Toxicity of a Furanocoumarin to Armyworms: A Case of Biosynthetic Escape from Insect Herbivores

Abstract. *When the linear furanocoumarin xanthotoxin, found in many plants of the families Rutaceae and Umbelliferae, was administered to larvae of* Spodoptera eridania, *a generalist insect herbivore, it displayed toxic properties lacking in its biosynthetic precursor umbelliferone. Reduced toxicity observed in the absence of ultraviolet light is consistent with the known mechanism of photoinactivation of DNA by furanocoumarins through ultraviolet-catalyzed cross-linkage of strands. Thus, the ability of a plant to convert umbelliferone to linear furanocoumarins appears to confer broader protection against insect herbivores.*

Linear furanocoumarins are benzo-2-pyrone compounds with a furan ring fused at the 6,7 positions. They have a limited distribution among plant families, which is typical of many secondary plant constituents (*1*). Reported only from members of the Leguminosae, Moraceae, Solanaceae, Pittosporaceae, Thymeleaceae, Compositae, Rutaceae, and Umbelliferae, the compounds occur with great regularity and diversity only in Rutaceae and Umbelliferae (*2, 3*). Biosynthetically, the furanocoumarins all appear to be derived from the precursor 7-hydroxycoumarin (umbelliferone, **1**) (*4*). Although umbelliferone and related hydroxycoumarins are also limited in distribution, they are reported from more than four times as many plant families as are the furanocoumarins (*2*). A single enzyme is responsible for the prenylation of umbelliferone to 6-dimethylallyllumbelliferone (demethylsuberosin, **2**), an established intermediate in the synthesis of linear furanocoumarins (*5*). I now report that a furanocoumarin is toxic to an insect herbivore and that this toxicity is not shared by its unprenylated precursor. This finding lends experimental support to the coevolutionary hypothesis that plants "escape" from adapted enemies by altering their chemical phenotype—that is, by evolving biogenetic pathways which produce ecologically novel secondary compounds (*6*).

I selected for bioassay experiments the southern armyworm, *Spodoptera eridania* (Cramer) (Lepidoptera: Noctuidae). The larva of this species is notoriously polyphagous; a list of recorded host plants encompasses almost 50 herbaceous species in a dozen families (*7*). I chose this generalist herbivore for toxicity tests because its adaptation for generalized feeding appears to involve broad-spectrum mechanisms for detoxication (*8*); toxicity of a compound to this species would suggest that the compound is likely to be toxic to a wide array of insect herbivores. Another reason for using *S. eridania* larvae is the report (*9*) that armyworms are unwilling or unable to thrive on parsnip (Umbelliferae: *Pastinaca sativa* L.), a plant known to contain linear furanocoumarins, but that they can grow normally on carrot (Umbelliferae: *Daucus carota* L.), a plant containing umbelliferone but lacking furanocoumarins (*10*).

Twenty larvae in each of five treatments were reared from egg to pupa in plastic petri dishes lined with moistened filter paper. The larvae were raised on a semisynthetic agar-based diet (*11*) to which was added either umbelliferone (Aldrich) or xanthotoxin (8-methoxypsoralen, **3**; Sigma) at concentrations of 0.1 or 1.0 percent (wet weight). These concentrations are within the range found in plants producing their own furanocoumarins (*12*). As a control, a group of larvae were given the artificial diet only. All larvae were raised under standard conditions [26.5°C during the day and 16.5°C at night, 75 percent relative humidity, and photoperiod (LD) 13.5:10.5; the chamber was illuminated by a General Electric Cool White bulb, which approximates daylight conditions]. Sixth instar larvae that had finished feeding were transferred to plastic boxes filled with soil, in which they pupated; pupal weights were recorded after sclerotization was complete.

I found no significant difference in larval development time, survivorship, or pupal weight between individuals raised on the control diet and those raised on either of the umbelliferone diets (Table 1). However, caterpillars raised on both the 0.1 and 1.0 percent xanthotoxin diets failed to mature past the second instar. The length of time spent in the first instar was prolonged over that of the control larvae and individuals that succeeded in molting were abnormally small. Death is unlikely to have resulted from acute starvation, since caterpillars on all diets produced fecal pellets daily throughout the experiment. I conclude, then, from these results that xanthotoxin inhibits development of armyworm larvae and that the ability of a plant to convert umbelliferone to linear furanocoumarins may confer upon it some degree of protection

Table 1. Growth of *Spodoptera eridania* larvae on artifical diets containing test chemicals. Values are means ± the standard error.

