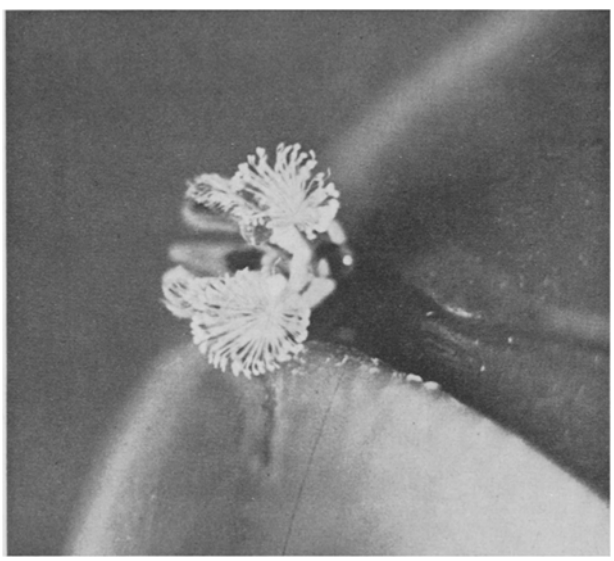
Dihydropyrrolizine Secretions Associated with Coremata of *Utetheisa* Moths (Family Arctiidae)

Male Tiger Moths (family Arctiidae) commonly possess scent organs in the form of brushes or inflatable coremata which are believed to be used for the dissemination of pheromones[1]. Some species are also known to utilise plants containing pyrrolizidine alkaloids as larval host plants. As an extension of our interest in the dihydropyrrolizine pheromones on the hair-pencils of butterflies of the subfamily Danainae[2] we have made a preliminary study of two such species of Arctiid moths, *Utetheisa pulchelloides* (Hamps.) and *U. lotrix* (Cram.), to see whether they secrete similar pyrrolizidine-derived dihydropyrrolizine pheromones.

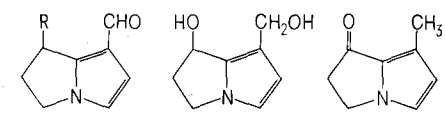
U. pulchelloides is found in south-eastern Australia, and its larvae are believed to live only, or very largely, on plants of the family Boraginaceae[3]. Heavy infestations occur on the two common weeds, *Heliotropium europaeum* and *Echium lycopsis*. When the body of the male moth is squeezed gently, the coremata are extruded (Figure) and can be detached, along with the associated genitalia. The coremata and genitalia were removed from 260 male moths collected in March in a field of *H. europaeum* and extracted with methylene chloride. From the extract, 1-formyl-7-hydroxy-6,7-dihydro-5H-pyrrolizine (I) was readily isolated by preparative thin layer chromatography. The mass spectrum of the isolate showed the expected molecular ion at *m/e* 151 and a fragmentation pattern characteristic of (I)[4]. The thin layer chromatographic behaviour and colour reactions of the isolate and its derivative (III), obtained by sodium borohydride reduction, were identical with those exhibited by authentic samples of (I) and 1-hydroxymethyl-7-hydroxy-6,7-dihydro-5H-pyrrolizine (III)[4]. The remainder of the carcasses of the male and the whole carcasses of female moths, separately extracted with methylene chloride, gave no detectable amounts of (I) or the other related dihydropyrrolizine derivatives found in Danaid butterflies[2]. The carcasses did, however, contain alkaloidal substances. When assayed by the technique used for pyrrolizidine alkaloids in plants[5], moths collected from a field of *H. europaeum* contained approximately 130 µg alkaloid per moth (corresponding to approximately 1.3% body weight after methanol extraction) of which 100 µg per moth was in the form of N-oxides. Heliotrine was a major constituent and heleurine and supinine minor constituents of the carcass extract. The other alkaloids of *H. europaeum*[6], lasiocarpine and europine, were not detected. Thus some selectivity occurs in the storage of alkaloids in the bodies of the moths. The coremata of moths collected from a field in which *E. lycopsis* was dominant also yielded (I). The bodies of these moths contained alkaloidal substances which were different from those of the moths collected from *H. europaeum*.

The species *Utetheisa lotrix*, which closely resembles *U. pulchelloides*, occurs in Queensland and northern New South Wales where its larvae are throught to live mostly on the leaves of *Crotalaria* species[3]. *U. lotrix* is best distinguished from *U. pulchelloides* by an examination of the genitalia. We have used a technique applicable in the field to identify dihydropyrrolizines secreted on the coremata and genitalia of male *U. lotrix* collected on *Crotalaria mucronata* ranging from Brisbane to Rockhampton. When extracted in groups of six to twelve moths, Brisbane collections contained mostly (I) and a small amount of 1-formyl-6,7-dihydro-5H-pyrrolizine (II). Collections from near Rockhampton, however, contained equal amounts of (I) and (II). Suspecting that the collections may have been mixed *U. pulchelloides* and *U. lotrix* (the species are known to overlap in this region), the field technique was adapted to examine the secretions of individual moths in such a way that the genitalia remained suitable for identification purposes. Of eight moths collected near Brisbane and studied in this way six contained (I) only, and two contained (I) and (II) in about equal amounts. All were identified by Dr. I. F. B. Common, as *U. lotrix*. Thus, on chemical grounds, *U. lotrix* can be divided into two groups. Whether the difference in secreted dihydropyrrolizines is due to a genetic difference or to a difference in larval food plant is not yet known. Some *Utetheisa* species, including *U. pulchelloides*, are believed to travel distances up to hundreds of miles, either wind-borne or as true migrants[7–10], and moths collected on *C. mucronata* may not necessarily have fed on this species in the larval stage.

