# Review of the Defensive Chemistry of Coccinellids

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## I. General Background

The history of interaction between coccinellid beetles and humans is long and varied. Known by many different common names (*e.g.* ladybirds, ladybugs, polka-dotted beetles), coccinellids are easily noticed among insects because of their bright colors and large appetites. In the Middle Ages they were dedicated to the Virgin Mary during religious ceremonies,[1] and today they are of interest both as beneficial insects and as potential agricultural pests. In addition, as more is learned about the chemistry of coccinellids, chemical ecologists are becoming more intrigued with these subjects of childhood rhyme. For example, recent evidence suggests that alkaloids produced by leaf-litter coccinellids are the source of some of the poisonous compounds found in the skin of dendrobatid frogs.[2]

The family Coccinellidae, belonging to the superfamily Cocujoidea, is divided into six subfamilies based on adult and larval morphology and diet. Adult beetles are 0.8−18 mm long with a flat ventral surface and long oval bodies, which can be up to three times as long as wide.[3] This family of beetles is extremely diverse, containing phytophagous, mycophagous, and carnivorous species. The family contains upward of 4000 species, among which are the familiar orange or red beetles (carnivorous) and the less famous dark species. The bright coloration of many coccinellids appears to be a form of aposematism, the warning of potential predators through visual display, sound, or odor. The darker beetles, smaller than their aposematic relatives, are sometimes used in the biological control of agricultural pests. Two phytophagous species (*Epilachna varivestis* and *Epilachna borealis*) feed on agricultural crops and are serious pests in North America.[4,5]

Although some coccinellids are known to aggregate, they are rarely exploited as a food source by other organisms. This may be attributed to the wide range of chemical defenses that the beetles possess, which they advertise by their bright coloration and by means of aposematic odors.[6] In a stimulating discussion of defensive odors, Rothschild noted that the distinctive odor of coccinellids is similar to the odor of aggregations of other aposematic insects, and she postulated that this odor may provide an olfactory warning to potential predators just as bright colors provide a visual warning.[6] The aposematism of coccinellids correlates with the presence of alkaloids in these beetles. In many coccinellid species, these alkaloids are deployed by a mechanism known as "reflex bleeding". This is a well-documented defense mechanism in which insects under attack emit hemolymph which is deterrent to potential predators.[6−10]

While earlier reviews have covered both the chemistry and biology of coccinellids,[1,3,10,11] our knowledge of the defensive chemistry of these beetles has grown significantly during the past decade. Herein we present an up to date review of the chemical defenses of coccinellid beetles, including accounts of the isolation and identification of alkaloids, synthetic work, biosynthetic studies, and bioassay results.

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Angela Glisan King received her undergraduate degree from the University of Pennsylvania. While majoring in chemistry, she conducted independent research on the chemical communication between saddle-back tamarins under the direction of Professor Amos B. Smith, III. After receiving her bachelors degree in 1990, she enrolled in the graduate program in chemistry at Cornell University. At Cornell she entered the research group of Professor Jerrold Meinwald and began research in synthetic organic chemistry as it related to chemical ecology. While at Cornell, she was both a Syntex Fellow and National Institutes of Health Predoctoral Trainee. Upon completion of her doctoral degree, Dr. Glisan King accepted a position at Wake Forest University, where she is currently teaching introductory chemistry. Her research interests are at the interface of chemical ecology and organic chemistry, particularly in the identification and synthesis of invertebrate defensive compounds.

Jerrold Meinwald, Goldwin Smith Professor of Chemistry at Cornell University, was born in New York, NY, in 1927. He graduated from Stuyvesant High School and attended Brooklyn and Queens Colleges before serving in the U.S. Navy as an Electronics Technician's Mate. His education was resumed at the University of Chicago (Ph.B. 1947, B.S. 1948) and continued at Harvard (M.A. 1950, Ph.D. 1952), where he worked with R. B. Woodward. A DuPont Postdoctoral Fellowship brought him to Cornell, where he has spent most of his subsequent career. He was a member of the group of scientists who founded the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi and served as an ICIPE Research Director from 1970 to 1977. At the request of the National Science Foundation, he organized the first Sino-American Symposium on the Chemistry of Natural Products, held in Shanghai in 1980. Along with his colleagues Thomas Eisner, Wendell Roelofs, and Jon Clardy, he is a founding member of CIRCE (the Cornell Institute for Research in Chemical Ecology). Most recently, Dr. Meinwald served as a Fellow of the Center for Advanced Study in the Behavioral Sciences at Stanford, California (1990–91). He was awarded the Tyler Prize for Environmental Achievement in 1990, and the Gustavus John Esselen Award for Chemistry in the Public Interest (Northeastern Section of the American Chemical Society) in 1991. The International Society of Chemical Ecology awarded him its Silver Medal in 1991. He has served three terms as a National Sigma Xi Lecturer (1965, 1975, 1992–94). Music is Dr. Meinwald's chief recreational activity. He studied flute with Arthur Lora (NY) and with James Pappoutsakis and Marcel Moyse (Boston). He founded the *Ithaca Baroque Ensemble* soon after arriving at Cornell, and whenever possible, arranges for chamber music performances (on flute, *flauto traverso*, or recorder) in connection with lectureships or scientific symposia. (Photo Copyright 1988 Harvey Ferdschneider.)

## *II. Azaphenalenes*

The aposematic coloration of some coccinellids, such as *Coccinella septempunctata*, has been considered to imply their chemical defense,[12] and a survey of >30 species of coccinellids indicates that aposematic coloration is linked to the presence of alkaloids.[13−17] Coupled with the deterrent effect of coccinellids' reflex bleeding, this coloration led to a study of coccinellid chemistry. This endeavor resulted in the isolation and identification of many alkaloids, among which are the azaphenalenes, a class of tricyclic amines and amine oxides which impart a bitter taste to many coccinellids.[18] To date, eight alkaloids of this type have been isolated from coccinellids, one of which may actually be a mixture of two closely related isomers. Coccinellid beetles appear to be the only natural source of alkaloids based on the azaphenalene skeleton.

### A. Isolation and Identification

#### *1. Coccinelline and Precoccinelline*

In a pioneering study, Tursch *et al*. reported the isolation of coccinelline (**1**) and precoccinelline (**2**) (Figure 1), the first of these alkaloids to be characterized, from the methanol extract of *C. septempunctata*.[18] The active components were purified by chromatography, progress being monitored by tasting

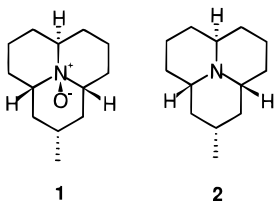
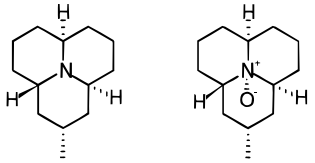


**Figure 1.** Coccinelline and precoccinelline.

of the fractions as well as by measuring repellency toward ants! Two compounds were obtained: a crystalline substance named coccinelline, which high-resolution mass spectrometry showed to have a molecular formula of $C_{13}H_{23}NO$, and the less polar precoccinelline, an amorphous compound of the molecular formula $C_{13}H_{23}N$.[18] Either reduction with ferrous sulfate or catalytic hydrogenation over $PtO_2$ converted coccinelline to precoccinelline. In a complementary transformation, precoccinelline could be oxidized to coccinelline by treatment with monoperphthalic acid, confirming the supposition that coccinelline is the *N*-oxide of precoccinelline. Precoccinelline formed a methiodide whose $^1$H NMR spectrum showed only three protons vicinal to the nitrogen atom (in addition to the *N*-methyl group protons). Since the $^1$H NMR spectrum of coccinelline itself showed neither olefinic nor N−H protons, it followed that coccinelline must be a tricyclic tertiary amine oxide. Coccinelline is devoid of optical activity, and the $^{13}$C NMR spectrum, which shows only eight signals, requires that the structure must have a plane of symmetry which passes through three of the carbon atoms. The azaphenalene-based structure **1**



**3** **4**

**Figure 2.** Hippodamine (**3**) and convergine (**4**).

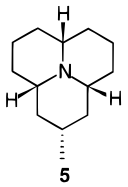
was established for coccinelline on the basis of a single-crystal X-ray diffraction experiment.[19]

### 2. Convergine and Hippodamine

A second free-base/*N*-oxide pair of azaphenalene alkaloids was isolated by the same research group from *Hippodamia convergens*.[20] Fractionation of beetle extract by chromatography afforded hippodamine (**3**) and convergine (**4**) (Figure 2). The mass spectrum of hippodamine revealed a molecular formula of $C_{13}H_{23}N$ and a fragmentation pattern almost identical to that of precoccinelline,[14] which suggested that these compounds were stereoisomers.[20] Convergine was readily reduced to hippodamine by treatment with lithium aluminum hydride. An analysis of the initial data collected for these alkaloids suggested (erroneously) that both alkaloids were racemic mixtures, since no optical rotations were observed. However, X-ray analysis showed convergine to have a chiral structure **4**, and since it had been shown that convergine could be reduced to hippodamine, the structure of hippodamine was also established. The absolute configuration of both alkaloids was also determined.[21] The optical rotations of these alkaloids were reexamined subsequent to the X-ray diffraction study. The reevaluation showed the alkaloids to have a small rotation that was strongly solvent dependent.

### 3. Myrrhine

Tursch and co-workers were also able to isolate and characterize an azaphenalene alkaloid, myrrhine, present in *Myrrha octodecimguttata*[9,13] (Figure 3). The alkaloid was again shown to have the molecular formula $C_{13}H_{23}N$ and a mass spectrum fragmentation pattern almost identical to those of precoccinelline and hippodamine.[13] Its infrared spectrum revealed the presence of "Bohlman bands",[22,23] characteristic of amines with C−H bonds antiperiplanar to the nitrogen's unshared pair of electrons. This observation suggested that myrrhine is the achiral amine **5**. This hypothesis was confirmed by the conversion of coccinelline, under Polonovski reaction conditions (acetic anhydride/dichloromethane),[24] to an unstable enamine which underwent catalytic hydrogenation to give a mixture of myrrhine and hippodamine, indicating that these two products differ only in their



**5**

**Figure 3.** Myrrhine.

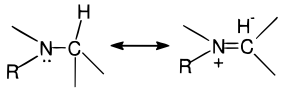


**Figure 4.** A proposed model for the basis of Bohlman bands in infrared spectra.

configuration at one stereocenter. Thus, the structure of myrrhine was established as **5**.[13]

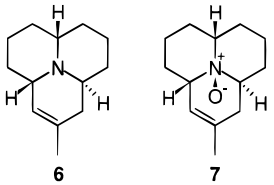
The study of Bohlman bands in the infrared spectra of coccinellid alkaloids has been of great value in determining the stereochemistry of both natural products and synthetic intermediates.[13,25] First reported as an empirical observation in 1958, Bohlman bands are $\nu_{CH}$ absorbances which occur at lower frequencies (∼2800−2600 $cm^{-1}$) than the normal aliphatic C−H stretching bands. They are attributable to the presence of at least two hydrogen atoms α and antiperiplanar to a nitrogen lone pair. Garraffo *et al.* proposed a model to explain these observations in which the nitrogen lone-pair electrons contribute to a resonance hybrid structure that weakens the α C−H bond as shown in Figure 4.[23] This model explains why the greatest Bohlman band effect is seen when there is a 180° dihedral angle between the nitrogen lone-pair $sp^3$ orbital and an antiperiplanar α hydrogen, since this orientation creates maximum overlap with the $\sigma$ bond of the α hydrogen and makes the contribution of the charged resonance structure more significant.

