomic relationship between these species is corroborated functionally in their defensive chemistry. Although feeding on species of three taxonomically diverse plant families, they display almost identical alkaloid patterns in their defensive secretions (Pasteels *et al.* 2001). Based on the unique biochemistry of PA sequestration studied in detail with *P. boucardi*, we suggested that once *Platyphora* became biochemically adapted to PAs of the lycopsamine type, this character became the common driving force for further host-plant selection (Hartmann *et al.* 2001). To confirm this suggestion, *P. eucosma* feeding on *Koanophyllon panamense* (Asteraceae, tribe Eupatorieae) and *P. bella* feeding on *Tournefortia cuspidata* (Boraginaceae), two more species belonging to PA clade, were analyzed for their ability to sequester and metabolize PAs. The results are compared to those obtained with *P. boucardi,* feeding on *Prestonia portobellensis* (Apocynaceae). In addition, *P. ligata*, a species closely related to *P. eucosma*, feeding on *Mikania micrantha* (Asteracae, tribe Eupatorieae), a host plant devoid of PAs, was included in the studies. *P. kollari* feeding on *Ipomoea batatas* (Convolvulaceae) and belonging to a different clade, outside the PA clade, was chosen as a control indicative of *Platyphora* species not related to those feeding on PA-containing plant taxa.

Materials and Methods

Insects

All species originated from Panama, except for *Platyphora kollari* from Brazil. *P. eucosma* (Stal) was collected on *Koanophyllom panamense* R. M. King & H. Robinson in Cerro Campana (alt. 800 m); *P. ligata* (Stal) and *P. bella* (Baly) in Cerro Jefe (850m), respectively, on *Mikania micrantha* H. B. K. and *Tournefortia angustifolia* (Kunth in Humb.) Bonpl. & Kunth. *P. kollari* (Stal) was collected in Fortaleza on *Ipomoea asarifolia* (Desr.) Roem & Schult. *P. eucosma, P. ligata* and *P. kollari* were reared for several generations in Brussels. *P. eucosma* and *P. ligata* fed on their original food plants grown in a greenhouse. *P. kollari* fed on *Ipomoea batatas* (L.) Lam. Some feeding experiments with *P. eucosma* and all experiments with *P. ligata* and *P. kollari* were performed with laboratory reared insects. Experiments with *P. bella* and some with *P. eucosma* were performed in Panama using field collected insects.

Tracer feeding experiments

All tracers feeding studies were performed as described previously (Hartmann *et al.* 2001). The ^{14}C-labelled tracers (1.5 10^5 cpm) were offered individually, painted on the surface of the respective host-plant leaves. The ^{3}H-labelled tracer was applied at a total radioactivity of 10^6 cpm. After termination of the experiments, beetles were dissected as described by Hartmann *et al.* (2001); and larvae, as specified by Pasteels *et al.* (2003). Secretions and tissues were preserved in methanol until analysis in Braunschweig.

P. eucosma experiments, [^{14}C]rinderine feeding (Panama): Five beetles received the tracer, painted on host-plant leaf-pieces, for 6 days (2 individuals) and 4 days (3 individuals), afterwards the beetles were kept on non-treated host-plant leaves for another 8 days. Secretions were collected at day 8 and 15 and were pooled. The beetles were dissected immediately after second secretion (day 15) was collected.

[^{14}C]Retronecine feeding (Panama): Four beetles received the tracer for 6 days (3 individuals) and 4 days (1 individual) and were further treated as described for the rinderine feeding.

[^{3}H]Isoretronecanol feeding (Brussels): Three beetles received each 10^6 cpm tracer painted on host plant pieces for 5 days and then transferred to untreated leaves. After 10 days, secretions were collected and the beetles killed and dissected.

P. bella experiments (Panama): Three beetles received [^{14}C]retronecine for 4 days and where then transferred to untreated leaves. Secretions were collected after 8 days and the beetles killed and dissected.

P. ligata experiments (Brussels): Three beetles each were fed with [^{14}C]rinderine and [^{14}C]retronecine, respectively. After 4 days the secretions were sampled and the beetles dissected. The experiment was repeated with 3 larvae that were killed and dissected 2 days after feeding.

Alkaloid analysis

Procedures for the extraction, separation and quantification of labeled alkaloids and their metabolites were exactly the same as described previously (Hartmann *et al.* 2001). Separation and identification of PAs by gas chromatography combined with mass spectrometry (GC-MS) was achieved according to Witte *et al.* (1993).

Alkaline hydrolysis of isoretronecanol esters

The alkaloid fraction obtained from the secretions of beetles which previously fed on [^{3}H]isoretronecanol was evaporated, dissolved in 100 µl 10% NaOH and hydrolyzed at 95°C for 120 min. The labeled necine base was identified by TLC and radiodetection in comparison to authentic reference compounds.

