not found in intact tissues, the properties of the naturally-present DIMBOA-Glc may be important in plant-insect interactions. Although DIMBOA was always a more active molecule, DIMBOA-Glc also showed feeding deterrent and toxic activities on *S. graminum*. Since DIMBOA-Glc may be hydrolyzed to DIMBOA upon disruption of cells during insect feeding[11], the toxic and feeding deterrent properties of both compounds may be relevant to plant protection. The effects of both compounds on aphids fed on artificial diets were observed at a concentration range similar to that found in wheat leaves (0.1 to 6.3 mmoles/kg of fresh weight). Since an inverse correlation between hydroxamic acid content of leaves and aphid infestation level has been found[4,5], it is possible that varieties resistant to aphids may be obtained by selecting those with higher hydroxamic acid content. It is difficult to predict the concentration of hydroxamic acid necessary to achieve plant protection against *S. graminum*. However, it has been found that plants with more than 4 mmoles/kg of fresh weight in their leaves are not susceptible to *S. graminum* and *Metopolophium dirhodum*[4,6].

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Defensive steroids from a carrion beetle (*Silpha americana*)[1]

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Summary. The defensive anal effluent discharged by *Silpha americana* in response to disturbance contains a mixture of steroids stemming from a glandular annex of the rectum. The compounds have been characterized as 15β-hydroxyprogesterone (**1**, principal component), 5β-pregnan-15β-ol-3,20-dione (**2**), 5β-pregnan-3α,15β-diol-20-one (**3**), 5β-pregnan-7β, 15β-diol-3,20-dione (**4**), 5β-pregnan-3α, 7β, 15β-triol-20-one (**5**), 5β-pregnan-16α-ol-3,20-dione (**6**), and 5β-pregnan-3α, 16α-diol-20-one (**7**), none previously found in insects. Bioassays with jumping spiders showed compounds **1** and **6** to be feeding deterrents at the 1 μg level.
Key words. Coleoptera; chemical defense; steroids; pregnanes; isoprenoids.

Carrion beetles of the genus *Silpha* (family Silphidae) characteristically emit a malodorous ooze from the anus when disturbed. The effluent is strongly alkaline, rich in ammonia[4], and presumed to be defensive. We found that in *Silpha americana* the fluid also contains a mixture of pregnanes, derived from a glandular annex of the hindgut. We here report on the structure of these compounds, and on their feeding deterrency to an arthropod predator.

Beetles were collected by the hundreds at baits (dead fish and chickens) near Ithaca, New York, and Lake Placid, Florida. The rectal gland, first noted by Leydig[5], was revealed by dissection. It consists of a blind sac, opening directly into the rectum, and an elaborately subcompartmented diverticulum of the sac. The entire gland is lined with membranous cuticle, continuous with the lining of the rectum itself. Treatment of beetles with aqueous potassium hydroxide dissolves away tissues and results in isolation of the lining. The relationship of gland to rectum, and particularly the subcompartmentalization of the diverticulum, is clearly evidenced in such preparation (fig. 1). In the untreated gland the diverticulum is contiguous with the glandular sac, and its subcompartments are tightly appressed.

Several hundred glands were dissected from beetles freshly killed by freezing. They were extracted whole with a 1:1 dichloromethane-methanol mixture (ca. 15 μl per gland). Direct analysis of the extract by gas chromatography (GC) and gas chromatography-mass spectrometry (GC–MS) proved impractical, since the chief components were of low volatility, and appeared to suffer thermal degradation. Trimethylsilylation of the extract also gave disappointingly complex results as a consequence of variable degreees of silylation, as well as of the thermal instability of some of the products. Thin-layer chromatography (TLC) of the extract avoided these difficulties, and revealed the presence of one component with strong UV absorption, along with about a half dozen additional components. A completely satisfactory TLC separation, however, was difficult to achieve. Finally, reverse phase high performance liquid chromatography (HPLC), using a C-18 column and 45% acetonitrile–55% water as solvent, permitted the preparative separation of seven pure compounds. Each of these components was isolated by lyophilization of the liquid phase, and each was then subjected to spectroscopic analysis (electron impact, chemical ionization, and high resolution mass spectrometry, ^{1}H NMR and UV spectroscopy).