### 4. Hippocasine and Hippocasine N-Oxide

Fractionation of *Hippodamia caseyi* methanolic extract allowed an examination of its alkaloid content.[16] *H. caseyi* was found to contain the previously identified hippodamine and convergine, as well as two new alkaloids, hippocasine (**6**) and hippocasine *N*-oxide (**7**) (Figure 5). The optically active hippocasine *N*-oxide was isolated and characterized as its hydrochloride. High-resolution mass spectrometry gave a molecular formula of $C_{13}H_{21}NO$, and the mass spectral fragmentation pattern of the hydrochloride was similar to that of the well-known 2-methylperhydro-9b-azaphenalene *N*-oxide system, except that each major fragment was 2 Da lighter, indicating the presence of a double bond or additional ring. The presence of a double bond was supported both by infrared and $^1H$ NMR spectroscopy. X-ray analysis of hippocasine *N*-oxide hydrochloride led to the assignment of structure **7**. Since hippocasine is transformed into **7** upon treatment with methanolic hydrogen peroxide, it could be assigned structure **6**.[16]

### 5. Propyleine

Continued efforts by Tursch *et al.* resulted in the isolation of a mixture of two additional coccinellid alkaloids.[17] Extraction of *Propylaea quatuordecimpunctata* gave a material named propyleine, which



**6** **7**

**Figure 5.** Hippocasine (**6**) and hippocasine *N*-oxide (**7**).

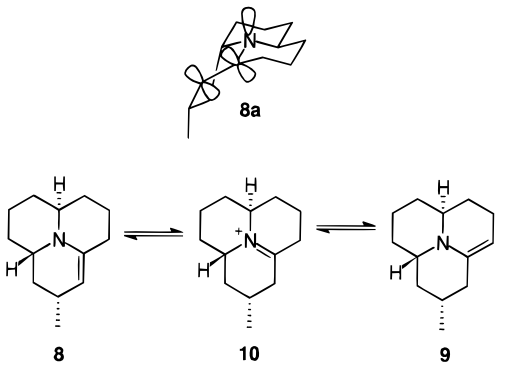



**Figure 6.** Propyleine (**8**), isopropyleine (**9**) and intermediate iminium salt **10**.

was subsequently shown to be a mixture of two isomeric enamines. High-resolution mass spectrometry showed a molecular formula of $C_{13}H_{21}N$, and the fragmentation pattern in the mass spectrum of propyleine hydrochloride was similar to that of precoccinelline (**2**), except each major fragment from propyleine was 2 Da lighter, indicating the presence of a double bond or additional ring. Under standard hydrogenation conditions, propyleine was reduced to precoccinelline, confirming the presence of a double bond within the precoccinelline carbon−nitrogen skeleton. The $^1$H NMR spectrum of propyleine gave a signal for one olefinic proton, suggesting the presence of an enamine system,[26] although the characteristic enamine ultraviolet absorption at 220−235 nm was not observed.[27] The only dehydroprecoccinelline structure predicted from models to lack the typical enamine ultraviolet absorption is that shown in formula **8a** (although the position of the methyl group is not fixed). In this enamine, the lone-pair electrons of the nitrogen atom is not in an orbital which is parallel to the p orbitals of the double-bond system, and thus this structure should not be expected to give rise to the usual enamine UV absorption. This argument provided the sole basis for the placement of the double bond in propyleine as shown in formula **8**.[17]

Upon considering the assignment of **8** as the structure of propyleine,[17] Mueller *et al.* were surprised that structure **9** was not also entertained, since deprotonation of the iminium salt **10**, which would have formed under the acidic isolation conditions, would be expected to give a mixture of **8** and **9** (Figure 6). To investigate this matter, a synthesis of racemic propyleine was carried out (*vide infra*).[28] This synthesis showed that the material isolated under acidic conditions from *P. quatuordecimpunctata* is actually a rapidly interconverting mixture of propyleine (**8**) and isopropyleine (**9**), with **9** compromising 75% of the mixture under the conditions of isolation.[28] It is now clear that since the original isolation conditions induce the formation of iminium salt **10**, neither the ratio of propyleine to isopropyleine occurring within the beetles nor the absolute configuration of these alkaloids is known.

## B. Azaphenalene Syntheses

All of the monomeric coccinellid azaphenalene alkaloids have by now been synthesized. Several of the syntheses of these defensive alkaloids make use

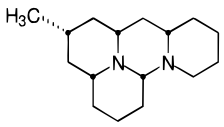
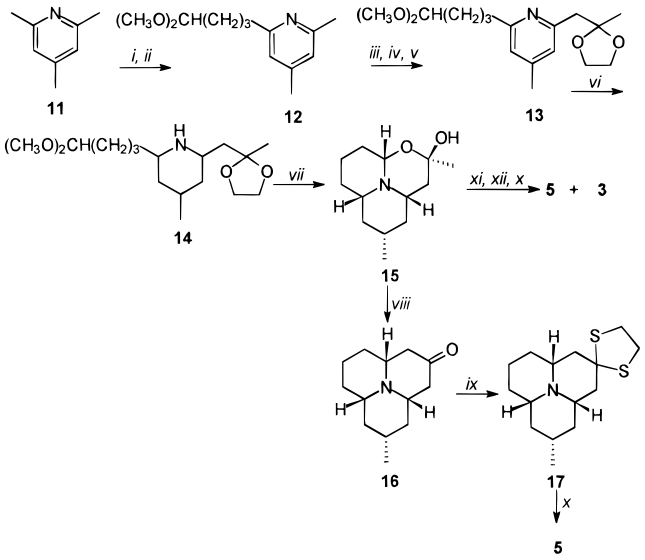


**Figure 7.** Dihydrodeoxyepiallocernuine.

**Scheme 1**

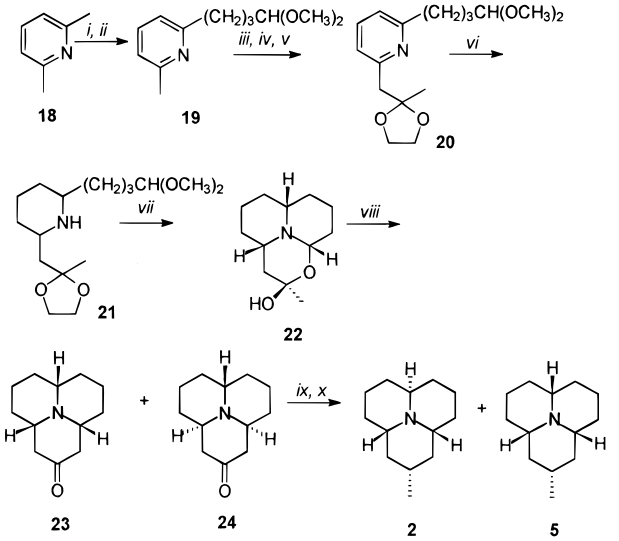



Reaction conditions: (i) PhLi; (ii) 3-bromopropionaldehyde dimethyl acetal; (iii) PhLi; (iv) $CH_3CN$, HCl; (v) ethylene glycol, TsOH; (vi) Na/isoamyl alcohol; (vii) dil. HCl; (viii) TsOH; (ix) TsOH, TEA, ethanedithiol; (x) Raney Ni/EtOH; (xi) pyrrolidine, AcOH; (xii) ethanedithiol, $BF_3$·etherate.

of the fact that the methyl group occupies the thermodynamically more stable equatorial position in each of the compounds **1**−**10**.[28−30] Ayer *et al.* used their experience with the total synthesis of (±)-dihydrodeoxyepiallocernuine[31] (Figure 7) to develop an approach used in the first synthesis of the 2-methylperhydro-9b-azaphenalene alkaloids myrrhine, hippodamine, and convergine, as outlined in Scheme 1.[29]

Formation of the monolithium derivative of 2,4,6-collidine (**11**) was followed by the addition of 3-bromopropionaldehyde dimethyl acetal to give **12**. Subsequent addition of phenyllithium produced an anion which reacted with acetonitrile to give a ketone upon workup, which was protected as the corresponding acetal **13**. Reduction with sodium and isoamyl alcohol afforded a mixture of saturated stereoisomeric amines **14**, which was chromatographed before removal of the protecting groups to afford racemic **15**. Warming of **15** with 2 equiv of *p*-toluenesulfonic acid produced a single product, ketone **16**, with the same configuration at all stereogenic centers as myrrhine (**5**). Due to the instability of **16**, this amino ketone was immediately converted to thioketal **17**, which was desulfurized with Raney nickel to give myrrhine (**5**). Oxidation with *m*-CPBA gave the corresponding *N*-oxide, identical to natural myrrhine *N*-oxide.[29] Interestingly, milder cyclization conditions (pyrrolidine, acetic acid) transformed **15** into a mixture of two stereomeric ketones. Formation of the corresponding thioacetals without purification, followed by desulfurization, gave a mixture of myrrhine (**5**) and (±)-hippodamine (**3**), which was converted to its *N*-oxide, (±)-convergine (**4**).[29] The overall yields of **5** and **3** from hemiketal **15** were, respectively, 33% and 23%.

**Scheme 2**



Reaction conditions: (i) PhLi; (ii) 3-bromopropionaldehyde dimethyl acetal; (iii) PhLi; (iv) $CH_3CN$, $H^+$; (v) ethylene glycol, TsOH; (vi) Na/isoamyl alcohol; (vii) dil. HCl; (viii) pyrrolidine, AcOH; (ix) MeLi, thionyl chloride; (x) $H_2$/Pt.

Using a similar approach, Ayer and Furuichi achieved the first synthesis of coccinelline (**1**) and precoccinelline (**2**).[30] 2,6-Lutidine (**18**) was converted into hemiketal **22** using the previously described strategy (Scheme 2).[29] Closure of the third ring by refluxing **22** in pyrrolidine and acetic acid gave a mixture of two ketones, **23** and **24**. A plausible mechanism for the epimerization of two stereocenters in this reaction is shown in Figure 8. After separation of the stereomeric tricyclic ketones, addition of methyl lithium followed by dehydration and reduction yielded myrrhine (**5**) from **23** and precoccinelline (**2**) from **24**. Precoccinelline was converted to coccinelline by reaction with *m*-chloroperbenzoic acid.

Stevens and Lee used the Robinson−Schöpf methodology[32−34] for an entirely different synthetic approach to the azaphenalenes (Scheme 3).[25] Their successful scheme employed the reaction of amine dialdehyde **25** with acetone dicarboxylic ester to generate the perhydro-9b-azaphenalene **26**, easily converted to ketone **24,** which would eventually yield precoccinelline and its corresponding *N*-oxide, coccinelline.

The dimethyl acetal of amine dialdehyde **25** was prepared in six steps from dimethyl malonate (Scheme 4).[25] In the first step, treatment of the malonic ester with acrolein gave aldehyde **27**, which was then protected as its dimethyl acetal before decarboxymethylation (NaCl, wet DMF, reflux) to afford **28**. Claisen condensation of **28** yielded keto ester **29**, which underwent decarbomethoxylation upon refluxing with sodium chloride in wet DMF followed by reductive amination to give dimethyl acetal **30**. Condensation with dimethyl acetonedicarboxylate (**31**) followed by hydrolysis at pH 2 produced only one stereoisomer of the tricyclic amine **26**. The configuration at the stereogenic centers was deduced from the absence of Bohlman bands in the infrared spectrum[22,23] along with $^{13}C$ NMR studies. Decarbomethoxylation produced ketone **24**, from which precoccinelline (**2**) was made by treatment with methylenetriphenylphosphorane followed by catalytic hydrogenation. The synthetic precoccinelline was transformed to coccinelline (**1**) by treatment with *m*-chloroperbenzoic acid.[25]

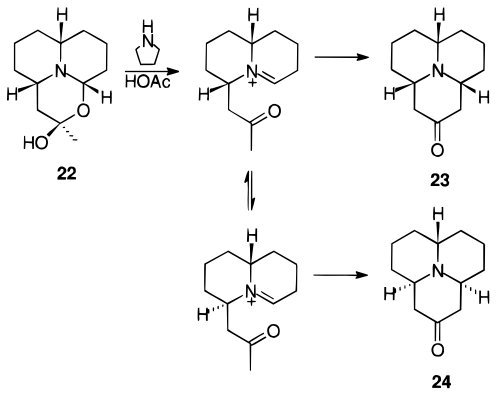
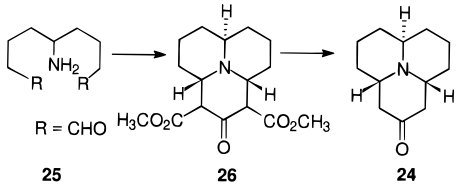
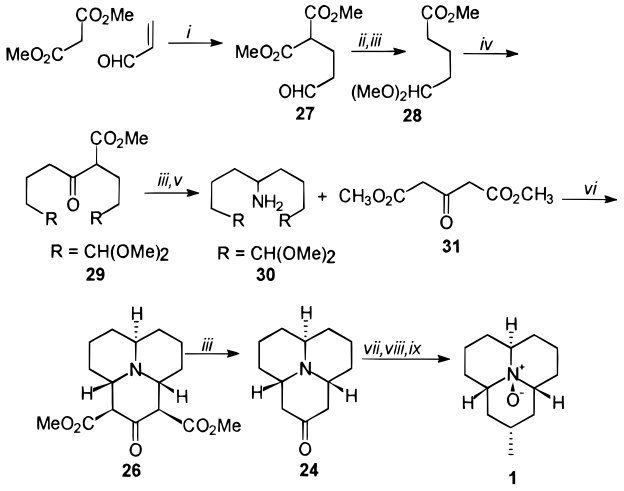


**Figure 8.** Twofold epimerization during formation of the azaphenalene skeleton.

**Scheme 3**

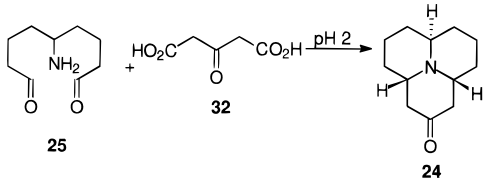


**Scheme 4**



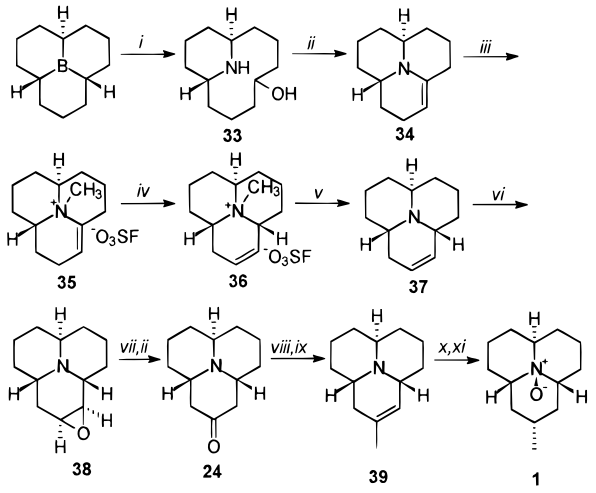
Reaction Conditions: (i) MeONa, $CH(OMe)_3$, $H^+$; (ii) TsOH, trimethyl orthoformate; (iii) NaCl, wet DMF, reflux; (iv) NaH; (v) $NH_4OAc$, $NaCNBH_3$, molecular sieves; (vi) $H^+$; (vii) $Ph_3P{=}CH_2$; (viii) $H_2$, Pd/C; (ix) m-CPBA.