Drug treatment (%)	Time to pupation (days)	Survivorship* (%)	Pupal weight (g)†
None	31.31 ± .39	75	0.230 ± .0001
Umbelliferone			
0.1	30.20 ± .47	70	0.221 ± .0013
1.0	32.38 ± .45	65	0.199 ± .0018
Xanthotoxin			
0.1	‡	0	
1.0	‡	0	

*A 2 by 3 test of independence with the use of the G-statistic shows that survivorship is independent of treatment for the control and the two umbelliferone diets ($G = 0.476$; $P > .50$). †A one-way analysis of variance shows that pupal weight on the umbelliferone and control diets are not significantly different ($F_{2,15} = 1.1$; $P > .10$). ‡No larvae survived to pupation.

Table 2. Growth of *Spodoptera eridania* larvae on artificial diets containing 0.1 percent (fresh weight) xanthotoxin in the presence and absence of ultraviolet light. Values are means ± the standard error.

Diet	Time to pupation (%)	Survivorship* (%)	Pupal weight (g)
	Ultraviolet light present		
Control	19.82 ± .19	85	0.392 ± .02
Xanthotoxin	†	0	†
	Ultraviolet light excluded		
Control	23.94 ± 0.31	80	0.343 ± .02
Xanthotoxin	37.50 ± 2.37	40	0.268 ± .01

*A two-way analysis of variance of survivorship shows that the interaction term is highly significant ($F_{1,4} = 81.0$; $P < .001$). †No larvae survived to pupation.

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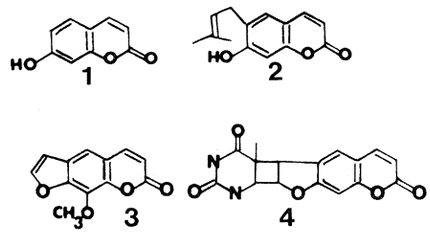
against herbivores, including those which can tolerate the unsubstituted hydroxycoumarins.

Umbelliferone and related hydroxycoumarins have antimicrobial and allelopathic properties (*13*). However, the presence of the furan ring imparts to linear furanocoumarins the ability to crosslink the strands of the DNA double helix (*14*). In the presence of ultraviolet light, linear furanocoumarins form cyclo-adducts of the type shown in structure **4** with pyrimidine bases of the two DNA strands. The ensuing interference with template function is manifested in a phototoxicity and, in this way, furanocoumarins can disrupt a number of biological processes in bacterial and fungal cells, DNA viruses, in vitro mammalian cells, organized plant tissues, and in vivo epidermal cells in birds and mammals (*15*). In view of the phototoxicity in other organisms, I conducted a second experiment to investigate the influence of ultraviolet light on the toxicity of xanthotoxin to armyworm larvae.

In each of four treatments, 20 larvae (two replicate groups of ten) were reared from eggs in individual ultraviolet transparent plastic dishes (7 cm in diameter by 3.5 cm in height), covered with perforated plastic caps and lined with filter papers; dishes were half-filled with moistened vermiculite when larvae were ready to pupate. In each treatment, the plastic dishes were placed in cardboard boxes (32.5 by 16 by 10 cm); each was fitted with a lid on which was placed either an ultraviolet-transparent plastic film (Saran Wrap) or a gelatin filter (Kodak Wratten 2A) of equivalent size (27 by 11 cm). The gelatin filter screens out almost all light of wavelengths less than 390 nm; the wavelengths that activate the furan double bond are in the range of 320 to 360 nm. Under each light regime, larvae were given either a normal control diet or 0.1 percent xanthotoxin diet. Larvae were raised under standard conditions (50 percent relative humidity, LD 16:8 at 25°C during the day and 16°C at night). The light bulb illuminating the environmental chamber was a General Electric Cool White bulb, which approximates daylight conditions in the ultraviolet range (*16*).

As before, I found that a 0.1 percent xanthotoxin diet was highly toxic to armyworms under daylight conditions (Table 2). No caterpillars survived to pupation and the few that reached the third and fourth instar were unable to move normally. In addition, their cuticle changed to an abnormal pink color—alteration of skin pigmentation is a common symptom of furanocoumarin sensitivity in mammals and birds (*15*). Since fecal pellet number is highly correlated with food consumption ($r = .98$) (*17*), I used as an index of consumption rate the number of fecal pellets produced by each caterpillar over the first instar. For armyworms surviving to the second instar on the xanthotoxin diet, the number of fecal pellets produced over first instar (74.32 ± 4.49) was not significantly different from the number produced by caterpillars on the control diet (64.38 ± 4.46; $t = 1.57$, $P < .10$). The same relationship held for fecal pellet number over the second instar. I conclude, therefore, that mortality in the early instars was due to toxicity and not to slow starvation.



