# Biosynthesis of Defensive Compounds from Beetles and Ants

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Many beetle and ant species owe their protection against predators to the presence of defensive compounds in their hemolymph or in specialized exocrine glands. A growing number of studies devoted to the elucidation of the biosynthetic pathways used by the insects to produce these compounds have been reported in recent years. An overview of these studies is presented.



## 1 Introduction

Although the biosynthetic capability of insects was long underestimated by both chemists and biologists, it is now recognized that many of these arthropods are able to produce a whole array of secondary metabolites ranging from acetogenins to terpenes, alkaloids, and others. Many of these compounds are involved in the communication systems of insects, either as pheromones or as allomones. Despite the large number of defensive compounds already isolated from insects, few studies have addressed their biosynthetic origin. This situation undoubtedly reflects the many problems that hamper this area of research. Finding the

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*The three senior authors of this Microreview have been working together in the field of insect defensive compounds for 33 years. So far, this collaboration has resulted in the joint publication of about 100 papers in international journals.*

*Pascal Laurent (right) was born in Brussels in 1975. He studied chemistry at the ''Université Libre de Bruxelles''. He received his masters degree in 1997 and his doctorate in 2001 with a thesis under the guidance of Professors J. C. Braekman and D. Daloze. He is now working in the field of the chemistry of bioactive secondary metabolites as associate researcher in the department of Organic Chemistry at ULB.*

*Jean-Claude Braekman (center right) was born in Brussels in 1942. He received his doctorate in organic chemistry (1968) from the ''Université Libre de Bruxelles''. After one year spent at McMaster University (Ontario) working on the biosynthesis of the Lycopodium alkaloids with Professors D. B. MacLean and I. D. Spencer (1970), he returned to Brussels where he joined the laboratory of Bio-organic chemistry created by Professor B. Tursch. There, first as associate researcher and then as senior researcher at F.N.R.S., he concentrated on the chemistry, bioactivity, and biosynthesis of secondary metabolites. He obtained a teaching position at the Faculty of Sciences of ULB in 1986. He is now full Professor and Director of the Department of Organic Chemistry of that University. He is a member of the Overseas Academy of Sciences of Belgium and General Secretary of the ''Société Royale de Chimie'' of Belgium.*

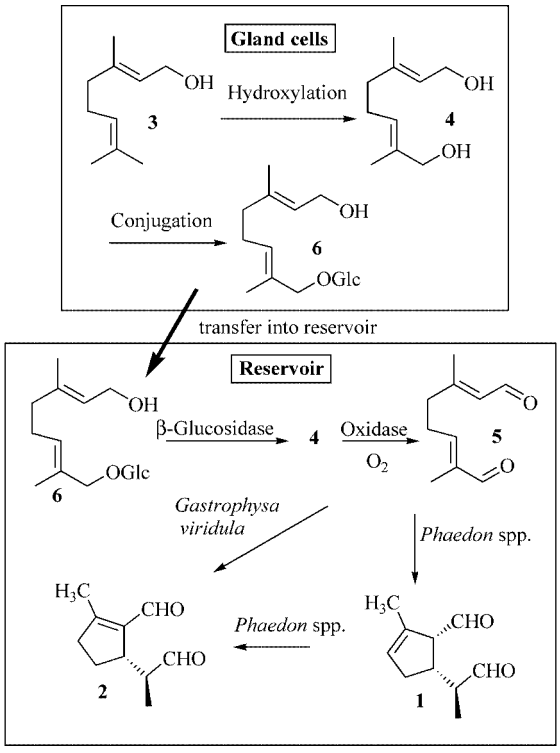
*Désiré Daloze (left) was born in Brussels in 1940. He studied at the ''Université Libre de Bruxelles'' and completed his Ph.D. thesis in 1966 in the laboratory of the late Professor G. Chiurdoglu under the supervision of Professor B. Tursch, working on the isolation and partial synthesis of pentacyclic triterpenes. After a two-year post-doctoral period with Professor H. G. Viehe in fluorine chemistry, he received a teaching position at the University of Brussels. In 1970, he joined B. Tursch who had just launched the Bio-organic chemistry laboratory with the aim of studying chemical communication in living organisms. His main research interest has been chemical ecology, with a strong emphasis on the defense mechanisms of marine invertebrates (soft corals and sponges) and insects (coccinellids, chrysomelids, and ants). He was for 12 years Editor of ''Chimie Nouvelle'', the journal of the ''Société Royale de Chimie''. He is now, together with Jacques-M. Pasteels, Editor-in-Chief of ''Chemoecology''.*

*Jacques-M. Pasteels (center left) was born in Brussels in 1939. He studied biology at the ''Université Libre de Bruxelles''. He received his Ph.D. in 1966 working in the exocrinology of termitophile beetles from Gabon, under the supervision of Professor P. P. Grassé (Paris). After post-doctoral stays with Professors D. Kishner (Chico, California) and M. S. Blum (Athens, Georgia), he received a permanent position at the ULB where he has been full Professor since 1979. His main research interest is the chemical and evolutionary ecology of insects, in particular of social insects and beetles. He has long field experiences both in the tropics (Africa, Papua New Guinea, Panama, South America) and in Alpine and sub-boreal regions. He is presently Editor-in-Chief of ''Chemoecology'', together with D. Daloze. He was President of the Belgian Royal Society of Zoology in 1987 and President of the International Society of Chemical Ecology (ISCE) in 1991. He was awarded the Silver Medal of the ISCE in 1996 and is a member of the Belgian Royal Academy of Sciences.*

**MICROREVIEWS:** This feature introduces the readers to the author's research through a concise overview of the selected topic. Reference to important work from others in the field is included.

right method of administering the labeled precursor(s) (feeding, injection, …) is one of the most important problems. As insects are complex organisms, how can we be sure that the precursor(s) will reach the site of biosynthesis intact? Also, the administration of relatively large doses of labeled material may lead to channel overload and disturb physiological processes. On the other hand, with insects, low incorporation rates are generally observed with little-advanced precursors such as acetate. Thus, in most cases radioactive precursors must be used, which necessitate both special laboratory facilities and the setup of degradation experiments in order to locate the label in the molecule.

This microreview will mostly focus on the biosynthetic work performed on the defensive compounds from ants, and chrysomelid and coccinellid beetles. Some excellent biosynthetic studies of defensive compounds from other groups of insects have been reported, but they will not be considered here, since they have been adequately covered in books and reviews.[1−4] We will also not consider sequestration without metabolic transformation by the insect. Moreover, only evidence based on tracer experiments will be covered here, excluding speculative biosynthetic schemes presented without experimental support.




