Identification and localization of volatile hairpencil components in male *Amauris ochlea* butterflies (Danaidae)[1]

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Summary. By gas-chromatography and mass-spectroscopy 7 volatile substances have been found on the abdominal hairpencils of *Amauris ochlea* males. 6 substances have been identified. The spatial distribution of compounds is correlated to the 5 types of hairs comprising the scent organ.

Extracts of male abdominal scent organs ('hairpencils') of several species of butterflies of the family Danaidae have been previously analyzed[4]. These analyses, however, have been aimed primarily at determining the presence or absence of a small group of compounds related to[5] and including 2,3-dihydro-7-methyl-1H-pyrrolizin-1-one (cf. table, structure **VII**). This heterocyclic ketone was first isolated from Lycorea ceres ceres[6] and later proved, for Danaus gilippus, to be an aphrodisiac pheromone necessary for successful courtship[7]. Although the ketone is the major volatile component of the hairpencils of a number of species, other components are always present[4,8]. Those which have been previously identified include a pyrrolizidine alkaloid[9], an acetophenone[4a], and various terpenoids, fatty acids and esters[6,10–12]. Pyrrolizidine alkaloids which are ingested by the males from plants have been shown to be precursors required for production of the ketonic pheromone[9,13,14]. The other components may have a variety of important functions; the long chain alcohols and esters, which are viscous oils, appear to serve as solvents for the actual pheromones[7]; other components may convey intraspecific messages or taken together may serve the purpose of species recognition.

The hairpencils and their secretions appear to be generally more complex in Amauris species than in Danaus species[13]. Those of Amauris ochlea have proved to be among the more interesting[15,16]. We here report the identification of 7 prominent volatile components of the hairpencils

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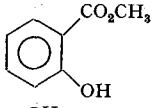
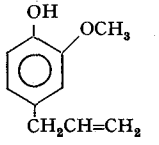
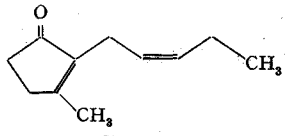
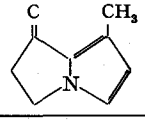
Volatile components from Amauris ochlea hairpencils

Formula (cf. figure 1)	Name	Structure	Approximate amount (μg) per male
I	n-Octanal	$CH_3(CH_2)_6CHO$	< 0.1
II	n-Nonanal	$CH_3(CH_2)_7CHO$	1.5
III	Hex-3-enoic acid	$CH_3CH_2CH{=}CHCH_2CO_2H$	30
IV	Methyl salicylate		< 0.1
V	Eugenol		0.6
VI	cis-Jasmone		0.3
VII	2,3-Dihydro-7-methyl-1H-pyrrolizin-1-one		< 0.1

of Amauris ochlea (Biosduval) as well as their topochemical distribution within the complex hairpencil system.

The butterflies were collected in Kenya at 3 different times (54 males in May 1974, 11 males in August 1975 and 3 males in August 1976). Hairpencils were expanded artificially, removed, and either placed directly in carbon disulfide or first separated into their various parts (see below) and then stored in carbon disulfide. Analyses were carried out after combining an extract with an additional carbon disulfide rinse of the glands and concentrating the resulting extracts. Gas chromatographic (GC) analyses were carried out on a 8′ by 2 mm glass column packed with 2% OV-17 on 100/120 mesh Gas Chrom Q, first utilizing a Varian 2100 GC with flame ionization detector and later, on selected samples, a computerized Finnigan 3300 GC mass spectrometer. Runs were programmed from 50 °C to 250 °C at 10 °C/min.

A typical chromatogram is reproduced in figure 1. The components (cf. table) were identified on the basis of identity of mass spectra of individual peaks to published spectra as well as coincidence of GC retention times to those of authentic samples[17a]. Amounts were determined by comparison of peak areas to calibration curves of area versus amount for the authentic samples[17b]. Similar results were obtained with all 3 groups of animals.

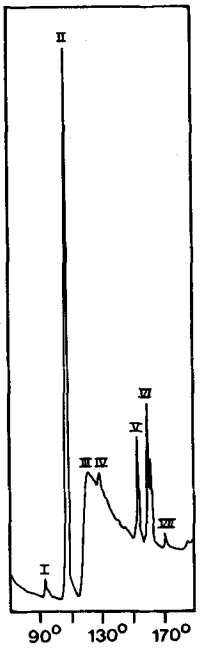


Fig. 1. Gas chromatogram of an extract of Amauris ochlea hairpencils. Components identified are designed by Roman numerals.