Results

Pyrrolizidine alkaloids in Koanophyllon panamense *and its leaf beetle* Platyphora eucosma

A previous alkaloid analysis of *K. panamense* revealed only small amounts of the simple necine bases isoretronecanol and trachelanthamidine (see Fig. 4) (Pasteels *et al.* 2001). *Koanophyllon* is closely related to the genus *Eupatorium* known to contain PAs of the lycopsamine type (Hartmann & Witte 1995). A re-examination of various plant organs from newly collected plant materials confirmed the presence of isoretronecanol and trachelanthamidine in leaves and inflorescences, but in addition, the latter were found to contain trace amounts of rinderine and lycopsamine. In roots, small quantities of O^7-methylbutanoylintermedine or one of its stereoisomers (RI, 2417; [M^+] m/z, 383) and the respective $O^{3'}$-acetyl derivative (RI, 2493; [M^+] m/z, 425) were found, but no other PAs could be detected.

Analysis of secretions of field collected *P. eucosma* beetles confirmed the presence of intermedine, lycopsamine and of beetle specific necine esters, such as the O^9-(2'-hydroxyisopentanoyl) esters of trachelanthamidine (RI

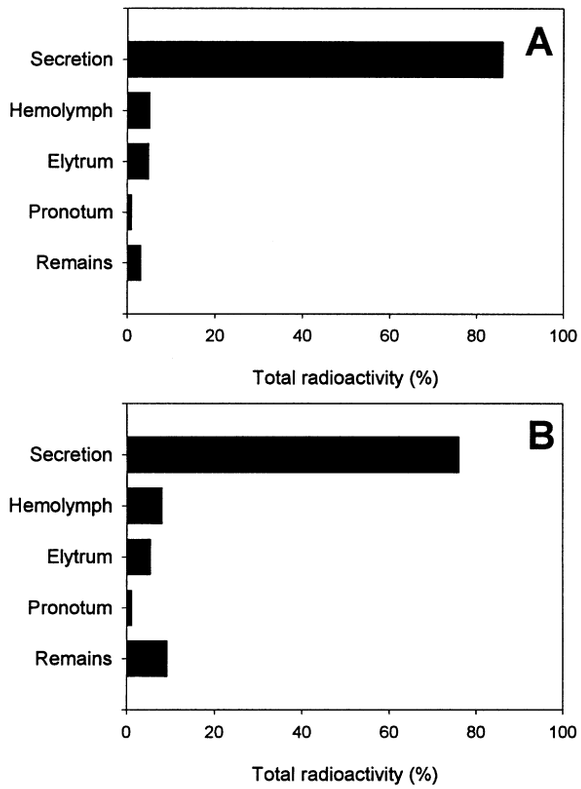

Fig. 1 Uptake and tissue distribution of radioactivity in adult beetles of *P. eucosma* which previously had been fed on (A) [^{14}C]rinderine ($n = 5$) and (B) [^{14}C]retronecine ($n = 4$). Each beetle was allowed to feed on host-plant leaf-pieces painted with $1.5 \cdot 10^5$ cpm tracer. After 4 to 6 days the beetles were transferred to untreated host-plant leaves and secretions were collected 2 and 8 days later and pooled. Beetles were killed and dissected immediately after the collection of the second secretions. Recovery of total radioactivity was 6.1% (rinderine) and 8.1% (retronecine)

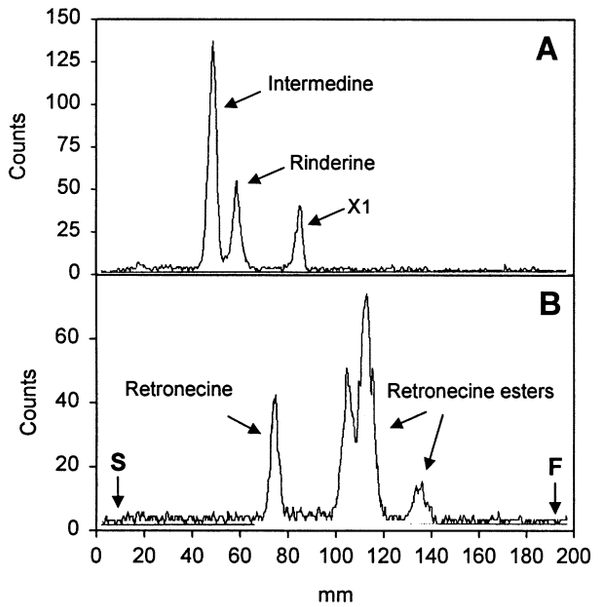

Fig. 2 Metabolism of [^{14}C]rinderine and [^{14}C]retronecine by *P. eucosma.* TLC separation of pooled defensive secretions collected 2 and 8 days after transfer of the beetles from tracer-treated leaves to untreated host-plant leaves. (A) Feeding of labeled rinderine; (B) feeding of labeled retronecine. See Fig. 1 for further details

1734; m/z [M^+] 241) and isoretronecanol (RI 1768; m/z [M^+], 241), the O^7-(2'-hydroxyisopentanoyl) esters of retronecine (RI 1868; m/z [M^+] 255) and of heliotridine (RI 1880; m/z [M^+] 255) as well as the respective O^9-(2'-hydroxyisopentanoyl) esters of retronecine (RI 1888; m/z [M^+] 255) and of heliotridine (RI 1916; m/z [M^+] 255).

