Paternal allocation of sequestered plant pyrrolizidine alkaloid to eggs in the danaine butterfly, *Danaus gilippus* [1]

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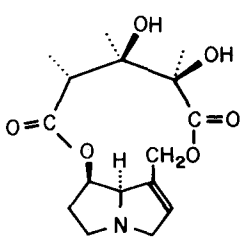
Summary. Pyrrolizidine alkaloid sequestered by adult male *Danaus gilippus* from plants is transferred in large measure to the female at mating, and by the female to the eggs. The eggs, presumably, are protected as a result. The male's courtship pheromone, danaidone, derived from the sequestered alkaloid, may function to advertise the male's alkaloid-donating capacity.

Key words. Lepidoptera; pyrrolizidine alkaloid; pheromone; sexual selection; nuptial gift; egg defense.

A specialized adult male behavior, the sequestration of pyrrolizidine alkaloids (PAs; for example **I**) from plants, appears to be commonplace in danaine butterflies (family Nymphalidae). Male danaines fly to PA-containing plants, sometimes aggregating on them, and feed systematically from the surface of damaged or senescent parts thereof [4, 5]. Chemical analyses showed the males to accumulate substantial systemic loads of PA through such behavior [4–6]. The sequestered alkaloids presumably protect danaines from predators, as has been documented for the closely related ithomiine butterflies [6].

Courtship studies had shown danaine males to produce pheromones based on the pyrrolizine skeleton, substances such as danaidone (**II**), which in the queen butterfly, *Danaus gilippus*, was proved experimentally to mediate male acceptance by the female [7]. Because of their structural similarities to PAs, it was suggested that pyrrolizine pheromones might be derived metabolically by the males from ingested alkaloid [8]. Such derivation has now been demonstrated for several danaines [9]. We here report that in *D. gilippus*, henceforth referred to as the queen, the male puts the acquired alkaloid to yet another use. It transfers the alkaloid in substantial measure to the female at mating, for eventual incorporation into the eggs. Such bestowal of a chemical defensive agent upon eggs, on the exclusive part of a male parent, had not previously been documented for Lepidoptera.



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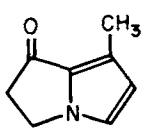
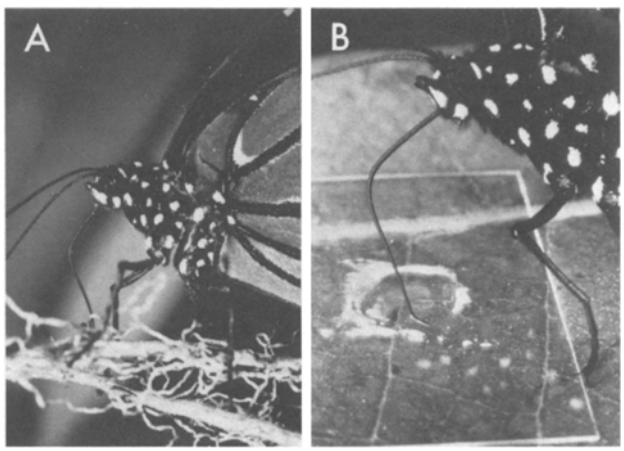


II

The queen in central Florida, where its behavior had been studied [7, 10], and where we obtained the founding members of our laboratory colony, has access to a number of PA-containing plants, including *Eupatorium capillifolium* and *Crotalaria spectabilis*. Males in the field can be lured readily to wilting uprooted *E. capillifolium* plants (fig., A), and are commonly seen feeding on the surface of dried seed pods of *C. spectabilis*. The latter plant contains monocrotaline (**I**) as its chief PA [11], the compound upon which we fed male queens in the laboratory. Given access to monocrotaline (in N-oxide form) in honey solution or as a crystalline powder, male queens avidly consume the offering (fig., B). They effect crystal ingestion by first liquifying the sample with fluid regurgitated from the proboscis, and then imbibing the solution. Transfer of PA in the queen from male to female and female to eggs was demonstrated in a single experiment. Four 7–10-day-old males (greenhouse-reared on *Asclepias syriaca, A. curassavica,* and *A. tuberosa*), each fed 4 days earlier on 1 mg monocrotaline N-oxide (offered in 25 µl of 4% honey/water solution; samples were consumed without visible residue), were released in a greenhouse together with comparably-reared virgin male and female queens not previously exposed to monocrotaline.