When armyworms were fed the 0.1 percent xanthotoxin diet in the absence of ultraviolet light, 40 percent successfully pupated and later emerged as normal adults. A two-way analysis of variance showed that the effect of ultraviolet light on survivorship is considerably greater in the presence of xanthotoxin than in its absence ($P < .001$). The lower pupal weight of the xanthotoxin-fed larvae may be a reflection of reduced consumption rates rather than furanocoumarin toxicity; even in the absence of ultraviolet light, xanthotoxin appears to act as a feeding repellent (*18*).

The fact that furanocoumarin toxicity to insects is at least partly eliminated by the exclusion of ultraviolet radiation is of interest with respect to the nature of the insect fauna of plants that contain furanocoumarins. During the summers of 1976 and 1977, I collected insects from the foliage of umbelliferous plants in Tompkins County, New York (*19*). Of the 14 species collected, ten were lepidopterous larvae that live in rolled leaves or webbed flower heads. These leaf rollers, representing three families of Microlepidoptera, were primarily oecophorids in the genera *Agonopterix* and *Depressaria*—genera frequently associated not only with the furanocoumarin-containing umbellifers but with plants of the families Rutaceae and Leguminosae (genus *Psoralea*) that also contain furanocoumarins (*20*). Because a rolled leaf fails to transmit detectable amounts of ultraviolet light (as tested in an ultraviolet spectrophotometer), it appears that the leaf-rolling habit, common to many Microlepidoptera, may have been a preadaptation for feeding on plants containing furanocoumarins.

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11. Modified from S. S. Rehr, P. P. Feeny, D. H. Janzen, *J. Anim. Ecol.* **42**, 405 (1973). The Vanderzant Modification Vitamin Mixture (0.3 g per 300 g of diet; ICN Pharmaceuticals, Inc., Cleveland, Ohio) was substituted for the vitamin solution described by Rehr *et al.* Insects were taken from a culture maintained in the Cornell Insectary by C. Wilkinson; whenever possible, egg clusters from a single female were used. Adults are deposited in the Cornell University Collection, Lot 1023, sublot 41c.
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16. Spectral data for filters from Kodak filters, Standard Book Number 0-87985-029-9, Eastman Kodak Company, 1970; fluorescent lamp spectral data from General Electric Lamp Information sheet 211-3066.
17. Sixteen freshly molted sixth instar armyworms were fed control diet for 2, 4, 8, or 24 hours for 3 days; each day, the amount of diet eaten (initial wet weight times the average percent dry matter–dry weight of portion uneaten) and the number of fecal pellets produced were recorded.
18. Sixth instar xanthotoxin-fed larvae produced on a daily basis approximately half the number of fecal pellets (6.15 ± 0.46) that the control larvae produced (12.76 ± 0.72), indicating that xanthotoxin markedly reduced feeding rate. The relation between feeding rate and pupal weight

was demonstrated experimentally as follows. Consumption rates of armyworms was artificially varied by feeding them control diet for 2, 4, or 8 hours per day. As indicated by fecal pellet number, the amount of food eaten over the instar was approximately equal for all three treatments ($F_{2,.5} = 0.51$, $P > .10$). The average number of fecal pellets produced daily was inversely correlated with pupal weight ($r = 0.613$, $P < .01$, $n = 18$). Since the number of fecal pellets is highly correlated with the amount of food eaten, this finding suggests that pupal weight is inversely correlated with feeding rate.

19. Specimens were deposited in Cornell University Collection, Lot 1023, sublot 41b. Microlepidoptera were prepared by R. Brown and S. Passoa and determined by J. G. Franclemont.
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21. Supported by NSF predoctoral fellowship grant (M.B.) and NSF research grant DEB 76-20114 (to P. P. Feeny). I thank C. F. Wilkinson and M. Root for the loan of armyworms; J. Christy for assistance with statistics; R. Root, L. Brattsten, and W. Blau for comments on an earlier version of this manuscript; M. Rausher for unpublished date, technical advice, and unremitting enthusiasm; and my adviser, P. P. Feeny, for general encouragement.

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Choice Behavior in Rhesus Monkeys: Cocaine Versus Food

Abstract. *Rhesus monkeys were allowed to choose between intravenous injections of cocaine and food reinforcement for lever pressing. A choice trial was available every 15 minutes continuously for 8 days. The animals chose cocaine almost exclusively, which resulted in high cocaine intake, decreased food intake, weight loss, and marked behavioral toxicity. The study provides evidence of the reinforcing efficacy of cocaine.*

Various techniques have been proposed to determine the relative strength of a variety of reinforcers. One method involves presenting the subject with a choice between two different reinforcement conditions. Typically the choice of one reinforcer postpones the opportunity to obtain the second reinforcer. The number of times one reinforcer is chosen compared to the total number of opportunities to choose can be viewed as an index of preference. Techniques have been developed that allow animal subjects to indicate a preference between two or more qualitatively different reinforcers such as food, drugs, or electrical brain stimulation (*1*, *2*).

