olites (ca. 0.5–1.0 mg). The spectra were obtained after several scans using a computer averaging technique.

Results. The NMR-spectra of the acetoxychlorobiphenyl metabolite is shown in Figure 1 and consists of 4 recognizable doublets which is consistent with a symmetrical structure. Thus the hydroxyl group must have been introduced into the 4′ position of the biphenyl nucleus and this structure is consistent with the chemical shifts and coupling constants shown for 4-acetoxy-4-chlorobiphenyl (Figure 1).

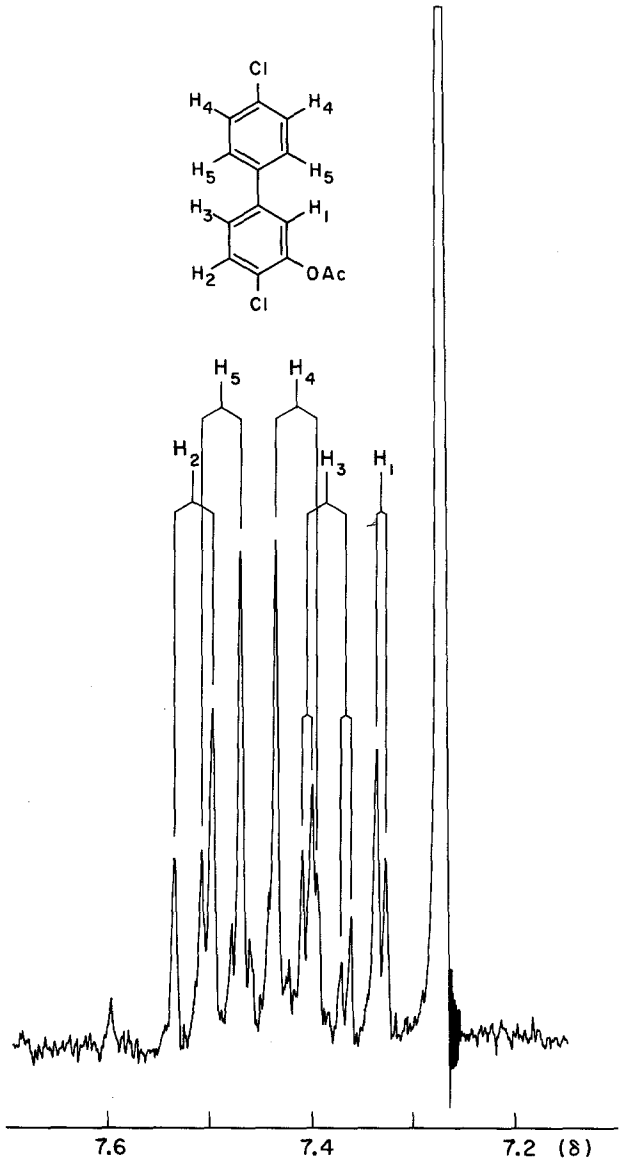

Fig. 2. NMR-spectrum of 3-acetoxy-4,4′-dichlorobiphenyl.

The NMR-spectra of the acetoxydichlorobiphenyl which was formed from feeding 4,4′-dichlorobiphenyl to rats is shown in Figure 2. The set of 2 doublets for the symmetrical H_4 and H_5 protons are easily recognized at δ 7.42 and 7.48 ppm. Since the hydroxyl group can only be introduced at the 2 or 3 position of the biphenyl ring the structure assignments shown in Figure 2 are consistent with hydroxylation at position 3 to give 3-acetoxy-4,4′-dichlorobiphenyl. H_1 is *meta* coupled with H_3; H_3 in turn gives a quartet due to *meta* coupling with H_1 and *ortho* coupling with H_2; H_2 appears as a doublet couplet only with H_3. These coupling constants would also be consistent for 2-acetoxy-4,4′-dichlorobiphenyl even though introduction of an hydroxyl group into the sterically-hindered 2 position would be less likely. The structures of both 4-acetoxy-4-chlorobiphenyl and 3-acetoxy-4,4′-dichlorobiphenyl were confirmed by unambiguous synthesis of the authentic compounds[12] which in turn, were identical to the two metabolites. The mechanism of the hydroxylation and further metabolic degradation of isomeric PCBs are currently under investigation.

Zusammenfassung. Identifizierung und Strukturaufklärung zweier Metaboliten von 4-Chorbiphenyl und 4,4′-Dichlorbiphenyl mittels NMR-Spektroskopie.