Scheme 1

## 2 Chrysomelid Beetles

Most chrysomelids or leaf beetles are strictly phytophagous and highly specialized in their feeding habits. Like other specialist herbivores, they are expected to use toxins from their host plants for their own chemical defense. Actually, the dependence of leaf beetles on the secondary chemistry of their host plants to produce their defensive compounds ranges from none to mere sequestration of unmodified plant toxins, with all intermediate possibilities, i.e. the plant compound may be slightly or profoundly modified by the beetles. Host-plant influence on leaf beetle defensive chemistry is often suggested by circumstantial evidence provided by the comparison of insect and plant toxins, but has in some instances been fully demonstrated by tracer experiments.

### 2.1 Biosynthesis of Toxins by Leaf Beetles

#### *2.1.1 Iridoid Biosynthesis*

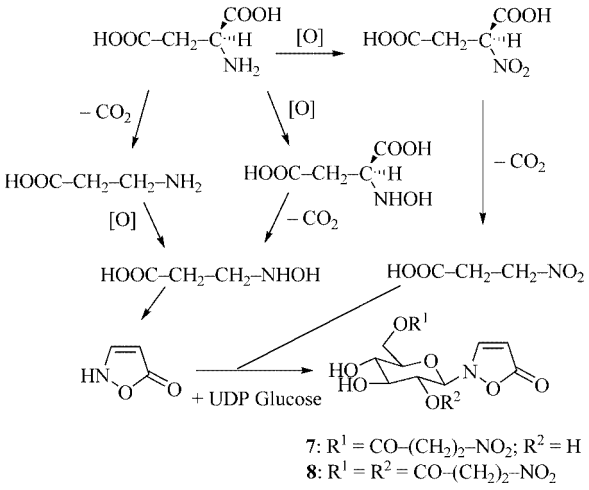
Larvae of many species in the Chrysomelina produce volatile methylcyclopentanoid monoterpenes (iridoids), such as plagiodial (**1**) and chrysomelidial (**2**) stored in the reservoirs of serial abdominal and thoracic exsertile glands.[5] In a series of elegant experiments using regio- and stereospecifically labelled deuterated precursors, the biosynthetic pathways leading to these monoterpenes have been studied in detail.[6−8] Larvae of *Phaedon armoraciae*, *Phaedon cochleariae*, *Gastrophysa viridula* and *Plagiodera versicolora* produce **1** and **2** from mevalonate by a sequence similar to that found for analogous compounds in plants (Scheme 1). Geraniol (**3**) undergoes a hydroxylation reaction to 8-hydroxygeraniol (**4**), which is further oxidized to 8-oxocitral (**5**). The mode of cyclization of the latter depends on the beetle species. In *G. viridula*, **2** is directly formed from **5**, while in both *Phaedon* spp. **2** is produced via **1** followed by isomerization of the endocyclic double bond (Scheme 1). The oxidation of **4** to **5** is catalyzed by an oxygen-dependent oxidase which stereospecifically removes the pro-(*R*) 1-H and 8-H of **4** (*Re* specificity).[9] Traces of 8-hydroxygeraniol (**4**) and its 8-*O*-β-D-glucoside **6**, as well as glucose, were found in the defensive secretions of *G. viridula* and *P. versicolora*, suggesting that **6** is the water-soluble form by which **4** is transported into the gland reservoir (Scheme 1).[10] This hypothesis has been recently confirmed by feeding experiments using thioglucoside substrates which are hydrolytically stable mimics of natural glucosides. Larvae of *Phaedon cochleariae* and *Gastrophysa viridula* rapidly accumulate the thioglucosides of 8-hydoxygeraniol in their defensive secretion, the 8-(*S*)-glucoside being preferred to its 1-(*S*) isomer by a factor of ten.[11] The transport system is highly specific since the thioglucoside of geraniol is not transported. This indicates that these larvae are able to use plant glucoside precursors in addition to their own pathways of biosynthesis of iridoids.[11]

It is interesting to mention that some rove beetles (Staphylinidae), which also use iridoids as defensive substances, utilize the same monoterpenoid precursors and follow a similar overall biosynthetic pathway to leaf beetles, but with small stereochemical differences.[12]

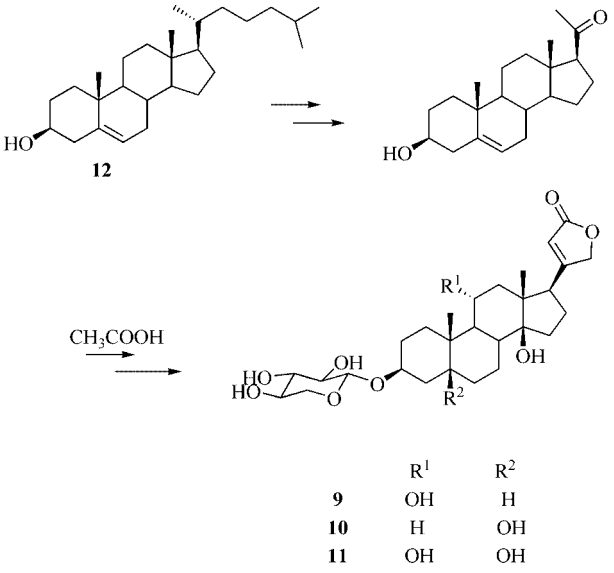
#### *2.1.2 $\Delta^3$-Isoxazolin-5-one and 3-Nitropropanoic Acid*

Adults of the subtribe Chrysomelina release from elytral and pronotal glands glucosides of $\Delta^3$-isoxazolin-5-one es-

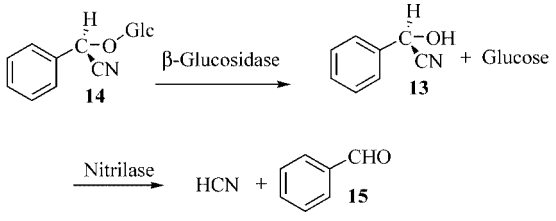
  




Scheme 2




Scheme 3




Scheme 4

terified with one or more 3-nitropropanoic acid moieties (e.g. **7** and **8**).[5,13] These compounds are not present in the food plants of the beetles, although they were reported from some legumes,[14] and free 3-nitropropanoic acid from molds.[15] By feeding adults of *Chrysomela tremulae* with L-[U-$^{14}$C]aspartic acid, Randoux et al. demonstrated that the beetles use this amino acid as precursor of both $\Delta^3$-isoxazolin-5-one and 3-nitropropanoic acid.[16] The biosynthesis of these moieties in leaf beetles appears to be similar to that reported for plants[17] and molds[15] and requires a series of oxidation steps and also the decarboxylation of L-aspartic acid as proposed in Scheme 2.