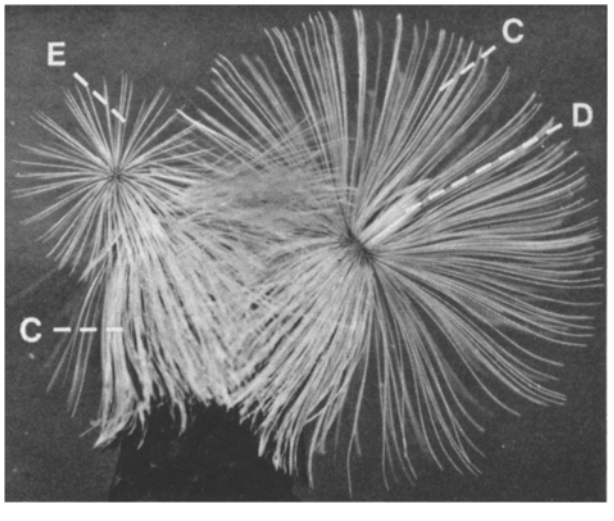


Fig. 2. Hairpencils of Amauris ochlea, artificially expanded to demonstrate their morphological complexity (cf. text). On the right side, long white hairs (C) form a sphere, in the centre of which cone hairs (D) are to be seen. On the left side, long white hairs (C) have folded backwards; the cone has opened to expose central hairs (E), which also form a sphere. In these stages black hairs (A) are lying close to the abdomen and are not visible in this photograph; hair type B is not detectable macroscopicllay.

17 a) Compounds **I, II, IV** and **V** were obtained from commercial sources. Compound **VI** was supplied by International Flavors and Fragrances. Compound **VII** was synthesized as previously described[6]. For compound **III**, the double bond position could not be ascertained from mass spectral data. It was therefore determined by *microozonolysis*[18] of the esterified (diazomethane) and isolated (micropreparative GC) component. GC/MS examination (Porapak Q column) of the ozonolysis product revealed the presence of propionaldehyde. b) The amount of **III** was estimated by treating the extract with diazomethane, and comparing the peak area of the resulting methyl ester of **III** with a calibration curve for methyl hexanoate.

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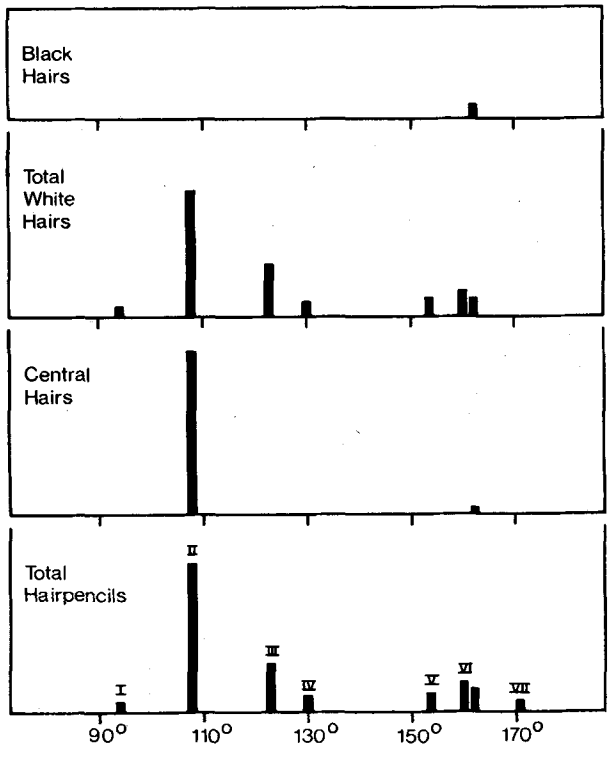

Fig. 3. Schematic representation of chromatograms of hairpencil parts. Line heights indicate relative peak heights (not areas) within each chromatogram; equal heights of the sace component in different chromatograms do not necessarily imply equal amounts. The peak appearing between peaks **VI** and **VII** was not identified. Compound **VII**, although present in the whole hairpencil extracts, could not be detected in extracts of separated hairpencil parts, possibly due to distribution in undetectable small amounts over all of the hairpencil parts.

With the exception of **VII**, these compounds occur widely in the plant kingdom[19]. In addition, compounds **I**[20a], **II**[20b] and **IV**[4b,20c] have been isolated from various insect species. Until now, compounds **III**, **V** and **VI** do not appear to have been identified in insects.