**Scheme 5**



Langlois *et al.*[35] have recently reported that the yield of **24** could be increased by treating amine **25** with acetonedicarboxylic acid (**32**) (Scheme 5) in place of the diester used by Stevens and Lee.[25] This return to Schöpf's original methodology avoided the low-yielding decarbomethoxylation step otherwise necessary to convert **26** to **24**.

**Scheme 6**

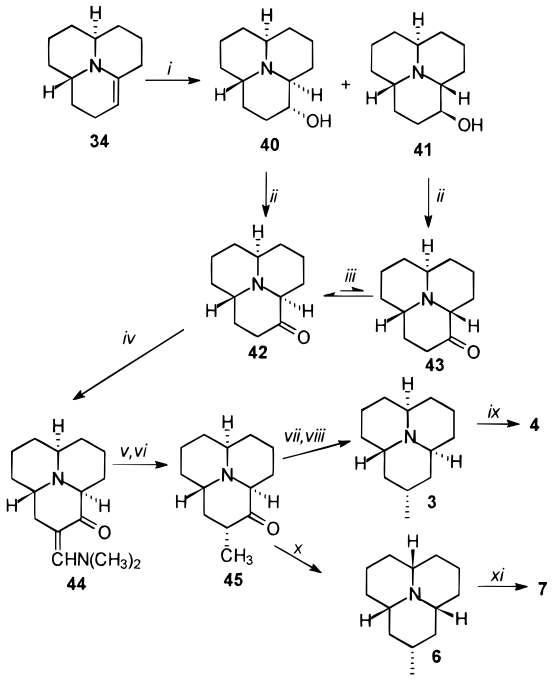


Reaction conditions: (i) N-chloro-O-(2,4-dinitrophenyl)hydroxylamine, $H_2O_2$/NaOH; (ii) $CrO_3/H_2SO_4$; (iii) $CH_3OSO_2F$; (iv) LDA; (v) LiSEt; (vi) TFA, $CF_3CO_3H$; (vii) Li, EDA; (viii) $Ph_3P{=}CH_2$; (ix) TsOH; (x) $H_2$, Pd/C; (xi) m-CPBA.

In a series of papers presenting still another approach to azaphenalene synthesis, Mueller and coworkers have reported the total synthesis of all of the known coccinellid azaphenalene alkaloids. The starting material for this work was perhydroboraphenalene, prepared by the hydroboration of 1,5,9-cyclododecatriene.[36−38] In the synthesis of coccinelline and precoccinelline (Scheme 6),[36,38] perhydroboraphenalene was treated with *N*-chloro-*O*-(2,4-dinitrophenyl)hydroxylamine (generated *in situ*) and hydrogen peroxide, yielding the unisolated amino alcohol **33**. Oxidation to the corresponding ketone produced the tricyclic enamine **34**.[39,40] Addition of methyl fluoro sulfate to enamine **34** produced eneammonium salt **35**, which gave the allylic ammonium salt **36** on treatment of **35** with lithium diisopropylamide. The allylic amine **37** was obtained by cleavage of the methyl group of **36** with lithium ethyl mercaptide. Stereoselective epoxidation from the less hindered face of amine **37** gave **38**, which underwent reductive opening to give an axial alcohol, which was oxidized to afford ketone **24**, an intermediate in Ayer and Furuichi's synthesis of coccinelline and precoccinelline.[30] A Wittig reaction was followed by isomerization of the resulting exocyclic olefin with *p*-toluenesulfonic acid to generate olefin **39**, which was hydrogenated to give precoccinelline (**2**), identical with an authentic sample. Treatment with *m*-chloroperbenzoic acid converted **2** into coccinelline (**1**).[36,38]

The tricyclic intermediate **34** was also used for the first syntheses of hippocasine and hippocasine *N*-oxide (Scheme 7),[37,38] and for a new synthesis of hippodamine and convergine, hydroboration−oxidation of **34** gave alcohols **40** and **41** in a 3:1 ratio. Oxidation of the mixture produced ketones **42** and **43**, which equilibrated to a separable 9:1 mixture of **42**:**43** upon treatment with mild base. To avoid N-alkylation while introducing a methyl group α to the carbonyl, **42** was treated with the Bredereck reagent[41] to generate vinylogous amide **44**. Reduction with "lithium bronze" to an unstable β-amino ketone was followed by catalytic hydrogenation to give **45**, a branching point in the synthesis of hippo-

**Scheme 7**

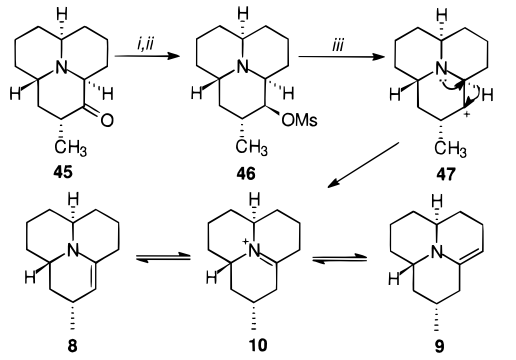


Reaction conditions: (i) $BH_3$:DMS, $H_2O_2$/NaOH; (ii) $CrO_3$/HOAc/$H_2SO_4$; (iii) $NaOCH_3$; (iv) $CH_3OCH[N(CH_3)_2]_2$; (v) lithium bronze; (vi) $H_2$/Pd; (vii) ethanedithiol/$BF_3$·etherate; (viii) Li/EDA; (ix) m-CPBA; (x) $NH_2NH_2$, TsCl, t-BuNHLi; (xi) $H_2O_2$.

damine and hippocasine. Although significant epimerization occurred in the Wolf−Kishner reduction of **45** to generate a 2:1 mixture of hippodamine (**4**) and its axial methyl isomer, reduction of the thioketal of **45** with lithium in ethylenediamine cleanly produced racemic hippodamine, which was transformed to its *N*-oxide by treatment with *m*-chloroperbenzoic acid. This same ketone was carried on to racemic hippocasine (**6**) via a Bamford−Stevens reaction of the derived tosyl hydrazone with lithium *tert*-butylamide. Hippocasine *N*-oxide was formed by treatment of **6** with hydrogen peroxide. Comparison showed the natural and synthetic samples of alkalkoids to be identical.[37]

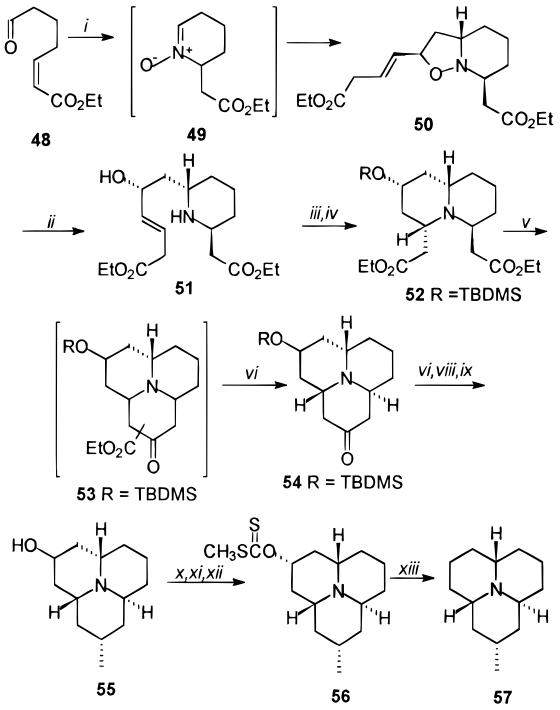
The only synthesis of propyleine (**8**) and isopropyleine (**9**) to date was performed by Mueller *et al*.[28] during their investigation of the structure of propyleine.[17] They had proposed that iminium salt **10** must have formed during the original isolation of the alkaloid, and that **10** must have given rise to a mixture of **8** and **9** after deprotonation. Synthetic **10** was prepared to test this hypothesis (Scheme 8). Ketone **45** was reduced using lithium aluminum hydride to give the corresponding alcohol, which was then converted to mesylate **46**. E1 conditions induced a hydride ion shift to generate iminium salt **10**, which deprononated to give a mixture of enamines (±)-**8** and (±)-**9** in a 1:3 ratio, presumably via the initial formation of cation **47**. The 60 MHz $^1$H NMR and IR spectra of this mixture were identical with those of the natural material. At 270 MHz, the $^1$H NMR signals of the individual isomers could be discerned. Upon addition of TFA, the $^{13}$C NMR signals collapsed to show a single immonium carbon resonance, indicating that both isomers formed the same immonium salt **10**.[28] Since the acidic condi-

**Scheme 8**



Reaction conditions: (i) LAH; (i i) MsCl, TEA; (iii) $K_2CO_3$, DMSO.

**Scheme 9**

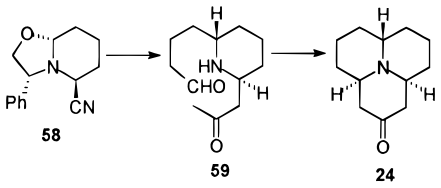


Reaction conditions: (i) $H_2NOH{\cdot}HCl$, $NaOAc{\cdot}3H_2O$, ethyl hexa-3,5-dienoate, 120°C; (ii) Zn, HOAc, EDTA; (iii) DBU, $C_6H_6$; (iv) TBDMSCl; (v) LDA; (vi) LiCl, DMF; (vii) $PH_3{=}CH_2$; (viii) $H_2$, Pd/C; (ix) $Et_4N^+F^-$; (x) NaH, imidazole; (xi) $CS_2$; (xii) MeI; (xiii) $Bu_3SnH$, AIBN

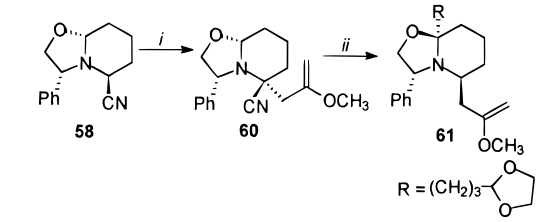
tions used to isolate propyleine would have caused either **8** or **9** to rearrange; the actual composition of the alkaloid(s) present in *P. quatuordecimpunctata* remains unknown.

A cycloaddition reaction between ethyl hexa-3,5-dienoate and a nitrone provides the foundation of a synthesis of (±)-*epi*-hippodamine (**57**) (Scheme 9).[42] Nitrone **49**, generated *in situ* by treatment of **48** with hydroxylamine, reacted with ethyl hexa-3,5-dienoate to give cycloadduct **50**. Reduction of **50** with zinc in acetic acid and ethylenediaminetetraacetic acid gave unstable piperidine **51**, which underwent conjugation, intramolecular Michael addition, and protection to give **52**. Dieckmann ring-closure afforded unisolated tricyclic amine **53**, which underwent deethoxycarbonylation to give **54**. A Wittig reaction of **54** with methylenetriphenylphosphorane gave the expected olefinic product, which was subjected to catalytic hydrogenation before removal of the silyl protecting group to give **55**. Deoxygenation of **55** proceeded

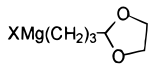
**Scheme 10**



**Scheme 11**



Reaction conditions: (i) LDA-THF,3-bromo-2-methoxypropene; (ii) TBDMSOTf,



through xanthate **56** to yield (±)-*epi*-hippodamine (**57**).[42]

Yue *et al.* achieved a fourth synthesis of precoccinelline,[43] by employing their "CN(*R,S*)" strategy for the stereoselective synthesis of *cis*- or *trans*-2,6-dialkylpiperidines.[44,45] This strategy uses the substitution of the CN group to obtain the *R* or *S* configuration of a chiral center α to a nitrogen atom. Their approach began with the (2*S*,6*R*)-2-cyano-6-oxazolopiperidine **58**, an easily prepared precursor (Scheme 10), and proceeded via a protected form of *trans*-disubstituted piperidine **59** to generate ketone **24**, an intermediate in Ayer's synthesis of **2**.[30]

Treatment of **58** with lithium diisopropylamide (Scheme 11) followed by addition of 2-methoxyallyl bromide afforded **60**. Reaction with TBDMSOTf and (1,3-dioxolanyl-2-propyl)magnesium chloride gave oxazolidinone **61** via a tandem alkylation/aza-Cope rearrangement.