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Volatile Ketones in the Hairpencil Secretion of Danaid Butterflies (*Amauris* and *Danaus*)

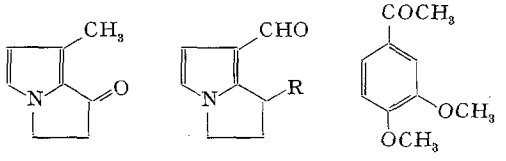
Chemical studies of the courtship pheromones of butterflies of the subfamily Danaidae[1–4], prompted by earlier behavioral investigation of these insects[5] have led to the isolation of a heterocyclic ketone, 2,3-dihydro-7-methyl-1*H*-pyrrolizin-1-one (I) from the glandular abdominal brushes ('hairpencils') of males of 3 species of the group. In 1 species, the Queen butterfly *Danaus gilippus*, which was studied in detail, the ketone functions as an aphrodisiac, administered by the males in flight to the antennae of the females[6,7]. The antennae are electrophysiologically sensitive to the ketone[8,9]. Other danaid butterflies are known[10] or presumed to court in a similar way, although it is now becoming apparent that the chemical repertory of the hairpencils is diversified. Culvenor et al.[11] have reported the isolation of the closely related heterocyclic aldehydes II and III from the males of several species. We here report on the hairpencil chemistry of nine species and subspecies of the genera ***Amauris*** and

Volatile ketones in hairpencil secretions

Species	Locality [a]	Components [b]	
		I	IV
Amauris albimaculata	U, K_1	+	–
A. crawshayi (= *oscarus oscarus* of authors)	U	–	–
A. echeria	K_1	+	–
A. niavius [c]	U, K_2	+	+
A. ochlea	K_3	+	–
A. tartarea	U, K_1	–	+ [d]
Danaus chrysippus dorippus	$K_{2,4}$	+ [e]	–
D. formosa mercedonia	U	–	–
D. limniace petiverana	U, K_2	+	–

[a] U, Uganda (Kampala area); K, Kenya (K_1, Kakamega; K_2, Mombasa; K_3, Shimba Hills; K_4, Kenya Highlands). [b] Identifications based on direct comparison with authentic samples by gas chromatography-mass spectrometry (gc-ms). [c] Ectraxts from specimens collected at 3 different times were combined. [d] Less than 2% of the total extract, compared to 15% in *A. niavius*, [e] Identification based on thin layer chromatography (tlc). Rf and color response to 2,4-DNPH indistinguishable from those of a synthetic sample of I.

Danaus. Some of these forms possess ketone I, while others have another volatile, aromatic ketone, 3,4-dimethoxyacetophenone (IV), present either with or without ketone I.



I II: R = H III: R = OH IV

The butterflies studied and their East African sources, as given to us by the suppliers of the insects, are listed in the Table. They were mailed to our laboratories in Seewiesen, Germany, and Ithaca, N.Y., where their hairpencils were removed and extracted with methylene chloride or carbon disulfide. Most butterflies arrived live or moribund and were vivisected, but hairpencils were also taken from specimens that arrived dead but still soft enough for dissection.

The extracts of the hairpencils were analyzed by thin layer chromatography (Eastman Chromagram Sheet 6060 Silica Gel with fluorescent indicator, developed with methylene chloride), and by gas chromatography-mass spectrometry (LKB-9000, 2 m × 0.25 mm glass column, 1% OV-1 on 80–100 mesh Gas Chrom Q). The occurrence of 3,4-dimethoxyacetophenone (IV) in an insect-derived secretion was noted for the first time in the course of this work. Naturally occurring I and IV were identified by direct comparison of their spectral and chromatographic properties with those of authentic synthetic samples. The Table summarizes the results thus obtained.

In the case of *A. niavius*, we observed a considerable variation in the ratio of these pheromonal constituents. Thus while ketone I and the acetophenone IV were found in approximately equal amounts in 3 of the 4 shipments of *A. niavius*, in the 4th shipment the ratio of I:IV was 1:10. Such fluctuations might result from differences in dietary intake of essential biosynthetic precursors of the constituents. Existing evidence suggests that danaid males may obtain alkaloidal precursors of I from plants that they visit after emergence as adults (e.g. species of the borraginaceous genus *Heliotropium*)[11–15]. A single male of *A. niavius* that we raised in the laboratory (on *Cynanchum vincetoxicum, syn. Vincetoxicum officinale*) was shown to have no detectable amounts of I in its hairpencils. Even IV might conceivably derive from an

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[19] Acknowledgment. We thank W. BAILEY and P. WHITE (Makerere, Uganda), and D. M. FANARA (Mombasa, Kenya) for supplying us with some of the species; G. HOWARTH, R. I. VANE-WRIGHT (London), and M. CLIFTON (Nairobi) for identification of the species; and RENATE LEFERT for assistance in the electrophysiological experiments in Seewiesen. Partial support by research grants from the National Institutes of Health (Grant Nos. AI-12,020 and AI-2908) is gratefully acknowledged.

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exogenous source. This compound has never been reported from an animal, but is known from the essential oil of an iris[16], and as a methylation product of the base hydrolyzate obtained from lignin[17].

