ly to means obtained in the field on many individuals with mercury thermometers (*4*). Such data further support the validity of reptile body temperatures obtained in laboratory thermal gradients (*7*).

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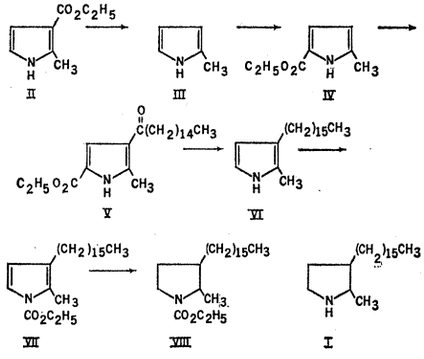
Fire Ant Venom: Synthesis of a Reported Component of Solenamine

Abstract. *2-Methyl-3-hexadecylpyrrolidine was synthesized. It has hemolytic activity as has solenamine, but it apparently does not have the insecticidal activity of the fire ant venom. As judged by gas chromatography, the structure is not a component of solenamine.*

The venom of the imported fire ant (*Solenopsis saevissima richteri* Forel) (*1*) possesses considerable insecticidal activity toward the boll weevil *Anthonomus grandis* Boheman and the rice weevil *Sitophilus oryzae* (L.). This venom is composed of two phases, namely an alkaline carrier in which fine droplets of a greater density are suspended (*1*). The latter material apparently is associated with the insecticidal activity and exhibits infrared absorption at 5.70 μ. Blum *et al.* (*1*) obtained this venom, referred to as "milked venom," by stroking the abdomens of major fire ant workers with a fine capillary until the stings were everted. The droplets were then collected in the capillary from the end of the sting.

From homogenates of whole ants, Adrouny *et al.* (*2*) prepared a material that possessed the potent necrotoxicity of the milked venom. This material (a mixture) was termed "solenamine" to avoid its premature identification with the toxin. Adrouny reported that solenamine was indeed the principal—and very likely the sole—toxic component of the venom. The consistent similarity in chemical and biological properties between milked venom and solenamine led him to expect the latter to perform also as an insecticide (*2*).

By gas chromatography, solenamine was found to be composed of two amines for which the structures 2-methyl-3-hexadecylpyrrolidine (I) and the corresponding 3-pyrroline were proposed (*2*). Blum and his associates (*1*) had ascribed the insecticidal activity to a carbonyl-bearing compound that Adrouny *et al.* did not mention.



Pyrrolidines and 3-pyrrolines have been previously investigated for their insecticidal activities. Studies of the common firebrat *Thermobia domestica* (Packard) with 2-substituted pyrrolidines and their corresponding 3-pyrrolines (*3*), as well as studies with nicotine, were reported.

To synthesize compounds with the two proposed structures, I obtained 2-methyl-3-hexadecylpyrrole from which the pyrrolidine could be obtained by hydrogenation and from which the 3-pyrroline could be prepared by reduction with zinc and acid. Addition of bromine to vinyl acetate, followed by addition of ethyl acetoacetate and ammonia, produced crude 2-methyl-3-(ethoxycarbonyl)-pyrrole (II); this compound was saponified and decarboxylated yielding 2-methylpyrrole (III) (*4*). The available α-position was blocked by phosgenation in the presence of a tertiary amine and then by treatment with ethanol. The resulting 2-methyl-5-(ethoxycarbonyl)-pyrrole (IV) has on several previous occasions been subjected to the conditions of electrophilic substitution, with the position of the new substituent being assigned *ortho* to the 2-methyl group (*4, 5*). The structural assignments apparently were based on mechanistic reasoning. In this case, treatment of 2-methyl-5-(ethoxycarbonyl)-pyrrole with palmitoyl chloride in the presence of aluminum chloride produced 2-methyl-3-palmitoyl-5-(ethoxycarbonyl)pyrrole (V) [melting point (m.p.), 110° to 111°C] in high yield (*6*). I justified the assignment of the 3-, rather than the 4-, position by the proton nuclear magnetic resonance (NMR) spectrum of the subsequently obtained 2-methyl-3-hexadecylpyrrole (VI). This latter intermediate was synthesized by saponification and decarboxylation to 2-methyl-3-palmitoylpyrrole (m.p., 65.5° to 66.5°C), which gave 2-methyl-3-hexadecylpyrrole upon reduction with lithium aluminum hydride. Purification by sublimation (100°C, 0.05 mm-Hg) produced a white crystalline solid (m.p., 48° to 49.5°C) which turned brick-red when exposed to air at ambient temperature overnight. The NMR spectrum revealed two aryl protons at 5.81 and 6.32 parts per million (ppm) (*6*). These same protons appear at 5.82 (β C-H) and 6.28 ppm (α C-H) in 2,3-dimethylpyrrole, but 2,4-dimethylpyrrole absorbs at 5.57 and 6.08 ppm (*7*). Thus there is no doubt that I have synthesized a 2,3-dialkylpyrrole, that is, electrophilic substitution occurred *ortho* to the methyl group of 2-methyl-5-(ethoxycarbonyl)-pyrrole.

All attempts to reduce 2-methyl-3-hexadecylpyrrole to the 3-pyrroline by known procedures with zinc and acid (*7, 8*) were uniformly unsuccessful. Although alkylpyrroles are very acid-labile, NMR spectra of protonated alkylpyrroles can be obtained if concentrated acid solutions of these pyrroles are used (9). However, an attempted reduction in concentrated acid was not successful.