### *2.1.3 Cardenolides*

Cardenolides characterize the defensive secretions of adults in the Chrysolinina.[18,19] None of these species feed on plants containing cardenolides, e.g. Asteraceae, Ranunculaceae, Apiaceae. Using [4-$^{14}$C]cholesterol and [1,2-$^3$H$_2$,23-$^{14}$C]cholesterol as tracers, Van Oycke et al.[20] demonstrated that *Chrysolina coerulans* produces its cardenolides, i.e. the xylosides of sarmentogenin (**9**), periplogenin (**10**), and bipindogenin (**11**), from cholesterol (**12**) by a pathway involving a $C_{21}$ intermediate as in plants[21] (Scheme 3).

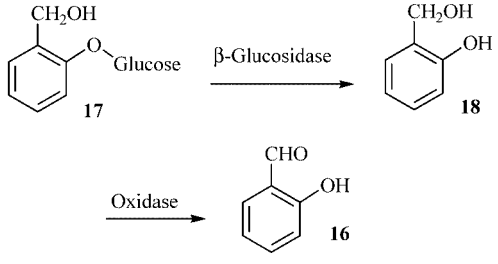
### *2.1.4 Cyanogenesis*

The larvae of Australian *Paropsis* and *Chrysophtarta*, feeding on *Eucalyptus*, secrete HCN, benzaldehyde and glucose from a pair of dorsal exsertile glands.[22] From lyophilized larvae of *Paropsis atomaria*, Nahrstedt and Davis isolated (*R*)-mandelonitrile (**13**) and its glucoside, prunasin (**14**).[23] HCN and benzaldehyde (**15**) were released after incubation of lyophilized larvae with nitrilase and β-glucosidase. These results point to a biosynthetic route of HCN production as in Scheme 4.[23] Adults, eggs, and pupae were shown to possess the same cyanogenesis ability as the larvae but in a much reduced way. No specific releasing organs of HCN are known in these life stages.

## 2.2 Biosynthesis from Sequestered Plant Metabolites

### *2.2.1 Salicylaldehyde*

When disturbed, instead of secreting iridoids (see above), larvae of *Chrysomela* species and of *Phratora vitellinae* release salicylaldehyde (**16**) from their abdominal and thoracic glands.[18] It has long been suspected that salicylaldehyde derives from salicin (**17**) present in their salicaceous host plants.[24,25] This was demonstrated by feeding *C. tremulae* larvae with [7-$^{14}$C] salicin (Scheme 5).[26] This conversion occurs extracellularly within the glandular reservoirs in which a β-glucosidase and an oxidase are secreted.[27] Whereas the β-glucosidase shows little substrate specificity, the oxidase is highly specific for salicyl alcohol (saligenin) (**18**)[27,28] and exhibits the same stereospecificity as the oxidase involved in the biosynthesis of iridoids (see above).[29]




Scheme 5

Salicyl alcohol functions as an electron acceptor yielding hydrogen peroxide, which does not accumulate in the secretion. The continuous formation of salicylaldehyde is similar to the operation of an enzyme reactor. The aldehyde steadily leaves the aqueous reaction medium, building an organic phase that reaches up to 15% of the total volume of the secretion.[28] A very efficient and specific transport mechanism of salicin from the gut to the gland reservoirs has been demonstrated using thiosalicin as tracer.[30]

The similarity between the enzymatic mediation of the final steps in the biosynthesis of iridoids (Scheme 1) and of the derivation of salicylaldehyde from salicin (Scheme 5) supports the hypothesis that the second metabolic process evolved from the first by small changes in only two preexisting mechanisms: (i) a change in the specificity of transport of plant glycosides to the glands,[30] and (ii) a change in the substrate specificity of the alcohol oxidase secreted in the glandular reservoirs, as originally suggested by Pasteels et al.[27]

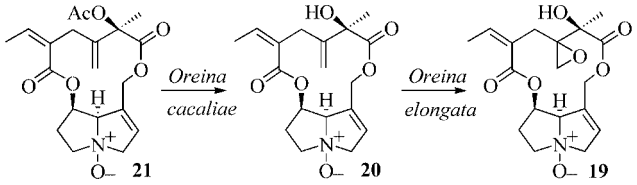
### 2.2.2 Isobutyrates and 2-Methylbutyrates

Some North American *Chrysomela* and the European *C. lapponica* larvae feeding on salicaceous plants secrete isobutyrates and 2-methylbutyrates besides salicylaldehyde. These esters, but not salicylaldehyde, also characterize the secretions of *Chrysomela* larvae feeding on *Alnus* or *Betula*.[31,32] They are of mixed insect/plant biosynthetic origin. The acid moieties are synthesized by the insect from amino acids (valine and isoleucine), but the alcohols are largely, if not exclusively, derived from the host plants. The biosynthesis of these esters in *C. lapponica* has been recently reviewed in detail by Schulz[4] and will not be repeated here.

### 2.2.3 Pyrrolizidine Alkaloids (PAs)

Members of two genera of leaf beetles, *Oreina* and *Platyphora*, use PAs as chemical defense. The way PAs are derived and, in some cases, partially metabolized, as well as the nature of the PAs, considerably differ in the two genera.

Sequestration of macrocyclic retronecine esters of the senecionine type of PAs[33] occurs in *Oreina* species feeding on *Senecio* and *Adenostyles* (Asteraceae, tribe Senecioneae).[19] In three species, PAs are produced in admixture with autogenous cardenolides (see above), but *O. cacaliae* relies on PAs only for its chemical defense.[34] In larvae, PAs are stored in the hemolymph and, to a lesser extent, in the integument.[35] In the adults, five to six times more PAs are stored in the hemolymph than in the pronotal and elytral secretions. However, PA concentration in the secretion reaches 0.1 to 0.3 mol/L which is about 2.5 orders of magnitude higher than that found in the body of the beetle.[36] In *Oreina* species, PAs are only sequestered and stored as *N*-oxides, implying a specific, active transport mechanism both in the gut and in the glands, as well as a mechanism preventing the spontaneous reduction of plant PA *N*-oxides in the gut.[37] PA sequestration in *Oreina* is selective, and selectivity is higher in the defensive secretions than in the hemolymph. *Oreina* leaf beetles show very little ability to transform plant PAs. Nevertheless, a PA not present in plants, called oreine (**19**) (13,19-epoxysenecionine *N*-oxide), was observed in the defensive secretion of *O. elongata occidentalis*.[38] It is most probably the epoxidation product of seneciphylline *N*-oxide (**20**). In *O. cacaliae*, *O*-deacetylation of acetylseneciphylline *N*-oxide (**21**) to seneciphylline *N*-oxide (**20**) was also reported (Scheme 6).[38]