A possible mechanism for this transformation is shown in Figure 9. Upon treatment of **60** with the Lewis acid TBDMSOTf, the cyano group could be removed, resulting in formation of an iminium ion, subject to attack by a Grignard reagent. At this point, the presence of a Lewis acid could cause a second iminium ion to form, resulting in cleavage of the five-membered ring. This iminium ion is posi-

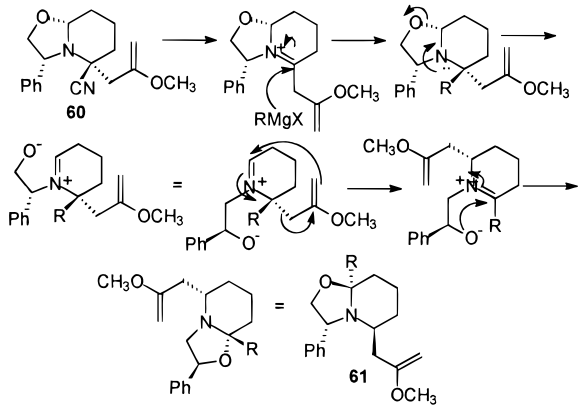
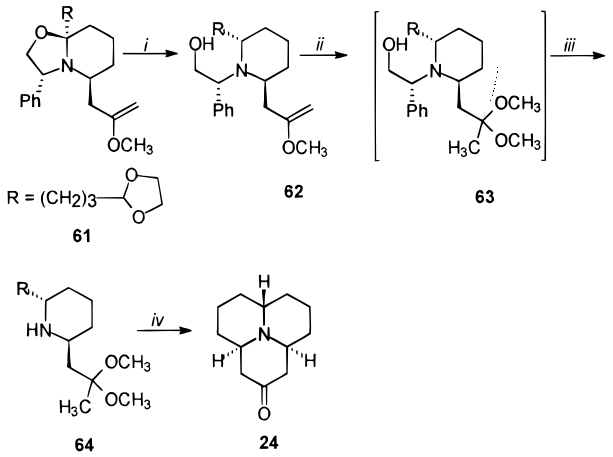


**Figure 9.** A proposed mechanism for the tandem alkylation/rearrangement.

**Scheme 12**




Reaction conditions: (i) LAH; (iii) formic acid,$CH_3OH$; (iii) $K_2CO_3$,10%Pd/C; (iv) l0% HCl, CSA.

tioned for an aza-Cope rearrangement, which, if followed by formation of a new five membered ring, would give the observed product.

The product of the tandem alkylation/aza-Cope rearrangement, **61**, underwent reductive ring cleavage with lithium aluminum hydride to yield the *trans*-disubstitued piperidine **62** (Scheme 12). A "one-pot" methanolysis and debenzylation transformed **62** to ketone acetal piperidine **64** via **63**. Ketone **24**, obtained from **64** by hydrolysis of the protecting groups and an intramolecular Mannich reaction, was converted to precoccinelline using Mueller's conditions.[36,37]

## *III. 9-Azabicyclo[3.3.1]nonanes*

The first naturally occurring alkaloid with the 9-azabicyclo[3.3.1]nonane skeleton to be characterized was pseudopelletierine (**65**, Figure 10), isolated from the bark of pomegranate trees.[46a,b] It was from pseudopelletierine by a long sequence of reactions that Willstätter first prepared cyclooctatriene in 1911.[47] Subsequently, other azabicyclononanes have been identified in both the plant and animal kingdoms. Two azabicyclononane (homotropane) alkaloids play a role in the chemical defense of coccinellid beetles.

### A. Isolation and Identification

Adaline (**66**, Figure 10) was first isolated by Tursch *et al.* from the European coccinellid, *Adalia bipunctata*, by fractionation of whole insect extracts.[48] Initial chemical and NMR spectroscopic studies showed adaline to be a bicyclic amino ketone. Single crystal X-ray diffraction, carried out on adaline hydrochloride, revealed the complete structure,[48] and

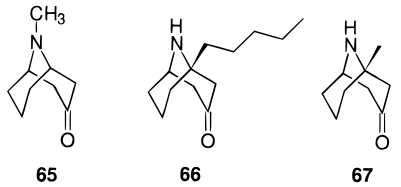



**Figure 10.** Pseudopelletierine (**65**), adaline (**66**), and euphococcinine (**67**).

X-ray analysis as well as analysis of its optical rotatory dispersion (ORD) spectrum established its absolute configuration.[49] Thus, natural adaline has been assigned the *R* configuration at C-1.

Euphococcinine (**67**, Figure 10), (+)-9-aza-1-methylbicyclo[3.3.1]nonan-3-one, a lower homologue of adaline, was first identified from an Australian seacoast plant, *Euphorbia atoto*, by Hart and co-workers[50] and was later found in coccinellid beetles.[51,52] The name euphococcinine reflects its isolation from both plants and insects.[52]

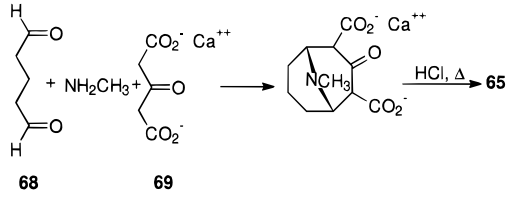
### B. Azabicyclononane Syntheses

Early synthetic work directed toward the synthesis of azabicyclononane alkaloids was based on the Robinson-Schöpf biomimetic strategy, involving tandem Mannich reactions.[32−33] Earliest example, pseudopelletierine (**65**) was produced by the reaction of glutardialdehyde (**68**) with methylamine and calcium acetonedicarboxylate (**69**) in accord with the proposed biosynthetic route (Scheme 13).[32]

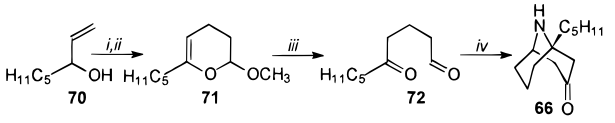
Tursch *et al.* employed a similar approach for the first synthesis of adaline (**66**) (Scheme 14).[49,53] Oxidation of 1-octen-3-ol (**70**) with Jones reagent followed by a cycloaddition reaction with methyl vinyl ether afforded the dihydropyran **71**. Acid hydrolysis to give ketoaldehyde **72** and a Mannich reaction with $\beta$-ketoglutaric acid and ammonium chloride gave (±)-adaline.[49]

A second synthesis of racemic adaline (Scheme 15) was completed by Medina *et al.* [54] Racemic amino nitrile **73**, prepared according to a standard procedure,[55] was converted to the 2,6-dialkylpiperidine **74**. Refluxing **74** in acidic methanol proceeded via inter-

**Scheme 13**

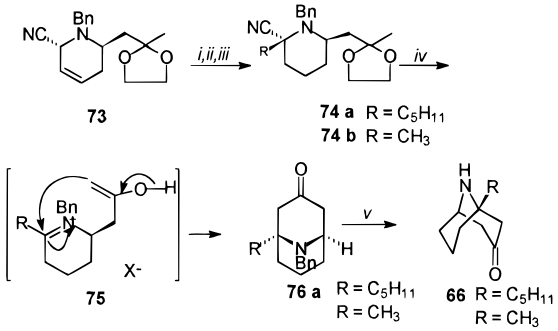



**Scheme 14**



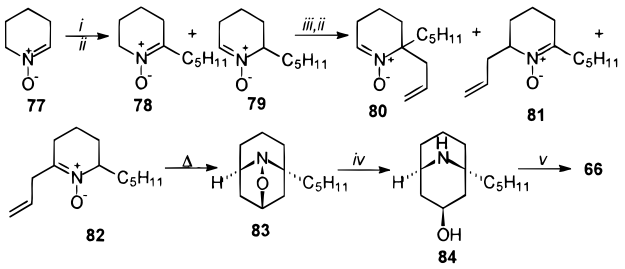

Reaction conditions: (i) $CrO_3$; (ii) Δ ,$H_3CO^-$; (iii) $H_2O/H^+$; (iv) $NH_4Cl$, $HO_2C$ $CO_2H$ .

**Scheme 15**



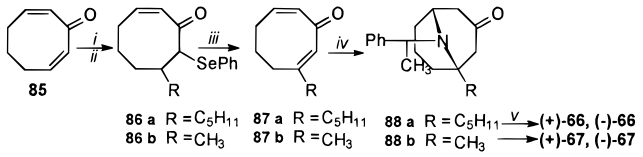

Reaction conditions: (i) $H_2$-Pd/C; (ii) LDA; (iii) RBr; (iv) 10 % HCl; (v) $H_2$-Pd/C-HCl-EtOH.

**Scheme 16**



Reaction conditions: (i) $C_5H_{11}MgBr$; (ii) HgO; (iii) allylMgBr; (iv) Raney Ni; (v) PCC.

**Scheme 17**



Reaction conditions: (i) RMgX, CuI; (ii) PhSeBr; (iii) $H_2O_2$-Py; (iv) (R)-(+)-α-methylbenzylamine; (v) $H_2$-Pd/C.

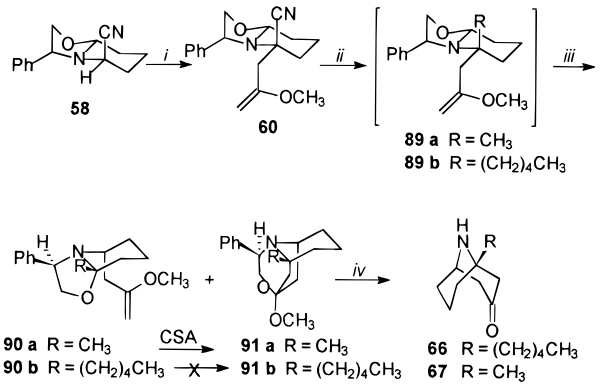
mediate **75**, producing *N*-benzyladaline (**76a**), which gave adaline (**66**) by catalytic debenzylation. The same methodology served for the synthesis of racemic euphococcinine (**67**).[54]

Another synthesis of racemic adaline was reported in 1980 by Gossinger and Witkop.[56] The reaction of nitrone **77** with *n*-pentylmagnesium bromide (Scheme 16) gave a hydroxylamine which was then oxidized to yield a mixture of nitrones **78** and **79**. This mixture was treated with allylmagnesium bromide followed by mercuric oxide, producing a mixture of nitrones **80**−**82**. Nitrones **80** and **81** underwent an intramolecular 1,3-dipolar addition upon heating to give isoxazoline **83**. Hydrogenolysis to **84** followed by oxidation provided racemic adaline (**66**).

The first asymmetric syntheses of adaline (Scheme 17) and euphococcinine employed a three-step, one-pot sequence of reactions.[57] Beginning with 2,7-cyclooctadienone (**85**), conjugate addition of the appropriate Grignard reagent and trapping of the resulting enolate ion with phenylselenium bromide produced intermediates **86a** and **86b**. Elimination to give dienones **87a** and **87b** proceeded after oxidation with hydrogen peroxide. A double Michael addition with (*R*)-(+)-α-methylbenzylamine gave a diastereomeric mixture for each of the adducts **88a** and **88b**. The diastereomers were separated by recrystallization, and hydrogenolysis of the chiral α-methylbenzyl group provided optically pure products. Upon hydrogenolysis, the solid isomer produced the natural product (−)-adaline (**66**), previously assigned the (1*R*,5*S*) configuration based on its ORD spectrum,[49] while the liquid enantiomer yielded the (1*S*,5*R*) enantiomer. Synthetic (1*R*,5*S*)-adaline showed the same optical rotation as the natural product, $[\alpha]_D$ −11°.[57]

The diastereomers of **88b**, used in the synthesis of euphococcinine, displayed a close correspondence to the **88a** pentyl series. In both cases, the solid Michael adduct was more polar than the liquid diastereomer, and hydrogenolyzed to the levorotatory base. Hill and Renbaum [57] use this correspondence to suggest that there is also a correspondence in configuration. Thus they surmise that the liquid

**Scheme 18**



Reaction conditions: (i) LDA-THF, 3-bromo-2-methoxypropene; (ii) TBDMSOTf, RMgX; (iii) $SiO_2$; (iv) $H_2$-Pd/C, aq HCl.

diastereomer of **88b**, which produced the dextrorotary euphococcinine enantiomer after hydrogenolysis, has the (1*S*,5*R*) configuration. The optical rotation of euphococcinine was reported as $[\alpha]_D$ +6° when it was first isolated,[50] and thus they conclude that the configuration of euphococcinine is (1*S*,5*R*).[57]