The major component in this mixture was the one exhibiting strong UV absorption (λ) EtOH/max 240 nm). High resolution MS established its molecular formula ($C_{21}H_{30}O_2$), and the low resolution MS data suggested that this compound might be a 15- or 16-hydroxylated progesterone[6]. Direct comparison of this material (MS, ^{1}H NMR, HPLC) with an authentic sample

of 15β-hydroxyprogesterone (**1**) showed the two samples to be indistinguishable. From the intensity of the UV absorption (240 nm) of the extract of defensive glands dissected from individual beetles, it could be estimated that each gland contains about 5–10 μg of **1**. Separate extraction of a sample of glandular diverticuli and glandular sacs showed that ca. 90% of the compound is present in the diverticulum. We suggest that the diverticulum is the glandular source of the steroid and that the sac is the storage reservoir from which the compound is added to the rectal contents. Analysis of rectal effluent did indeed show presence of the compound.

The remaining six compounds (**2**–**7**) also proved to be hydroxylated, 20-ketopregnanes, varying only in oxidation state of ring A, and in hydroxylation pattern. In each case, the structure and stereochemistry were deduced chiefly on the basis of a detailed analysis of the NMR data, comparisons of observed chemical shifts of the angular methyl groups with calculated values, and, to a limited extent, partial synthesis. Figure 2 gives the structures of all seven of the steroids which we have isolated from the glandular extract. While none of these compounds appears to have been found previously in insects, only **3**, **4**, and **5** were previously unknown. Compounds **2**, **4**, **5** and **7** were estimated from amounts collected by HPLC to be present in the gland at 25–50% of the level of **1**, while amounts of **3** and **6** were less than 10% of that of **1**.

Spectral data for the compounds were as follows:

15β-Hydroxyprogesterone (**1**): High resolution MS *(m/z)* 330.2203 (calc. for $C_{21}H_{30}O_3$. 330.2195). EIMS *(m/z)* 330 (0.5%), 312 (10), 231 (8) 124 (13), 109 (13), 107 (17), 105 (21), 95 (14), 93 (17), 91 (36), 81 (15), 79 (30), 77 (21), 71 (20), 67 (18), 55 (23), 43 (100), 41 (17). CIMS *(m/z)* 371 (5%), 359 (20), 331 (100), 313 (46), 295 (7). ^{1}H NMR ($CDCl_3$) δ5.73 (1H, s), 4.30 (1H, m), 2.14 (3H, s), 1.23 (3H, s), 0.94 (3H, s). ^{1}H NMR (C_6D_6) δ5.84 (1H, m), 3.78 (1H, m), 1.86 (3H, s), 0.95 (3H, s), 0.73 (3H, s). UV λ_{max}, 240 log ε 4.03. The ^{1}H NMR spectrum of the isolated sample was indistiguishable from that of authentic 15β-hydroxyprogesterone (**1**).

5β-Pregnan-15β-ol-3,20-dione(**2**): High resolution MS (*m/z*) 332.2363 (calc. for $C_{21}H_{32}O_3$, 332.2351). EIMS (*m/z*) 332 (1.4%), 314 (15), 299 (7), 271 (4), 233 (5),, 215 (17), 121 (21), 119 (17), 111 (11), 109 (24), 107 (30), 105 (26), 101 (18), 100 (81), 95 (26), 93 (28), 91 (30), 85 (12), 81 (39), 79 (30), 77 (16), 71 (26), 69 (13), 67 (25), 55 (42), 43 (100), 41 (32). CIMS (*m/z*) 373 (10), 361 (28), 333 (99), 315 (100), 297 (23), 271 (6), 100 (14), 85 (5). ^{1}H NMR ($CDCl_3$) δ 4.31 (1H, m), 2.68 (1H, dd, J = 14,4), 2.49 (1H, dd, J = 9,9), 2.30 (1H, ddd, J = 6,15,15), 2.13 (3H, s), 1.05 (3H, s), 0.90 (3H, s).