Scheme 6

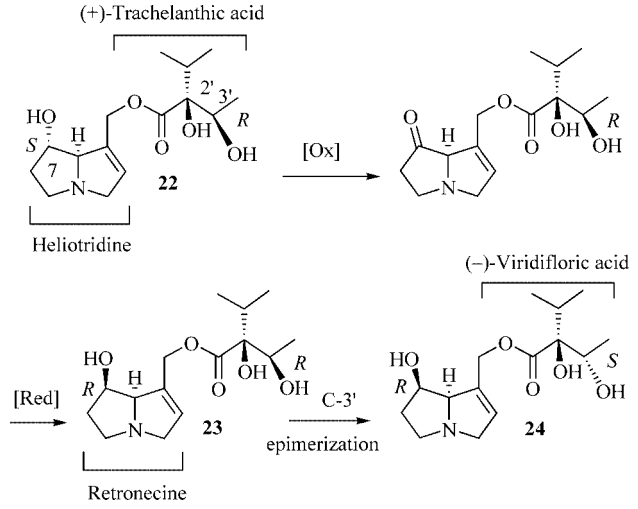
Chemical defense based on plant-derived PAs in *Platyphora* species differs remarkably from that just described in *Oreina*, and obviously evolved independently. Only open-chain retronecine esters of the lycopsamine type PAs[33] are sequestered by beetles feeding either on Apocynaceae, Boraginaceae or Asteraceae (tribe Eupatorieae).[39,40] These compounds, always found in admixture with triterpene saponins (see below) in the defensive secretions, are sequestered and stored as tertiary PAs, not as *N*-oxides. In the adults, the defensive secretion is the only storage compartment. PA concentration in the secretion reaches 5 to 20 mM, which is considerably lower than the PA concentration in *Oreina* leaf beetles, reflecting at least in part the fact that PAs are present in the food plants of *Platyphora* in concentrations two to three orders of magnitude lower than those reported in the food plants of *Oreina*. *Platyphora* leaf beetles show a much higher ability to transform plant PAs than *Oreina* species and even a capacity to produce their own PAs from sequestered plant necin bases. This was demonstrated by feeding adults of *P. boucardi* with appropriate radiolabelled or deuterated tracers.[41] *P. boucardi* specifically epimerizes rinderine (**22**) to its stereoisomers intermedine (**23**) and lycopsamine (**24**), i.e. C-7 epimerization of heliotridine base to retronecine via a ketone intermediate as in a pyrrolizidine alkaloid sequestering lepidopteran[42] and C-3′ epimerization of the necic acid (+)-trachelanthic acid to (−)-viridifloric acid (Scheme 7).

Even more remarkable is the ability of *P. boucardi* to esterify sequestered simple necine bases such as retronecine (**25**) with propanoic, lactic, or 2-hydroxyisovaleric acids (Scheme 8) and to transform triester alkaloids found in the host plant into monoesters of the lycopsamine type and thus to generate PAs not found in plants.[41,42] Larvae showed the same ability to sequester and metabolize plant PAs as the adults. However, no specific storage compartment was found in the larvae, in which PAs appear to be homogeneously distributed in the hemolymph and tissues.[43]

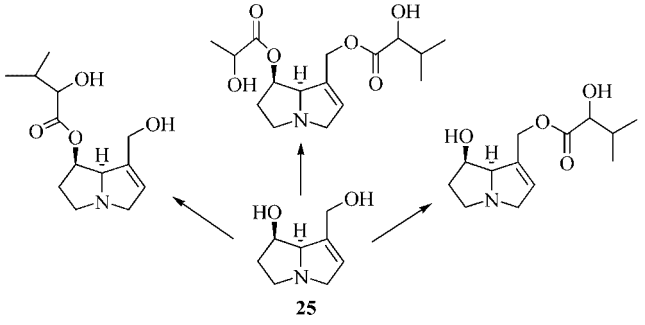
### 2.2.4 Oleanane Triterpene Saponins

Quite recently, several triterpene saponins of the oleanane group were found in the secretions of defensive glands from




Scheme 7

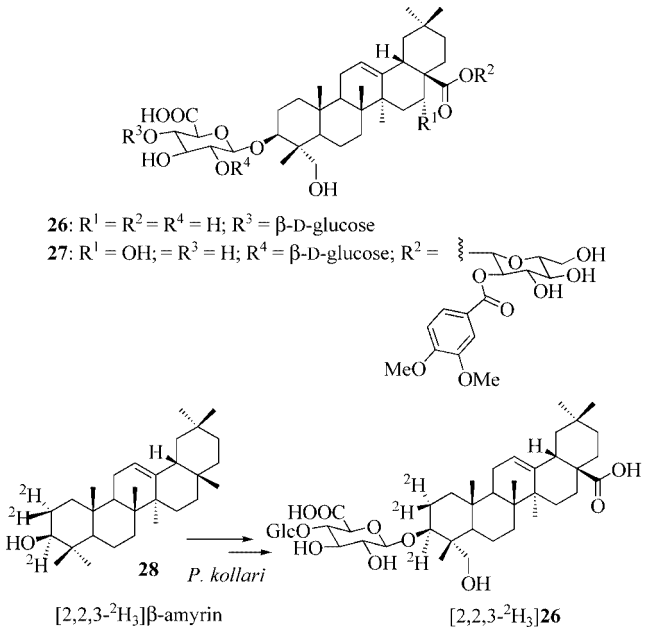



Scheme 8

adult beetles belonging to the genera *Platyphora*, *Leptinotarsa* and *Desmogramma* (e.g. **26** and **27**).[44−46] This discovery was intriguing since insects are unable to biosynthesize pentacyclic triterpenes, and since the host plants of these beetles were found to be devoid of these saponins.[44] We have now shown that feeding *P. kollari* adults with [2,2,3-$^2H_3$]β-amyrin (**28**) leads to the production of up to 40% [2,2,3-$^2H_3$]**26** by the beetles (Scheme 9).[47] This result indicates that the oleanane saponins found in these chrysomelid beetles are produced from β-amyrin, which is a constituent of the epicuticular waxes of many plants.[48] Basically, this strategy is similar to that of the Chrysolinina beetles, which produce cardenolides from ubiquitous phytosterols (see above). The oxidation and conjugation enzymes required to convert β-amyrin to these saponins are common in living organisms since they usually take part in the detoxification of xenobiotics.