By employing the "CN(R,S)" method, Yue and coworkers were able to successfully control stereochemistry during a recent asymmetric synthesis of adaline and euphococcinine (Scheme 18), and determine the absolute configuration of natural (+)-euphococcinine by the synthesis of its enantiomer.[45]

Chiral nitrile **58**, which was prepared according to a standard procedure,[44] was deprotonated with LDA and allowed to react with 3-bromo-2-methoxy-1-propene[45] to produce the alkylated nitrile **60** with retention of configuration. The methyl or pentyl substituents were introduced by sequential addition of TBDMSOTf and methylmagnesium iodide. Reactive intermediate **89a** rearranged to give desired **90a** as well as **91a**. Compound **90a** and any trace amounts of **89a** which remained could be converted to desired **91a** by treatment of the crude reaction mixture with camphorsulfonic acid. Treatment of **60** with TBDMSOTf and *n*-pentylmagnesium bromide produced a mixture of **90b** and **91b**, with no trace of **89b** remaining. The proposed pathway for the con-

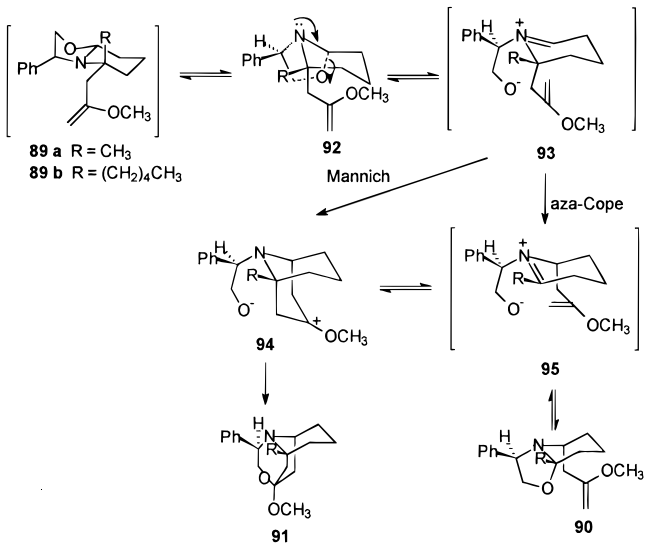


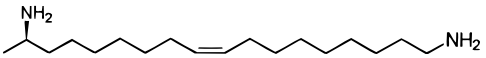
**Figure 11.** The proposed mechanism for conversion of **89** to **90** and **91**.

version of **89** ultimately to **90** and **91** is shown in Figure 11. Surprisingly, **90b** could not be converted into **91b**. Hydrolysis of the intramolecular ketal and subsequent hydrogenolysis of the chiral appendage were performed in one pot to yield (−)-euphococcinine (**67**) from **91a** and (−)-adaline (**66**) from intermediate **91b**.

## IV. Harmonine

### A. Isolation and Identification

Harmonine was first isolated by the acetylation and fractionation of a basic extract obtained from *Harmonia leis conformis*.[58] High-resolution mass spectrometry of the resulting bis-acetyl derivative revealed a molecular formula of $C_{22}H_{42}NO_2$; the infrared spectrum showed the presence of at least one secondary amide (3300, 1645, and 1500 $cm^{-1}$). NMR analysis indicated two $NHCOCH_3$ groups, a disubstituted double bond, 11 isolated methylenes, plus two methylenes adjacent to an $sp^2$ carbon atom. Further spectroscopic and chemical studies established the structure of the natural product as (*Z*)-1,17-diaminooctadec-9-ene (**96**). The absolute stereochemistry of harmonine, shown to be 17-*R*, was determined through analysis of lanthanide induced shifts in the $^1H$ NMR spectrum of **96** and has been confirmed by a subsequent synthesis.[59]

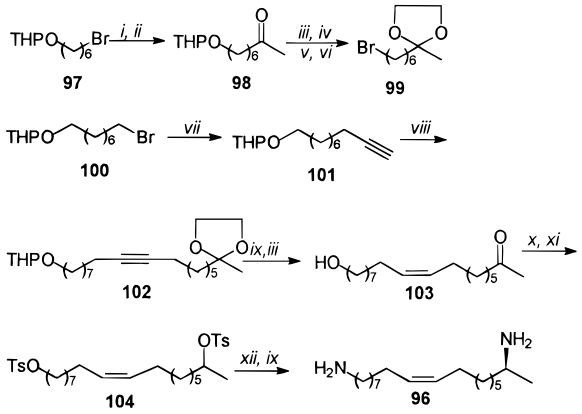


**96**

### B. Harmonine Synthesis

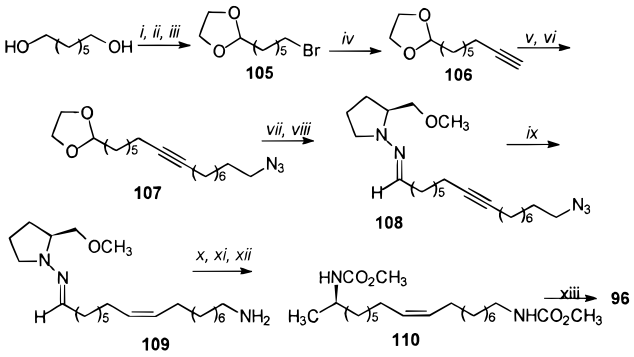
Soon after the structure of harmonine was determined,[58] a synthesis of the racemic alkaloid was completed (Scheme 19).[59] The synthesis began with the addition of the Grignard reagent derived from the THP ether of 6-bromo-1-hexanol (**97**) to acetaldehyde, followed by oxidation with PCC to give ketone **98**. After removal of the THP group, conversion of the alcohol to the bromide and protection of the carbonyl group yielded bromide **99**. Coupling of **100** with acetylene in the presence of $LiNH_2/NH_3$ gave **101,** which was alkylated with bromide **99** to produce

**Scheme 19**



Reaction conditions: (i) $Mg/CH_3CHO$; (ii) PCC; (iii) $HCl/CH_3OH$; (iv) MsCl/pyr; (v) NaBr; (vi) ehtylene glycol, $H^+$; (vii) acetylene/$LiNH_2$; (viii) n-BuLi/**99**; (ix) $H_2$/Lindlar; (x) $NaBH_4$; (xi) TsCl/pyr; (xii) $NaN_3$.

**Scheme 20**



Reaction conditions: (i) HBr; (ii) PCC; (iii) ethylene glycol, PPTS; (iv) lithium acetylide-EDA complex; (v) n-BuLi, 1,6-bromochlorooctane; (vi) $NaN_3$; (vii) HCl/acetone; (viii) SAMP; (ix) $H_2$/Lindlar; (x) $CH_3Li$; (xi) $ClCO_2CH_3$; (xii) Li/$NH_3$; (xiii) TMSI

intermediate **102**. Semihydrogenation introduced the $\Delta^9$-*Z*-double bond and was followed by removal of the two protecting groups to give hydroxy ketone **103**. Reduction with $NaBH_4$ gave the expected diol, which was converted into ditosylate **104**. The final transformation to **96** was achieved by treatment of **104** with $NaN_3$, followed by catalytic hydrogenation.

An elegant asymmetric synthesis of harmonine has also been reported by Enders and Bartzen (Scheme 20).[60] In this work, the stereogenic center at carbon 17 was introduced in the last step by the enantioselective SAMP/RAMP hydrazone method.[60] 1,7-Heptanediol was converted to the corresponding bromohydrin, followed by oxidation of the remaining hydroxyl group to the aldehyde with PCC. Subsequent protection of the carbonyl with 1,2-ethanediol gave acetal **105**. Coupling of **105** with lithium acetylide−ethylenediamine complex produced terminal acetylene **106**. Alkylation of **106** with 1,6-bromochlorooctane, followed by the displacement of the primary chloride substituent with $NaN_3$ gave azide **107**. Hydrolysis of the acetal preceded conversion of the aldehyde to its SAMP hydrazone (*S*)-**108** by reaction with (*S*)-1-amino-2-(methoxymethyl)pyrrolidine (SAMP). Hydrogenation over Lindlar's catalyst generated both the $D^9$-*Z*-double bond and the primary amino group of **109** without affecting the CN double bond of the hydrazone. Nucleophilic addition of methyllithium to the CN double bond in **109** was followed by a two step process to produce the protected diamine **110**. The methoxycarbonyl group of the hydrazino moiety allowed reductive cleavage of the N−N bond by lithium/ammonia without epimerization.[61] Cleavage of the carbamate groups with trimethylsilyl iodide gave **96** with an overall yield of 5% (>97% ee).[60]

## V. Pyrrolidines, Piperidines, and Aromatic Amines

An analysis of the alkaloids present in *Cryptolaemus montrouzieri* revealed that in addition to euphococcinine,[50] there were two unidentified major components, one of which was identified by Brown and Moore.[51] The high-resolution mass spectrum of the acetylated alkaloid revealed the molecular formula of the natural material to be $C_9H_{17}NO$. Both reduction with lithium aluminum hydride and cata-

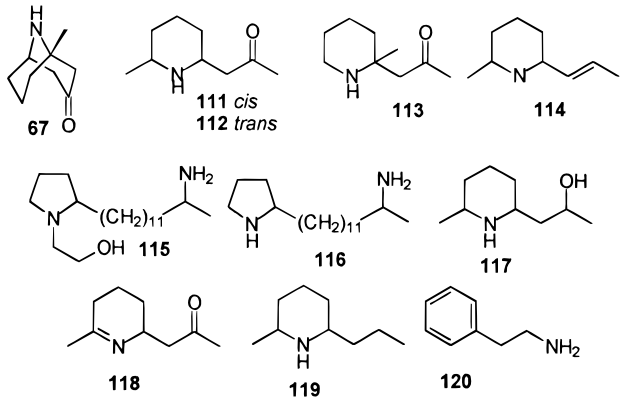



**Figure 12.** Alkaloids identified in *E. varivestis* and *Cryptolaemus montrouzieri*.

lytic hydrogenation showed an incorporation of two hydrogen atoms, indicating the presence of a carbonyl group but no further unsaturation. This spectroscopic evidence suggested that the alkaloid was 1-(6-methyl-2-piperidyl)propan-2-one (**111**) (Figure 12) or possibly **113**, although **111**, similar to pinidine (**114**), was thought to be more likely in view of its likely polyketide origin. Wolff−Kishner reduction of the alkaloid gave a product identical to dihydropinidine, and the synthesis of 1-(6-methyl-2-piperidyl)propan-2-one from 2,6-lutidine yielded a material indistinguishable from **111**. Catalytic reduction of 1-(6-methyl-2-pyridyl)propan-2-one, known to give *cis* products, produced **111** but no other isomer, showing the stereochemistry of the alkyl substituents in **111** to be *cis*. The other major component from *Cryptolaenus montrouzieri* has the same molecular weight as **111**, to which it readily isomerizes (presumably through an elimination/addition mechanism) and is thought to be *trans*-1-(6-methyl-2-piperidyl)propan-2-one (**112**). Due to its instability and the small quantities available, this compound has not yet been completely characterized.[51]

The extract of the Mexican bean beetle, *Epilachna varivestis*, was shown to contain a complex mixture of alkaloids, with the composition of the mixture varying with the developmental stage of the beetles.[62,63] Attygalle *et al.* identified eight alkaloids in eggs, larvae, and adult beetles (Figure 12), two of which, **115** and **116**, were previously uncharacterized.[62] The uncharacterized alkaloids were present in the eggs, larvae, and adults; **115** was the most abundant alkaloid in all three of these stages, but **111**, **117**−**120**, and **67** were found only in adults. Euphococcinine (**67**) and the two pyrrolidine alkaloids (**115** and **116**) comprised over 90% of the defensive alkaloid mixture in adults. An analysis of the high-resolution mass spectrum of **115** showed a molecular formula of $C_{19}H_{39}N_2O$; the base peak showed the molecular formula $C_6H_{12}NO$, and a methyl group and a primary alcohol were evident. The compound was resistant to hydrogenation and was thus concluded to be monocyclic. The alkaloid was shown to contain an uninterrupted chain of 17 carbons by its reduction to *n*-heptadecane skeleton by high temperature hydrogenolysis. Further chemical and NMR studies revealed the complete structure of this compound to be that shown in formula **115**. A comparison of the mass spectral and infrared data of the other new

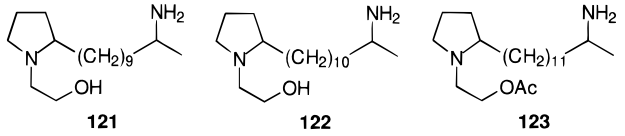



**Figure 13.** Additional alkaloids found in *E. varivestis*.

alkaloid and its derivatives with that of **115** led to its being assigned structure **116**. A third new alkaloid related to **115** and **116** was not fully characterized.[62] The more volatile alkaloids were identified by comparison of their spectral data with literature spectra (**111**, **119**, **120**, **67**) or on the basis of chemical studies (**117** and **118**). The dialkylpiperidine **111** had been identified previously from *Cryptolaenus montrouzieri*.[51] Although a number of 2,6-dialkylpiperidines are common in ant venoms,[64,65] **117** has not been previously found in nature.[62] The configurations of **111**, **117**, and **119** were assigned as *cis* by comparison of their gas chromatographic retention times with synthetic standards, prepared by reduction of the appropriate 2,6-disubstituted pyridines with sodium/ethanol and sodium borohydride. This reaction is known to give mixtures of *cis* and *trans* products with the *cis* isomer dominating.[62,66]

A particularly interesting study of *Epilachna varivestis* alkaloids aimed at determining the changes in alkaloid content which occur at various developmental stages.[63] Eggs, larvae, and pupae were found to have similar alkaloid profiles and were also found to contain two previously uncharacterized lower homologues of **115**, **121**, and **122** (Figure 13). The *O*-acetate of **115**, **123**, was also detected, although this compound may have been an artifact from the method of isolation. An unsaturated alkaloid related to **115** was isolated. It appeared that the unsaturation was present in the ring but the position of the double bond could not be determined.