5β-Pregnan-3α, 15β-diol-20-one (**3**): High resolution MS (*m/z*) 334.2525 (calc. for $C_{21}H_{34}O_3$, 334.2508). EIMS (*m/z*) 330 (> 0.1%), 316 (15), 298 (9), 216 (9), 201 (9), 121 (12), 111 (11), 100 (61), 95 (23), 93 (24), 91 (21), 83 (18), 81 (31) 79 (24), 71 (27), 69 (17), 67 (24), 57 (25), 55 (41), 43 (100), 41 (35). CIMS (*m/z*) 375 (4%), 363 (11), 335 (6), 333 (7), 331 (3), 329 (3), 317 (39), 299 (100), 281 (24), 257 (8). ^{1}H NMR ($CDCl_3$) δ 4.28 (1H, m), 3.65 (1H, m), 4.45 (1H, dd, J = 9,9), 2.23–2.13 (2H, m), 2.12 (3H, s), 2–1 (18H), 0.95 (3H, s), 0.86 (3H, s). ^{1}H NMR (C_6D_6) δ 3.85 (1H, m), 3.35 (1H, m), 1.86 (3H, s), 0.96 (3H, s), 0.84 (3H, s).

5β-Pregnan-7β, 15β-diol-3,20-dione (**4**): High resolution MS (*m/z*) 348.3217 (calc. for $C_{21}H_{34}O_4$, 348.2300). EIMS (*m/z*) 348 (1%), 330 (2), 312 (2), 291 (8), 273 (9), 269 (11), 255 (9), 145 (17), 121 (13), 119 (18), 107 (25), 105 (25), 100 (70), 95 (27), 93 (27), 92 (26), 81 (30), 79 (26), 71 (27), 67 (2), 55 (39), 43 (100). CIMS (*m/z*) 389 (5%), 377 (23), 349 (47), 331 (40), 313 (74), 295 (13), 271 (100). ^{13}C NMR δ 169.0, 80.7, 77.8, 72.7, 70.9, 65.9, 62.2, 45.7, 44.4, 41.7, 41.0, 40.6, 37.8, 35.9, 35.7, 34.7, 32.4, 23.6, 27.9, 16.7. ^{1}H NMR ($CDCl_3$) δ 4.42 (1H, ddd, J = 7,5,3), 3.79 (1H, ddd, J = 12,11,6), 2.50 (1H, dd, J = 14,15), 2.43 (1H, dd, J = 10,10), 2.3–1.3 (16H), 2.13 (3H, s), 1.19 (1H, dd, J = 6,11), 1.08 (3H, s), 0.95 (3H, s). ^{1}H NMR (C_6D_6) δ 4.25 (1H, m), 3.03 (1H, ddd, J = 5,9,10), 2.63 (1H, ddd, J = 6,15,13), 2.15 (1H, dd, J = 9,9) 2.1–0.8 (H), 1.87 (3H, s), 1.62 (2H, ddd, J = 10,11,11), 1.04 (3H, s), 0.61 (1H, dd, J = 6,11).

5β-Pregnan-3α, 7β, 15β-triol-20-one (**5**): High resolution MS (*m/z*) 350.2453 (calc. for $C_{21}H_{36}O_4$, 350.2457). EIMS (*m/z*) 350 (1%), 332 (2), 314 (3), 293 (4), 275 (4), 271 (4), 257 (5), 253 (9), 215 (11), 121 (16), 199 (16), 109 (19), 107 (24), 105 (25), 100 (27), 95 (32), 93 (31), 91 (27), 81 (32), 79 (29), 71 (19), 67 (26), 55 (44), 43 (100), 41 (34). CIMS (*m/z*) 391 (8%), 379 (16), 351 (15), 348 (10), 347 (7), 345 (7), 334 (16), 333 (66), 331 (15), 329 (9), 316 (23), 315 (100), 313 (14), 298 (15), 297 (66), 273 (14), 255 (38). ^{1}H NMR ($CDCl_3$) δ 4.38 (1H, ddd, J = 7,5,3), δ 3.77 (1H, ddd, J = 10,11,5), 2.41 (1H, dd, J = 10,10), 2.3–2.15 (1H, m), 2.12 (3H, s), 2.1–0.9 (H), 0.97 (3H, s), 0.90 (3H, s). ^{1}H NMR (C_6D_6) δ 4.24 (1H, ddd, J = 7,5,3), 3.21 (2H, m), 2.65 (1H, ddd, J = 15,13,13), 2.12 (1H, dd, J = 15,13), 2.07 (1H,