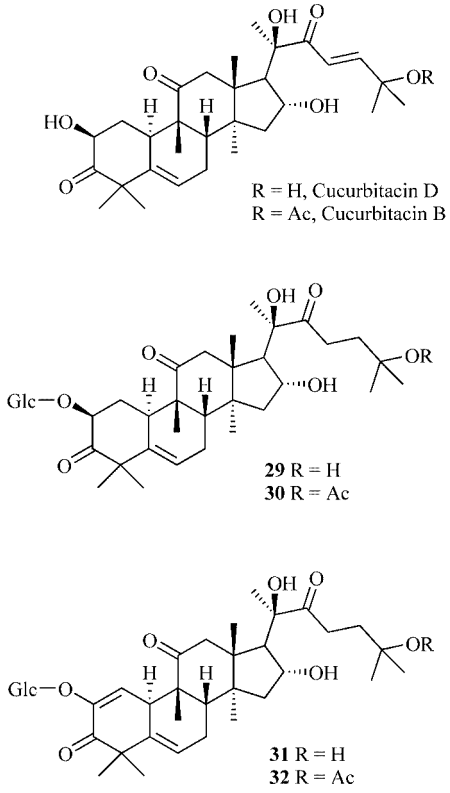
### *2.2.5 Cucurbitacins*

When feeding on deterrent Cucurbitaceae, some leaf beetles of the subfamily Galerucinae sequester and store large amounts of cucurbitacins (e.g. cucurbitacins B and D) in their blood and tissues, and these act as defensive compounds against predation.[49−52] In some beetles, those compounds are partially metabolized.[49,53] The four glucosidic products **29**−**32** were indeed isolated from the whole-




Scheme 9

body extract of *Diabrotica undecimpunctata howardi* and *D. virgifera virgifera* after feeding the beetles with cucurbitacin D (Scheme 10). As those insects had been reared on a cucurbitacin-free diet before feeding, this result demonstrates that the glucosylation, hydrogenation, desaturation, and acetylation reactions take place in vivo in the beetle.[54]

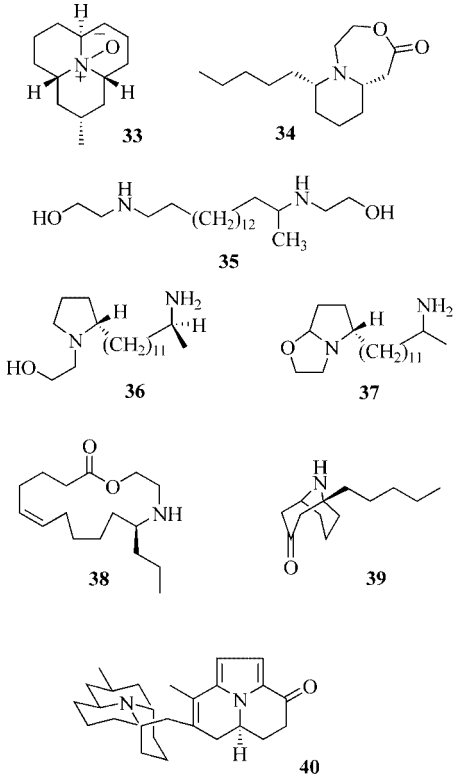



Scheme 10

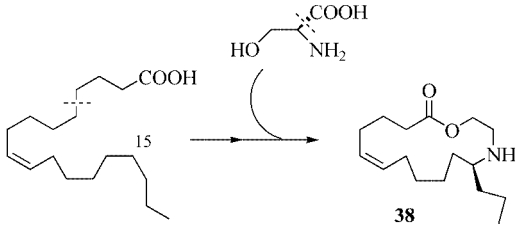
## 3 Coccinellid Beetles

The family Coccinellidae comprises over 5200 species worldwide. Most of these beetles are carnivorous, but some are phytophagous or mycophagous. If we except ladybirds which can easily camouflage themselves, a lot of these insects are characterized by beautiful red-orange colors and are known to form dense aggregations during dormancy. In spite of these characteristics, which could increase risks of predation, these beetles are rarely exploited as food sources by predators. Thanks to pioneering studies of Tursch and co-workers in the early 1970s, it is now well established that many coccinellids owe their protection, at least in part, to the presence of repellent and, in some cases, toxic alkaloids in the hemolymph emitted during reflex bleeding. Up to now over 50 alkaloids have been isolated and characterized from ladybirds, including perhydroazaphenalenes (e.g. coccinelline **33**), piperidines (e.g. calvine **34**), acyclic amines (e.g. signatipennine **35**), pyrrolidines (e.g. **36** and **37**), azamacrolides (e.g. epilachnene **38**), homotropanes (e.g. adaline **39**) and "dimeric" alkaloids (e.g. exochomine **40**) as depicted in Scheme 11.[55,56] Despite the structural diversity of ladybird alkaloids, most of their skeletons can be seen as a chain of carbon atoms joined at one or more sites to a nitrogen atom. In some cases such as calvine (**34**),[57] signatipennine (**35**),[58] pyrrolidines **36**[59] and **37**,[60] and epilachnene **38**,[61] an ethanolamine moiety completes the structure.



Scheme 11

Inspection of the structure of epilachnene (**38**), the major azamacrolide secreted by defensive glandular hairs of the pupa of the Mexican bean beetle *Epilachna varivestis*,[61] suggested that the ethanolamine is derived from serine while the 14-carbon backbone could arise from an unsaturated fatty acid like oleic acid. Attygalle et al. tested this possibility by feeding larvae of *Epilachna varivestis* with a specifically deuterated oleic acid, (*Z*)-[9,10-$^2H_2$]octadec-9-enoic acid. Feeding experiments with [2-$^{13}C$,$^{15}N$]-labeled L-serine and [2,3,3-$^2H_3$]L-serine were also carried out. Subsequent GC/MS and GC/IR analyses of the collected pupal defensive secretion revealed good incorporation of all three precursors, establishing the biogenetic origin of the alkaloid's entire carbon/nitrogen skeleton. Its formation implies the loss of four carbon atoms from the carboxyl end of oleic acid (presumably through 2 β-oxidations) and the addition of β-aminoethanol generated from serine (Scheme 12).[62]



