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CONSTITUENTS OF THE VENOM OF A SOUTH AFRICAN FIRE ANT (*SOLENOPSIS PUNCTATICEPS*)

2,5-DIALKYLPYRROLIDINES AND -PYRROLINES, IDENTIFICATION AND SYNTHESIS

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Abstract—Structures of a series of 2,5-dialkylpyrrolidines and -pyrrolines isolated from the South African fire ant, *Solenopsis punctaticeps*, are determined by gas chromatography-mass spectrometry and confirmed by synthesis using the Hofmann–Löffler reaction on the corresponding primary amine.

INTRODUCTION

Because of the potency of their stings, many large species of ants in the genus *Solenopsis* are referred to as fire ants. The venoms of these species are potently necrotizing[1] and hemolyzing,[2] and are singular in containing only trace amounts of proteinaceous constituents.[3] Fire ant venoms are dominated by a series of 2-methyl-6-alkylpiperidines[3-6] which appear to be primarily responsible for the necrotic lesions[1,7] that result from their subdermal injection into mammals. Examinations of a large number of South American species have so far demonstrated that the 2-methyl-6-alkylpiperidine theme is a constant character of fire ant venoms.§

In this paper we analyze the venom of *Solenopsis punctaticeps*,[8] a South African species in this worldwide genus.[9] This species does not belong to the group of true fire ants that is primarily limited in its distribution to the New World tropics. Instead, it appears to be related to the diminutive species in this genus which generally form small cryptic colonies and are not renowned for their stinging propensities. We have observed that workers of this species can indeed sting, but aside from slight itching and erythema, the reaction of humans to the venom is mild compared to that encountered with the stings of true fire ants. As shown below, the venom of *S. punctaticeps* does not contain any of the dialkylpiperidines identified in fire ant venoms, but rather, is fortified with 2,5-dialkylpyrrolidines and -pyrrolines.

DISCUSSION

The pure venom, which was collected as described earlier,[10] consists of a two-phase system in which fine droplets are suspended in a "carrier", in much the same way as venoms of true fire ants. Based on glc analysis of model compounds (see below) approximately 60 μg of the basic components were obtained from each ~0.5 mg ant (12% of the "dry" weight).

Figure 1 shows a methylene chloride extract of the venom injected directly on a 10% SP-1000 liquid phase. Mass spectra of the later-eluting components (peaks 5, 6 and 8) exhibit certain similarities to spectra of 2,6-dialkylpiperidines from other species of fire ants,[3-6] namely, two intense peaks resulting from α-cleavage of the side chains and weak molecular ions of odd mass. Acetylation, using pyridine and acetic anhydride, caused peaks 5, 6 and 8 to elute about 50° higher than the corresponding free bases on the less polar OV-17 phase, confirming their secondary amine character. In this case, consideration of the combination of ions from the free bases leads to the conclusion that the substances are pyrrolidines rather than piperidines. Thus peaks 5 and 6 show mass spectra (Figs. 2 and 3, respectively) that allow their easy formulation as isomers **1** and **2** respectively.

The mass spectrum of peak No. 8 shows analogous

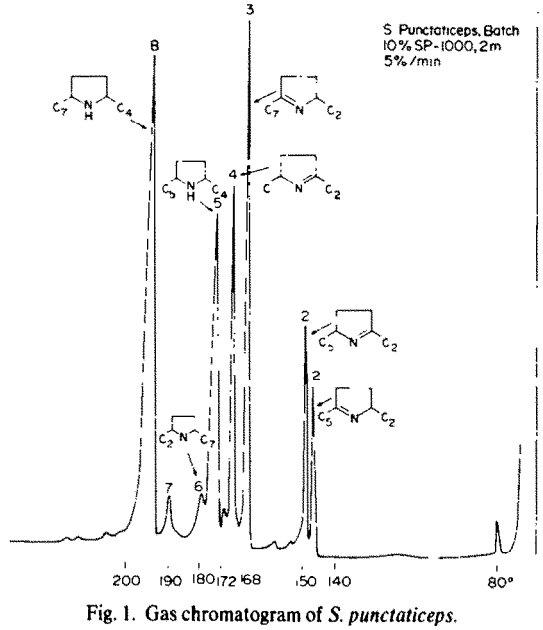

Fig. 1. Gas chromatogram of *S. punctaticeps*.