These results indicate the ability of *K. panamense* to synthesize necine esters, but still leave open the origin of the plant alkaloids found in field-collected *P. eucosma* (Pasteels *et al.* 2001). In the laboratory, *P. eucosma* readily fed on *K. panamense* inflorescences, which could be a PA source in the field.

Uptake and metabolism of labeled rinderine and retronecine by P. eucosma

Tracer experiments with [^{14}C]rinderine and [^{14}C]retronecine were performed to verify the ability and efficiency of the beetle to metabolize and accumulate these alkaloids. The amount of total radioactivity recovered from the secretions and the various tissues 14 days after feeding is illustrated in Fig. 1. Upon feeding of [^{14}C]rinderine, more than 85% of the total radioactivity recovered from the beetles is located in the secretions (Fig. 1A). In the case of [^{14}C]retronecine, the respective proportion accounted for more than 75% (Fig. 1B). Only a small fraction of total radioactivity (generally less than 5 to 10%) was detected in the hemolymph and residual body parts, indicating an extremely efficient transfer of the absorbed tracers into the defensive glands and secretions. The beetles sequestered about 6% (rinderine feeding) and 8% (retronecine feeding) of the tracer supplied with the food-plant leaves.

The extracts were separated by TLC (Fig. 2) and the relative abundance of the metabolites evaluated (Table 1). Rinderine was largely epimerized to intermedine which in the secretions accounted for more than 60% of the radioactivity (Table 1). The minor metabolite X1 (Fig. 2A) shows the same R_f value as 3'-acetylintermedine or one of its stereoisomers. With the exception of the hemolymph which still contained 15% of unchanged rinderine, the small amount of total radioactivity recovered from the various beetle tissues was attributed to intermedine.

Retronecine was largely metabolized to the beetle specific retronecine esters (Hartmann *et al.* 2001). A typical TLC-profile is illustrated in Fig. 2B. More than 80% of radioactivity recovered from the secretion accounted for retronecine esters (Table 1). In the hemolymph and the body tissues, non-metabolized retronecine was the major compound (Table 1). Again, however, it should be considered that the radioactivity recovered from the beetle's body accounted for less than 25% of total recovered radioactivity (Fig. 1B).

Table 1 Tissue distribution of radioactively labeled metabolites in adults of *Platyphora eucosma* which previously had been fed on [^{14}C]rinderine and [^{14}C]retronecine, respectively

	Relative abundance of radioactivity (%)				
	Fed on [^{14}C]rinderine		Fed on [^{14}C]retronecine		
Tissue	Intermedine	Rinderine	X1	Retronecine	Retronecine esters
Secretions	62	23	15	17	83
Hemolymph	85	15	nd	75	25
Elytra	>95	nd	nd	51	49
Pronotum	>95	nd	nd	not determined[a]	
Remains	>95	nd	nd	>95	<5

[a]Recovery of total radioactivity too low. nd, not detected

Metabolism of labeled isoretronecanol by P. eucosma

Since the stereoisomeric necine bases isoretronecanol and trachelanthamidine (see Fig. 4) were the only alkaloids detected in the above-ground parts of the beetle's food plant *Koanophyllon panamense*, feeding experiments with [^{3}H]isoretronecanol were performed to see whether (i) *P. eucosma* is able to transform this simple necine base into retronecine or (ii) to esterify this necine as indicated by the occurrence of the respective esters in the secretions of wild caught beetles (see above). The result was unequivocal (Fig. 3). TLC analysis of secretions collected from beetles that had received [^{3}H]isoretronecanol showed a labeled peak (Fig. 3B), which upon alkaline hydrolysis yielded a compound which co-migrated with authentic isoretronecanol (Fig. 3C). None of the other necine bases such as retronecine/heliotridine or supinidine were detectable (Fig. 3A), indicating that the beetles are able to esterify isoretronecanol but unable to transform isoretronecanol in one of the other necine bases. Total uptake of labeled isoretronecanol was low (ca 1%); 43% of the ingested radioactivity was recovered from the secretions as isoretronecanol ester (Table 2), most likely the 2-hydroxyisopentanoyl ester that has been identified in the secretions of wild caught beetles. No free isoretronecanol could be recovered from the secretions. The isoretronecanol ester was also the major compound in the body tissues except the hemolymph, which contained almost exclusively the unchanged necine base. An unknown metabolite (Table 2, X) with an R_f value similar to that of retronecine, but according to co-chromatography definitely not identical with this necine base, was detectable in substantial amounts only in the remains.