A Captive *Danaus gilippus* male, feeding on roots of *Eupatorium maculatum* (family Asteraceae), a pyrrolizidine alkaloid-containing plant. Male *Danaus* in nature procure their pyrrolizidine alkaloid by such visitation to senescent or damaged parts of pyrrolizidine-alkaloid producing plants. *B D. gilippus* male feeding on a crystalline offering of pyrrolizidine alkaloid (monocrotaline N-oxide).

The four experimental males eventually mated, and upon uncoupling were immediately frozen and stored for subsequent chemical analysis. Their female partners were individually caged, offered honey/water solution, and allowed to oviposit on potted *A. syriaca* plants until death. Eggs produced by each female were collected and weighed every three days, then stored frozen for analysis. For control purposes, two sets of comparable egg samples were collected, from two females mated with monocrotaline-unfed males. Males, females, and the pooled egg output of each female, were analyzed for monocrotaline content. The gas chromatographic technique used for the purpose has been described [12].

In contrast to the control samples, which consistently showed no detectable levels of monocrotaline, the experimental samples all contained substantial quantities of the compound: males (209 ± 41 µg), females (27 ± 12 µg), eggs (345 ± 51 µg) ($\bar{x} \pm$ SEM). Given the zero value for the controls, all alkaloid recovered from females and eggs must have stemmed from the males. The summed alkaloid in females and eggs therefore provides a minimal estimate of the amount of monocrotaline transmitted by the male at mating. This alkaloidal 'gift', on average (372 ± 43 µg; range 320–501 µg), amounted to an astonishing 64% (range 55–77%) of the male's initial monocrotaline load (estimated by summing the alkaloid contents of male, female, and eggs). The female's efficiency of transfer was even higher: the amount of monocrotaline recovered from eggs corresponded to 93% (range 83–98%) of the quantity calculated to have been received by the female at mating. Despite the variable number of eggs produced per female (range 228–423), the monocrotaline content per egg for the four experimental samples was relatively constant (range 0.9–1.2 µg/egg; 0.2–0.3% of wet weight).

Evidence was obtained indicating that males transfer PA to females by seminal infusion. Four virgin males, each fed 1 mg monocrotaline N-oxide (2 were given the alkaloid in dilute honey/water solution as before; the other 2 received crystalline alkaloid in the hollow of glass depression slides), were dissected 19–20 days later, and their component parts analyzed for monocrotaline content. As is apparent from the table, the alkaloid level was highest in that region of the reproductive system comprised of the simplex (the median ejaculatory duct) and aedeagus (the intromittent organ). This region, presumably the first to void its contents into the female at mating, contained fully 45% (range 26–65%) of the total alkaloid recovered from the males. The initial intake of monocrotaline by the males can be expected to have been efficient (methylene chloride rinsings of 10 depression slides from which males had individually imbibed 1 mg crystalline monocrotaline N-oxide offerings proved alkaloid free). The fact that the analyzed males still retained, as late as 19–20 days after feeding, an average of 79% of the 1 mg monocrotaline they had been given, attests to the long-term alkaloid storage capability of these insects.

Distribution of monocrotaline in virgin queen males fed 1 mg monocrotaline N-oxide (values given as $\bar{x} \pm$ SEM; N = 4 butterflies).

	Monocrotaline µg	% wet weight
Head and thorax	221 ± 57	0.16
Wings	104 ± 25	0.26
Abdomen minus reproductive tract	98 ± 33	0.26
Reproductive tract:		
Testes and vas deferens	< 4	< 0.09
Accessory glands	< 4	< 0.13
Duplex	11 ± 4	0.26
Simplex and aedeagus	355 ± 47	1.62
Total	789 µg	

While we did not perform predation tests with queen eggs per se, it seems reasonable to assume that these should derive protection from their alkaloidal endowment. PAs are provenly deterrent to arthropod predators such as spiders [6, 13], and they have been shown to protect eggs against coccinellid beetles in another lepidopteran, the moth *Utetheisa ornatrix*, whose eggs also contain PAs [12].