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§J. G. MacConnell, M. S. Blum, W. F. Buren, R. N. Williams and H. M. Fales, *Toxicon* in press.

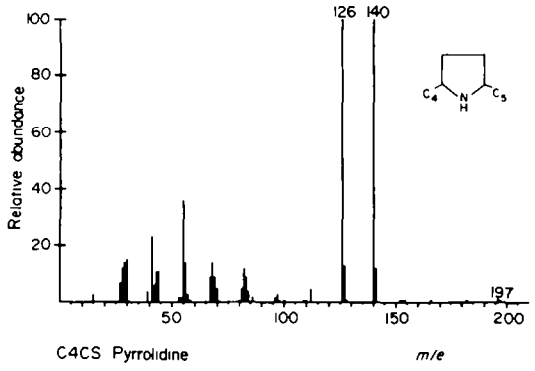


Fig. 2. Mass spectrum of 2-butyl-5-pentylpyrrolidine.

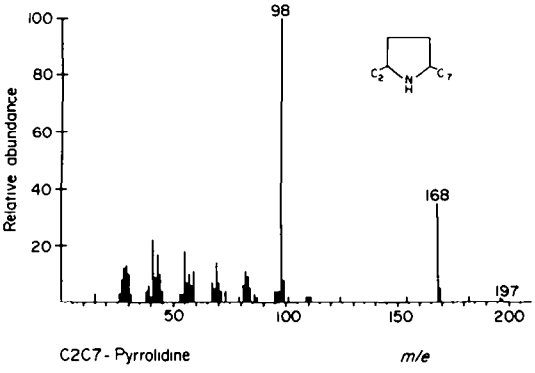
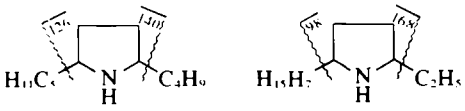


Fig. 3. Mass spectrum of 2-ethyl-t-heptylpyrrolidine.



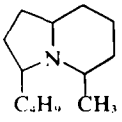
m.w. 197 m.w. 197

1 2

α-cleavage ions at m/e 126 and 168 and a molecular ion at m/e 225 so it is the C_4, C_7-substituted homolog. Traces of a C_2, C_5-substituted homolog were also observed in a batch from an earlier colony.

Spectra of the N-acetyl derivatives of these saturated pyrrolidines show weak molecular ions, ready cleavage of the side chains from both positions and finally, loss of ketene from these ions, confirmed by intense metastables.

Interestingly, a compound assigned structure 1 had been identified earlier in pheromones produced by Pharaoh's ants.[11] Identification was based on its mass spectrum, substantially the same as Fig. 2, as well as its apparent biogenic relationship[11] to pheromone 3. We have sought, but find no evidence in our ant species, for octahydroindolizine structures such as 3.



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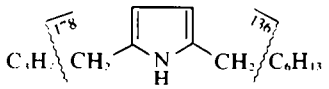
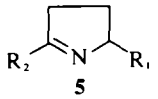
Examination of the side-chains of the various components reveals that they differ by 2-carbon units thus suggesting their origin from acetate. If this is so, they should not be branched. Carbon-skeleton hydrogenolysis[12] over 1% Pd on Gaschrom P at 320° confirmed this fact; only n-C_{11}–C_{15} hydrocarbons identified by retention time and mass spectra were obtained, along with a series of pyrroles containing all the expected side chains including those of the unsaturated components in peaks 1–4 (see below). The pyrroles, which predominated over the alkanes in our catalytic system, were easily identified by their intense molecular ions and ions due to benzylic cleavage of either side chain (4).