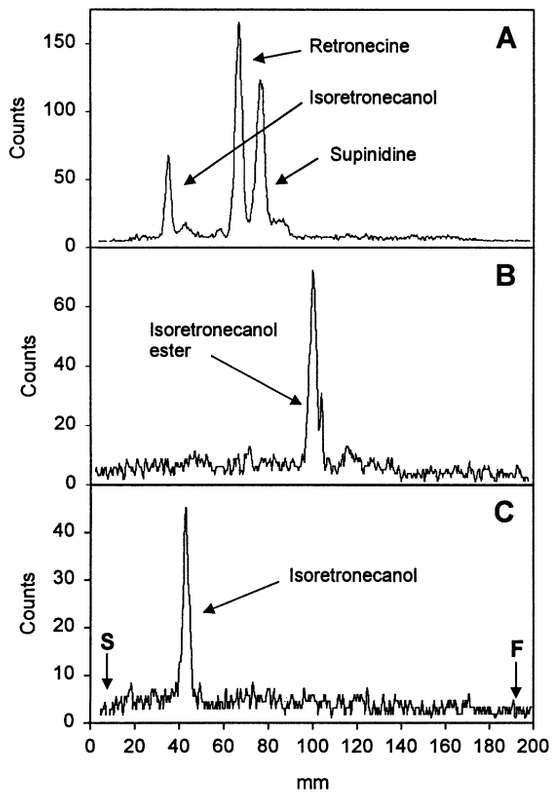

Fig. 3 Metabolism of [^{3}H]isoretronecanol by *P. eucosma.* TLC separation of pooled defensive secretions collected 14 days after transfer of the beetles from tracer-treated leaves to untreated host plant leaves. (A) Reference compounds; (B) Pooled defensive secretions of 3 beetles; (C) defensive secretions after alkaline hydrolysis

Uptake and metabolism of labeled retronecine by P. bella

P. bella beetles were only available for a feeding experiment with [^{14}C]retronecine. The beetles were fed and analyzed in the same way as described above for *P. eucosma.* The beetles synthesized retronecine esters (Table 3) which showed the same TLC migration as the retronecine esters produced by *P. eucosma* (Fig. 2). The individuals used in this experiment appear to be less efficient in their capability of transferring the alkaloid into the defensive secretions. However, it should be taken into consideration that the experiment was carried out with field-collected beetles that did not feed very well. There was no chance to repeat the experiment with more active specimens. However, the experiment confirmed the ability of *P. bella* to synthesize the beetle-specific retronecine esters.

