and the Squibb Institute of Medical Research for samples of authentic reference compounds.
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(Z)-1,17-Diaminooctadec-9-ene, a novel aliphatic diamine from Coccinellidae

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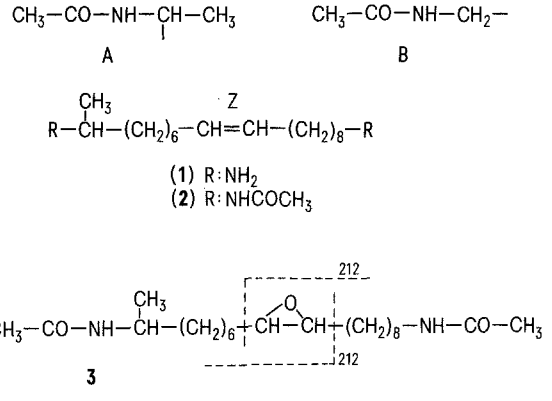
Summary. The isolation and structure determination of (Z)-1,17-diaminooctadec-9-ene from several species of Coccinellidae is reported.
Key words. Coccinellidae; (Z)-1,17-diaminooctadec-9-ene; *Hippodamia convergens; Harmonia leis conformis.*

Ladybugs (Coccinellidae) have few natural enemies. This can be attributed in part to the fact that, when molested, these beetles emit hemolymph droplets at their joints. This well-described process, known as reflex bleeding, has been shown to constitute an efficient protection to would-be predators[3,4]. It has been demonstrated that the repellent properties of the fluid of several species of Coccinellidae result from the presence of alkaloids[5]. Until now, the structures of only a few of them have been determined. Structurally, these basic derivatives contain the 2-methyl-perhydro-9b-azaphenalene[5-7], homotropane[8,9] or piperidine ring systems[9].

In our previous survey of several species and varieties of ladybugs for alkaloids, we reported the presence of a very polar, Dragendorff positive, compound in *Adonia variegata, Harmonia 4-punctata* and *Semiadalia-11-notata*[5]. In this paper we wish to describe the structure determination of this novel derivative (**1**), as well as its isolation from two other species. *Hippodamia convergens* and *Harmonia leis conformis.*

About 500 beetles of this latter species were treated as described previously[5]. The crude basic fraction was acetylated with the mixture pyridine/acetic anhydride 2:1. Two successive chromatographies on alumina (eluent: ethyl acetate) gave amorphous diacetylharmonine **2** ($[\alpha]_D = +3°$ ($CHCl_3$, c = 0.53)) homogeneous by tlc in at least 3 different eluents. High resolution mass spectroscopic analysis of **2** establishes the molecular formula to be $C_{22}H_{42}N_2O_2$. The presence of one or more secondary amide groups is evident from the IR spectrum (bands at 3300, 1645 and 1500 cm^{-1}). The 270 MHz ^{1}H-NMR spectrum ($CDCl_3$-TMS) exhibits signals suggestive of two $NHCOCH_3$ groups (two 3H singlets at δ 1.97 and 1.96, two 1H broad multiplets at 5.44 and 5.75 disappearing slowly on D_2O treatment) one of which is part of substructure A (3H doublet at δ 1.12 coupled to a 1H multiplet at δ 3.26 itself coupled to the NH multiplet at δ 5.44), while the second is part of substructure B (2H double triplet at δ 3.24 coupled to the NH multiplet at δ 5.75).

In addition, this spectrum shows signals attributable to a disubstituted double bond (2H triplet at δ 5.37), 11 aliphatic methylenes (22H, broad singlet at δ 1.30) and two methylenes adjacent to a sp^2 carbon atom (4H multiplet at δ 2.00). All



these data strongly suggest that **2** is a C_{18} aliphatic long-chain bearing, besides a double bond, two acetylamino groups at C-1 and C-17, respectively. This was further substantiated by the mass spectrum which shows characteristic fragment ions at m/z 280 and 294 corresponding to the loss, from the molecular ion, of substructure A and B, respectively. Successive losses of CH_2 units from these fragment ions clearly confirm the presence of an aliphatic long-chain structure. The presence in the IR spectrum of **2** of a CH out of plane bending band at 725 cm^{-1} together with a lack of absorption at about 970 cm^{-1} indicates that the geometry of the double bond is Z. The position of this double bond follows from the mass spectral analysis of the epoxide **3** obtained by treatment of **2** with m-chloroperbenzoic acid. Indeed, it is well known that long-chain epoxides undergo preferential cleavage α to the epoxy group, producing identifiable fragments that indicate the position of the original double bond in the chain[10].

