

- 5 A.F.P.M. De Goeij, T.H.J. Ververgaert and J. Van Steveninck Clin. chim. Acta 62, 287 (1975).
- 6 A.W. Girotti, Biochem. biophys. Res. Commun. 72, 1367 (1976).
- 7 R. Strom, C. Crifò, S. Mari, G. Federici, I. Mavelli and A. Finazzi-Agrò, Physiol. Chem. Phys. 9, 63 (1977).
- 8 A. Finazzi-Agrò, M.B. Fadda, G. Floris, M.R. Dessì and C. Crifò, Experientia 34, 181 (1978).
- 9 J. Maral, K. Puget and A.M. Michelson, Biochem. biophys. Res. Commun. 77, 1525 (1977).
- 10 J. Goa, J. clin. Lab. Invest. 5, 218 (1953).
- 11 H. Lück, in: Methods of Enzymatic Analysis, 2nd edn, p.886. Ed. M.V. Bergmeyer. Verlag Chemie/Academic Press, London-New York 1974.
- 12 A. Concetti, P. Massei, G. Rotilio, M. Brunori and E.A. Rachmilewitz, J. Lab. clin. Med. 87, 1057 (1976).
- 13 N. Hasty, P.B. Merkel, P. Radlick and D.R. Kearns, Tetrahedron Lett. 1972, 49.
- 14 A. Rigo, P. Viglino, and G. Rotilio. Analyt. Biochem. 68 (1975).
- 15 R.E. Heikkila, F.S. Cabbat and G. Cohen, J. biol. Chem. 251, 2182 (1976).
- 16 M. Younes and V. Weser, Biochem. biophys. Res. Commun. 78, 1247 (1977).
- 17 B. Goldberg and A. Stern, J. biol. Chem. 251, 6468 (1976).
- 18 S. Mc Mahon, S.G. Sullivan and A. Stern, Biochim. biophys. Acta, submitted.
- 19 R.C. Bray, S.A. Cockle, E.M. Fielden, P.B. Roberts, G. Rotilio and L. Calabrese, Biochem. J. 139, 43 (1974).
- 20 A. Finazzi-Agrò and I. Mavelli, XV Congr. Soc. It. Biofis. Biol. Mol., Abstr., p. 18, 1978.
- 21 A. Finazzi-Agrò, C. Giovagnoli, P. De Sole, L. Calabrese, G. Rotilio and B. Mondovi, FEBS Lett. 21, 183 (1972).

Pyrrolizidine alkaloid storage in African and Australian danaid butterflies

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Summary. 8 species of African and Australian danaid butterflies, captured in the field, were analyzed and found to contain pyrrolizidine alkaloids. It is suggested that these alkaloids, which are gathered by the adults from plants, contribute significantly to the chemical defences of the danaids.

Danaid butterflies feed on exudates and on withered parts of plants containing pyrrolizidine alkaloids^{3,4} (figure). These phytochemicals are partially metabolized by the males of many species of this family and are converted into dihydropyrrolizines⁵⁻⁷ which are used as pheromones during courtship behaviour^{8,9}. Recently it was shown that adult male and female *Danaus plexippus* (L), which do not produce such pheromones but still feed on plants containing pyrrolizidine alkaloids, are able to retain the alkaloids unmodified in their bodies for extended periods¹⁰. It was therefore suggested that pyrrolizidine alkaloids, as well as acting as pheromone precursors, may also be 'stored' by danaid butterflies as a protection against predators¹⁰.

We report here on a chemical examination of 57 danaid butterflies, representing 5 African and 3 Australian species of 3 genera (*Amauris*, *Danaus*, *Euploea*). Our results (table) indicate that 'storage' of pyrrolizidine alkaloids occurs in both sexes. The butterflies were captured in the field (in Kenya/East Africa and Queensland/Australia) and kept alive without access to alkaloids for periods ranging from 2 to 21 days in order to ensure that alkaloids detected in their bodies were in fact 'stored' and not recently ingested gut contents.

The diversity of alkaloids found confirms that danaids use various plant sources of pyrrolizidine alkaloids. They have been reported to visit and feed on plants in the families Boraginaceae, Asteraceae, Leguminosae and Apocynaceae^{3,4}, and pyrrolizidine alkaloid representatives of all these plant families were found in the bodies of the butterflies. However, it remains to be investigated whether or not the various danaid species prefer certain pyrrolizidine alkaloids and if the different types of pyrrolizidine alkaloids are equally appropriate as pheromone precursors and for storage.