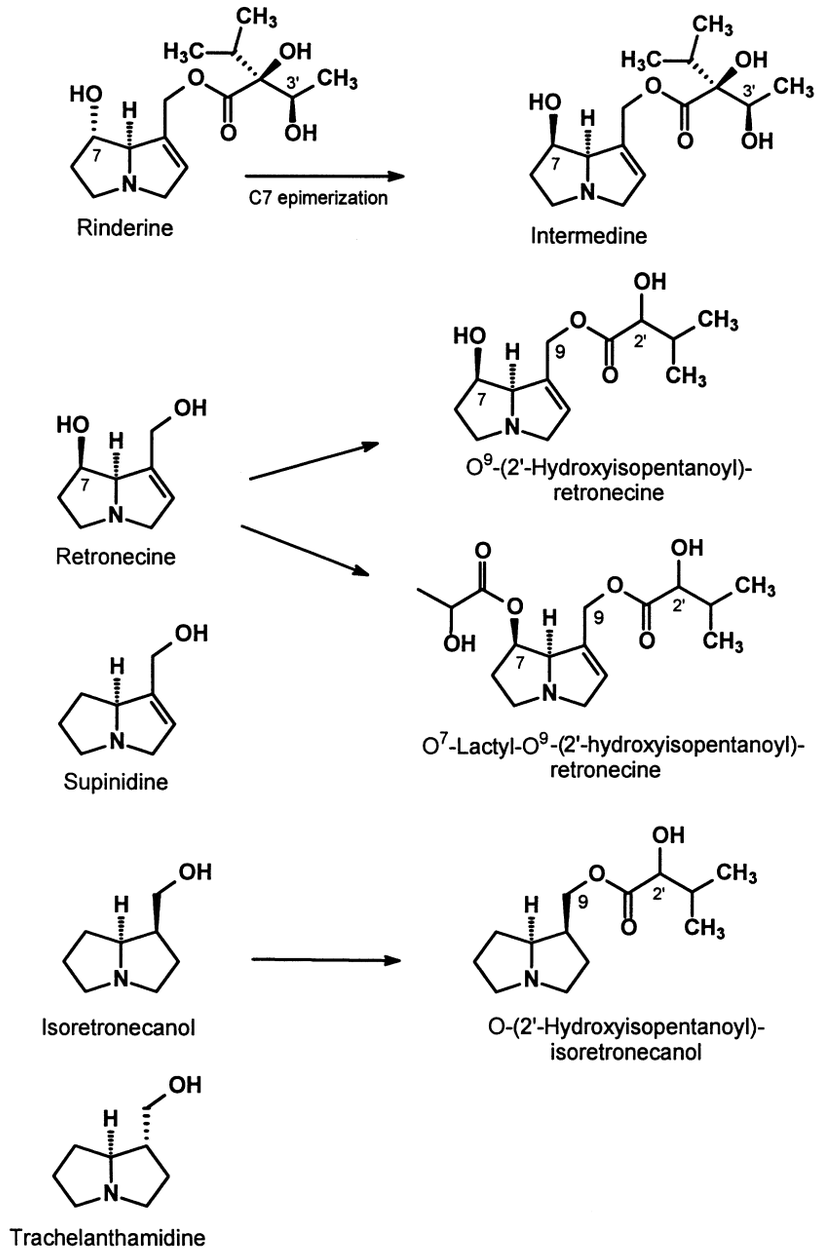

Fig. 4 Transformations of rinderine, retronecine and isoretronecanol catalyzed by *Platyphora* leaf beetles adapted to pyrrolizidine alkaloids of the lycopsamine type

Handling of pyrrolizidine alkaloids by P. ligata and P. kollari

P. kollari does not belong to the beetles of the PA clade and feeds on *Ipomoea asarifolia* and *I. batatas* (Convolvulaceae), two species that do not contain PAs. All efforts to feed *P. kollari* with radioactively labeled PAs failed. Rinderine or retronecine painted on the host-plant leaf (1 µg/2 cm^2) appeared to be strongly deterrent and prevented any feeding.

P. ligata belongs to the PA clade but was shown to be devoid of PAs (Pasteels *et al.* 2001). It feeds on *Mikania micrantha* (Asteraceae, Tribe Eupatorieae) a species in which we did not find any PAs (Hartmann, unpublished results). In contrast to *P. kollari,* beetles and larvae of *P. ligata* were not deterred by PAs and easily fed on host-plant leaves painted with radioactively labeled PAs. However, in experiments performed in the same manner as described above for *P. eucosma* neither labeled rinderine nor

Table 2 Tissue distribution and relative abundance of radioactively labeled metabolites in adults of *Platyphora eucosma* which previously had been fed on [^{3}H]isoretronecanol. Total recovery of radioactivity from secretions and body extracts was ca 1% of radioactivity supplied

	Total radioactivity (%)	Relative abundance of radioactivity (%)		
		Isoret	Isoret ester	X
Secretions	43	nd	100	nd
Hemolymph	17	100	nd	tr
Elytra	13	20	80	nd
Pronotum	8	tr	>90	tr
Remains	21	35	45	20

Isoret, isoretronecanol; nd, not detected; tr, traces

Table 3 Tissue distribution and relative abundance of radioactively labeled metabolites in adults of *Platyphora bella* which previously had been fed on [^{14}C]retronecine ($n = 3$). Each beetle was allowed to feed on host plant leaves painted with $1.5 \cdot 10^5$ cpm tracer for 4 to 6 days, then the beetles were transferred to untreated host-plant leaves and secretions were collected 2 and 8 days later and pooled. Beetles were killed and dissected immediately after the collection of the second secretion. Recovery of total radioactivity was 1.9%

	Total radioactivity (%)	Rel. abundance of radioactivity (%)	
		Retronecine	Retronecine esters
Secretions	11	nd	>95
Hemolymph	25	nd	>95
Elytra	19	nd	>95
Pronotum	3	not determined[a]	
Remains	42	40	60

[a]Recovery of total radioactivity too low. nd, not detected

Table 4 Feeding of [^{14}C]rinderine and [^{14}C]retronecine to adults and larvae of *Platyphora ligata*. Insects were fed individually with $1.5 \cdot 10^5$ cpm each. Secretions (adults) and potential surface radioactivity (larvae) were collected three days after feeding; afterwards individuals were killed, dissected and preserved till analysis. $n = 3$ per trial; nd, not detected

	Relative abundance (% of tracer fed)		
Tracer fed	Secretions or larval surface	Hemolymph	Carcass
Adult beetles I			
Rinderine	nd	nd	0.7
Retronecine	nd	nd	9.8
Adult beetles II			
Rinderine	nd	nd	<0.2
Retronecine	<0.05	<0.05	<0.3
Larvae			
Rinderine	nd	nd	0.6
Retronecine	nd	<0.05	1.0

labeled retronecine were metabolized by larvae and adult beetles. There was absolutely no transfer and accumulation of alkaloid into the beetles' defensive secretions (Table 4). Generally the accumulation of radioactivity in the bodies was far below 1%. Only in one experiment with labeled retronecine fed to adult beetles, almost 10% of radioactivity could be recovered from the carcass, but again nothing from the secretions (Table 4). Besides unaltered retronecine, only polar metabolites were detected in body extracts.

