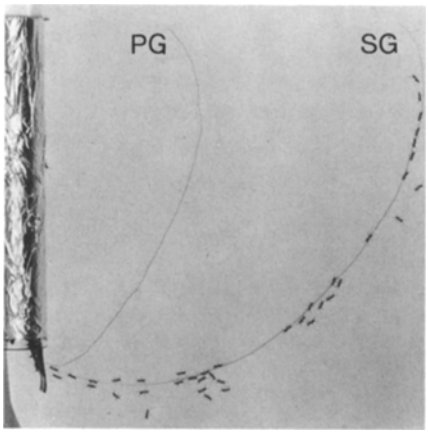

Fig. 2. Two artificial trails are drawn with a crushed sternal gland *SG* and a poison gland *PG*. *Onychomyrmex* workers follow only the sternal gland trails

O. sp. 1 (Australian National Insect Collection), *O. hedleyi*, and *O. sp. 2* (ANIC) (spp. 1 and 2 are currently undescribed). They all possess the sternal gland and we could demonstrate that in all species (except *O. doddi*, of which we had only preserved material) this gland secretes a recruitment and trail pheromone. Furthermore, we found that response to this pheromone is in part species-specific. *Onychomyrmex hedleyi* and *O. sp. 2* (which frequently occur sympatrically) do not readily follow each others trails, though both follow the sternal gland trails of *O. sp. 1*, with which neither occurs sympatrically. On the other hand, *O. sp. 1* responds to trails drawn with sternal gland secretion of *O. hedleyi*, but its response to *O. sp. 2* trails was considerably weaker.

We also investigated several other species of the tribe Amblyoponini (*Amblyopone australis, A. longidens, A. reclinata, A. pallipes, Mystrium camillae; Myopopone castanea*); in none of these did we detect the sternal gland. The gland is also absent in *Cerapachys* and *Sphinctomyrmex*, two genera belonging to the ponerine tribe Cerapachyini which also forage by group raiding expeditions. The trail communication in these species is organized in part by pheromones from the poison gland and the pygidial gland [4]. Thus it appears that this major pheromone-producing sternal gland is unique to the genus *Onychomyrmex*.

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Anthraquinones and Anthrones: Occurrence and Defensive Function in a Chrysomelid Beetle

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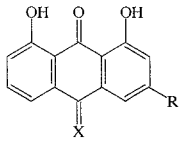
Anthraquinones and their derivatives frequently occur among aphids and scales [1] but these compounds have been reported in other insects only twice. In both of the latter cases (*Laetilia coccidivora*, a pyralid moth and *Chrysolina brunsvicensis*, a chrysomelid beetle) evidence strongly suggests that the insects acquire their tricyclic compounds directly through the diet [2]. We now report that larvae of the elm leaf beetle, *Pyrrhalta luteola*, derive protection against predators through a mixture of anthraquinones and anthrones and further, that these compounds appear to be biosynthesized in the insect's body.

Larvae of *P. luteola* in the United States often form dense, exposed populations on their host plants and although they are frequently encountered by small arthropoda predators such as ants, they are rarely preyed upon. Ants such as *Monomorium minimum* and *Iridomyrmex humilis* sometimes inflict wounds upon the larvae, but upon contacting the chrysomelid's bright yellow hemolymph, they quickly relinquish the attack and intensively groom themselves. Moreover, when larval hemolymph is applied onto normally acceptable foods, the ants refuse to feed.

To determine the chemical nature of their feeding deterrents, 600 larvae were soxhlet-extracted in pentane and extracts were bioassayed for activity in laboratory colonies of ants. The yellow extract, which exhibited strong feeding deterrency for ants was concentrated and the residue was chromatographed on Sephadex LH-20 with chloroform. The resulting single yellow band was chromatographed on silica gel, yielding 7 mg of bright yellow material. Selected ion monitoring of the temperature-programmed mass spectrum (Varian MAT 445 mass spectrometer equipped with a Varian SS200 computer) of the mixture showed the presence of two distinct compounds. One compound (*1*) (Fig. 1) had important ions at *m/z* 240 (100%, M^+), 212 (22%, M−CO), 184 (18%, M−2CO), 128 (10%, M−4CO) and 92 (14%) and the other (*2*) had important ions at *m/z* 254 (100%, M^+), 226 (25%, M−CO), 198 (15%, M−2CO), 142 (6%, M−4CO) and 99 (6%), suggesting a dihydroxyanthraquinone and a methyl dihydroxyanthraquinone [3]. The UV-Vis spectrum had λ_{max}^{EtOH} at 427, 367 (sh), 281, and 252 nm. Treatment with dilute NaOH produced a bathochromic shift of the absorption band at 427 to 510 nm, characteristic of 1,8-dihydroxy-9,10-anthraquinones [4]. The 90-MHz NMR spectrum (Varian EM-390) of the natural mixture had the expected complex aromatic signal from $\delta = 7.0$–8.0 ppm and a singlet at $\delta = 12.20$ ppm had the same chemical shift observed for hydrogen-bonded phenolic protons in an authentic sample of 1,8-dihydroxy-9,10-anthraquinone (*1*). Also in the spectrum were a singlet at $\delta = 2.48$ ppm for an aromatic methyl group, and a pair of equally intense singlets at 12.23 and 12.10 ppm, matching values obtained from an authentic sample of 3-methyl-1,8-dihydroxy-9,10-anthraquinone (*2*) (Fig. 1). In addition, several complex aromatic signals from $\delta = 6.7$ to 7.0 ppm, along with singlets at 12.42 and 4.37 ppm suggested the presence of the 9-anthrone of *1* as well as a pair of less intense singlets at 12.50 and 12.33 ppm and singlets at 4.32 and 2.42 ppm suggesting the 9-anthrone of *2*. These values matched the chemical shifts