m/e 221

4

Formation of the pyrroles and n-alkanes also proves that the side chains are not both located on the same alpha carbon atom of the pyrrolidine nucleus. This fact is confirmed by consideration of the properties of the two isomeric pairs of unsaturated components found in peaks 1–4 (Fig. 1). These compounds exhibited more complex mass spectra (e.g. Figs. 4 and 5) and showed relatively more intense molecular ions that indicate one additional point of unsaturation. From their early elution on the polar SP-1000 phase relative to their saturated counterparts, they were considered to be Δ^1-pyrrolines (5). The



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latter, lacking NH groups, should elute earlier since they are incapable of H-bonding to the liquid phase. Further proof of the existence of the C=N linkage in both pairs of isomers was obtained by ready reduction of these components in the mixture to their saturated analogues using sodium borohydride or borodeuteride, the latter to allow distinction from endogenous saturated components. However, reduction of the unsaturated isomers in peaks 1 and 2 (Fig. 1) gives not one but two glc peaks of nearly equal intensity separated by 5°. Both peaks show identical mass spectra corresponding to the trace of C_2,C_5-substituted pyrrolidine isolated from an earlier collection. Reduction of the components of peaks 3 and 4 likewise gave two glc peaks that show mass spectra identical to that of the C_2, C_7-substituted pyrrolidine in peak 6. These pairs of compounds obtained on reduction are obviously *cis* and *trans* isomers, proving unequivocally that the Δ^1-pyrrolines from which they originate are substituted in the 2,5- rather than 2,2-position.

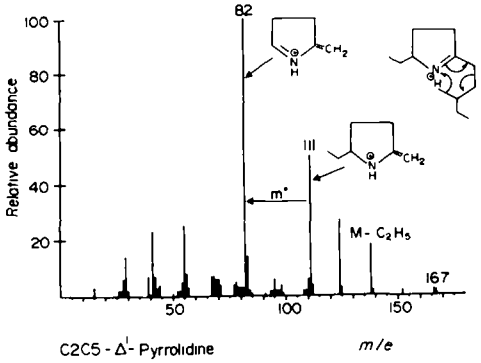


Fig. 4. Mass spectrum of 2-ethyl-5-pentyl-5-pyrroline.

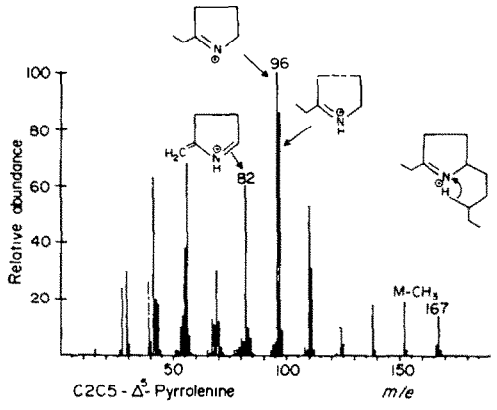
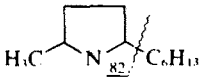


Fig. 5. Mass spectrum of 2-ethyl-5-pentyl-1-pyrroline.

Reduction to nearly equal amounts of *cis* and *trans* isomers suggests that there is little energy difference in the transition state leading to the *cis* and *trans* forms of the 2,5-disubstituted pyrrolidine ring. This is in some contrast to the piperidine series[3] where reduction of Δ^1-2,6-disubstituted piperideines provides overwhelmingly the *cis* form.

Interestingly, on acetylation with pyridine and acetic anhydride these unsaturated amines also appear as acetates on gc–ms. These enamine amides may form under the anhydrous conditions used or they may form in the flash heater of the gas chromatograph itself. Under Schotten–Baumann conditions (aqueous alkali) ketoamides are the expected product.[13]

Finally, analysis of the mass spectra from peaks 1 and 2 allows a tentative assignment to the location of the double bond toward the appropriate side chain. Thus, scans of peak 1 (Fig. 4) show a molecular ion at *m/e* 167, an intense ion at *m/e* 82 and an odd-electron ion at *m/e* 111. The ion at *m/e* 82 might be interpreted as indicating that this component contained 1- and 6-carbon chains attached at the 2- and 5-positions (**6**).