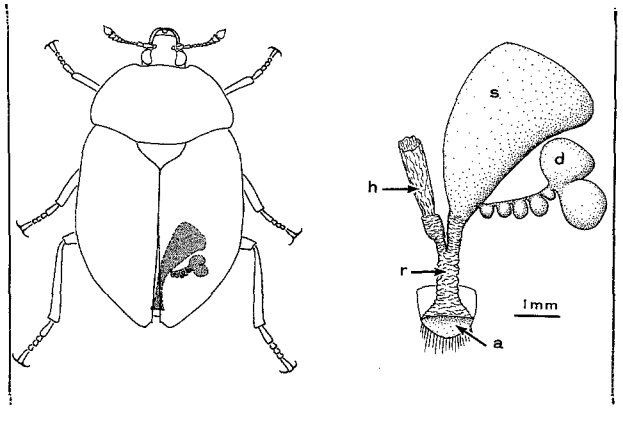

Figure 1. *Silpha americana* showing position of gland, and (right) enlarged view of gland and associated hindgut (KOH-treated preparation consisting of cuticular linings only); s = glandular sac; d = glandular diverticulum; h = anterior portion of hindgut; r = rectum; a = anus.

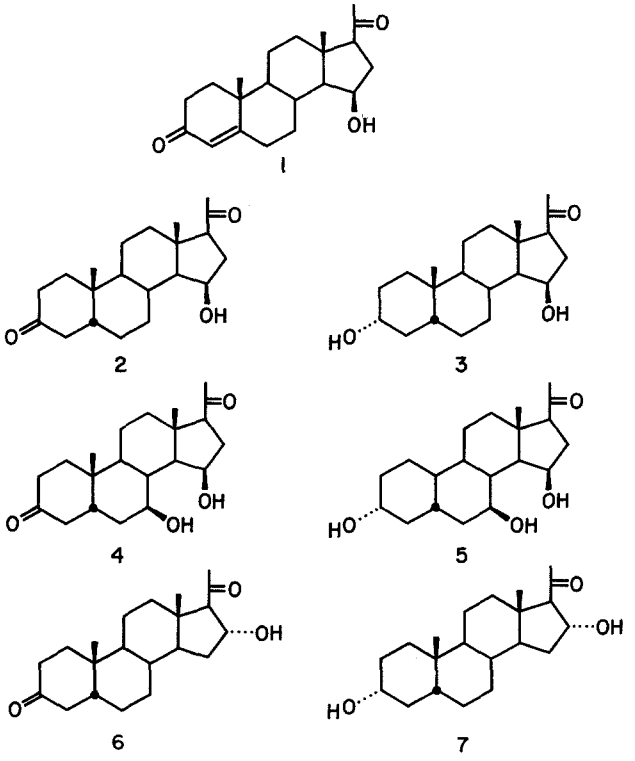

Figure 2. Pregnanes from rectal gland of *Silpha americana*.

ddd, J = 15,11,7), 1.85 (3H, s), 1.75 (1H, m), 1.64 (1H, ddd, J = 11,11,11), 1.6–0.1 (H), 1.06 (3H, s), 0.60 (3H, s), 0.60 (1H, dd, J = 6,11).

5β-Pregnan-16α-ol-3,20-dione (**6**): High resolution MS (*m/z*) 332.2363 (calc. for $C_{21}H_{32}O_3$, 332.2351) EIMS (*m/z*) 332 (0.2%), 314 (3), 299 (4), 271 (3), 233 (3), 232 (3), 215 (5), 111 (16), 109 (12), 107 (16), 105 (18), 101 (21), 100 (83), 95 (17), 93 (21), 91 (27), 85 (36), 81 (25), 79 (29), 71 (7), 55 (40), 43 (100). CIMS (*m/z*) 373 (10), 361 (26), 333 (100), 315 (72), 297 (18), 273 (4), 271 (2), 255 (4), 232 (2), 233 (2), 101 (18), 100 (54). ^{1}H NMR ($CDCl_3$) δ 4.82 (1H, m), 2.67 (1H, dd, J = 15,13), 2.53 (1H, d, J = 6), 2.32 (1H, ddd, J = 15,15,6), 2.17 (1H, dddd, J = 15,6,3,2), 2.15 (3H, s), 2.00 (1H, ddd, J = 15,5,2), 2.0–1.0 (16H), 1.00 (3H, s), 0.63 (3H, s). ^{1}H NMR (C_6D_6) δ 4.78 (1H, m), 2.40 (1H, d, J = 6), 2.30 (1H, dd, J = 15,13), 2.11 (1H, dddd, J = 15,6,3,2), 1.98 (1H, ddd, J = 15,15,6), 1.95 (1H, ddd, J = 15,5,2), 1.90 (3H, s), 1.7–0.5 (16H), 0.63 (3H, s), 0.48 (3H, s). This material was indistinguishable (on the basis of its ^{1}H NMR spectrum and chromatographic behavior) from an authentic sample of **6** prepared by catalytic hydrogenation of 16α-hydroxyprogesterone.