We have not found any reported observations on a specific functional use of the coremata in species of *Utetheisa*. The secretion of the compounds (I) and (II) by *U. pulchel-*



Extruded coremata of *U. pulchelloides*.



(I) R=OH
(II) R=H

(III)

(IV)

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loides and *U. lotrix* creates a strong possibility that, in these species at least, the structures have a function closely allied to that of the hair-pencils of Danaid butterflies. It is thus possible that (I) and (II) play a role in the mating of *Utetheisa* similar to that established for the related ketone (IV) in *Danaus gilippus berenice*[11].

Zusammenfassung. Die Duftorgane der männlichen Bärenspinner *Utetheisa pulchelloides* und *U. lotrix* (Fam. Arctiidae) scheiden Dihydropyrrolizine aus, die im Typ den Pheromonen von Schmetterlingen der Subfamilie Danainae (Fam. Papilionidae) gleichen. Wahrscheinlich handelt es sich um Derivate von Pyrrolizidin-Alkaloiden, die in den Wirtsplanzen der Raupen in hoher Konzentration vorkommen.

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CSIRO Division of Animal Health, Animal Health Research Laboratory, Private Bag No. 1, P.O. Parkville (Victoria, Australia), 17 November 1971.

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Relationship Between Lipolysis and Storage of Diphenylhydantoin in the Adipose Tissue

In a previous paper it was reported that increased lipolysis results in a more marked storage of phenobarbital in adipose tissue[1]. Since diphenylhydantoin (DPH), an anticonvulsant drug, also accumulates in fat[2,3], it was considered important to establish if its uptake by adipose tissue is influenced by the degree of lipolysis.

Materials and methods. Male Sprague Dawley rats (average body weight 180 g) were used throughout all the experiments.

In vivo studies. DPH at 100 mg/kg body wt. was given s.c. to fed or fasted rats and the animals were sacrificed at different times after the administration. The concentration of DPH was measured in plasma and in adipose tissue (epididymal and perirenal).

In vitro studies. The study of the accumulation of DPH in adipose tissue was carried out according to the procedure previously described for phenobarbital[1]. The release of DPH from adipose tissue was studied according to the following procedure: epididymal adipose tissue, cut into small pieces and pooled, was preincubated for 1 h at 37 °C in Krebs Ringer phosphate at pH 7.4, containing 3% albumin and DPH 53 µg/ml. This adipose tissue (400–600 mg) preloaded with the drug was filtered, washed and added to 4 ml of medium, free of DPH, in the presence or absence of noradrenaline. The incubation was carried out at 37 °C, with gentle shaking, for 1 h and at the end of this period the DPH remaining in the adipose tissue was measured.

Determinations of DPH in 1 ml of plasma or 400 mg of adipose tissue were carried out according to the method of MORSELLI[4]; free fatty acids (FFA) were measured according to DOLE[5], with minor modifications. Triglycerides were determined according to VAN HANDEL, ZILVERSMIT and BOWMANN[6].

Results. DPH injected s.c. is rapidly absorbed in plasma reaching a peak at 30 min and 20 min, respectively, in fed and fasted rats. However, the rate of disappearance of this drug from plasma was quicker in fasted than in fed animals. Accumulation of DPH in both epididymal and perirenal adipose tissue was more rapid in fasted than in fed rats (Table I), although an interpretation of this phenomenon is difficult because of the different kinetics of plasma DPH in the two types of experimental conditions used.

The results obtained in vitro are more conclusive. Table II shows that DPH uptake by the adipose tissue of fed rats is proportional to the DPH concentration in the medium. Table III indicates that DPH accumulation in adipose tissue increases with the time of incubation. It was also found that the DPH content in adipose tissue was higher both per g of weight and per g of triglycerides, when lipolysis was increased by the presence of noradren-

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Table I. Levels of diphenylhydantoin (DPH) in plasma and adipose tissue after a single injection of DPH (100 mg/kg s.c.) to fed or to overnight fasted rats

Time[a] (min)	Fed rats DPH (µg/ml) or g ± S.E.			Fasted rats DPH (µg/ml) or g ± S.E.		
	Plasma	Adipose tissue		Plasma	Adipose tissue	
		Epididymal	Perirenal		Epididymal	Perirenal
10	13.5 ± 0.1	—	—	16.3 ± 0.3	—	—
20	16.2 ± 0.3	11.4 ± 0.6 (0.70)	12.0 ± 0.4 (0.74)	23.6 ± 1.8	7.7 ± 0.8 (0.32)	12.3 ± 0.3 (0.52)
30	18.4 ± 1.2	15.7 ± 1.1 (0.88)	15.6 ± 1.1 (0.84)	12.6 ± 0.9	21.3 ± 1.4 (1.69)	22.7 ± 1.2 (1.80)
60	16.3 ± 0.3	16.4 ± 0.9 (1.00)	16.4 ± 0.9 (1.00)	10.4 ± 1.2	26.1 ± 0.9 (2.50)	29.3 ± 1.1 (2.81)
120	12.6 ± 1.6	25.1 ± 1.4 (1.99)	25.5 ± 1.1 (2.04)	9.3 ± 0.1	10.9 ± 1.2 (1.17)	12.7 ± 0.1 (1.36)
240	3.6 ± 1.4	9.9 ± 3.7 (2.75)	9.7 ± 0.4 (2.69)	8.1 ± 0.5	—	—
360	0.1 ± 0.0	—	—	1.2 ± 0.1	—	—

[a] Time elapsed between DPH administration and sacrifice of the animals. In brackets: the ratio between adipose tissue and plasma levels of DPH.