Illicit use of cocaine and other psychomotor stimulant drugs has been increasing in the past several years. Cocaine has been termed the ultimate euphorigenic whose preference by the drug connoisseur is undisputed (*3*). Numerous studies have shown that animals will readily learn to press a lever in order to obtain an intravenous injection of cocaine (*4*). In monkeys given unlimited access to intravenous cocaine, irregular periods of high intake result in marked behavioral toxicity similar to that seen in human users of intravenous stimulants (*5*, *6*). Experimental analogs of human psychomotor stimulant abuse may be obtainable using animal subjects and may offer new insights into this problem.

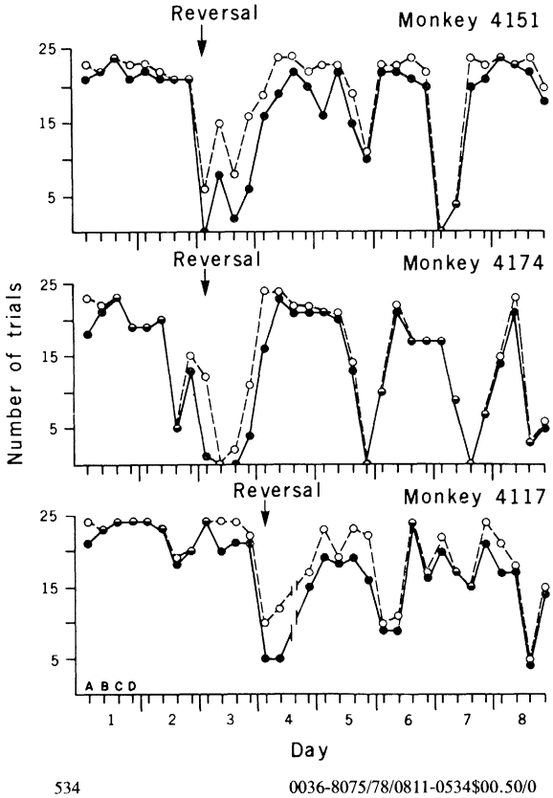

Fig. 1. Number of drug choices and total trials completed by each of three rhesus monkeys during 8 days of testing. The data for each day are divided into 6-hour blocks, where *A* indicates 12:00 p.m. to 6:00 p.m., and so forth. Arrows indicate the point at which stimulus light color and reinforcement condition pairings were reversed. The break in the graph for monkey 4117 indicates loss of data due to equipment failure.

The purpose of this study was to examine the relative reinforcing strength of cocaine in a competitive situation in which a qualitatively different reinforcer was also available. Rhesus monkeys were allowed to choose either an intravenous injection of cocaine or a small amount of food. The anorexigenic properties of cocaine have been documented (*7*) and may be a bias in favor of drug choices. However, monkeys self-administering cocaine under conditions of limited access eat relatively normally. The experimental design allowed access to both reinforcers 24 hours a day, but limited the number of choice trials within that period. Since no other food source was available to the animals, food was considered to be a competitive reinforcer.

Three adult male rhesus monkeys with prior experimental and drug histories were used as subjects. Under phencyclidine-pentobarbital anesthesia, each animal was surgically prepared with a permanently indwelling venous catheter and outfitted with a stainless steel restraining harness (*5*). Throughout the study, the animals were individually housed. The drug was injected by a peristaltic pump located behind the cubicle. Cocaine hydrochloride was dissolved in saline so that a concentration of 0.3 mg (as the salt) per kilogram of body weight would be delivered in 1 ml of fluid in 8 seconds. A pellet dispenser (BRS/LVE PDC) mounted on the outside of the cage delivered five 1-g food pellets (Noyes) into a trough on the inside of the cage. Two response panels, each with three stimulus lamps and a single primate lever (BRS/LVE PRL-001), were also mounted on the inside of the cage, one on each side of the food trough. All contingencies were controlled by solid-state behavioral programming equipment. Data were collected in the form of digital counts and cumulative recordings of lever-pressing behavior. Sessions were run continuously for 8 days. At 12:00 p.m. each day, data were recorded, pump and feeder reservoirs were refilled, and the animal's catheter was checked for patency.

The choice procedure was similar to one previously reported (*2*) except that

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