The binate hairpencils of A. ochlea consist of 5 different hair types (cf. figure 2)[15,16]. Black hairs (A) lie on the outer lateral sides of bundles of white hairs. A layer of another hair type (B) separates black hairs from the long white hairs (C) which make up the major part of the organ. The long white hairs produce pheromone-transfer-particles which are disseminated onto the female during courtship flight[21]. Enclosed by the long white hairs are the central hairs (E), which are separated from the rest of the organ by a tightly closing cone made of another hair type (D).

In view of the morphological complexity of this organ, it seemed desirable to determine whether some of the chemical components were localized in particular parts of the hairpencils. Interestingly, a spacial distribution of the chemicals was found. Preparations analyzed were a) black hairs (A), b) total white hairs (B–E), and c) central hairs (E). Chromatograms of extracts of these preparations are represented schematically in figure 3. The biological meaning of the topochemical distribution of the different compounds is under investigation.

Electrophysiological experiments, now in progress, show the olfactory effectiveness of all 7 characterized compounds. Although the compounds have not been tested in behavioral experiments, their stimulatory power and their distinct distribution on the complex scent organ supports the idea that at least certain ones act as pheromones. In addition, it is quite possible that they play a major role in species recognition.

Analyses in progress with indoor raised A. ochlea indicate that the production of some of the volatile hairpencil components depends on the ingestion by males of precursors from plants. This, as well as the influence of contacts between abdominal and alar scent organs on the chemical components found in the hairpencils, shows similarity to Danaus species[14,22].

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Increased membrane-bound polyribosome fraction in the brains of rats with hereditary diabetes insipidus

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Summary. Rats homozygous for hypothalamic diabetes insipidus were found to have a significantly higher concentration of membrane-bound polyribosomes than their heterozygous littermates. There was no difference in the concentration of free polyribosomes.

Hereditary hypothalamic diabetes (D.I.) occurs in rats of the Brattleboro strain. Animals that are homozygous for D.I. lack the ability to synthesize vasopressin, in contrast to their heterozygous littermates[3,4]. Vasopressin has been reported to be implicated in memory processes[5]. Support for this notion comes from findings suggesting that homozygous D.I. rats have memory deficits in comparison with their heterozygous littermates[5]. Since membrane-bound brain polyribosomes may also play a role in memory consolidation[6], we decided to examine the polyribosome distribution between brain cytoplasm and brain membranes of homozygous and heterozygous D.I. rats.

Materials and methods. 9 homozygous and 10 heterozygous male D.I. rats of the Brattleboro strain (obtained from TNO, Zeist, The Netherlands) were housed in groups of 4–5. The rats were 3 months old. The homozygous rats weighed approximately 250 g, whereas the heterozygous ones weighed 350 g. The experiment was conducted according to a randomized block design. The rats were killed by decapitation, and the brains were rapidly removed and placed on dry ice. Cerebellum and pineal gland were removed and discarded. The brain was weighed and rinsed in ice-cold homogenization buffer. All subsequent procedures were performed at 0–4 °C.

Free polyribosomes were prepared according to the method of Bloemendal et al.[7]. The brains were homogenized separately and centrifuged at 12,000 × g for 20 min. In the resulting supernatant, protein was determined according to the method of Lowry et al.[8]. For the preparation of membrane-bound polyribosomes, the intial 12,000 × g sediment was rehomogenized in $^2/_3$ of the original homogenization volume, after which deoxycholate and Triton X-100 were added to a final concentration of 0.5% (w/v) and 1% (v/v), respectively, followed by the procedure of Bloemendal et al.[7]. Absorbances (A) of polyribosome suspensions at 236, 260 and 280 nm were measured with a Zeiss Spectrophotometer Q II using 1 cm quartz cuvettes. 0.3 ml fractions of the polyribosomal preparations were analyzed on 10 ml 15–35% (w/v) linear sucrose gradients, which were centrifuged at 4 °C for 1 h at 40,000 rpm in a SB 283 rotor. After centrifugation, the absorbance of the gradients at 260 nm was continuously monitored using a Gilford Spectrophotometer 240 (2-mm-cuvet). The results were analyzed by means of a randomized blocks analysis of variance.

Results and discussion. The brains of homozygous D.I. rats were lighter than those of heterozygous rats (mean ± SEM: 1.36 ± 0.01 and 1.55 ± 0.03, respectively; $U = 5$, $p < 0.002$, 2tailed Mann-Whitney U-test).