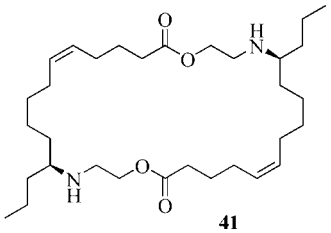
Scheme 12

This biosynthetic process thus includes a fatty acid amination step, implying a functionalization of the C-15 methylene group in oleic acid so as to introduce a C−N bond in place of a nonactivated C−H bond. Analytical evidence from experiments in which *Epilachna varivestis* pupae were fed with (*Z*)-[$^2H_{17}$]octadec-9-enoic acid and (*Z*)-[$^2H_{15}$]octadec-9-enoic acid, indicate that only the C-15 methylene group of oleic acid is involved in the process leading to C−N bond formation in epilachnene (**38**). Hydroxylation of oleic acid at C-15 (or at the corresponding site after chain shortening), oxidation to the corresponding ketone, Schiff-base formation by reaction with serine or a serine-related amine, and stereospecific imine reduction [natural epilachnene occurring as a single enantiomer with (*S*) configuration at its stereogenic center[63]] would thus lead to the alkaloid after lactonization.[64] Interestingly, labeled epilachnene was also obtained, although with a much lower efficiency, when the beetles were fed with [$^2H_{35}$]stearic acid. The loss of twelve deuterium atoms during the process (eight during the fatty acid chain shortening, two in the formation of the double bond and two when the alkyl chain is aminated) supports the postulated biosynthetic scheme.[64]

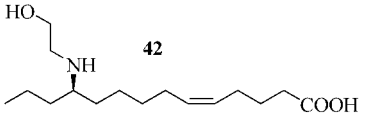
In pupae of *Subcoccinella 24-punctata* the secretion of the glandular hairs consists largely of the three macrocycles (e.g. **41**) which correspond to the three possible dimers of the two unsaturated (ω−3)-(2-hydroxyethylamino) acids **42** and **43** (Scheme 13).[65]

In contrast, the pupal secretion of the coccinellid beetle *Epilachna borealis* is composed principally of a combinatorial library of several hundred macrocyclic polyamines (e.g. **44** in Scheme 14).[66] This new family of natural products is characterized by macrocyclic polylactones which are

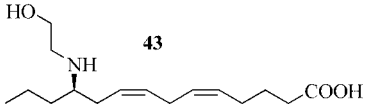
  



**41**

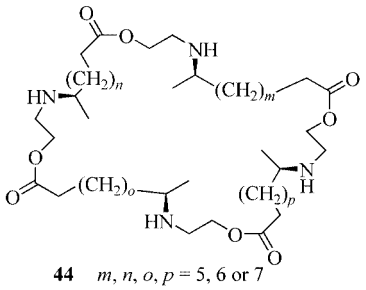


**42**

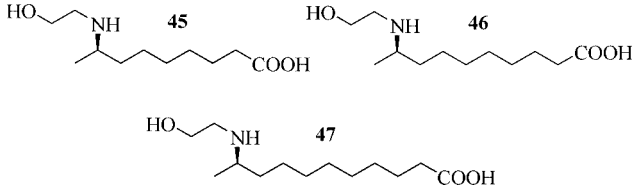


**43**

Scheme 13



**44** *m*, *n*, *o*, *p* = 5, 6 or 7



**45** **46**



**47**

Scheme 14

derived from oligomerization of three homologous saturated (ω−1)-(2-hydroxyethylamino) acids **45**−**47**. These building blocks are incorporated into the oligomers in random fashion.

Interestingly, the fresh secretion of *E. borealis*, does not contain detectable amounts of the monomeric azamacrolides, indicating that the oligomerization is a well-controlled process. This is also supported by the fact that the pupal secretions of *E. varivestis* and *Subcoccinella 24-punctata* contain almost exclusively one type of macrocycle from their set of building blocks while *E. borealis* utilizes a set of similar monomers to produce a large library of macrocycles. Thus the oligomerization and cyclization of the fatty acid derived building blocks is species-specific and carefully controlled.[66]

The autogenous production of alkaloids by ladybirds has also been unambiguously proved in three other cases. In the tribe Coccinellini, various alkaloids have been isolated but none are more characteristic of these beetles than the 2-methylperhydro-9b-azaphenalenes.[55,56]

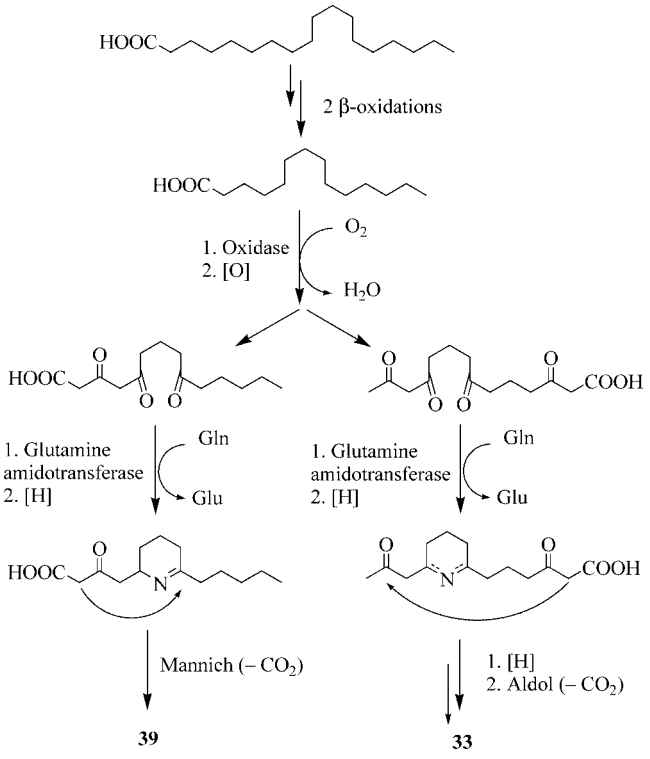
To elucidate the biosynthesis of coccinelline (**33**), adults of *Coccinella septempunctata* were fed with [1-$^{14}$C]- and [2-$^{14}$C]acetate and were found to incorporate these precursors into coccinelline (**33**). This result, together with degradation experiments on the labeled samples of coccinelline, fully proves a polyacetate origin for the alkaloid. Moreover, the specific activities measured after the degradation reactions also excluded a biogenetic scheme involving the condensation of six acetate units and subsequent methyl addition, by *S*-adenosylmethionine for example.[67]

The same type of pathway was suggested for adaline (**39**), the major alkaloid of beetles of the genus *Adalia*.[67,68] This hypothesis was recently proved by feeding *Adalia bipunctata* adults with [1-$^{14}$C]- and [2-$^{14}$C]acetate. The $^{14}$C-labelled adaline isolated in these experiments was submitted to a degradation scheme, which furnished benzoic acid containing the C-3 carbonyl carbon atom of adaline. Since the radiolabel was present only in the benzoic acid obtained from adaline after feeding the beetles with [1-$^{14}$C]acetate at a level corresponding approximately to 16% of the activity of the intact alkaloid (in the feeding experiment with [2-$^{14}$C]acetate, no radioactivity was observed for benzoic acid), it can be concluded that all of the alkaloid carbon atoms arise from seven acetate units.[69]