Discussion

Within the diverse genus *Platyphora* a well defined distinctive clade, the "PA clade", includes all species known to sequester PAs (Termonia *et al.* 2002). The five species analyzed in detail all display the same PA composition in their secretions and store PAs exclusively in the pro-toxic tertiary form (free base) (Pasteels *et al.* 2001). Detailed tracer studies with *P. boucardi* (Hartmann *et al.* 2001) revealed that the insects possess at least three specific biochemical activities to handle and maintain plant-acquired PAs: (i) O^7- and to some extent $O^{3'}$-epimerization of rinderine to intermedine and lycopsamine the major stereoisomers found in the secretions of all wild caught PA sequestering *Platyphora* beetles (Pasteels *et al.* 2001); (ii) esterification of free retronecine with aliphatic 2-hydroxy analogues of valine and alanine, i.e. formation of "beetle PAs"; (iii) efficient accumulation of plant-acquired PAs and self-synthesized retronecine esters in the defensive glands against a steep concentration gradient. A more than 100-fold concentration difference was found to exist between hemolymph and defensive secretions. Moreover, larvae of *P. boucardi* catalyze the same metabolic reactions as shown for adults, although they do not possess the defensive glands of adults. They store the PAs in their bodies and are able to transfer the alkaloids during metamorphosis via pupae into the defensive secretions of adults (Pasteels *et al.* 2003).

The tracer feeding studies with *P. eucosma* confirmed the results obtained with *P. boucardi.* The two beetle species feeding on host plants from different plant families

(i.e., Apocynaceae and Asteraceae, tribe Eupatorieae, respectively) show the same biochemical specificities. Unfortunately with *P. bella*, a species feeding on a Boraginaceae, a complete analysis was not possible due to the limited availability of individuals. However, at least the formation of the beetle PAs from retronecine takes place in the same manner as with the two other species.

The consistent sequestration behavior supports the view that all PA-sequestering species of the "PA clade" are derived from a common ancestor adapted to plant species containing PAs of the lycopsamine type. During speciation they may have become adapted to species of those plant families that have in common PAs of the lycopsamine type. This suggests that not taxonomic relations, but a common chemical character may have governed host-plant selection during the leaf beetle's speciation. In this context, *P. ligata* appears to be a revealing species. Adults and larvae of *P. ligata* easily feed on host-plant leaves painted with labeled PAs, but they are unable to accumulate PAs in their defensive secretions and did not show any of the specific metabolic reactions described for the PA-sequestering species. Probably this species lost its ability to maintain and metabolize plant-acquired PAs after it secondarily switched from a PA-containing host plant to its present host *Mikania micrantha* which, even though belonging to the tribe Eupatorieae, was found devoid of PAs. Just the sensory tolerance to PAs seems to have survived. This is supported by the observation that *P. kollari*, a related species from outside the PA clade refused to feed on host-plant leaves painted with PAs.

The inability of *P. ligata* to metabolize and accumulate plant-acquired PAs convincingly corroborates the specificity of these processes in the PA-adapted *Platyphora* species. It also suggests that these processes are sustained under selection pressure, which in the case of PA sequestration could have two different causes: (i) an ecological one, determined by the pressure of predation and (ii) a physiological one, determined by the need for safe maintenance and compartmentation of the pro-toxic alkaloids. It is impossible to decide explicitly which of the two causes are contemporarily more important. However in comparison to other PA-sequestering insects, we have an intriguing situation. The host plants of the five PA-adapted *Platyphora* species have in common very low alkaloid contents (Hartmann *et al.* 2001, Pasteels *et al.* 2001). On the other hand, we have well adapted insects with specific mechanisms to handle plant-acquired PAs and even synthesize their own esters from necine bases. Secretions from wild caught beetles show rather low PA concentration, generally ranging from 2 to 7 mM (Pasteels *et al.* 2001), which is more than 50- to 100-fold lower than the levels found in defensive secretions of *Oreina* leaf beetles (Rowell-Rahier *et al.* 1991, Pasteels *et al.* 1996, Hartmann *et al.* 1997). The tracer feeding studies demonstrate that the potential of *P. boucardi* to concentrate plant-acquired PAs (rinderine) and the beetle-synthesized retronecine esters in their defensive secretions is considerably higher (almost 40 mM) than what is realized in the field (Hartmann *et al.* 2001).