Our data prompt further speculation about the function of PA-derived male pheromones in danaine butterflies. The earlier argument, that males can offer proof to females by way of such pheromones of the magnitude of their alkaloidal load, and indirectly, therefore, of their alkaloid-sequestering ability [14], can now be amplified. Courting males, it seems, could use the pheromone for direct quantitative advertisement of the alkaloidal 'gifts' they have in store, providing a basis by which the female might exercise mate choice. In the queen butterfly, in fact, males deprived of adult access to PAs and therefore devoid of the PA-derived pheromone, danaidone (**II**), are distinctly less successful in courtship than danaidone-endowed counterparts [7]. We suggest that sexual selective strategies comparable to that of the queen will be found to be commonplace in danaines, and shown to be the adaptive concomitants of paternal egg-endowment mechanisms. The widespread occurrence of pyrrolizine pheromones in danaines [4] supports this prediction, as does the occurrence of PAs in field-collected females [6, 15], since in many species only males routinely visit PA-containing plants [4, 5]. Analyses by Brown [6] of danaine and ithomiine butterflies demonstrated the presence of PAs in the male reproductive system and eggs of several species, including the queen, suggesting that PA transfer during mating may be widespread in both these groups.

Courtship and egg-allocation strategies comparable to those of the queen butterfly occur also in moths of the family Arctiidae. PA-derived male pheromones have been demonstrated in several of these [4], and in two species, *Utetheisa ornatrix* and *Cisseps fulvicollis*, the male also bestows dietary PA upon the eggs [12, 16]. However, in both these species the female herself contributes PA to the eggs.

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Plakorin, a potent Ca^{2+}-ATPase activator from the Okinawan marine sponge *Plakortis* sp.

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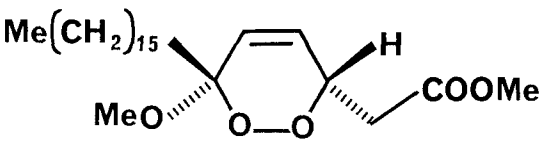
Summary. A new cyclic peroxide, plakorin, which is a potent sarcoplasmic reticulum (SR) Ca^{a+}-ATPase activator has been isolated from the Okinawan marine sponge *Plakortis* sp., its structure was elucidated on the basis of spectral data.
Key words. Sponge; plakorin; *Plakortis* sp.; cyclic peroxide; Ca^{2+}-ATPase activator.

The Ca^{2+}-ATPase in sarcoplasmic reticulum (SR) membrane plays a key role in muscle relaxation by energized Ca^{2+}-pumping from the cytoplasm into the lumen of SR[2]. In our continuing studies on pharmacological tools[3,4] for resolving the molecular mechanism of excitation-contraction coupling in skeletal and cardiac muscle, and on other bioactive compounds[5–8] from Okinawan marine organisms, we encountered extracts of the Okinawan marine sponge *Plakortis* sp. which exhibited remarkable activation of SR Ca^{2+}-ATPase activity. In this paper we describe the isolation and structure elucidation of a new cyclic peroxide, plakorin (**1**), as a powerful Ca^{2+}-ATPase activator.

The SR Ca^{2+}-ATPase was prepared from rabbit skeletal white muscle by the method of Meissner et al.[9]. The technique of measurement of the Ca^{2+}-ATPase activity was carried out as previously described[10]. The sponge *Plakortis* sp. was collected at Kerama Islands, Okinawa, and kept frozen until used. The methanol extracts of the sponge were partitioned between ethyl acetate and water. The ethyl acetate soluble fraction was subjected to silica gel column chromatography (hexane/ethyl acetate, 4:1) followed by preparative silica gel TLC (hexane/ethyl acetate, 3:1). Plakorin (**1**)[11] was obtained as a colorless oil, $[\alpha]_D^{27}$ + 44.3° (C = 0.2, $CHCl_3$), in 0.007% yield (wet wt) together with but-3-enolide (**2**, 0.003%).



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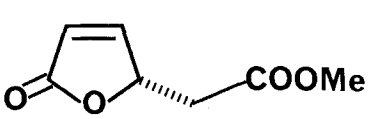

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