In vitro incubation assays using ladybird tissues have permitted a better understanding of the biosynthesis of adaline (**39**) and of coccinelline (**33**) in *Adalia bipunctata* and *Coccinella septempunctata*, respectively.[70] In a first series of experiments, two-spotted ladybird tissues were used and several potential sources of nitrogen were tested (Gln, Glu, Ala, Lys, $NH_4Cl$). The results of these incorporations, using [2-$^{14}$C]acetate as radioactive precursor, clearly showed that Gln is by far the best nitrogen donor in the biosynthesis of adaline, probably under the action of a glutamine amidotransferase. The specific incorporation rate (0.12%) of the adaline sample from this experiment was 42 times higher than that from an experiment carried out without an added source of nitrogen (0.0029%).[70] The subsequent assays were conducted to distinguish between a fatty acid and a polyketide pathway for the biosynthesis of **39**. Since it is known that the fatty acid pathway requires oxygen (in contrast to the polyketide pathway), a comparison of the results of incubation experiments in the presence and in the absence of oxygen could shed some light on this question. The results showed that oxygen is indeed essential to the biosynthesis of **39**, since the specific incorporation rate measured for the adaline sample in the absence of oxygen (0.00021%) was much lower than that of the control (0.12%). This conclusion was reinforced by the results of another assay, carried out in the presence of oxygen, but with addition of 2-octynoic acid, a well-known inhibitor of fatty acid biosynthesis. In this case, too, the specific incorporation rate of adaline dropped significantly, pointing to a fatty acid rather than a polyketide pathway for the biogenesis of **39**.[70] Finally, with the aim of identifying the ladybird tissue responsible for alkaloid formation, adults of

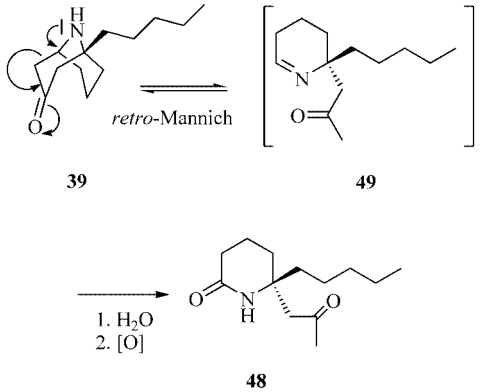
  

*Coccinella septempunctata* were carefully dissected to separate the fat body from the other tissues. The comparison of the specific incorporation rates of incubation experiments with whole-ladybird tissues (0.10%), excized fat body (0.21%), and remaining tissues (0.011%), demonstrated that the biosynthesis of coccinelline (**33**) takes place in the fat body.[70] An updated biosynthetic scheme for these two molecules is presented in Scheme 15.




Scheme 15

Finally, a biosynthetic scheme has been proposed to explain the co-occurrence of adaline (**39**) and adalinine (**48**) in *Adalia bipunctata* and in *A. decempunctata*.[71] (−)-Adaline (**39**), being a β-amino ketone, could undergo a *retro*-Mannich reaction leading to the imine **49**, which, after addition of water, followed by oxidation, would afford (−)-adalinine (**48**) (Scheme 16).[71] This hypothesis was tested by feeding two-spotted ladybirds with (−)-[$^2H_{11}$]adaline.




Scheme 16

GC/MS studies of the defensive secretion produced by these beetles showed that both deuterated adaline and adalinine were present in these insects and that the eleven deuterium atoms were located, as expected, in the pentyl chain of the alkaloids. Interestingly, GC/MS studies of the hemolymph of ladybirds fed with (+)-[$^2H_{11}$]adaline showed the presence of labeled and unlabeled adaline and of unlabeled adalinine, but no deuterated adalinine could be detected. This indicates that the transformation of adaline into adalinine is stereospecific and that the enzymes which catalyze this transformation recognize only the natural (−) enantiomer of adaline. Moreover, even if the exact mechanism of this transformation cannot be firmly established yet, these results clearly demonstrate a biogenetic relationship between (−)-adaline and (−)-adalinine, the latter being formed, at least in part, from the former.[69]

## 4 Ants

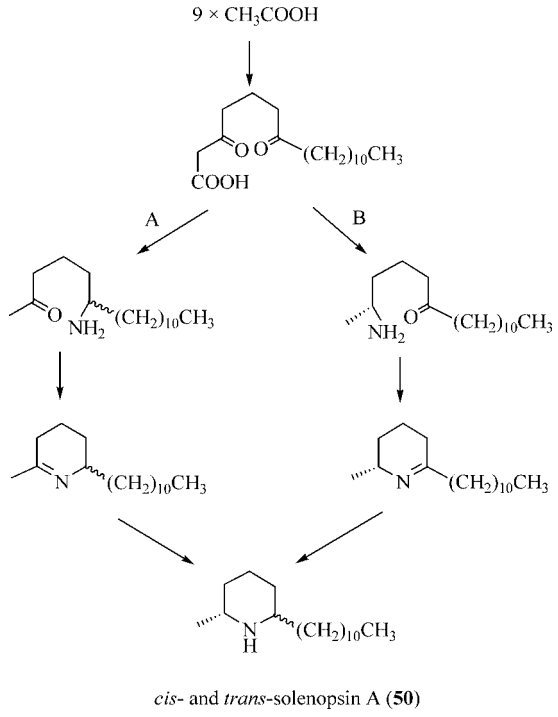
Many ant species use chemicals for defensive and offensive purposes. In this context, the poison gland which is attached to the sting plays a major role. In most of the ant subfamilies, the constituents of the poison gland are proteinaceous. In *Formica* ants, it is formic acid. The biosynthesis of this toxic compound, studied by Hefetz and Blum,[72] has been reviewed several times[1,2,73] and will not be discussed here. In some species of the Myrmicinae and Pseudomyrmecinae, the proteinic venoms have been replaced by alkaloids.[73,74] Although ant alkaloids have been the subject of many structural and synthetic studies,[73,74] only the biosynthesis of the solenopsins and the tetraponerines has been studied so far using tracer experiments.