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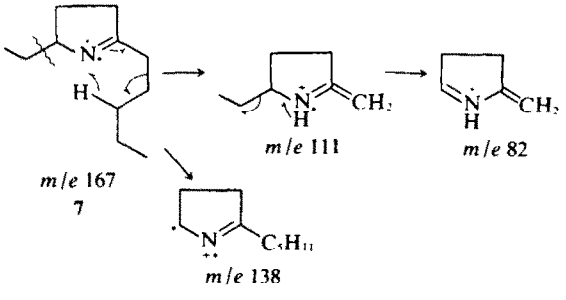
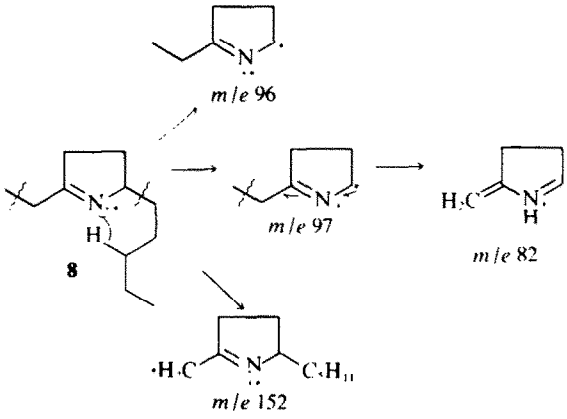
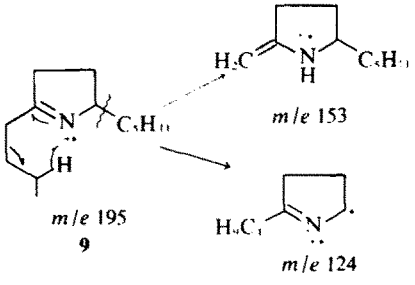
However, this seemed unlikely since no corresponding saturated component was observed on reduction with sodium borohydride. Alternatively, if 2- and 5-carbon chain substituents were present and if in addition the double bond were directed toward the 5-carbon side chain (**7**), a McLafferty rearrangement of the γ-hydrogen would lead to the ion of *m/e* 111. Subsequent α-cleavage of the 2-carbon side chain from this ion would provide an ion of *m/e* 82 and a metastable ion at apparent mass 60.6



confirms this fragmentation. Simple allylic cleavage of the other side chain would account for the prominent ion at *m/e* 138 (M–Et).

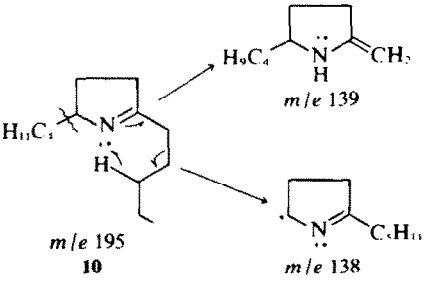


The mass spectrum of the isomer (**8**) in peak 2 reveals intense ions at *m/e* 96 and 97 (Fig. 5), the former presumably resulting from analogous allylic cleavage of the C_5 side chain in structure **8** and the latter from the same cleavage with a hydrogen transfer to nitrogen forming the allylic radical ion of *m/e* 97. This ion then loses a Me group to produce *m/e* 82 as in the previous case. Simple allylic cleavage of the C_2 side chain produces on ion at $M - Me_3$ (*m/e* 152).



Peaks 3 and 4 containing the C_2, C_7 homologues show mass spectra containing ions entirely analogous to those of peaks 1 and 2 respectively. They are, therefore, assigned the structures shown in Fig. 1.

