Anthraquinones in the Leaf Beetle *Trirhabda geminata* (Chrysomelidae)

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Hydroxylated anthraquinones have been found to accumulate in different developmental stages of the chrysomelid beetle *Trirhabda geminata*. Eggs, larvae and adults were analyzed by HPLC and GC-MS. Each developmental stage analyzed contained 1,8-dihydroxy-3-methyl-anthraquinone (chrysophanol) and 1,8-dihydroxyanthraquinone (chrysazin). No anthraquinones were detected in the faeces of *T. geminata*. The level of stored anthraquinones did not change during starvation. In the host plant of this specialist herbivore, the brittlebush *Encelia farinosa* (Asteraceae), anthraquinones were not detected. Possible biological functions of anthraquinones stored in *T. geminata* are discussed.

Introduction

The leaf beetle *Trirhabda geminata* Horn (Chrysomelidae) is a specialist herbivore that lives on the brittlebush *Encelia farinosa* Gray (Asteraceae) which is a common plant in the deserts of the southwestern United States. In previous studies the adaptation of *T. geminata* to the toxic constituents of *E. farinosa*, in particular to the chromene derivative encecalin has been reported (Wisdom, 1982; Paine *et al.*, 1993; Kunze *et al.*, 1995 b). The fate of plant derived chromenes in *T. geminata* has also been described (Kunze *et al.*, 1995 a). In the present study we report on the occurrence of hydroxylated anthraquinones in different developmental stages of the chrysomelid beetle *T. geminata* and discuss possible advantages of anthraquinone accumulation for the leaf beetles.

Materials and Methods

Beetles and plant material

Eggs and adults of *T. geminata* as well as live specimens of *Encelia farinosa* were collected at Box Spring Mountain, Riverside, California, U.S.A., in April and May 1994. The beetles were maintained in the laboratory at room temperature (ca. 25 °C) and were allowed to feed freely on *Encelia farinosa* for 8 hours prior to their use in experiments.

Experimental design

Groups of beetles (*n*=3 in each case) were starved for 0, 3, 6, 12 and 24 hours on wet tissue paper. Haemolymph was collected from 5 non-starved beetles of both sex using a glass microcapillary which was passed carefully through the integument of the beetles without damage of the gut and transferred into acetone. Frass and eggs were also collected and freeze dried. Freeze dried beetles, frass, eggs and leave samples were ground and extracted three times for 24 h with acetone. The solvent was evaporated under vacuum and the residue was dissolved in a known amount of methanol and filtered prior to HPLC analysis and GC-MS analysis. The acetone solution of the haemolymph was filtered and analysed by HPLC.

Larvae

Larvae of *T. geminata* were collected during an expedition to Baja California (Mexico), in the

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nearhood of Moctezuma, Mexico (exact localities of collections compare Table I). Larvae were immediately immersed into methanol during the expedition. Larvae that had been stored in methanol were subsequently extracted three times with methanol and the solvent of the combined extracts was evaporated under vacuum. The residue was dissolved in a known amount of methanol and filtered prior to HPLC analysis and GC-MS analysis.

Sample analysis

The HPLC system was from Knauer (Berlin, F. R. G.) equipped with a multi wavelength detector (Knauer). Samples were injected on a Eurospher 100-C18 column (Knauer) (125 x 4 mm, 5 μm poresize) and separated using a linear binary gradient consisting of solvent A [H_2O, adjusted with *ortho*-phosphoric acid to pH 2] and solvent B [methanol] (from 100% A to 100% B in 45 min followed by an isocratic segment at 100% B for 15 min). Flow was at 1 ml min^{-1}, detection at 254 nm. Chrysazin and chrysophanol were quantified using the external standard method. Chrysazin and chrysophanol were calculated as emodin (2-methyl-4,5,7-trihydroxyanthraquinone (Roth, Karlsruhe, F. R. G.).

The GC-MS system was a Carlo Erba Mega 5160 gas chromatograph equipped with a quartz column (30 m-0.32 mm; DB-1, J&W Scientific, CA) which was directly coupled to a quadruple mass spectrometer (Finnigan MAT 4515). Conditions: injector 250 °C, temp. prog. 150–300 °C, 6 °C min^{-1}, split ratio 1:20, carrier gas He 0.5 bar.

Results and Discussion

Eggs, larvae and adults of *T. geminata* were shown to contain two anthraquinone derivatives that were identified based on their typical mass spectra as well as by comparison with known standards. The mass spectrum of the first anthraquinone exhibited characteristic ions at *m/z* 240 (100%, M$^+$), 212 (17%, M − CO), 184 (18%, M − 2CO), 128 (10%) and 92 (10%) indicating that the compound was 1,8-dihydroxyanthraquinone (= chrysazin, compare Fig. 1) (Howard *et al.*, 1982). The mass spectrum of the second anthraquinone showed fragments at *m/z* 254 (100%, M$^+$), 226 (11%, M − CO), 197 (9%), 152 (10%), 141 (6%), 127 (8%), 115 (8%) suggesting that compound 2
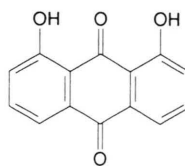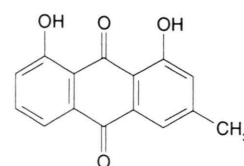
Chrysazin (**1**)   Chrysophanol (**2**)

Fig. 1.

was 1,8-dihydroxy-3-methylanthraquinone (chrysophanol, compare Fig. 1) (Howard *et al.*, 1982). The identity of both compounds was confirmed by comparison with spectra of known standards. No anthraquinone derivatives, however, were detected in the host plant *E. farinosa*.