In the palaearctic *Oreina* leaf beetles, at least one species (*O. cacaliae*) gave up its autogenous defense in favor of PA sequestration. Substitution of autogenous defense by host-plant acquired defense in *O. cacaliae* emphasizes the great potential of PAs as defense chemicals. In fact, it was shown that plant-acquired PAs provide *O. cacaliae* leaf beetles with a better protection from predation by birds than do autogenously synthesized cardenolides (Rowell-Rahier *et al.* 1995). This is supported by examples of other PA-adapted insect species. PA-storing neotropical ithomiine butterflies are well protected against predation by the tropical orb spider *Nephila*. Butterflies containing PAs are immediately cut out of the web by the spider and liberated unharmed, whereas insects devoid of PAs are eaten (Eisner & Meinwald 1987, Masters 1990, Trigo *et al.* 1993, Trigo *et al.* 1996). Arctiid moths protect their eggs by endowing them with PAs against predators such as coccinellid beetles (Dussourd *et al.* 1988), larvae of the green lacewing (*Ceraeochrysa cubana*, Neuroptera) (Eisner *et al.* 2000), and ants (Hare & Eisner 1993). It is not known which PA concentration is needed to assure protection. The low PA concentration needed to prevent *P. kollari* from feeding, may indicate a sensitive deterrent role of PAs. In PA-adapted lepidopterans, PAs are often phagostimulants. In the polyphagous caterpillar of the arctiid moth *Estigemene acraea*, a highly sensitive taste receptor cell for PAs exists in the lateral galeal sensillum which respond to PA concentrations down to less than 10^{-9} mol · l^{-1} (Bernays *et al.* 2002). It is still not known whether in non-adapted insects similarly low concentration may cause feeding deterrence. *Platyphora* leaf beetles are protected by saponins. Is there an ecological advantage for the alkaloid-sequestering *Platyphora* species to store PAs in addition to saponins in comparison to *Platyphora* species simply secreting saponins? Are the relatively low PA concentrations found in *Platyphora* substantial enough to guarantee a protective function? More ecological evidence is needed to answer these questions and to elucidate the defensive functions of PAs of *Platyphora* leaf beetles.

Acknowledgements

The studies were supported by grants from the Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie to TH and from the Belgian Fund for Joint Basic Research (2.4560.00) to JMP. Logistical support provided by the Smithsonian Research Institute facilitated field portions of this study.

References

Bernays EA, Chapman RF, Hartmann T (2002) A highly sensitive taste receptor cell for pyrrolizidine alkaloids in the lateral galeal sensillum of a polyphagous caterpillar, *Estigmene acraea*. J Comp Physiol A 188**,** 715–723.

Dobler S, Mardulyn P, Pasteels JM, Rowell-Rahier M (1996) Host-plant switches and the evolution of chemical defense and life history in the leaf beetle genus *Oreina*. Evolution 50, 2373–2386

Dussourd DE, Ubik K, Harvis C, Resch J, Meinwald J, Eisner T (1988) Biparental defensive endowment of eggs with acquired plant alkaloid in the moth *Utetheisa ornatrix*. Proc Natl Acad Sci USA 85, 5992–5996

Eisner T, Eisner M, Rossini C, Iyengar VK, Roach BL, Benedikt E, Meinwald J (2000) Chemical defense against predation in an insect egg. Proc Natl Acad Sci USA 97, 1634–1639

Eisner T, Meinwald J (1987) Alkaloid-derived pheromones and sexual selection in Lepidoptera. Pp 251–269 *in* Prestwich GD, Blomquist GD (eds) Pheromone Biochemistry. New York, Academic Press

Hare JF, Eisner T (1993) Pyrrolizidine alkaloid deters ant predators of Utetheisa ornatrix eggs: Effects of alkaloid concentration, oxidation state, and prior exposure of ants to alkaloid-laden prey. Oecologia 96, 9–18

Hartmann T (1999) Chemical ecology of pyrrolizidine alkaloids. Planta 207, 483–495

Hartmann T, Ober D (2000) Biosynthesis and metabolism of pyrrolizidine alkaloids in plants and specialized insect herbivores. Pp 207–244 *in* Leeper FJ, Vederas JC (eds) Topics in Current Chemistry: Biosynthesis – Aromatic Polyketides, Isoprenoids, Alkaloids, vol 209. Berlin, Springer

Hartmann T, Theuring C, Schmidt J, Rahier M, Pasteels JM (1999) Biochemical strategy of sequestration of pyrrolizidine alkaloids by adults and larvae of chrysomelid leaf beetles. J Insect Physiol 45, 1085–1095

Hartmann T, Theuring C, Witte L, Pasteels JM (2001) Sequestration, metabolism and partial synthesis of tertiary pyrrolizidine alkaloids by the neotropical leaf-beetle *Platyphora boucardi*. Insect Biochem Mol Biol 31, 1041–56