5β-Pregnan-3α, 16α-diol-20-one (**7**): High resolution MS (*m/z*) 334. 2517 (calc. for $C_{21}H_{34}O_3$, 334.2508). EIMS (*m/z*) (0.1%), 316 (3), 301 (2), 111 (11), 109 (8), 107 (11), 105 (8), 101 (37), 100 (100), 95 (10), 93 (12), 91 (10), 85 (19), 81 (13), 79 (11), 55 (13), 43 (37), 41 (12). CIMS (*m/z*) 375 (4%), 363 (9), 335 (19), 333 (5), 317 (15), 315 (6), 299 (100), 281 (9), 101 (14), 100 (21). ^{1}H NMR ($CDCl_3$) δ 4.81 (1H, m), 3.62 (1H, m), 2.50 (1H, d, J = 7), 2.15 (3H, s), 1.95 (1H, m), 1.9–1.1 (18H), 0.98 (1H, ddd, J = 14,14,3), 0.90 (3H, s), 0.60 (3H, s).

Silpha americana proved distinctly unacceptable to predators. A captive Swainson's thrush (*Hylocichla ustulata* ♂) which over a period of weeks was offered some 500 arthropods of over 100 species[8], including many edible forms, treated *Silpha* as it did other chemically protected beetles (lampyrids, meloids, cantharids, coccinellids), rejecting them after pecking (N = 3, of which two survived), or leaving them untouched (N = 12). Another such thrush (♂), offered *S. americana* interspersed with edible controls in the form of mealworms (six feeding sessions spanning 8 days; feeding protocol as previously described for comparable tests[8,9]), ate all the mealworms (N = 61), but merely pecked (N = 3, none injured) or ignored (N = 27) the *Silpha*. A captive toad (*Bufo americanus*) offered a comparable *Silpha*/mealworm regimen (3 feeding sessions over a period of 6 days) ate all the mealworms (N = 32) but only three of 15 *Silpha*. Most of the latter were ignored (N = 10) or spat out promptly after being taken into the mouth (N = 2); one of the three *Silpha* eaten was actually reingested after being thus rejected. *Silpha* proved invulnerable also to jumping spiders. 20 beetles that were given to an equal number of individually-caged *Phidippus regius*, a jumping spider readily capable of coping with undefended prey the size of *S. americana*, were all released unharmed after being briefly seized by the spiders, in response to which the beetles always discharged anal fluid.