1: R = H, X = O
2: R = CH_3, X = O
3: R = H, X = H_2
4: R = CH_3, X = H_2

Fig. 1. Anthraquinones (*1, 2*) and anthrones (*3, 4*) from *P. luteola*

observed in the NMR spectra of authentic samples of 1,8-dihydroxy-9-anthrone (*3*) and 1,8-dihydroxy-3-methyl-9-anthrone (*4*) (Fig. 1), respectively, which were prepared by $SnCl_2$ reduction of *1* and *2* [5]. Comparison of the intensities of the hydrogen-bonded phenolic signals of all four compounds with the anthrone methylene signals near 4 ppm (*3* and *4*) and the aromatic methyl signals near 2.4 ppm (*2* and *4*) suggests that compounds *1*, *2*, *3*, and *4* are present in the approximate ratio 2:1:1:1.

The identities of compounds *1*, *2*, *3* and *4* were corroborated by gas chromatography [6] of their bis-trimethylsilyl derivatives. Each of the compounds was detected in samples of hemolymph from larvae but none of the four compounds could be found in either acidified or nonacidified extracts of the leaves of the host plant (*Ulmus pumila*). Natural concentrations of a mixture of the compounds produced roughly a 40% reduction in feeding when tested against starved laboratory colonies of the fire ant, *Solenopsis invicta*.

These findings suggest that anthrones and anthraquinones play a significant role in deterring potential predators of *P. luteola*. Additional tests are needed to determine if the anthraquinones are externalized only through wounds and if the larvae are protected by other defensive mechanisms.

It is noteworthy that insects relying upon benzoquinones and naphthoquinones for defense invariably isolate these reactive compounds in chitin-lined reservoirs, away from sensitive body tissues. How *P. luteola* avoids deleterious effects from its apparently freely-circulating anthraquinones is not obvious, and certainly merits further study.

A defensive role has recently been ascribed for carminic acid, an anthraquinone in the scale insect, *Dactylopius confusus*, and it has been suggested that anthraquinones may serve a similar function in other Homoptera [7]. Our results support the view that the use of anthraquinones in defense may be widespread but at this juncture it would be premature to conclude that these compounds function only as deterrents of predators. For instance, compound *1* and related anthraquinones have been shown to be potent cytostatic agents against a number of microorganisms [8], and it is possible that they may protect their bearers against certain diseases or for that matter, parasites as well.

The derivation of the elm leaf beetles' anthraquinones is unknown. We could not detect anthrones or anthraquinones in leaves upon which *P. luteola* feeds, nor have these compounds been reported in phytochemical studies of the Ulmaceae [9]. Although anthraquinones are probably biosynthesized by *P. luteola* it is possible that symbiotic microorganisms may actually be responsible for the production of these compounds.

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Development of a Serum-free Medium for Cultivation of Insect Cells

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A promissing method in biological pest control is the use of specific insect pathogenic viruses, especially nuclear polyhedrosis viruses (Baculoviridae). Due to their occurrence in nature in a high amount, the application of these viruses for pest control will not cause a contamination of the environment with new substances. A further advantage of these viruses is their well-known specificity and the use of a virus preparation similar to a chemical insecticide [1].

A prerequisite for the use of virus preparations as a control agens is a dependable inexpensive method for virus production. In addition to the replication of viruses in living insects the in vitro technology – the replication of viruses in cell cultures – is of great importance. By such an in vitro technology not only the production of insect cells in large volumes and their use for virus production has to be feasible, but also the establishment of a simple and inexpensive culture medium.

Culture media for lepidopterous cell lines consist of a basic medium containing inorganic salts, amino acids, vitamins, carbohydrates, and protein hydrolysates, and a supplement of 5–20% foetal calf serum (FCS) [2, 3]. This high amount of FCS causes an expensive culture medium. Therefore, under these conditions an in vitro production of nuclear polyhedrosis viruses as a biopreparation for pest control is beyond commercialization.

We tried to develop a simple and inexpensive culture medium, initiating the substitution of FCS.

IPLB-Sf cells, growing in TC10 medium with 10% FCS [3], were used as model system. After reduction of the FCS to a final concentration of 5% in the medium, we added different compounds (used in nutrition broths for bacteria and fungi) to a concentration of 0.5%. Only egg yolk emulsion (E) showed an enhancement of cell growth. After four passages we used TC10 medium without FCS, but with 1% E. The cell reproduction rate was the same as in the medium with 10% FCS (Fig. 1) and to some degree reduced compared to the medium with 5% FCS and 0.5% E. This substitution of FCS by egg yolk showed no negative influence on cell replication; in contrast, as far as tested (200 passages) the rate of cell reproduction was unaltered.

Further experiments showed that Sf cells could be cultured in this serum-free medi-

* Prof. Dr. Klaus Weissermel dedicated to his 60th birthday