Two days after emergence from the pupal stage, adult insects began to show increase in the amount of euphococcinine and a concurrent decrease in the amount of **115** present.[63] None of the alkaloids detected at any stage could be found in the beetles' food plant, *Phaseolus vulgaris*, indicating that the entire array of Mexican bean beetle alkaloids is of insect rather than plant origin.[63]

## *VI. Azamacrolides*

### A. Isolation and Identification

Microscopic examination showed the pupae of *Epilachna varivestis* to be covered with glandular hairs, each having a droplet of oil at its tip.[67] Attack by ants upon the pupae revealed that an attacking ant tended to back away and cleanse itself upon contact with the oil. In view of the defensive role of these droplets, Attygalle and co-workers undertook a chemical analysis of this secretion, which resulted in the identification of a novel family of alkaloids, the azamacrolides.[67] The oil was collected in capillary tubes and analyzed directly by coupled gas-chromatography/mass spectrometry. Five compounds (**124**−**128**) were identified from the secretion (Figure 14), with epilachnene (**124**) comprising over 90% of

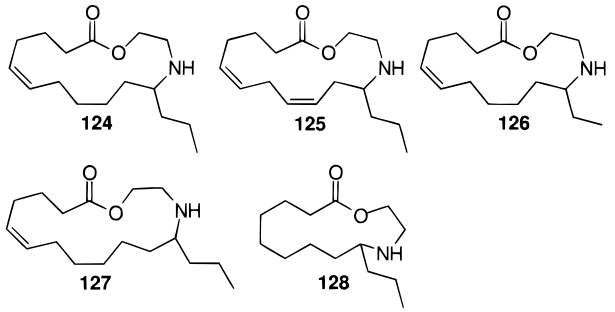



**Figure 14.** The azamacrolides, isolated from *E. varivestis* pupae.

the volatile material. Although mass spectral data were collected for all components, subsequent chemical and NMR studies focused on epilachnene, since this component was available in the largest quantities. The molecular formula of epilachnene was shown to be $C_{16}H_{29}NO_2$ by high-resolution mass spectrometry and the base peak in the mass spectrum corresponded to the loss of a $C_3H_7$ moiety. The gas-phase IR spectrum of epilachnene indicated a carbonyl group (1753 $cm^{-1}$) typical of an ester or lactone as well as a C−O−C band (1152 $cm^{-1}$) and a weak *Z*-olefin absorbance at 3011 $cm^{-1}$.[68] It was confirmed that the carbonyl group was not present as a ketone or aldehyde by the failure of epilachnene to react with *N*,*N*-dimethylhydrazine. Catalytic microhydrogenation showed that epilachnene contained only one carbon−carbon double bond and was thus monocyclic. Acetylation gave a tertiary carboxamide (IR absorbance at 1745 $cm^{-1}$), showing epilachnene itself to be a secondary amine. $^1H$ NMR analysis of epilachnene revealed the presence of two olefinic protons as well as an *n*-propyl group. On the basis of the facile loss of a $C_3H_7$ fragment in the mass spectrum, the propyl side chain was thought likely to be attached to a carbon atom adjacent to the nitrogen atom. The $^1H$ NMR spectrum also revealed two adjacent low-field methylene groups, one attached to an oxygen atom and the other attached to the nitrogen. The correlated spectroscopy (COSY) spectrum allowed the assignment of the remaining structural details as shown in formula **124**. Epilachnene is a 15 membered macrocyclic lactone, possessing both a secondary amine and a *Z*-double bond within the ring. One chiral center is present, α to the amine, although the configuration at this one stereogenic center has not been determined.[67]

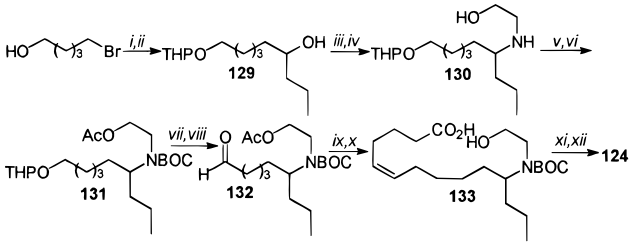
The remaining components of the *E. varivestis* secretion were assigned structures on the basis of a comparison of their mass spectral data with data obtained from epilachnene. The second largest component, epilachnadiene (**125**), had a molecular ion at *m*/*z* 265 and a fragment ion at *m*/*z* 222, indicating the presence of a second double bond within the macrocyclic ring of epilachnene. Hydrogenation of a mixture of epilachnene and epilachnadiene gave only one product, epilachnane, supporting the presence of a second double bond. The placement of the second double bond within the ring was tentatively assigned as shown in **125** on the basis of the assumption that the compound was biosynthesized by chain-shortening of an unsaturated acid such as linoleic or linolenic acid, which would result in a skipped diene.[67] This assignment has since been confirmed by the recent synthesis of **125** by Rao and Kumar.[69] The structural assignment of norepilachnene (**126**) was based on its facile loss of an ethyl moiety in the mass spectrum, instead of the propyl loss seen for epilachnene. Homoepilachnene (**127**) shows one additional methylene group in the macrocycle, whereas **128** is a saturated macrocycle containing one methylene less than epilachnene. On the basis of its mass spectral fragmentation pattern, this saturated alkaloid appears to be 9-propyl-10-azacyclododecan-12-olide.[67] The configuration at the stereogenic center in each of the azamacrolides has not yet been determined.

## B. Azamacrolide Synthesis

The structures of all five of the azamacrolides have been confirmed by the synthetic efforts of Rao and Kumar.[69,70] The synthesis of the major secretory component, epilachnene, began with the protection of 1,5-bromopentanol (Scheme 21) followed by formation of a Grignard reagent and addition to butanal to produce alcohol **129**.[70] Conversion to the mesylate and treatment with ethanolamine gave amino alcohol **130**, which was doubly protected to give **131**. Removal of the THP ether and oxidation to the corresponding aldehyde gave **132**, which underwent a Wittig reaction and ester hydrolysis to give hydroxy acid **133**. Lactonization and deprotection gave racemic epilachnene (**124**). The spectral data observed for the synthetic material were in agreement with those of the natural product.[70]

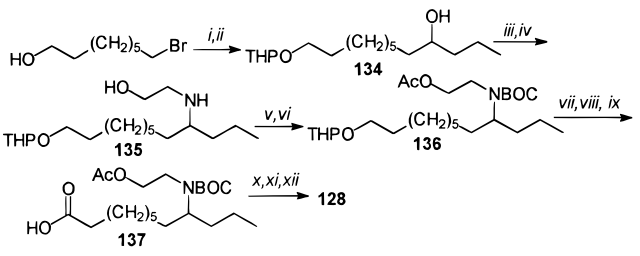
9-Propyl-10-azacyclododecan-12-olide (**128**) was obtained by a similar approach (Scheme 22).[70] 1,8-Bromooctanol was protected before reaction with magnesium, followed by treatment with butanal. The resulting alcohol **134** was converted to its mesylate, which gave amino alcohol **135** upon treatment with

**Scheme 21**



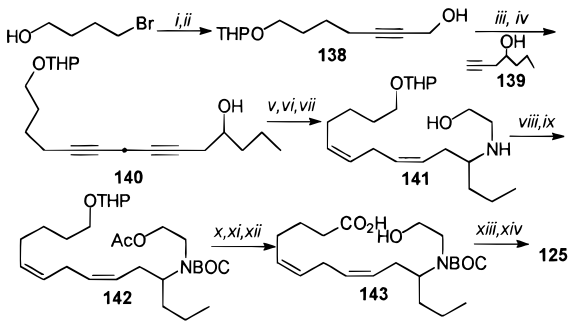

Reaction conditions: *i*, DHP; *ii*, Mg, butanal; *iii*, MsCl, TEA; *iv*, ethanolamine; *v*, $BOC_2O$, TEA; *vi*, $Ac_2O$, DMAP, pyr.; *vii*, PPTS; *viii*, PCC; *ix*, NaH, $Ph_3P^+CH_2(CH_2)_3CO_2HBr^-$, DMSO; *x*, NaOH; *xi*, 2,4,6-trichlorobenzoyl chloride/TEA/DMAP; *xii*, TFA.

**Scheme 22**



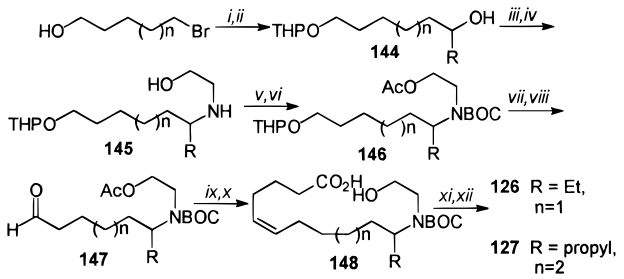

Reaction conditions: (i) DHP; (ii) Mg, butanal; (iii) MsCl, TEA; (iv) ethanolamine; (v) $BOC_2O$, TEA; (vi) $Ac_2O$, DMAP, pyr.; (vii) PPTS; (viii) PCC; (ix) $Ag_2O$; (x) $K_2CO_3$, MeOH; (xi) 2,4,6-trichlorobenzoyl chloride/TEA/DMAP; (xii) TFA.

**Scheme 23**



Reaction conditions: (i) DHP; (ii) $LiNH_2$, propargyl alcohol; (iii) n-BuLi, MsCl, LiBr; (iv) EtMgBr, CuCl; (v) $H_2$, $Pd/CaCO_3$; (vi) MsCl, TEA; (vii) ethanolamine; (viii) $BOC_2O$; (ix) $Ac_2O$, pyr, DMAP; (x) PPTS; (xi) PCC; (xii) $Ag_2O$, NaOH; (xiii) 2,4,6-trichlorobenzoyl chloride, TEA, DMAP; (xiv) TFA.

**Scheme 24**



Reaction conditions: (i) DHP; (ii) Mg, butanal (or propanal); (iii) MsCl, TEA; (iv) ethanol amine; (v) $BOC_2O$, TEA; (vi) $Ac_2O$, DMAP, pyr.; (vii) PPTS; (viii) PCC; (ix) NaH, DMSO, $Ph_3P^+CH_2(CH_2)_3CO_2HBr^-$; (x) NaOH; (xi) 2,4,6-trichlorobenzoyl chloride/TEA/DMAP; (xii) TFA.

ethanolamine. After the addition of two protecting groups to give **136**, cleavage of the THP ether and oxidation with PCC and silver oxide afforded acid **137**. Acetate hydrolysis followed by lactonization and removal of the BOC group gave the desired racemic lactone **128**.[70]

The synthesis of **125** began with the alkylation of propargyl alcohol with protection of 1,4-bromobutanol (Scheme 23) to give alcohol **138**.[69] Conversion to the bromide preceded the C-alkylation of **139** to yield diyne **140**. Semihydrogenation to give the *cis,cis*-diene was followed by conversion to the mesylate and subsequent treatment with ethanolamine to afford amino alcohol **141**. A double protection to **142** followed by removal of the THP ether and oxidation with PCC and silver oxide gave hydroxy acid **143**. Ring closure and deprotection proceed as in previously described examples to give epilachnadiene (**125**).[69]

Homoepilachnene (**127**) and **126** were synthesized by a similar approach (Scheme 24).[69] Grignard reaction of the THP ether of bromo alcohol **144** with butanal or propanal was followed by formation of the mesylate and treatment with ethanolamine to give **145**. Formation of the corresponding carbamate and acetylation gave **146**. Removal of the THP ether and oxidation with PCC was followed by a Wittig reaction and hydrolysis to give **148**. Ring closure and removal of the BOC protecting group afforded **127** and **126**.[69]

## *VII. Dimeric Alkaloids*

A new class of coccinellid alkaloids was recognized in 1992 with the discovery of a dimeric alkaloid containing the common 2-methylperhydro-9b-azaphenalene skeleton linked to a novel azanaphthylene partner.[71] Two additional alkaloids of this type have been subsequently characterized.[72,73]

