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CYCASIN IN THE ENDANGERED BUTTERFLY *EUMAEUS ATALA FLORIDA*

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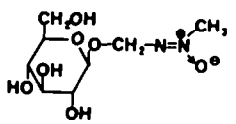
Key Word Index—*Eumaeus atala florida*; *Lycaenidae*; *Zamia floridana*; Cycadaceae; cycasin; methylazoxymethanol; toxin; sequestration.

Abstract—The strikingly beautiful aposematic hairstreak butterfly, *Eumaeus atala florida*, its pupa and brightly coloured, gregarious larva, all contain cycasin, the β-D-glucoside of methylazoxymethanol (MAM), which is also present in the food plant. It is suggested that the sequestration and storage of this toxin by the butterfly protects it from potential predators and that its various, highly specialized, warning attributes indicate a long association with the plant host.

INTRODUCTION

The Cycadales, a primitive group of gymnosperms, are characterized by their ability to synthesize and accumulate glycosides of methylazoxymethanol (MAM), a potent toxin and carcinogen [1]. When cycad material is ingested by vertebrate animals, the toxin is liberated from the glycoside by the hydrolytic cleavage of the glycosidic link [2, 3]. The red and yellow larva of the scintillating Lycaenid butterfly, *Eumaeus atala*, feeds on the young foliage of the cycad *Zamia floridana*. Hitherto, a study of this species has posed insuperable difficulties since it was too rare to sacrifice for research purposes and at one time was thought to be extinct [4, 5]. It was rediscovered in 1959 and became surprisingly abundant in the few extremely localized colonies in Dade County, Florida, where it occurs. Its survival is still threatened by urban development. By using captive stock reared by Mr. Ron Boender, we can now report on the sequestration of cycasin (**1**), the β-D-glucoside of MAM, by *E. atala*. The captive stock should be available for the re-introduction of the butterfly in suitable areas where the food plant is not at risk.



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RESULTS AND DISCUSSION

The concentrations of cycasin found in leaves of *Z. floridana* are given in Table 1, and those found in *E. atala* at different stages of development and in different organs of the adult are given in Tables 2 and 3 respectively.

The concentration of cycasin both in the imago (Tables 2 and 3) and pupa is surprisingly high compared with the amounts of secondary plant toxins found in other aposematic species. For example, the imago of *Danaus plexippus* weighs approximately 450 mg and contains 0.1 mg of cardenolides [6], while the pupa of *Zerynthia* (270 mg) contains 0.15 mg of aristolochic acid [7].

Table 1. Mean concentration of cycasin in leaves of *Zamia floridana* ($n = 6$)

	% fresh wt	% dry wt
Young leaf	0.044	0.21
Mature leaf	0.0026	0.008

Table 2. Concentrations of cycasin in *Eumaeus atala*

	Wt (mg)	Wt of cycasin (mg)	% cycasin
Larva 1	52	none detected	0
Larva 2	51	0.014	0.027
Larva 3	93.5	0.013	0.014
Pupa 1	207	1.42	0.69
Pupa 2	262	0.89	0.34
Pupa 3	147	1.31	0.89
Adult 1	62	0.83	1.3
Adult 2	54	0.96	1.8
Adult 3	16	0.16	1.0

Dry wt used for larvae and adults. Fresh wt used for pupae.

Since the caterpillar feeds only on the younger fronds of foliage, it is significant that these contain more of the toxin (Table 1) than the older leaves. It is also interesting that cycasin is distributed fairly evenly between wings and body while in the larger Monarch the cardiac glycosides are concentrated in the wings and especially the wing scales [6, 8].

One other species of Lepidoptera, a Tiger Moth, *Seirarctia echo* feeds on *Z. floridana* in Florida. It has been shown [9] that its gut contains β-glucosidase and it is able to avoid MAM intoxication by glucosylating or re-

Table 3. Distribution of cycasin between organs in adult *Eumaeus atala*

	Organ	Wt (mg)	Wt of cycasin	% cycasin in organ(s)
Insect 1	Abdomen	13.5	0.03	0.2
	Head/thorax/wings	23	0.11	0.48
Insect 2	Wing	7.5	0.16	2.1
	Head/thorax/abdomen	29	0.64	2.2

glucosylating liberated MAM to cycasin. The cycasin so formed is stored by the larva in the haemolymph.

There has been more opportunity to study the relatively abundant Costa Rican *E. minyas*, and DeVries [10] reports that oviposition is communal, the imago exudes a defensive fluid with a pyrazine-like warning scent [11], pupation is gregarious and the conspicuous orange–brown pupa stridulates. Both species exhibit the 'tenacity of life' so characteristic of warningly coloured butterflies. Such a plethora of aposematic attributes suggests a very ancient association between *Eumaeus* and the cycad host plants.

Warning colouration is virtually unknown among Lycaenids (apart from *Eumaeus*), despite the fact that many 'Blues' feed on highly poisonous plants, including a cycad in Australia. They usually metabolize the toxic secondary plant substances [12] and their larvae feeding on, for instance, mistletoe or cyanogenic *Lotus* exhibit the acme of crypsis. *Eumaeus atala* is a classical example of the hazardous aposematic life-style [13] that sequestration and storage of plant toxins impose on an insect herbivore.

EXPERIMENTAL

Plant and insect material. Zamia floridana A.DC. leaves were obtained from plants growing at R.B.G., Kew; ♀ accession no. 062-73.00402 and ♂ accession no. 627-60.62701. Specimens of *Eumaeus atala florida* Roeber were supplied by Mr. Ron Boender and Mr. Clive Farrell.

Gas chromatographic analysis of cycasin. The analysis was based on that described by De Luca *et al.* [1]. Leaf (400 mg fr. wt) and insect (< 300 mg fr. or dry wt) material was homogenized in 80% aq. MeOH (200 mg/3 ml) and centrifuged at 2000 rpm for 5 min. This process was repeated × 3. The extracts were taken to dryness under red. pres. at 22° and 200 μl of trimethylsilylation reagent (Sigma-Sil-A) added and shaken. After 30 min at 22° 1 μl was injected directly into the gas chromatograph. The retention time of cycasin was 10 min. The compound in the extracts corresponding with cycasin co-chromatographed with the pure compound. The concn of cycasin in the extracts was determined by comparison with the pure compound. Incubation of the pure compound and extracts in 3 ml of water with almond β-glucosidase (Sigma G-8625) (0.5 mg) at 22° for 12 hr caused total disappearance of the cycasin peak and an increase in free glucose.

The column was a 1.5 m × 4 mm glass column packed with 3% OV-1. Column oven temp. was 200°, FID oven temp. was 240°, N_2 carrier gas flow rate was 40 ml/min.

Paper chromatography. Extracts were prepared in 80% aq. MeOH (200 mg/ml) and shaken overnight. Cycasin was detected on descending paper (Whatman no. 1) chromatograms developed in *n*-BuOH–HOAc–H_2O (4:1:1) by aniline–diphenylamine reagent [14] and resorcinol–HCl [15].

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NOTE ADDED IN PROOF

Examination of additional live material shows that *E. atala* also has a typical pyrazine-like odour.