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.

Quantities of ecdysones in various animals from natural waters

Whole animal	Stage	Weight extracted (kg)	Ecdysone	Ecdysone concentration (mg/kg)
Homarus americanus[14]	Postmolt	5	Ecdysterone	0.006
Jasus lalandei[2]	Intermolt	1,000	Ecdysterone	0.002
	Intermolt	3,000	2-Deoxycrust-ecdysone	0.00007
Callinectes sapidus[3]	Premolt 'Green'	25	Inokosterone	0.005
	Premolt 'Peeler'	25	Inokosterone	0.020
			Ecdysterone	0.004
	Postmolt 'Soft Shell'	25	Ecdysterone	0.280
			Makisterone A	0.024
Mytilus edulis[15]	—	—	N.I.[a]	—
Carcinus maenas[15]	Intermolt	—	N.I.	—
Crangon vulgaris[16]	Intermolt	3,000	N.I.	—

[a] Not identified.

were separated from the shell and extracted 3 times with *n*-butanol in a blender. The combined butanol extracts were concentrated at 55 °C under vacuum, yielding a red oil. The latter was taken up in ethyl acetate and extracted 3 times with water. The water extracts were backwashed 3 times with ethyl acetate and concentrated to dryness under an N_2 stream. The residue of the aqueous extract was stirred first with cold petroleum ether, then with cold acetone. The acetone extract was filtered and evaporated to dryness.

Thin layer chromatography (TLC) of a small portion of this material on silica gel with chloroform/methanol/acetone (6:2:1) as eluent gave 3 radioactive spots; one of which corresponded exactly with the retention time of an ecdysterone standard. This fraction was subsequently isolated by preparative TLC to give the crude ecdysterone. Structural identification was completed by adding 1 mg of ecdysterone standard to the crude ecdysterone mixture and acetylating with acetic acid – pyridine at room temperature for 2 h[11]. Radio TLC of the resulting mixture demonstrated that it contained 4 radioactive products which co-chromatographed with the 4 products produced on acetylation of authentic ecdysterone. These results were further verified by scintillation counting of the active fractions on the TLC plate.

Recently it has been reported that injection of ecdysterone into both normal and destalked intermolt lobsters induced precocious molting[12]. This work supports our results that ecdysterone, by itself, or in combination with other ecdysones, is playing a major role in the molting process of the lobster.

Injection of ecdysterone into other arthropods such as horseshoe crabs, barnacles, scorpions, and spiders also initiates molting[13]. These results and those of the extraction and identification of ecdysterone from both insects[9] and crustaceans (Table), strengthen the hypothesis that ecdysterone is a general arthropod molting hormone[17].

Résumé. L'ecdystérone, hormone de mue des insectes, a été extraite de homards femelles (*Homarus americanus*) venant de muer. Sa concentration moyenne est de 6μg/kg de homard vivant. L'assimilation de cholestérol (4-^{14}C) par des homards femelles avant la mue et la biosynthèse d'ecdystérone à partir de ce précurseur est démontrée.

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Adaptive Metabolic Variation of Chromosome Forms in Mole Rats, *Spalax*

Speciation through chromosomal rearrangement is widespread in animals[1], yet the significance of chromosome variation is still largely speculative and needs further elucidation. MAYR[2] suggested that it may act both as an isolating mechanism and a protection for favourable supergenes, as initially pointed out by WALLACE[3]. Evidence suggesting association between karyotypic variation and ecophysiological adaptation(s)