### A. Exochomine

Material from the extraction of 2500 specimens of the European coccinellid, *Exochomus quadripustulatus*, was taken into dilute hydrochloric acid and purified by chromatography.[71] Exochomine isolated in this manner was crystallized as its hydrochloride salt. The high-resolution mass spectrum of this compound showed its molecular formula to be $C_{26}H_{36}N_2O$; the base peak at $m/z$ 192 indicated the presence of a methylperhydro-9b-azaphenalene ring system.[71] The 26 signals in the $^{13}C$ NMR spectrum were comprised of 19 $sp^3$, six $sp^2$, and one carbonyl carbon. The carbonyl group was in the form of a conjugated ketone (d 187.2; IR $u_{CO}$ 1650 $cm^{-1}$). The $^1H$ NMR spectrum revealed two methyl groups, three methines $\alpha$ to a nitrogen atom, an isolated diastereotopic methylene, and two deshielded vinylic methines coupled to each other. A structure fitting this data would be a hexacyclic alkaloid made up of a methylperhydro-9b-azaphenalene skeleton, tethered to a previously uncharacterized unsaturated tricyclic system.[71]

A single-crystal X-ray diffraction study of exochomine established the structure and absolute configuration to be that shown in formula **149**. It is of interest that the absolute configuration of all the stereogenic centers in the perhydro-9b-azaphenalene moiety of **149** are the same as those in hippodamine and convergine.[71]

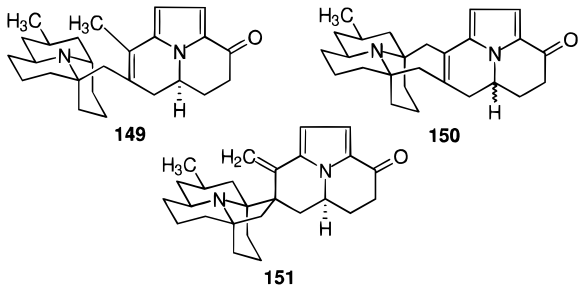
### B. Chilocorine A

An analysis of the acid-soluble components extracted from *Chilorcorus cacti* and from droplets of blood emitted by these beetles during "reflex bleeding" has yielded new examples of dimeric alkaloids closely related to exochomine.[72,73] One of the three major components of the extract, chilocorine A, was isolated by flash chromatography. High-resolution mass spectrometry determined its molecular formula to be $C_{26}H_{34}N_2O$. The ultraviolet absorption spectrum of chilocorine A indicated an extended conjugated system ($l_{max}$ 337 nm), closely related to that of exochomine (**149**) ($l_{max}$ 215, 235, 252, 336 nm),[71] suggesting that the two alkaloids had very similar chromophores. The $^{13}C$ NMR spectrum showed seven $sp^2$ hybridized carbon atoms, six in carbon−carbon double bonds and the remaining one as a carbonyl group. Chilocorine A must be heptacyclic on the basis of its molecular formula and the number of double bonds. Through a series of NMR experiments (DEPT, HMQC, DQ-COSY, TOCSY, HMBC) the structure of chilocorine A was determined to be that given in formula **150**. However, the configuration at the asymmetric center in the azaphenalene ring was not established.[73]

### C. Chilocorine B

More recently a second dimeric alkaloid from *Chilocorus cacti*, chilocorine B, was isolated and

shown to be isomeric with chilocorine A by high-resolution mass spectrometry. The mass spectrum also suggested that this isomer is derived from the same two tricyclic moieties.[73] The base peak at *m/z* 191 was identical to that of **150**, but different from that of exochomine. A series of NMR experiments (DEPT, HMQC, DQ COSY) showed the two previously identified subunits to be linked in a spirocyclic fashion, allowing for two possible diastereomers. Single-crystal X-ray diffraction analysis fully determined the structure and relative stereochemistry of this new alkaloid to be that shown by **151**.[73]




## VIII. Biosynthesis

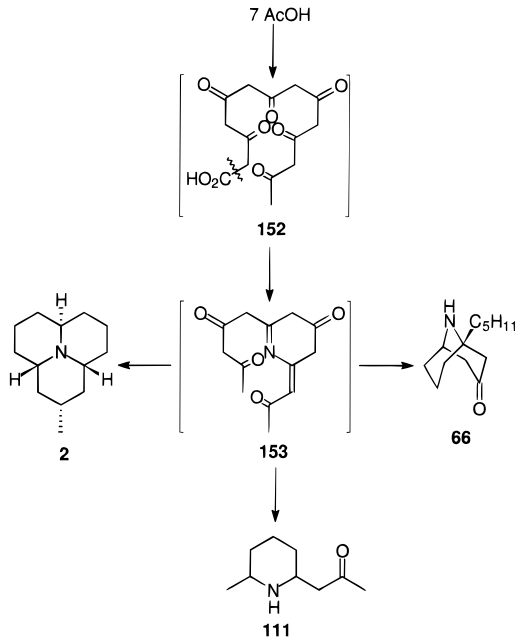
Table 1 lists the more than two dozen well-characterized alkaloids that have been found in coccinellid beetles. What these diverse structures have in common is an unbranched chain of carbon atoms joined in one or more places to one or more nitrogen atoms. It therefore becomes of interest to ask whether this structural theme is a consequence of shared biosynthetic origins. In fact, little is known about the biosynthesis of the alkaloids found in coccinellid beetles.

Coccinelline (**1**) and precoccinelline (**2**) have been found in the beetles' eggs, larvae, and adults, but have not been detected in the aphids upon which these beetles feed.[13] Thus, it appears clear that the alkaloids must be synthesized by the beetles themselves. All of the azaphenalene alkaloids possess an unbranched 13 carbon backbone, joined in three places to a single nitrogen atom. The linear condensation of seven acetate units (Scheme 25) would give intermediate **153**, which could go on to generate all of the azaphenalene alkaloids. A biosynthetic relationship between adaline (**66**) and the coccinelline-type alkaloids was suggested in 1973,[48] and both Pasteels *et al.*[13] and Ayer and Browne[1] have suggested that adaline is produced by a similar pathway (Scheme 25). This hypothesis gained support in 1982 with the isolation of *cis*-1-(6-methyl-2-piperidyl)-propan-2-one (**111**) from *Cryptolaemus montrouzieri*.[51] A compound of this type could be generated from **153**, the proposed key intermediate common to both adaline and precoccinelline.

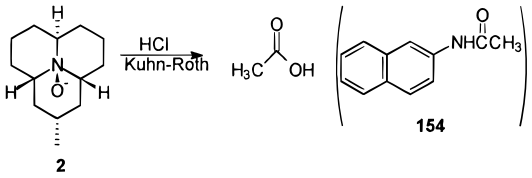
**Table 1. Coccinellid Beetle Alkaloids of Endogenous Origin**

| species | compound | ref(s) |
|---|---|---|
| *Adalia decempunctata* | **66** | 9,13,49 |
| *Adalia pantherina* L. | **66** | 48 |
| *Adalia quadrimaculata* Scopi | **66** | 48,49 |
| *Adalia bipunctata* | **66** | 9,13,48,49 |
| *Adonia variegata* | **96** | 58,59 |
| *Anisostica novemdecimpunctata* | **3** | 9,13,21 |
| *Cheilomenes propinqua* | **1,2** | 13 |
| *Chilocorus cacti* | **150,151** | 72,73 |
| *Coccinella californica* | **1,** | 13 |
| *Coccinella pentempunctata* | **1,2** | 9,13 |
| *Coccinella quatuordecimpunctata* | **1,2** | 9,13 |
| *Coccinella septempunctata* | **1,2** | 9,13,14,18,19 |
| *Coccinella transversoguttata* | **1,2** | 16 |
| *Coccinella unidecimpunctata* | **1** | 9,13 |
| *Coleomegilla maculata* | **2 or 5** | 1,15 |
| *Cryptolaemus montrouzieri* | **67,111** | 51 |
| *Epilachna varivestis* | **67,111, 115−128** | 52,62,63,67,74 |
| *Exochomus quadripustulatus* | **149** | 71 |
| *Harmonia leis conformis* | **96** | 58,59 |
| *Harmonia quatruorpunctata* | **96** | 58,59 |
| *Hippodamia caseyi* | **3,4,6,7** | 16 |
| *Hippodamia convergens* | **3,4,96** | 13,20,21,58,59 |
| *Micrapsis hexadecimpunctata* | **2** | 13 |
| *Myrrha octadecimguttata* | **5** | 9,13 |
| *Propylaea quatuordecimpuncata* | **8** | 9,13,17,26 |
| *Semiadalia novemdecimnotata* | **96** | 58,59 |

**Scheme 25**




**Scheme 26**




To explore experimentally the biosynthetic pathway by which coccinellids produce the azaphenalene alkaloids, Tursch *et al.* carried out feeding experiments using $^{14}$C-labeled precursors with *Coccinella septempunctata*.[13] Beetles were fed labeled acetate, either $^{14}CH_3CO_2Na$ or $CH_3{}^{14}CO_2Na$, and were found to incorporate these precursors into the coccinelline they produced. The isolated $^{14}$C-labeled coccinelline hydrochloride was submitted to a Kuhn−Roth oxidation (Scheme 26).[13] The resulting acetic acid, corresponding to carbon atoms C-2 and C-10 of coccinelline, was isolated as its 2-aminonaphthalene derivative. If the linear combination pathway shown in Scheme 25 was correct, the acetic acid isolated from the coccinelline derived from $^{14}CH_3CO_2Na$ would have one-seventh (14.3%) of the specific activity of the intact coccinelline, while coccinelline derived from

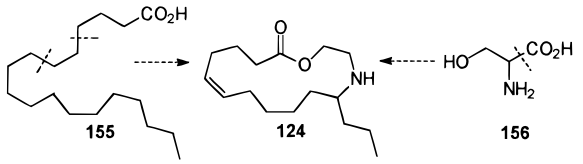



**Figure 15.** Possible biosynthetic route to epilachnene.

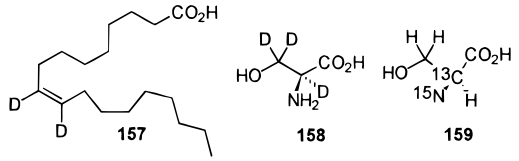



**Figure 16.** Isotopically labeled material used in biosynthetic studies with *E. varivestis.*

$CH_3{}^{14}CO_2Na$ would produce acetic acid possessing one-sixth (16.7%) of the intact coccinelline specific activity. If instead the pathway involved the linear combination of six acetates followed by the addition of a methyl group from another source, the specific activity of the acetic acid isolated from $^{14}CH_3CO_2Na$ or $CH_3{}^{14}CO_2Na$-derived coccinelline would be 0% and 16.7%, respectively. Since the radiolabel was present in the acetic acid obtained from feeding the beetles either $^{14}CH_3CO_2Na$ or $CH_3{}^{14}CO_2Na$ at approximently the same level (~16% of the activity in the intact coccinelline), it can be concluded that all of the alkaloid's carbons arise from acetate units.[13] These results are consistent with predictions based on a pathway involving the linear combination of seven acetate units, and it can be concluded that the azaphenalenes are of polyketide origin.[1,13]

The novel structure of the azamacrolides[67] implies an interesting synthetic route. The major azamacrolide, epilachnene (**124**), has already been described as a macrocylic lactone, based on a 14-carbon unbranched chain, and an ethanolamine moiety. These features suggested that the 14-carbon atom chain could arise from a 2-fold oxidative two-carbon chain shortening of an unsaturated fatty acid (Figure 15), such as oleic acid (**155**).[74] The ethanolamine moiety was envisioned to originate from serine (**156**). Interestingly, this would imply that the fatty acid would have to undergo amination at an unactivated site.