Previous reports have emphasized the predominance of males among butterflies seen feeding on pyrrolizidine alkaloid plants^{3,4}. It was therefore interesting to find that the samples of females we analyzed all contained alkaloids (albeit generally less than the male samples) indicating that, while they are apparently less frequent feeders, some

of them at least acquire and store pyrrolizidine alkaloids in nature.

D. formosa (sample of 3 males) was the only species without detectable amounts of pyrrolizidine alkaloids in the male sex. It (like *D. plexippus*) has been previously reported not to possess a pyrrolizidine alkaloid-derived pheromone on its hairpencils¹². However, W. Schäfer¹³ recently detected a pyrrolizidine metabolite in some (not all) hairpencil extracts of *D. formosa*. Since the bodies of females of this species contained alkaloids it seems likely that the males



Danaus chrysippus (male) applying a droplet (arrow) to withered *Heliotropium steudneri* (Boraginaceae) in order to extract pyrrolizidine alkaloids for ingestion. This is a common strategem of danaids in their quest for pyrrolizidine alkaloids.

Pyrrolizidine alkaloids found in danaid butterflies captured in the field*

Species	Sex	Number of butterflies	Known alkaloid - free period (days)	Main alkaloids (µg/butterfly)**			
				M	L	S	U
African							
<i>Amauris ochlea</i> Boisduval	♀	2	4	60	-	-	50
<i>Amauris ochlea</i> Boisduval	♂	2	7	105	-	-	-
<i>Amauris echeria</i> Stoll	♂	2	5	-	-	30	-
<i>Danaus (Tirumula) petiverana</i> Doubleday	♀	2	19	-	traces***	-	-
<i>Danaus (Tirumula) petiverana</i> Doubleday	♀	8	5	45	-	-	-
<i>Danaus (Tirumula) petiverana</i> Doubleday	♂	5	21	125	traces***	-	-
<i>Danaus (Tirumula) petiverana</i> Doubleday	♂	2	7	-	40	270	-
<i>Danaus (Tirumula) formosa</i> Godman	♀	2	5	-	-	12	-
<i>Danaus (Tirumula) formosa</i> Godman	♂	3	5	-	-	-	-
<i>Danaus (Anosia) chrysippus</i> L	♀	3	15	10	30	-	-
<i>Danaus (Anosia) chrysippus</i> L	♀	3	10	40	160	-	-
<i>Danaus (Anosia) chrysippus</i> L	♂	3	15	150	220	-	-
<i>Danaus (Anosia) chrysippus</i> L	♂	2	10	15	115	-	-
Australian							
<i>Euploea sylvester</i> Fabricius	♂	4	2	-	135	-	-
<i>Danaus (Anosia) affinis</i> Fabricius	♂	5	2	30	55	-	-
<i>Danaus (Tirumula) hamatus</i> MacLeay	♂	9	2	-	270	-	-

* Hairpencils of the males had been removed prior to extraction of the alkaloids by standard procedures¹¹. ** M=monocrotaline, L=lycopsamine (or diastereoisomer), S=senecionine, U=usaramine. Identified and quantitated by comparison of their gas chromatographic (GC) behaviour and characteristic mass spectra (MS) with those of authentic samples. Lycopsamine, monocrotaline and usaramine were also converted into methyl and butylboronate derivatives for GC/MS examination. GC/MS was performed on a Varian MAT III GC/MS using a 1.5 m x 2 mm glass-lined stainless steel column packed with 1% SE 30 on Chromosorb W, mesh size 80-100 with temperature programming from 180° to 230°C at 6°/min and carrier gas (helium) flowing at 15 ml/min. *** Less than 5 µg.

analyzed by us, as well as those analyzed by Meinwald et al.¹², had not had access to plants containing pyrrolizidine alkaloids prior to capture.

The predator-repellent qualities of several pyrrolizidine alkaloids have been confirmed recently by Eisner et al.¹⁴ and the pyrrolizidine alkaloids 'stored' by danaids therefore probably serve a defensive role although this has not yet been tested.