In the mass spectrum of **3**, a characteristic ion (75%) is observed at m/z 212 implying a 9,10 double bond in **2**. A further proof of the position of the double bond was afforded by oxidative cleavage of **2** by treatment with $NaIO_4$ in the presence of catalytic amounts of $KMnO_4$[11]. CH_2N_2 esterification of the resulting acidic mixture generates two methyl esters that were separated by preparative GC and identified by their character-

istic IR, MS and ^{1}H-NMR spectra as methyl 8-acetylaminononanoate and methyl 9-acetylaminononanoate.
In conclusion, all these results point to structure **2** for the diacetylderivative and thus the natural compound is necessarily the corresponding diaminoderivative **1**.
Comparison of the spectral properties of **2** with those of the diacetylated derivative of the polar Dragendorff positive compound, previously reported in the basic extracts of *Adonia variegata*, *Harmonia 4-punctata* and *Semiadalia 11-notata*[4] shows that **1** is also present in these species. Moreover, **1** could also be isolated besides hippodamine and n-octylamine from a basic extract of *Hippodamia convergens*, surprisingly devoid of convergine[12].
The synthesis of **1** in order to evaluate its biological activities is under way, as well as the determination of its absolute configuration.

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Selectivity of action between pyrethroids and combined DDT-pyrethroid insecticides on Na^+ influx into mammalian neuroblastoma

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Summary. Several of the most active synthetic pyrethroid insecticides in the presence of sea anemone toxin II, induced a dose related influx of sodium ion into the C9 mouse neuroblastoma. The influx of sodium ion into this mammalian cell did not take place with a DDT analogue, EDO and several new combined DDT-pyrethroid insecticides, although these have been reported to cause excess sodium influx into arthropod axons, related to their insecticidal activity. This difference between species in the action of the new insecticides at the nerve sodium channel explains their low mammalian toxicity.
Key words. C9 cell; NIE 115 neuroblastoma; $^{22}Na^+$ influx; pyrethroid insecticides; combined DDT-pyrethroid insecticides.

Jacques and co-workers[2] have examined in detail the influx of $^{22}Na^+$ into rat C9 cell and NIE 115 adrenergic neuroblastoma. They have demonstrated that micromolar amounts of synthetic pyrethroid insecticides stimulated the entry of $^{22}Na^+$ into these cells via the Na^+ channel. To induce this influx of the sodium ion they have shown the requirement for synergy of the added pyrethroids with toxins specific to the gating system of the C9 cell and the NIE 115 neuroblastoma Na^+ channels. Without these toxins e.g. veratridine, batrachotoxin, or sea anemone toxin II, the pyrethroids did not stimulate the $^{22}Na^+$ ion influx. The C9 cell has a special property in that its Na^+ channels which are electrophysiologically silent, can be chemically opened by these toxins. The NIE 115 neuroblastoma is a electrophysiologically fully active nerve cell.
Two new series of insecticidally active compounds have been reported[3,4], whose design was based on the combination of DDT and pyrethroid structures. The new insecticides have exceptionally low acute mammalian toxicity when compared with the synthetic pyrethroids. In this note we report the results of $^{22}Na^+$ influx experiment for the new structures and one DDT analogue, which also gives a low mammalian toxicity.

Results and discussion. EDO (GH149, table), an insecticide isosteric with DDT was shown by electrophysiological measurements to have an effect on Na^+ flux an arthropod (lobster) axon[5] similar to DDT itself. In this preparation it inactivated the closure of the Na^+ channel and caused a delay in the falling phase of the Na^+ mediated potential. This effect was blocked by tetrodotoxin. In the present experiments using the method reported previously[2] no stimulation of the $^{22}Na^+$ influx took place in the C9 cell and the NIE 115 neuroblastoma preparations, even on application of high concentrations (2.0 mmol) of EDO in the presence of sea anemone toxin II.
Four of the new DDT-pyrethroid compounds (table) were then tested in these two preparations. Listed values in the table show, that GH401, GH414 and GH601 have insecticidal activities intermediate between those of Kadethrin and Deltamethrin. However, similarly to EDO, these combined DDT-pyrethroid structures, added to a concentration of 0.1 mmol, did not stimulate the influx of $^{22}Na^+$ into the mammalian nerve cell preparations, in the presence of sea anemone toxin II.
In the experiments on the synthetic pyrethroids all had the 3,3-dimethylcyclopropane structure disubstituted at the 1,2-position of the ring. We could not correlate (table) the reported[2] rate of influx of ^{22}Na induced by the addition of the pyrethroids, with insecticidal activity measured in the Australian sheep blowfly (*Lucilia cuprina* W.), an insect we used previously[4] for the ranking of insecticidal activity.
In C9 cell preparations in which the Na+ channels are not capable of activation by electric stimulation, it was observed[2] that the pyrethroids activated the channel in a manner similar to that of veratridine which is known to slow down the inactivation process of a functional Na^+-channel[2]. In these preparation the increase of the rate of Na^+ influx could be stopped by tetrodotoxin, which was shown however, to exert its action at a different receptor to the pyrethroids. In that study it was also demonstrated that the Na^+ influx experiments in presence of sea anemone toxin II, closely simulated electrophysiological measurements of the delay in the falling phase of Na^+ mediated potentials in sodium channels induced by pyrethroids, in