Chromatograms from an earlier collection showed an intense peak eluting between No. 2 and No. 3 that contained the C_4, C_5 side chain isomers of **7** and **8**. Since the side chains are nearly the same length in this case little gc separation was achieved. In these isomers δ-hydrogens are available on *both* side chains so that when the double bond is directed toward the C_4 side (**9**), an ion due to the McLafferty rearrangement is expected at *m/e* 153 (M-42). Allylic cleavage of the C_5 group should give an ion at *m/e* 124. Its isomer (**10**) should show corresponding



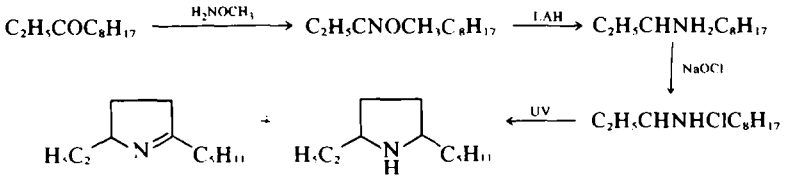
ions at *m/e* 139 (M-56) and *m/e* 138. Close inspection of scans from the front and back of the peak shows variations in the intensity of these ions which allows the tentative assignment of structure 10 to the earliest eluting isomer.

These fragmentations are similar but not identical to those assigned to Δ^1-piperideines found in related species.[3] In the pyrrolines, it is apparently not necessary to invoke extensive hydrogen migration as it was to explain the base peak occurring at *m/e* 110 in 2-methyl-6-n-undecyl-Δ^1-piperideine.

Synthesis

The above structure assignments are based entirely on mass spectral similarities with the known 2,6-dialkylpiperidines and since insufficient venom was available for further degradative studies, confirmation of the structures was sought through synthesis.

Synthesis of the C_2, C_5 homologue was carried out as follows:

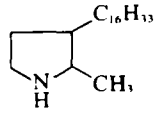


LAH reduction of the methoxime of 3-undecanone, in contrast to the oxime, provided a good yield of 3-undecylamine. Conversion to the N-chloroamine also proceeded well, the product being identified by gc–ms. Few examples of the Hofmann–Löffler reaction on primary N-chloramines exist because of their tendency to disproportionate in weakly acid solutions. However, Schmitz and Murawski have shown that the reaction proceeds well in concentrated acid using ferrous salts as initiators.[14] In our hands, photolysis gave better results. In such cases a pyrrolidine is expected rather than a piperidine[15] and the reaction proceeded to give a mixture of 2 isomers (*cis* and *trans*) in 60% yield as judged by gc–ms with some of the primary amine being recovered.† The two isomers were produced in about equal amounts and had the same retention times and mass spectra as those obtained by borohydride reduction of the C_2,C_5-unsaturated component of the venom. Further purification proved difficult (the compounds appear to oxidize easily in air) and only a few mg of product were finally isolated. However, the isomers can be separated on alumina; that eluting earliest corresponds to that of shortest GC retention time on SP-1000 or Pennwalt phase (Experimental). Nmr spectra of the pure isomers were very similar, even in the region of the protons α to the nitrogen and no assignment of stereochemistry was possible on this basis. A crystalline tosylate of the early-eluting isomer (m.p. 78–80°) was prepared and its nmr shows all expected resonances (Experimental) but unfortunately it exists as a twinned crystal and X-ray diffraction is not feasible.

The early-eluting isomer was very tentatively assigned the *cis* stereochemistry by analogy with the known *cis*- and *trans*-2,6-dimethylpyrrolidines, samples of which were kindly donated to us by Prof. R. Hill (Chemistry Department, University of Georgia) in the form of their crystalline picrates, m.p. 116–118° (lit. 120–1)[13] and 125–126° (lit. 132.5–134.5)[13] for the *cis* and *trans* isomers respectively. The *cis* isomer elutes earlier (10.7 min) than the *trans* (11.7 min) on a 10% Pennwalt column at 110°. On the same column at 180°, the synthetic C_2,C_5-pyrrolidines elute at 29.2 min and 30.5 min and their stereochemistry may correspond, although such an extrapolation is obviously hazardous. An unequivocal synthesis of either isomer is still our goal, but correlation with the natural venom will be difficult since the retention time of the trace of saturated C_2, C_5 base is obscured by other components. Confirmation of the structures of the unsaturated components is also desirable and this should be straightforward (nmr) once sufficient material has been isolated or synthesized.