The solenopsins are toxic 6-alkyl-2-methylpiperidines, e.g. *cis*- and *trans*-solenopsin A (**50**), which are produced by the poison gland of the so-called "fire ants" (*Solenopsis* spp.).[75] Their biosynthesis was studied by feeding 3000 to 4000 *Solenopsis geminata* ants with sodium [1-$^{14}$C]- and [2-$^{14}$C]acetate. Isolation of the radioactive solenopsins, dilution with "cold" synthetic material and degradation of these radioactive samples demonstrated that the solenopsins are formed from an 18-carbon polyacetate chain, which can afford the solenopsins (**50**) by either of the two pathways (route A or B) described in Scheme 17.[76]

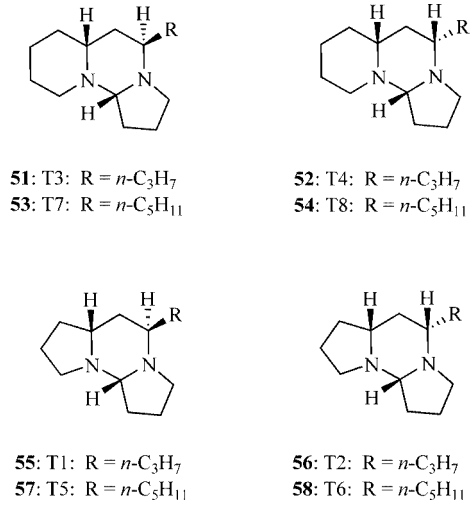
The tetraponerines are a family of alkaloids produced by the New Guinean ant *Tetraponera* sp. Eight tetraponerines have been described so far. They can be divided into two groups depending on the structure of the tricyclic skeleton which we refer to as "6,6,5" (**51**−**54**) and "5,6,5" (**55**−**58**) (Scheme 18).[77]

In each group, the four alkaloids differ from each other by the length of the alkyl chain and/or the configuration at the carbon atom bearing the alkyl chain.[77] The biosynthesis of two of these alkaloids, T8 (**54**) and T6 (**58**), belonging to the two different subgroups was studied. Administration of sodium [1-$^{14}$C]acetate and [2-$^{14}$C]acetate, L-[U-$^{14}$C]glutamic acid, γ-amino[U-$^{14}$C]butanoic acid, L-[U-$^{14}$C]ornithine hydrochloride and [1,4-$^{14}$C]putrescine dihydrochloride

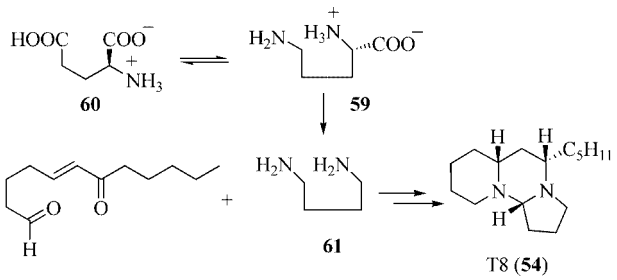



Scheme 17



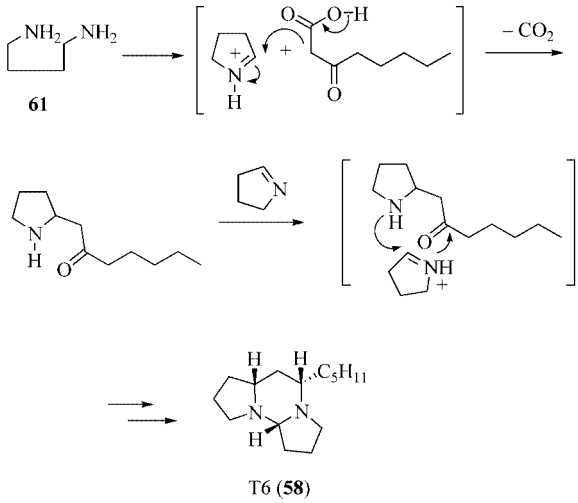

Scheme 18

to *Tetraponera* ants, followed by isolation and chemical degradation of the labeled T8 and T6 led to the conclusion that these compounds have a mixed biosynthetic origin. The pyrrolidine ring of T8 (**54**) originates from ornithine (**59**), via glutamic acid (**60**) and putrescine (**61**), whereas the remaining carbon atoms derive from a polyacetate chain




Scheme 19

arising from six acetate units (Scheme 19).[78] In contrast, both pyrrolidine rings of T6 (**58**) come from putrescine (**61**), with the remaining carbon atoms deriving from a seven-carbon moiety of polyacetate origin (Scheme 20).[79] These results show that two different pathways are operating in the biosynthesis of the two closely related tetraponerine skeletons in the same organism.




Scheme 20

## 5 Conclusions

In this review we have summarized recent knowledge on the biosynthetic pathways operating for the production of defensive compounds in some beetle and ant species.

What is most characteristic about the chemical defense of the phytophagous chrysomelid beetles is its diversity and its dependence on host-plant chemistry. In many cases (some PAs, cucurbitacins, ...), the compounds are directly taken up from the host plants, with few if any modifications. In the Chrysolinina (cardenolides) and *Platyphora* (oleanane saponins) the compounds are produced by the beetles through transformation of phytosterols or β-amyrin, respectively. De novo pathways are also operating in this group of insects, e.g. isoxazolinone glucosides and nitropropanoic acid deriving from aspartic acid, or iridoids originating from mevalonate. It should be pointed out that the structures of all leaf beetle defensive compounds isolated so far are identical or closely related to known plant compounds, even if they are biosynthesized by the beetles.

Moreover, in several cases (cardenolides, nitropropanoic acid, iridoids) there is evidence that the biosynthetic pathways are identical in beetles and plants.

In contrast, the typical defensive chemistry of coccinellid beetles is dominated by alkaloids that are produced by the beetles themselves. The results presented here demonstrate that our knowledge of the biosynthesis of ladybird defensive compounds is still very limited, only four alkaloids (coccinelline, adaline, adalinine, epilachnene) having been studied from this point of view using tracer experiments. But despite the structural diversity of ladybird alkaloids, the biosynthetic pathways followed for the synthesis are closely related. The biosynthetic data available so far indicate that they have a fatty acid origin. In one case, the amino acid pool was shown to be also involved, since serine is the precursor of the ethanolamine moiety of epilachnene. It is likely that this situation also pertains to other ladybird alkaloids such as calvine and the pyrrolidines, which also encompass an ethanolamine moiety.

Finally, the poison gland chemistry of many ant species is dominated by nitrogenous compounds (proteins or alkaloids as in some Myrmicinae and Pseudomyrmecinae). Whereas the solenopsins are formed exclusively by a polyacetate pathway, the tetraponerines result from a mixed biosynthesis, namely a combination of polyacetate (presumably fatty acid) and amino acid pathways.

The frequent implication of a fatty acid pathway in the biosynthesis of insect alkaloids seems to depart from the situation prevailing in plants, where most of the alkaloids are derived from amino acids (even if some piperidine alkaloids like pinidine and coniine have a fatty acid origin). On the other hand, the biosynthetic pathways leading to several non-alkaloidic compounds were found to be comparable in insects and in plants. However, more studies are needed to increase our knowledge in this field.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