Danaids are generally considered to be unpalatable to most potential predators and they are involved in many mimetic relationships. Based on studies with *D. plexippus*^{15,16} it has been assumed that cardiac glycosides (= cardenolides), sequestered by the larvae from their host plants (Asclepiadaceae, Apocynaceae, Moraceae), are the danaids main chemical defence against predation. However, larvae of several danaid species (e.g. *Amauris*) are reported to feed on Asclepiadaceae lacking cardiac glycosides¹⁷. Furthermore several danaid species seem not to store cardiac glycosides¹⁸, and even in *D. plexippus* not all individuals of a population contain cardiac glycosides¹⁶. On the other hand, ingestion and 'storage' of pyrrolizidine alkaloids seems to be common in adult danaids of both sexes. We therefore believe that pyrrolizidine alkaloids are an important element in the chemical defence system of danaids. It is even possible to envisage pyrrolizidine alkaloids to be the original, and perhaps principal chemical defence elements for danaids and that the use of cardiac glycosides is a more recent addition evolved in certain danaid groups only¹⁹.

Various other Lepidoptera show a close relationship to plants containing pyrrolizidine alkaloids. Many arctiid moths store pyrrolizidine alkaloids derived from their larval food plants²⁰⁻²² and Rothschild et al.^{20,21} were the first to equate this storage capacity with their diurnal habit and warning colouration. Many Arctiidae, Ctenuchiidae, and Ithomiidae also feed as adults on withered pyrrolizidine alkaloid-containing plants and Pliske³ has suggested that they too may be gathering these phytochemicals for their defense (and, in some cases, as male pheromone precursors²³).

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- Max-Planck-Institut für Verhaltensphysiologie, D-8131 See-wiesen, Federal Republic of Germany.
- T. E. Pliske, Environ. Ent. 4, 455 (1975).
- J. A. Edgar, Phil. Trans. r. Soc. Lond. B 272, 467 (1975).
- D. Schneider, M. Boppré, H. Schneider, W.R. Thompson, C.J. Boriack, R.L. Petty and J. Meinwald, J. comp. Physiol. 97, 245 (1975).
- J. A. Edgar, C. C. J. Culvenor and G. S. Robinson, J. Aust. ent. Soc. 12, 144 (1973).
- M. Boppré, R.L. Petty, D. Schneider and J. Meinwald, J. comp. Physiol. 126, 97 (1978).
- T. E. Pliske and T. Eisner, Science 164, 1170 (1969).
- U. Seibt, D. Schneider and T. Eisner, Z. Tierpsych. 31, 513 (1972).
- J. A. Edgar, P. A. Cockrum and J. L. Frahn, Experientia 32, 1535 (1976).
- C. C. J. Culvenor and L. W. Smith, Aust. J. Chem. 8, 556 (1955).
- J. Meinwald, C. J. Boriack, D. Schneider, M. Boppré, W. F. Wood and T. Eisner, Experientia 30, 721 (1974).
- W. Schäfer, unpublished GLC-data.
- T. Eisner, W. E. Conner, K. Hicks and D. Aneshansley, unpublished.
- T. Reichstein, J. von Euw, J. A. Parsons and M. Rothschild, Science 161, 861 (1968).
- L. P. Brower, Sci. Am. 220, 22 (1969).
- M. Rothschild, T. Reichstein, J. von Euw, R. Aplin and R. R. M. Harman, Toxicon 8, 293 (1970).
- N. A. Marsh, C. A. Clarke, M. Rothschild and D. N. Kellett, Nature 268, 726 (1977); M. Rothschild and N. Marsh, Ent. exp. appl. 24, 437. (1978); Ned. Ent. Ver. Amsterdam, Proc. 4th Insect/Host Plant Symposium.
- M. Boppré, Ent. exp. appl. 24, 64 (1978).
- R. T. Aplin, M. H. Benn and M. Rothschild, Nature 219, 747 (1968).
- M. Rothschild, in: Insect-Plant Relationships, p. 59. Ed. H. F. van Emden. Blackwell Scientific Publications, Oxford 1972.
- C. C. J. Culvenor and J. A. Edgar, Experientia 28, 627 (1972).
- J. A. Edgar, C. C. J. Culvenor and T. E. Pliske, J. Chem. Ecol. 2, 263 (1976).