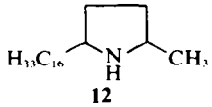
CONCLUSION

It is interesting to note that a pyrrolidine structure **11** had been proposed initially for the venom of ordinary fire ants by Adrouny in 1966[16] on the basis of little apparent evidence.



11

Sonnet synthesized this material[17] and showed that it was completely different from any constituents in the venom. As expected, the mass spectrum of **11** fails to show an intense peak for loss of the $C_{16}H_{33}$ side chain. We had also considered the pyrrolidine structure **12** in discussing the chemistry of the imported fire ant[18] but were able to dismiss it on straightforward mass spectral grounds.



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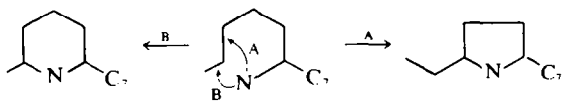
It is attractive to consider that both pyrrolidines and piperidines may originate from the same or homologous precursors by cyclization to different positions of, for example, a double bond. However, this would require the existence of 2-propyl-6-alkylpiperidines to preserve the



†A trace by-product (2%) in the synthesis was a pyrroline identical in retention time and mass spectrum to that assigned the structure shown by ion 7 on the basis of its mass spectrum, but insufficient compound was available for further characterization.

relationship bwith 2-butyl-5-alkylpyrrolidines such as **1**, and none has been identified to date.

It was noted above that the various components of this venom differ by one or two 2-carbon fragments, presumably derived from acetate. The total carbon number, adding one to accommodate hypothetical decarboxylation, is 12, 14 and 16, seemingly related to the naturally-occurring fatty acids, but the absence of an analogue from the ubiquitous C-18 oleate/stearate militates against this suggestion. An analogous pathway, however, has been experimentally verified by Leete[19] for coniine, a piperidine from hemlock.

EXPERIMENTAL

Ant colonies were excavated at Morton's Drift near Pietermaritzburg, Natal, South Africa. Pure venom was collected in 0.5 μl capillary tubes by "milking" worker ants.[10] The venom was transferred to "Spectrograde" pentane and the extracts were employed directly for gc–ms analyses.

Spectra were run on an LKB-9000 gc-mass spectrometer. Gas chromatographed conditions are given in the figures. Mass spectra were taken at 70 eV and 60 μA ionizing current. The source and separator were maintained at 290°C and samples were chromatographed at 6–8°/min.

For analytical purposes three GC columns were used: (1) 2m × 2.5 mm i.d. 10% SP-1000 on 80–100 mesh Supelcoport (Supelco, Bellafonte, Pa.) at 100° (28 psi), (2) 4m × 2.5 mm i.d. 10% Pennwalt 223 on KOH-washed 80–100 mesh Gas Chrom D (Lawshe Inst. Co., Bethesda, Md.) at 110° (30 psi), (3) Column 2 operated at 180° (30 psi). On column 1 n-$C_{14}H_{30}$ = 5.2 min, n-$C_{16}H_{34}$ = 16 min and 3-undecanone = 13.5 min. On column 2, n-C_8H_{18} = 10.2 min, n-C_9H_{20} = 18.7 min. On column 3, n-$C_{12}H_{26}$ = 18 min, n-$C_{13}H_{28}$ = 28.4 min.

3-Undecanone O-methyloxime. Methoxyamine hydrochloride (24 g, 0.29 mole) was combined with 3-undecanone (40 g, 0.24 mole) and 100 ml of 10% NaOH. EtOH was added to give a clear soln and the mixture refluxed for 1 hr. Evaporation of the alcohol and extraction with ether produced 42 g (90%) of the oily oxime whose identity was confirmed by the lack of CO absorption (IR) and gc–ms. Both *syn* and *anti* isomers of identical ms were observed whose retention times relative to starting ketone were 0.77 and 0.84 on column 1. The compound showed a molecular ion at *m/e* 199 (10%) and intense peaks at *m/e* 170 (20%, M – Et) and *m/e* 101 (100%, M – C_7H_{14}, McLafferty rearrangement).