Hartmann T, Witte L (1995) Pyrrolizidine alkaloids: chemical, biological and chemoecological aspects. Pp 155–233 *in* Pelletier SW (ed) Alkaloids: Chemical and Biological Perspectives, vol 9. Oxford, Pergamon Press

Hartmann T, Witte L, Ehmke A, Theuring C, Rowell-Rahier M, Pasteels JM (1997) Selective sequestration and metabolism of plant derived pyrrolizidine alkaloids by chrysomelid leaf beetles. Phytochemistry 45, 489–497

Masters AR (1990) Pyrrolizidine alkaloids in artificial nectar protect adult ithomiine butterflies from a spider predator. Biotropica 22 (3), 298–304

Pasteels JM, Dobler S, Rowell-Rahier M, Ehmke A, Hartmann T (1995) Distribution of autogenous and host-derived chemical defenses in *Oreina* leaf beetles (Coleoptera: Chrysomelidae). J Chem Ecol 21, 1163–1179

Pasteels JM, Rowell-Rahier M, Randoux T, Braekman JC, Daloze D (1988) Pyrrolizidine alkaloids of probable host-plant origin in the pronotal and elytral secretion of the leaf beetle *Oreina cacaliae*. Entomol Exp Appl 49, 55–58

Pasteels JM, Rowell-Rahier M, Ehmke A, Hartmann T (1996) Host-derived pyrrolizidine alkaloids in *Oreina* leaf beetles: physiological, ecological and evolutionary aspects. Pp 213–225 *in* Jolivet P, Cox ML (eds) Chrysomelidae Biology, vol 2, Ecological studies. Amsterdam, Academic Publishing

Pasteels JM, Termonia A, Windsor D, Witte L, Theuring C, Hartmann T (2001) Pyrrolizidine alkaloids and pentacyclic triterpene saponins in the defensive secretions of *Platyphora* leaf beetles. Chemoecology 11, 113–120

Pasteels JM, Theuring C, Witte L, Hartmann T (2003) Sequestration and metabolism of pro-toxic pyrrolizidine alkaloids by larvae of the leaf beetle *Platyphora boucardi* and their transfer via pupae into the defensive secretions of adults. J Chem Ecol (in press)

Plasman V, Braekman JC, Daloze D, Luhmer M, Windsor D, Pasteels JM (2000a) Triterpene saponins in the defensive secretion of a chrysomelid beetle, *Platyphora ligata*. J Nat Prod 63, 646–9

Plasman V, Braekman JC, Daloze D, Windsor D, Pasteels JM (2000b) Triterpene saponins, quaternary ammonium compounds, phosphatidyl cholines, and amino acids in the pronotal and elytral secretions of *Platyphora opima* and *Desmogramma subtropica*. J Nat Prod 63, 1261–4

Plasman V, Plehiers M, Braekman JC, Daloze D, de Biseau JC, Pasteels JM (2001) Chemical defense in *Platyphora kollari* Baly and *Leptinotarsa behrensi* Harold (Coleoptera: Chrysolemidae). Hypothesis on the origin and evolution of leaf beetles toxins. Chemoecology 11, 107–112

Rowell-Rahier M, Pasteels JM, Alonso MA, Brower LP (1995) Relative unpalatability of leaf beetles with either biosynthesized or sequestered chemical defence. Anim Behav 49, 709–714

Rowell-Rahier M, Witte L, Ehmke A, Hartmann T, Pasteels JM (1991) Sequestration of plant pyrrolizidine alkaloids by chrysomelid beetles and selective transfer into defensive secretions. Chemoecology 2, 41–48

Termonia A, Pasteels JM, Windsor DM, Milinkovitch MC (2002) Dual chemical sequestration: a key mechanism in transitions among ecological specialization. Proc R Soc London B 269, 1–6

Trigo JR, Brown KS, Jr., Witte L, Hartmann T, Ernst L, Barata LES (1996) Pyrrolizidine alkaloids: Different acquisition and use patterns in Apocynaceae and Solanaceae feeding ithomiine butterflies (Lepidoptera: Nymphalidae). Bot J Linn Soc 58, 99–123

Trigo JR, Witte L, Brown KS, Jr., Hartmann T, Barata LES (1993) Pyrrolizidine alkaloids in the arctiid moth *Hyalurga syma*. J Chem Ecol 19, 669–679

Van Oycke S, Braekman JC, Daloze D, Pasteels JM (1987) Cardenolide biosynthesis in chrysomelid beetles. Experientia 43, 460–462

Witte L, Rubiolo P, Bicchi C, Hartmann T (1993) Comparative analysis of pyrrolizidine alkaloids from natural sources by gas chromatography-mass spectrometry. Phytochemistry 32, 187–196

Received 20 September 2002; accepted 18 November 2002.