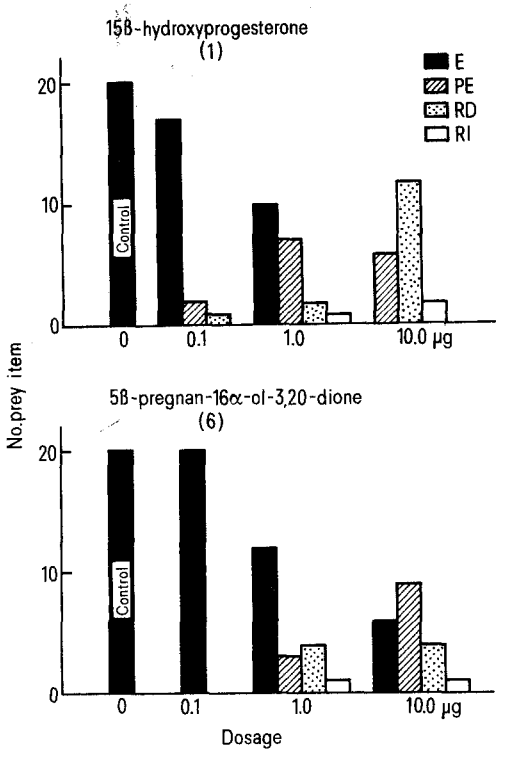

Figure 3. Fate of individual food items (freshly killed *Drosophila*), pretreated by topical addition of pregnanes (**1** and **6**) at dosages indicated, and offered to jumping spiders (*P. audax*). E = eaten; PE = partially eaten; RD = rejected after delay; RI = rejected immediately; sample size = 20 per dosage.

Evidence was obtained by bioassay of two of the *Silpha* steroids (**1** and **6**) with *Phidippus audax*, a smaller relative of *P. regius*, that these compounds are potent feeding deterrents. The testing procedure, used previously with other compounds and described in detail elsewhere[10], involved offering individual spiders standardized food items (freshly killed *Drosophila*), either treated by topical addition of steroid, or left untreated by such addition (controls). Fate of item was recorded as follows: *eaten* (reduced to a compacted mass of solid remains), *partially eaten* (part of body recognizably intact); *rejected uneaten after delay* (> 30 sec); and *rejected immediately* (< 30 sec). Both steroids were tested at three dosages (0.1, 1.0, 10.0 µg). Application to *Drosophila* was effected in dichloromethane solution (1 µl); controls were treated by application of solvent only. The results (fig. 3) show activity by both compounds, even at the 1 µg level, indicating that such steroids could account, in part at least, for the invulnerability of *Silpha* to spiders. To what extent the steroids contribute to the protectedness of the beetle against other predators remains unknown. Also unknown is the extent to which other chemical factors in *Silpha*, such as fecal components of the anal effluent, or carrion contaminant of the beetle's body, contribute to the beetle's defense. While a large number of glandular isoprenoids has been found among insects[11], steroidal defensive agents are of relatively restricted distribution. They have, however, been reported from beetles of three families, including Dytiscidae (whose steroids include some well-known vertebrate hormones[12]), Chrysomelidae (which produce cardenolides[13]), and Lampyridae (which contain insectan analogues of bufodienolides, called lucibufagins, in the blood[8,14]). It is generally accepted that insects lack the biochemical capacity for de novo production of steroids[15]. Whether *Silpha* derives its 20-ketopregnanes from cholesterol (potentially available in quantity in its carrion diet), with possible metabolic involvement of symbiotic microorganism, remains an intriguing unknown. Not all Silphidae produce defensive pregnanes. A species of another genus, *Necrodes surinamensis*, has a rectal gland seemingly homologous to that of *Silpha*, that secretes a mixture of fatty acids and novel isoprenoids[9].

We found *S. americana* to be eaten by captive field mice (*Peromyscus leucopus*), without ill effects (six beetles fed to each of two mice, male and female, over a period of one and several days respectively). It is conceivable that under natural conditions protracted intake of *Silpha*, with its contained pregnanes, could induce maladaptive endocrine imbalance in predators such as mice.

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(Z)-1,17-Diaminooctadec-9-ene, a novel aliphatic diamine from Coccinellidae

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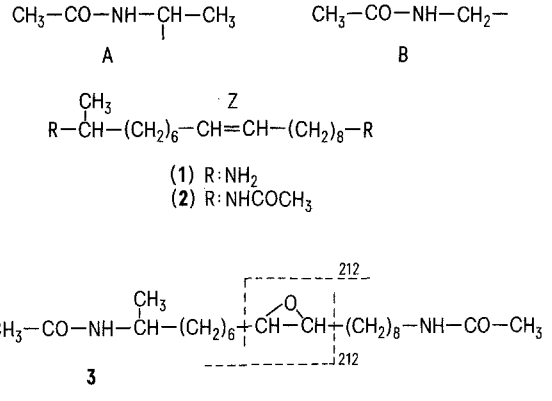
Summary. The isolation and structure determination of (Z)-1,17-diaminooctadec-9-ene from several species of Coccinellidae is reported.
Key words. Coccinellidae; (Z)-1,17-diaminooctadec-9-ene; *Hippodamia convergens; Harmonia leis conformis.*

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-