This pyrrole was quite resistant to hydrogenation with a rhodium catalyst at 3 atm; these conditions are sufficient to reduce 2,5-dimethylpyrrole, for example (*10*). Reaction of the pyrrole with potassium, followed by treatment with ethyl chloroformate, produced 1-(ethoxycarbonyl)-2-methyl-3-hexadecylpyrrole (VII) (purified by column chromatography and sublimation at 100°C and 0.05 mm-Hg; m.p., 35.5° to 36.5°C). This compound was easily hydrogenated to the pyrrolidine (VIII) with 5 percent rhodium-on-alumina catalyst in ethanol at 3 atm. The ethoxycarbonyl group was removed with hydrobromic acid, and the pyrrolidine (I) was purified by sublimation (80°C at 0.025 mm-Hg; m.p., 34° to 37°C). Gas-phase chromatography of this material revealed two components (~4 : 1) with close retention times. The NMR spectrum of this mixture showed the absence of aryl and olefinic absorption.

Chemical analyses were satisfactory for the pyrrolidine itself, an *N*-phenylurea derivative (obtained in 92.5 percent yield), and for an oxalic acid salt as well. Hence I believe that the minor component is the *trans* isomer.

The pyrrolidine exhibited hemolytic properties when tested with human, chicken, and pigeon blood. Solenamine was also hemolytic in a test with rabbit blood (*2*). However, residue tests on houseflies, *Musca domestica* (L.), both DTT-susceptible and -resistant, showed no insecticidal action, although fire ant venom is reported to be active against this species of insect. Adrouny, by means of a gas-chromatographic comparison of my material with solenamine, demonstrated that 2-methyl-3-hecadecylpyrrolidine (*cis* or *trans*) is not a component of the insect extract, solenamine. The retention time of one of the components of solenamine is quite close to the peaks of that of the pyrrolidine mixture; the 3-pyrroline, or a structure closely related to these, may yet be correct.

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3 May 1967

Interferon Binding: The First Step in Establishment of Antiviral Activity

Abstract. *Chick cells incubated at 1°C with interferon fail to develop antiviral activity, but this activity appears subsequent to a 7-hour incubation at 37°C after removal of interferon by repeated washings. Treatment with actinomycin D blocks the development of the latter activity. Cells incubated with interferon at 1°C for up to 1 hour and then washed and incubated for 2 hours at 37°C develop a degree of antiviral activity proportional to the concentration of interferon at initial incubation; at any concentration, the antiviral activity increased with the duration of initial incubation at 1°C, but a maximal response was reached at 10 or 20 minutes. Treatment with trypsin after incubation with interferon at 1°C inhibited development of antiviral activity. Interferon is rapidly bound to a superficial cell site, and this binding is necessary for development of antiviral activity in chick cells.*

The induction of antiviral activity in cells by interferon is a complex process. The first step takes place at 1°C; the second step, which apparently involves RNA and protein synthesis, requires that cells treated with interferon be incubated for some time at 37°C before virus infection (*1*). I studied the first step. When incubated with interferon at 1°C and then washed, the cells developed resistance to viral infection after incubation at 37°C for 1 hour or more. Because there is no detectable uptake of interferon by cells during the initial incubation period (*2*), interferon possibly initiates a series of reactions which lead to antiviral activity but does not itself bind to cells. My studies show, however, that the development of antiviral activity subsequent to exposure of cells to interferon at 1°C requires the binding of interferon to a superficial cell site.

Monolayers of chick embryo fibroblasts (CEF) were prepared (*3*). Semliki forest virus (SFV) was prepared in CEF treated with actinomycin D. Plaque assays of SFV titers were performed on CEF (*3*). Each milliliter of the solution of partially purified interferon contained 10,000 units of interferon and 179 μg of protein, a unit being the amount which inhibited development of SFV plaques by 50 percent (*4*).

The CEF monolayers were incubated with or without 3 units of interferon per milliliter for 4 hours at 1°C or 37°C and then washed six times with cold Eagle's medium. Some monolayers incubated at 1°C or 37°C were immediately infected with SFV at a virus : cell multiplicity of 40 : 1. The virus was adsorbed at 37°C for 1 hour; the cells were washed six times and incubated in Eagle's medium with 10 percent calf serum for 7 hours, at which time the log phase of virus growth ended. The monolayers and fluids were then frozen, thawed, and assayed for virus. No antiviral activity was present in cells kept at 1°C (Table 1), while those at 37°C had developed strong antiviral activity.

In the same experiment, other monolayers incubated at 1°C or 37°C with or without interferon for 4 hours were washed and then incubated for an additional 7 hours at 37°C. The cells were then infected and treated as in the other group. In the cells incubated with interferon at 1°C, the antiviral activity was approximately as strong as that in other cells incubated with interferon for 4 hours at 37°C (Table 1). Cells incubated with actinomycin D fail to develop antiviral activity when subsequently incubated with interferon (*3*). When actinomycin D was added to cells incubated with interferon at 1°C and then washed, no antiviral activity developed (Table 1). My results confirmed those of other workers who showed that after incubation of cells with interferon at 4°C a period of incubation at 37°C was required for development of antiviral activity (*5*). In addition, my findings showed that, whatever interferon-cell interaction had taken place in the cold, the development of antiviral activity could be blocked by actinomycin D.

The nature of the cell-interferon interaction at 1°C was further delineated by the following experiments. Various concentrations of interferon were incubated with CEF at 1°C for 1 to 60 minutes. The cells were washed 6 times with 10 ml of cold medium, incubated at 37°C for 2 hours, and then infected with SFV. No antiviral activity was present in the tissue cultures after the 2-hour incubation at 37°C; this indicated that no release of previously