

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 recogni-

zable 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).

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<sub>4</sub> and H<sub>5</sub> protons are easily recognized at  $\delta$  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<sub>1</sub> is *meta* coupled with H<sub>3</sub>; H<sub>3</sub> in turn gives a quartet due to *meta* coupling with H<sub>1</sub> and *ortho* coupling with H<sub>2</sub>; H<sub>2</sub> appears as a doublet couplet only with H<sub>3</sub>. 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<sup>12</sup> 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-Chlorbiphenyl und 4,4'-Dichlorbiphenyl mittels NMR-Spektroskopie.

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27 November 1973.

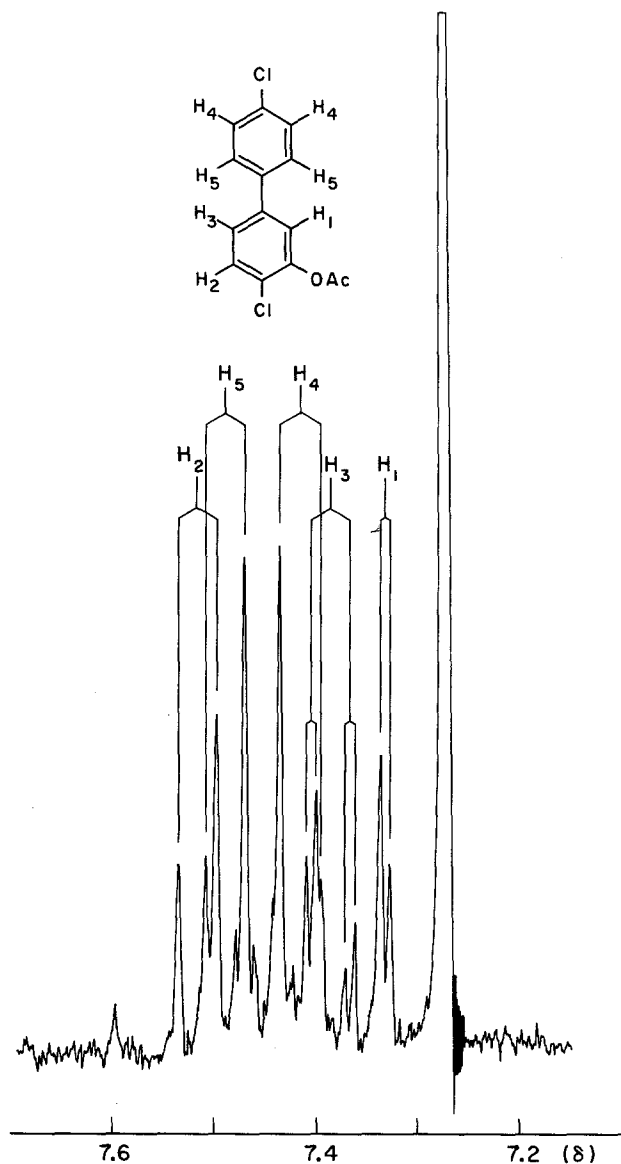


Fig. 2. NMR-spectrum of 3-acetoxy-4,4'-dichlorobiphenyl.

<sup>12</sup> O. HUTZINGER, S. SAFE and V. ZITKO, unpublished results.

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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<sup>1-4</sup>, prompted by earlier behavioral investigation of these insects<sup>5</sup> 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<sup>6,7</sup>. The antennae are electrophysiologically sensitive to the ketone<sup>8,9</sup>. Other danaid butterflies are known<sup>10</sup> 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.<sup>11</sup> 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



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

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<sup>18</sup>, in sharp contrast to the pheromonal extracts of the species studied earlier<sup>1-4</sup>. We plan to pursue this analytical work further, in the hope that the obviously complex chemical language of these species will eventually be elucidated<sup>19</sup>.

*Zusammenfassung.* Extrakte von Duftpinseln männlicher afrikanischer Schmetterlinge der Gattungen *Amau-*

*ris* 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<sup>1</sup>. 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*<sup>2</sup>, and the female marine crab, *Callinectes sapidus*<sup>3</sup>. 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  $\alpha$ -ecdysone. However, very little work has been accomplished on ecdysterone biosynthesis in crustacea. KING and SIDDALL<sup>4</sup> have shown that the shrimp, *Crangon nigricauda*, and the crab, *Uca pugilator*, convert  $\alpha$ -ecdysone to ecdysterone very efficiently during premolt and molting periods. The uptake and turnover of <sup>14</sup>C cholesterol in Y organs of the crab, *Hemigrapsus nudus*, were studied as a function of molt cycle by SPAZIANI and KATER<sup>5</sup>. 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' x 3/8" Poragel PN columns<sup>6</sup>. 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<sup>7</sup>. 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<sup>8,9</sup>. 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-<sup>14</sup>C] cholesterol in 0.3 ml of peanut oil<sup>10</sup>. After 16 h, the animal was sacrificed and the blood (46 ml) withdrawn. The muscle and viscera

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<sup>5</sup> E. SPAZIANI and S. B. KATER, *Gen. comp. Endocr.* 20, 534 (1973).

<sup>6</sup> D. A. SCHOOLEY and K. NAKANISHI, in *Modern Methods of Steroid Analysis* (Ed. E. HEFTMANN; Academic Press, New York, N.Y. 1973), p. 37.

<sup>7</sup> N. IKEKAWA, F. HATTORI, J. RUBIO-LIGHTBOURN, H. MIYAZAKI, M. ISHIBASHI and C. MORI, *J. Chromat. Science* 10, 233 (1972). — H. MIYAZAKI, M. ISHIBASHI and C. MORI, *Analyt. Chem.* 45, 1164 (1973).

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<sup>9</sup> D. H. S. HORN, in *Naturally Occurring Insecticides* (Eds. M. JACOBSON and P. G. CROSBY; Marcel Dekker, New York, N.Y. 1971), p. 333. — K. NAKANISHI, XXIII vol. *Int. Congr. Pure and Applied Chemistry* (Butterworths, London 1971), vol. 3, p. 27.

<sup>10</sup> 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.