3-Aminoundecane. The above methoxime (42.2 g, 0.21 mole) in 100 ml THF was added slowly to 25 g of LAH in 200 ml of distilled THF and allowed to reflux overnight. Decomposition of the complex with 50% NaOH and evaporation of the solvent gave 3-aminoundecane (21.6 g, 60%) which was characterized as the hydrochloride and recrystallized from water, m.p. 108–110°. The mass spectrum showed a weak molecular ion at *m/e* 171 and intense peaks at *m/e* 142 (37%, M – Et) and *m/e* 58 (100%, M – C_8H_{17}); retention time 0.72 relative to 3-undecanone on column 1.

N-Chloro-3-aminoundecane. The above amine (5 g, 0.029 mole) was stirred at room temp. for 30 min in 100 ml of 4–6% NaOCl. After extraction with ether and evaporation of the solvent the chloroamine was obtained in 89% yield (5.3 g) and characterized by gc–ms, retention time 1.27 relative to 3-undecanone on column 3. A very weak molecular ion was observed at *m/e* 205 and stronger chlorine-containing peaks were observed at *m/e* 176 (30%, M-Et), *m/e* 92 (100%, M–C_8H_{17}).

2-Ethyl-5-pentylpyrrolidine. The chloroamine (1.7 g) in 5 ml conc H_2SO_4 was irradiated for 15 min under N_2 in a quartz tube using a Model PCOX1 UV source (UV Products, Inc., San Gabriel, CA.). Longer times resulted in decomposition of the product. Crushed ice was added and the soln made basic with 40% NaOH. Extraction with chloroform produced a brown oil whose volatile components were shown by GC–MS on 10% SP-1000 to consist of about 40% of 3-aminoundecane and 60% of a 1:1 mixture of the *cis* and *trans* pyrrolidines, (retention times 0.46 and 0.50 respectively, relative to 3-undecanone on column 1 and 1.03 and 1.07 relative to n-$C_{13}H_{28}$ on column 3). A trace (~2%) of the compound assigned the structure **7** was recognized by its early retention time on column 1 and mass spectrum (spectrum 4). Presumably it is formed by oxidation of the pyrrolidine.

Chromatography on alumina with benzene produced the pyrrolidine mixture (0.25 g), the 3-undecylamine being retained by the column and subsequently eluted with EtOAc. A second chromatogram on alumina with benzene allowed the separation in poor yield of the two isomers, that eluting earlier (gc) on 10% SP-1000 and Pennwalt eluting first on alumina. The nmr of this substance ($CDCl_3$) showed peaks at δ1.12 (CH_3 of side chains), δ1.70 (CH_2 of C_2–C_4 of the pentyl side chain), δ2.10 (CH_2 of pyrrolidine ring and methylene groups next to ring), δ3.52 (α hydrogens). The peaks assigned to the α hydrogens were very close to those assigned to the same protons in the mixture of isomers and differentiation or assignment of stereochemistry on this basis was not possible. This compound was converted to a tosylate in pyridine, m.p. 78–80°, that showed a very weak molecular ion at *m/e* 323, and intense peaks at *m/e* 294 (M–Et) and *m/e* 252 (M–C_5H_{11}). Bands were observed in $CDCl_3$ in the nmr at δ0.91 (6H, $2CH_3$), δ1.33 (CH_2 of C_2–C_4 of pentyl side chain), δ1.55 (CH_2 of side chains next to ring), δ1.8 (CH_2 of pyrrolidine ring), δ3.5 (α hydrogens), δ2.45 (CH_3 of tosyl group), δ7.31 and δ7.75 (tosyl H). Removal of the tosylate group with lithium aluminum hydride in THF provided the early-eluting (GC and alumina) isomer in good yield. Unfortunately, the tosylate existed as a twinned crystal (Dr. J. Silverton, this laboratory) and was not amenable to X-ray analysis.

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