Finally, it must be noted that there are other less volatile compounds in all of these secretions, many of which have not been characterized. The extract from *A. niavius*, for example, shows as many as 33 components[18], in sharp contrast to the pheromonal extracts of the species studied earlier[1-4]. We plan to pursue this analytical work further, in the hope that the obviously complex chemical language of these species will eventually by elucidated[19].

Zusammenfassung. Extrakte von Duftpinseln männlicher afrikanischer Schmetterlinge der Gattungen *Amauris* und *Danaus* wurden chemisch analysiert. Zwei Substanzen wurden isoliert: ein neues aromatisches Keton (3,4-dimethoxyacetophenon) und ein schon von anderen Danaiden bekanntes heterozyklisches Keton.

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Lobster Molting Hormones: Isolation and Biosynthesis of Ecdysterone

Crustacea in general have distinct molting glands (Y organs) comparable to those of insects. However, in the lobster, *Homarus americanus*, no structure comparable to the Y organ or any molting gland has been demonstrated to be present[1]. We felt that this physiological difference between lobsters and the other members of their class might also be reflected in the chemistry of the lobster molting hormone, both in its structure and biosynthesis.

Although ecdysterone has been extracted and identified as the molting hormone of many species of insects, it has been found in only a few selected crustacea. Ecdysterone has been isolated in low concentrations from the marine crayfish, *Jasus lalandei*[2], and the female marine crab, *Callinectes sapidus*[3]. The ecdysone concentration was reported to be much higher in post-molt crabs than in premolt (see Table).

In insects the biosynthetic precursors of ecdysterone are cholesterol and α-ecdysone. However, very little work has been accomplished on ecdysterone biosynthesis in crustacea. KING and SIDDALL[4] have shown that the shrimp, *Crangon nigricauda*, and the crab, *Uca pugilator*, convert α-ecdysone to ecdysterone very efficiently during premolt and molting periods. The uptake and turnover of ^{14}C cholesterol in Y organs of the crab, *Hemigrapsus nudus*, were studied as a function of molt cycle by SPAZIANI and KATER[5]. Labeled derivatives of cholesterol were found to co-chromatograph with ecdysone standards, but the derivative concentrations were too low for additional analyses and their identity as ecdysones was speculative.

We report here the isolation of a lobster molting hormone, ecdysterone, and the first definitive study of the uptake and biosynthetic conversion of cholesterol to ecdysterone in crustacea.

Premolt female lobsters were collected at Woods Hole, Massachusetts and kept in sea water aquaria until molting occurred. Their sample weights ranged from 475–525 g. 10 freshly molted females were ground up in a blender in methanol, Soxhlet extracted, and filtered. The resulting solution was extracted with hexane to remove the majority of lipids. Three counter-current distributions were then performed: butanol/water (1:1); chloroform/methanol/water (1:1:1); and chloroform/ethanol/water (1:1:1) followed by repeated liquid chromatography on deactivated (20% water) silicic acid with chloroform/ethanol (5:1) as eluent. Standardization of the columns for ecdysone separations was accomplished by high pressure liquid chromatography using two 2′ × 3/8″ Poragel PN columns[6]. Silylation of the ecdysone containing fractions with trimethylsilylimidazole (TMSIM) was followed by gas chromatography on a 2% SE-30 on Gas Chrom Q, 6′ glass column at 280 °C column temperature[7]. The addition of aliquots of more TMSIM at 15 min intervals while heating at 80 °C, followed by gas chromatographic analysis, revealed the transformation of the penta-TMS derivative of ecdysterone to the hexa-TMS derivative. By the use of gas chromatography less than 50 ng of ecdysterone can be detected. Final structure proof was accomplished by mass spectrometry[8,9]. We are able to isolate 55% of the molting hormone by this procedure as determined from ecdysterone spiked samples. The average quantity of ecdysterone found was 3 μg per freshly molted 500 g female lobster.

The procedure and results of the biosynthetic studies are as follows. The blood sinus of a premolt female lobster (512 g) was injected with 10 μC of [4-^{14}C] cholesterol in 0.3 ml of peanut oil[10]. After 16 h, the animal was sacrificed and the blood (46 ml) withdrawn. The muscle and viscera

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[10] The T. C. U. group has experienced a high degree of success in inducing the molting condition in lobsters by eye stalk ablation. Aritene, a form of microcrystalline collagen, was used as a hemostat (Aricon, Inc., Box 85, Fort Worth, Texas 76101, USA). Progress towards molt was followed by *x*-raying the gastroliths. Usually 30–45 days was required for an animal with no initial gastroliths to molt. For lobsters in the 400–500 g range a length of about 1.6 cm for the gastroliths indicated an imminent onset of molting.