Amounts of chrysazin in the larvae of *T. geminata* (3.3 nmol/larva or 35.3 nmol/larva, compare Table I) were approximately two times higher than those of chrysophanol (1.5 nmol/larva or 16.5 nmol/larva respectively, compare Table I). The large quantitative variation of the amounts of anthraquinones detected in the different samples of larvae analyzed (Table I) is possibly due to different developmental stages of the larvae that were not taken into consideration during collection.

Eggs of *T. geminata* were likewise shown to contain chrysophanol and chrysazin. Chrysophanol, however, was by far the dominating component (80.3% of all detected anthraquinones) (data not shown).

Whereas faeces of *T. geminata* lacked anthraquinones, chrysazin and chrysophanol were detected in the haemolymph of adults of *T. geminata*. When adults that had been starved for 0, 3, 6 or 24 hours

Table I. Distribution, concentration and absolute amounts of anthraquinones in larvae of *T. geminata*.

Abundance of anthraquinones [nmol/larva]		
Compound	Sample 1* (n = 6)	Sample 2* (n = 59)
1	3.3	35.3
2	1.5	16.5

* Both samples were collected in February 1993, sample 1 60 km ONO of Moctezuma, Sonora, Mexico; sample 2 14 km away from Mactezuma, Sonora, Mexico. Larvae were extracted as groups and not on an individual basis. Numbers of compounds follow Fig. 1.
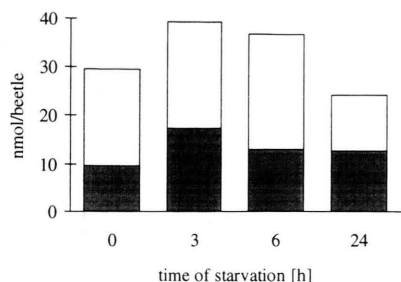
Fig. 2. Accumulation of anthraquinones in crude extracts of adult beetles of *T. geminata* after different length of starvation ($n = 3$ in each case; grey areas represent chrysazin, white areas chrysophanol).

on wet tissue paper were analyzed, the total amounts of anthraquinones in the resulting extracts were similar (24.3–39.3 nmol/beetle, compare Fig. 2) irrespective of the time of starvation. Since chrysophanol and chrysazin were not detected in the host plant *E. farinosa* they are considered to have been synthesized *de novo* either by the beetles or by endosymbiontic microorganisms as suggested for example for anthraquinones in scale insects (Kayser, 1985).

Accumulation of the anthraquinones chrysazin and chrysophanol has also been described in other members of the tribe Galerucini (subfamily Galerucinae, family Chrysomelidae): Howard *et al.* (1982), for example, reported on the detection of chrysophanol and chrysazin in larvae of *Pyrrhalta luteola* (=*Xanthogaleruca luteola*), whereas Hilker *et al.* (1992) reported on the occurrence of chrysophanol, chrysazin and dithranol in *Galerucella tenella*, *G. pusilla*, *G. calmariensis* and *G. lineola* and *Hydrogaleruca nymphaeae* and *Lochmea suturalis* (all members of the tribe Galerucini).

The ecological function of the anthraquinones found in *T. geminata* lies probably in protection of the beetles against predators. It is possible, for example, that anthraquinones protect the eggs of *T. geminata* as well as of other members of the tribe Galerucini against predatory ants. Howard *et al.* (1982) were able to demonstrate that a natural mixture consisting of four anthraquinones and their corresponding anthrones that are accumulated by the chrysomelid beetle *Pyrrhalta luteola* causes a significant feeding reduction in laboratory colonies of the fire ant *Solenopsis invicta*. Hilker and Schulz (1991) reported on the feeding deterrent activity of chrysophanol against the ant *Myrmica ruginodis*. In addition to anti-predatory effects the anthraquinones of *T. geminata* may also be involved in protection against microorganisms since these compounds are known to inhibit the growth of various bacteria (Cudlin *et al.*, 1976). Another biological function of anthraquinones could lie in a protection of the beetles against birds. Hilker and Köpf (1994), for example, demonstrated that the feeding deterrent activity of crude extracts derived from *Galeruca tanaceti* towards the great titmouse (*Parus major*) and the fir titmouse (*Parus ater*) was caused by the presence of chrysophanol. Based on the various bioactivities reported for anthraquinones it is possible that chrysazin and chrysophanol are also involved in the defense of *T. geminata* against *Perillus splendidus* (Pentatomidae) which has been reported as major predator of these beetles (Hogue, 1970). Further experiments are needed to test this hypothesis.

Acknowledgements

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