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Pregnanes from defensive glands of a belostomatid bug[1]

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Abstract. The aquatic bug *Abedus herberti* (Hemiptera: Belostomatidae) secretes a mixture containing four pregnanes (desoxycorticosterone (**I**), pregnenolone (**II**), progesterone (**III**), and 3α-hydroxy-pregn-5-en-20-one (**IV**)) from its cephalic glands. Pregnanes had previously been characterized from the defensive glands of aquatic beetles (Dytiscidae) and shown to be deterrent to fish. It may be specifically under predation pressure from fish that *A. herberti* and Dytiscidae evolved their comparable defenses.

Key words. Hemiptera; C_{21}-steroids; chemical defense; vertebrate hormones; parallel evolution.

Numerous freshwater arthropods are protected against predation by distasteful or otherwise deterrent glandular products (see review by Scrimshaw and Kerfoot[2]). Of particular interest has been the characterization of steroids, primarily pregnanes (C_{21}-steroids), from the prothoracic defensive glands of dytiscid beetles[2]. Experimental evidence indicates that these steroids serve specifically for protection against fish[3-5]. We here report a remarkable case of chemical evolutionary parallelism, the production of pregnanes by *Abedus herberti*, an aquatic hemipteran (Belostomatidae).

Belostomatid bugs, including adults and nymphs, have a pair of small sac-like glands, oriented lengthwise in the cranium to the sides of the midline, and opening by two small pores near the base of the beak. The glands are lined with cuticle and bear glandular cells on their ventrolateral surface[6,7]. In *A. herberti* these glands produce a viscous white fluid, which the bugs discharge when they are manually disturbed. The fluid oozes from the pores, spreading over the head and into the coxal cavities of the forelegs.

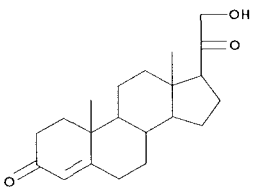
To obtain secretion for analysis, adult *A. herberti* of both sexes, collected from a number of streams in Pima County, Arizona, were held by hand and their emitted fluid was taken up in calibrated capillary tubes (output per individual ≈ 3.5 µl). The capillaries were crushed, the residue extracted with methylene chloride, and the extract dried over magnesium sulfate. Gas chromatographic analysis (Varian 2100 chromatograph, flame ionization detector, 3% OV-17 on 100/120 Gaschrom Q in a 3 m × 6 mm glass column) indicated the presence of a single major component (>90%) and a number of minor components (~1% each). Gas chromatographic/mass spectral analysis (Finnegan 3000 Series GCMS) gave mass spectra corresponding closely to that reported for desoxycorticosterone (cortexone, DOC) in the case of the major component, to pregnenolone for two of the minor components, and to progesterone for a third[8].

A portion of the methylene chloride solution was evaporated and the residue taken up in acetonitrile. Preparative high performance liquid chromatography (Waters µ-Bondapac C-18 column, 55% acetonitrile/45% water, UV detection at 254 nm) provided a sample of the major component, identified as desoxycorticosterone (**I**) by direct comparison of its 80 MHz proton NMR, infrared, and ultraviolet absorption spectra with those of an authentic sample. Co-chromatography (TLC and GC) with the authentic sample confirmed this identification.

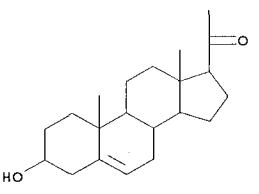
The least polar HPLC fraction contained a mixture of three of the minor components, two of which were identified as pregnenolone (**II**) and progesterone (**III**) by GC/MS analysis (including co-injection with authentic samples). The third component, isolated by preparative thin layer chromatography on a 0.25 mm silica gel plate, had a proton NMR spectrum similar to that of pregnenolone, but with its C-3 proton appearing as a narrow 5-line multiplet at δ 4.04. This observation, indicative of a 3α-hydroxyl group, suggested the compound to be 3α-hydroxypregn-5-en-20-one (**IV**). Direct comparison with an independently synthesized sample of this pregnenolone epimer confirmed this identification[9].

The steroidal output of *A. herberti* is substantial. An extract from the secretion of 29 bugs yielded 3 mg of purified desoxycorticosterone (**I**), corresponding to ~0.1 mg per individual. This is in line with the amount of this chemical reported for the defensive glands of dytiscid beetles (0.03–0.40 mg)[2].

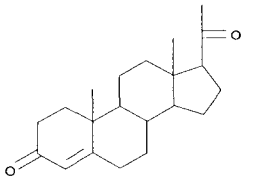
The concordance of the *A. herberti* secretion with that of dytiscid beetles is not complete. Of the four steroids isolated from the bug, only the principal component (**I**) has been found in the beetles. This difference, however,



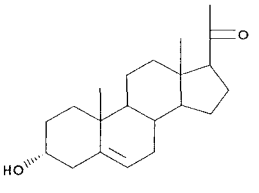
I



II



III



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should not obscure the close similarity of the defensive chemistry of these aquatic insects. One other species of *Abedus*, *A. indentatus*, which we observed at various localities in California, also emits a white secretion from its cephalic glands when disturbed.

While direct evidence for the defensive function of the *A. herberti* glands remains to be obtained, desoxycorticosterone (**I**) had previously been shown to be a potent feeding deterrent to fish[5]. Fish, in fact, could have been the major predators that forced the evolution of the steroidal glands of belostomatids and dytiscids.

Insects are unable to synthesize steroids, except by modification of other steroids[10]. In dytiscids and belostomatids, which are both predaceous, the common precursor of the defensive pregnanes could be cholesterol. What the two groups could therefore be envisioned to have evolved in parallel, is simply the capacity to degrade the C_{27} steroidal side chain.

Terrestrial insects also use steroids for defense. The compounds include, among others, cardenolides and lucibufagins, which the insects may produce endogenously or acquire from the diet[11–14]. Pregnanes themselves as exocrine defensive agents in terrestrial insects have been reported only from certain silphid beetles[15,16]. Dytiscids and belostomatids are not permanently restricted to water, but fly as adults (they are commonly taken at lights at night). One wonders whether the pregnanes serve them also for defense against aerial predators (birds, bats?), or against diving predators (birds?) under water.

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