Biosynthetic studies using isotopically labeled precursors were performed to test these hypotheses (Figure 16).[74] Larvae of *E. varivestis* were allowed to feed on bean plants to which deuterium-labeled oleic acid, (*Z*)-9-[9,10-$^2H_2$]octadecenoic acid (**157**) or deuterium-labeled L-serine (**158**) or $^{13}C$,$^{15}N$-labeled serine (**159**) had been applied. Droplets from the glandular hairs were collected (after pupation) as previously described, and the collected azamacrolides were compared to material collected from insects reared on a normal diet.[67] GC/MS and GC/IR analyses of the secretion revealed that 50% of the epilachnene obtained from individuals fed labeled **157** had incorporated the deuterium label, and that the deuterium atoms were located at the *cis* $D^5$ double bond, as anticipated. Feeding studies with deuterium-labeled L-serine (**158**) also showed incorporation into 5% of the recovered epilachnene. This indicated that the two-carbon chain was derived from serine, but was not evidence that serine was also the source of the nitrogen atom. To determine this, larvae were fed $^{13}C$,$^{15}N$-labeled L-serine (**159**). Again, the recovered epilachnene showed incorporation of both labels (at a level of 20%), confirming that the entire ethanolamine moiety is serine-derived. While it is not clear at this time whether the serine undergoes decarboxylation before or after the amination of the oleic acid moiety occurs, the building blocks responsible for the biosynthesis of epilachnene by *E. varivestis* are now clearly defined.[74]

## IX. Bioactivity

The first coccinellid alkaloids to be identified were isolated during a search for the compounds that gave coccinellid beetles their bitter taste. In fact, progress during the purification of coccinelline and precoccinelline was followed by tasting fractions.[18] Coccinelline was found to be responsible for the bitter taste of *C. septempunctata*, although not for the species odor.[18] To investigate the effect of this compound on potential invertebrate predators, purified alkaloid was assayed as a means of chemical defense against ants. Water containing coccinelline at a concentration of 0.5% was almost completely repellent to thirsty ants (*M. rubra*), and was somewhat deterrent even at a concentration of 0.1%. These concentrations are well below the level of coccinelline in beetle hemolymph. Direct contact with an ant's mouthparts is not needed to observe the effects of coccinelline. Coccinelline-impregnated filter paper placed around a food source will prevent ants from reaching the food.[18] Convergine, another azaphenalene alkaloid, was reported to show very similar activity (~1.5 times as active against *M. rubra*).[1,9] Early assays also demonstrated that coccinelline-containing beetles are not eaten as readily as their alkaloid free counterparts by quail, which indicates the azaphenalene alkaloids may also be effective against birds.[9]

The first reported assay to test the effectiveness of an azabicyclononane alkaloid as a means of chemical defense used enantiomerically pure samples to study the relationship between the absolute configuration of an alkaloid and its antifeedent activity. Hill and Renbaum[57] assayed both the natural and unnatural antipodes of adaline as feeding deterrents against imported fire ants, *Solenopsis invicta*. The ants were starved for 3 days before exposure to filter paper saturated with oil containing varying concentrations of adaline. The ants walked freely over the paper, but refused to eat the adaline-containing oil. Although adaline did not repel the ants in these experiments, it did appear to be a feeding deterrent, with a significant effect at $10^{-3}$ M. No statistical difference was observed between the effects of the enantiomers.

Another interesting study concerning the bioactivity of azabicyclononane alkaloids correlated adult age, unpalatability, and azabicyclononane alkaloid content. Eisner *et al.* showed that recently emerged *E. varivestis* adults were eaten by jumping spiders (*Phidippus regius*) despite their reflex bleeding behavior. On the other hand, most 7-day-old beetles were rejected by the spiders, for the most part unharmed.[52] Analytical studies correlated this feeding deterrence with the amount of euphococcinine

**Table 2. Coccinellid Defensive Compounds of Exogenous Origin**

| species | compound | source of compound | ref(s) |
|---|---|---|---|
| *Coccinella septempunctata* | senecionine (**160**) | *Aphis jacobaeae* | 85,86 |
| *Coccinella septempunctata* | intergerrimine (**161**) | *Aphis jacobaeae* | 85,86 |
| *Coccinella septempunctata* | senecivernine (**162**) | *Aphis jacobaeae* | 85,86 |
| *Coccinella septempunctata* | retrorsine (**163**) | *Aphis jacobaeae* | 85,86 |
| *Hyperaspis trifucata* | carminic acid (**164**) | Coccidae, *Dactylopius* spp. | 88 |

present in the beetle. Euphococcinine is not present in the larvae or newly emerged adults. However, the concentration of this homotropane alkaloid slowly builds up in both males and females, reaching a maximum of approximately 5 $\mu$g/droplet of blood in 1−2 weeks.[52]

A recent series of experiments has compared the chemical defense of two coccinellid species, *Adalia bipunctata* and *Coccinella septempunctata*. These beetle species each synthesize a differrent alkaloid for use in the reflex bleeding response. *A. bipunctata*, a Batesian mimic of *Coccinella septempunctata*, produces adaline,[48,49] while *C. septempunctata* relies on coccinelline and precoccinelline for its chemical defense.[18,32,76] In 1991, Holloway *et al.* examined the amount of blood emitted by *C. septempunctata* and its alkaloid content and found that while there was considerable variation among individuals, the amount of fluid emitted was amazingly high, up to 20% of the fresh body weight.[76] de Jong and co-workers performed a similar study to quantify both the amount of blood emitted by *A. bipunctata* and the amount of adaline this blood contained.[75] As was the case with *C. septempunctata*, researchers found significant variation among individual *A. bipunctata* beetles, both in the amount of blood emitted and in the alkaloid content. There was a significant sexual difference in the alkaloid content of blood samples, with males having a higher alkaloid titer except on the first postwinter hibernation bleeding. On average, the concentration ($\mu$g/mg) of alkaloid found in *A. bipunctata* was 6−8 times greater than the concentration of coccinelline found in *C. septempunctata*. This difference may reflect a greater efficacy of coccinelline as means of chemical defense.

Marples *et al.*[77] showed that the ingestion of coccinelline has severe consequences for birds. Adaline is mildly distasteful to birds, but is not as effective a feeding deterrent to birds as is coccinelline. When ants (*Myrmica rubra*) were offered pieces of dead, adaline-containing *A. bipunctata*, 50% of the pieces were rejected. When *C. septempunctata* were offered, all of the pieces were rejected, indicating the greater chemical protection of this species.[9] Thus, it has been postulated that the concentration of adaline in *A. bipunctata* is much greater than the concentration of coccinelline in *C. septempunctata* to compensate for adaline's lower repellency/toxicity.[75] These studies have provided the basis for an ongoing discussion of the evolution of aposematic coloration and chemical mimicry in these coccinellids.[75,76] It is worth noting that adaline may offer better protection than coccinelline against predators other than those tested (birds and ants) or against parasitization, the largest biotic mortality factor in adult coccinellids.[75,78]

The only published studies concerning the bioactivity of harmonine are preliminary results[59] which indicate that harmonine is a potent antifeedent compound against ants (*Myrmica rubra*), with a concentration threshold at $10^{-4}$ M.

As previously mentioned, the isolation of azamacrolides from *Epilachna varivestis* was motivated by the initial observation of the defensive action of the beetle's pupal secretion.[67] Foraging ants investigate pupae until contact with the oil droplets secreted by glandular hairs is made. At that instant, the ants forego further investigation and cleanse themselves. Unpublished preliminary results[79] show that synthetic 9-propyl-10-aza-cyclododecan-12-olide, a minor component identified in the secretion, serves as a feeding deterrent to ants (monomorium sp), but not as effectively as the intact secretion. The number of ants feeding at a well of 10% honey water at a specified time was recorded when the rim of the well had been treated with test solutions of this azamacrolide and with controls. Both the synthetic material and natural secretion reduced the number of feeding ants, although the synthetic material did not reduce the numbers as drastically as did the intact secretion.[79]

## X. Other Chemical Defense Mechanisms

Coccinellids do not rely solely on the synthesis of alkaloids for defense against predation. Nor are predacious arthropods and vertebrates the only dangers these beetles face. There are, in fact, a wide variety of chemical mechanisms which contribute significantly to the survival of these beetles. In some instances, coccinellid beetles utilize compounds produced by other organisms for their own defense (Table 2).

### A. Pyrrolizidine Alkaloid Sequestration

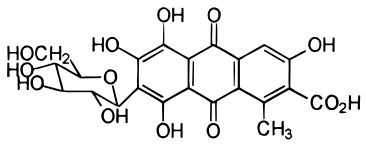
Pyrrolizidine alkaloids (PA's), are widely distributed among certain groups of plants, including the Asteraceae, Boraginaceae, and Fabaceae. These alkaloids have been shown to be involved in chemical defense and communication among a variety of arthropods, and are well-known antifeedent compounds.[80−84]

*Senecio jacobaea* is a plant that synthesizes a variety of PA's in its roots,[85] from which they are transferred to the shoots via the phloem.[86] The phloem contains several pyrrolizidine alkaloids, including senecionine, integerrimine, senecivernine, and retrorsine (**160**−**163**). *Aphis jacobaeae* Schrank (Aphididae) is a specialist on *Senecio*, and colonies have been found on three different species. Furthermore, coccinellids (*Coccinella septempunctata*) frequently infest these aphid colonies. Host plants, aphids, and the predatory coccinellids were therefore analyzed by gas chromatography/ mass spectrometry (GC/MS) to determine their respective PA content.[87]

Analysis of both *Aphis jacobaeae* and its honeydew showed a PA profile typical of the plant on which it was feeding. The PA concentration reached levels of 3.5 mg/g fresh weight in aphids. Coccinellids feeding on aphid colonies raised on *S. jacobaeae* also sequestered considerable amounts of PA's, with the PA pattern closely resembling that of the respective aphid and host plant. The PA concentration reached even higher levels (4.9 mg/g fresh weight) in the predatory coccinellids. These values amounted to 10−50% of the beetles' endogenous alkaloid (e.g. coccinelline) levels, which reach 10.5 mg/g.[86]

## B. Carminic Acid Sequestration

Despite their importance in coccinellid beetles, alkaloids are not the most commonly encountered type of organic compound that insects may use for defensive purposes. Glycosides, lactones, quinones, and a wide variety of simple aliphatic repellents have all been documented as insect defensive agents.[88] An intriguing example of a nonalkaloid defensive compound is carminic acid (**164**), a red dye, produced by cochineal bugs (Coccidae, *Dactylopius* spp.,), which has been shown to be a feeding deterrent to ants.[89]



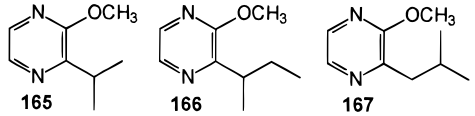

**164**

This anthraquinone, however, is not deterrent to all insects, and three insect species, one of which is a coccinellid beetle, *Hyperaspis trifucata,* are now known to feed on cochineals and to use the acquired carminic acid for their own defense. These interactions are of particular interest since most insects which acquire defensive compounds acquire them from plant rather than from animal sources.[89]

In a study of the fate of carminic acid in *Hyperaspis trifucata,* larvae were reared on a diet of cochineal bugs. When provoked, these beetles would emit several droplets of red fluid, determined to be hemolymph, by reflex bleeding. Analysis of these droplets showed on average 0.17% carminic acid content. Assays against ants verified that the reflex bleeding is an effective deterrent.[90]

## C. Methoxyalkylpyrazines as Warning Odorants

Rothschild's novel idea of coccinellid beetles using volatile odorants,[6] in addition to their bright coloration, to warn potential predators of their chemical defenses bore fruit in a recent survey.[91] In 1981, Moore and Browne reported the presence of alkoxyalkylpyrazines in Australian coccinellids.[92] Together with Rothschild, they extended this work and surveyed many species of plants and insects for these pyrazines, which they postulated to act as warning odors. Fourteen species of coccinellids were analyzed for the presence of 2-methoxy-3-isopropylpyrazine, 2-methoxy-3-*sec*-butylpyrazine, and 2-methoxy-3-isobutylpyrazine (**165**−**167**), and in nine of the surveyed species at least one of the pyrazines was detected by selected-ion monitoring (SIM) with GC/MS. Thus the researchers have concluded that pyrazines are commonly used by aposematic invertebrates as warning odorants.




**165** **166** **167**

**Figure 17.** 2-Methoxyalkylpyrazines found in coccinellids.

## D. Cardenolides

Oleander (*Nerium oleander* L.) is a plant well protected from predation by the presence of toxic cardenolides contained within its tissues, yet several types of aposematic insects feed on the plant. The larvae of *Coccinella septempunctata* L. and *C. undecimpunctata* L. feed upon aphids (*Aphis nerii* Fonscolcombe) which ingest the oleander tissue.[93,94] *Aphis nerii* Fonscolcombe has been shown to contain many cardiac glycosides, which it obtains from the host plant, including stropeside, adynerin, and odoroside H. While *C. septempuctata* does not apparently sequester the alkaloids from the aphids upon which it feeds, *C. undecimpuncata*, which preyed on alkaloid-laden *Aphis nerii* Fonscolcombe, was found to retain the toxins originating within the host plant, perhaps for use against predators. The small amount of cardenolides within the coccinellid prevented identification of the specific cardenolides it contained.[93,94]

## E. Unidentified Defensive Agents

In 1973, Pasteels *et al.* provided evidence that coccinellids are equipped with lines of defense other than alkaloids.[9] Three species of coccinellids which lack alkaloids were offered to foraging ants. Of the three species (*Aphidecta obliterata, Rhizobius litura,* and *Subcoccinella 24-punctata*), only *A. obliterata* was acceptable to the predators; the other two were rejected. Since these beetles do not contain any alkaloids, a different type of compound, as yet uncharacterized, must be responsible for the deterrency.

# *XI. Conclusion*

Of the more than 4300 identified species of coccinellid beetles, only a small percentage has been studied chemically. From these few examples, however, many new alkaloids have been discovered. These compounds have not only provided biologists with the opportunity to study chemically mediated behavior, but have also stimulated organic chemists by posing a number of analytical, synthetic, and biosynthetic challenges. Continued research on the chemistry and biology of this fascinating group of beetles promises to give significant new insight into the field of insect chemical ecology.

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