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THE CHEMISTRY OF FIRE ANT VENOM

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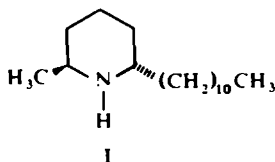
Abstract—Five alkaloids, three *trans*-2-methyl-6-alkylpiperidines, and two unsaturated analogues, have been identified in the venom of the red form of the fire ant, *Solenopsis saevissima*. The compounds are represented by the structural formulae I–V. Each has been prepared synthetically. These compounds, which appear to be the sole constituents of the venom, are believed unique among animal venoms.

THE red form of the fire ant, *Solenopsis saevissima*, occurs in the United States from North Carolina to Texas. The ant's trivial name derives from the potency of its venom, which exhibits pronounced hemolytic,[1] insecticidal, and antibiotic[2] activity. Of greater chemical interest, it is the only known non-proteinaceous venom delivered by bite or sting.[3]

In 1966, structures were proposed[4] for two active constituents in the venom. 2-Methyl-3-hexadecylpyrrolidine and the corresponding Δ^3-pyrroline were proposed by Adrouny as being the substances responsible for the toxic effects of this venom.[4] A year later, Sonnet[5] published a synthesis of the pyrrolidine and showed that neither the *cis* nor the *trans* form was present in the ant venom. In response to Sonnet's work, we have submitted this venom to a thorough chemical reexamination. In a preliminary report,[6] we proposed structure I (or its mirror image), *trans*-2-methyl-6-*n*-undecylpiperidine, for the component of lowest molecular weight (253) in the venom. The trivial name solenopsin A was proposed for this compound. We now present evidence that the composition of the five constituents of this venom can be summarized by structures I–V (absolute stereochemistry not yet known).



I

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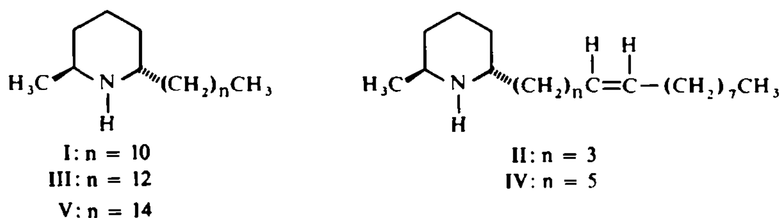


Pure venom was collected as described earlier.[2] The material gives positive spot tests for secondary amines (Experimental). Gas chromatography of the total venom (after drying its hexane solution over sodium sulfate), using a glass column of 3% OV-1 (methyl siloxane) on 100/120 mesh Chromosorb W at 180° (Experimental), revealed the presence of five constituents with retention times of 6·4, 11·6, 13·0, 23·9, and 27·0 minutes (Fig 1). Integration of the peak areas gave the following percentages

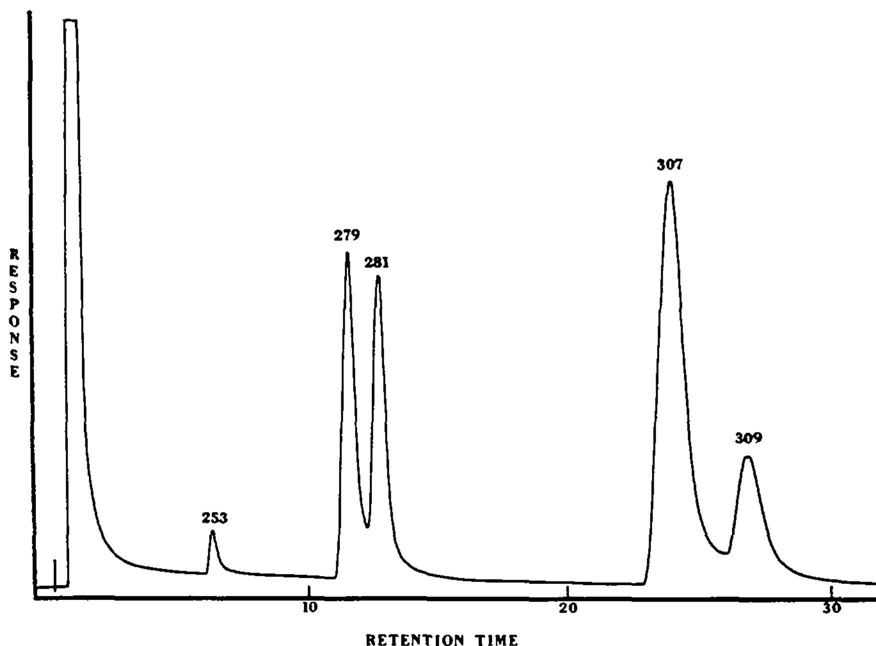


Fig 1. Gas Chromatogram of Fire Ant Venom. The horizontal scale is calibrated in minutes.

for the five components (in order of increasing retention time): 1·25, 15·1, 17·6, 44·2, and 21·8. Combined gas chromatography-mass spectrometry allowed determination of the molecular weights as 253, 279, 281, 307, and 309, not by the parent ions which were often absent, but *via* the P-1 and P-15 ions. The corresponding molecular formulae are $C_{17}H_{35}N$, $C_{19}H_{37}N$, $C_{19}H_{39}N$, $C_{21}H_{41}N$, and $C_{21}H_{43}N$, respectively, as confirmed by high resolution mass spectral measurements. By far the most intense signal in each of the five mass spectra appears at *m/e* 98, corresponding to the fragment $C_6H_{12}N^+$. Fig 2 depicts the mass spectrum of solenopsin A. The molecular weights

were confirmed by chemical ionization mass spectrometry, using methane as the reactant gas. Because this technique produces cations of lower energy than those produced by normal electron impact, it is particularly suitable for the study of compounds whose parent peaks are very weak or absent. In the present case, the most intense signal in the spectra of the five venom components appears at P + 1 (*m/e* 254, 280, etc.). *Via* electron impact, the parent peak (P) is only about 0·2% of the base peak

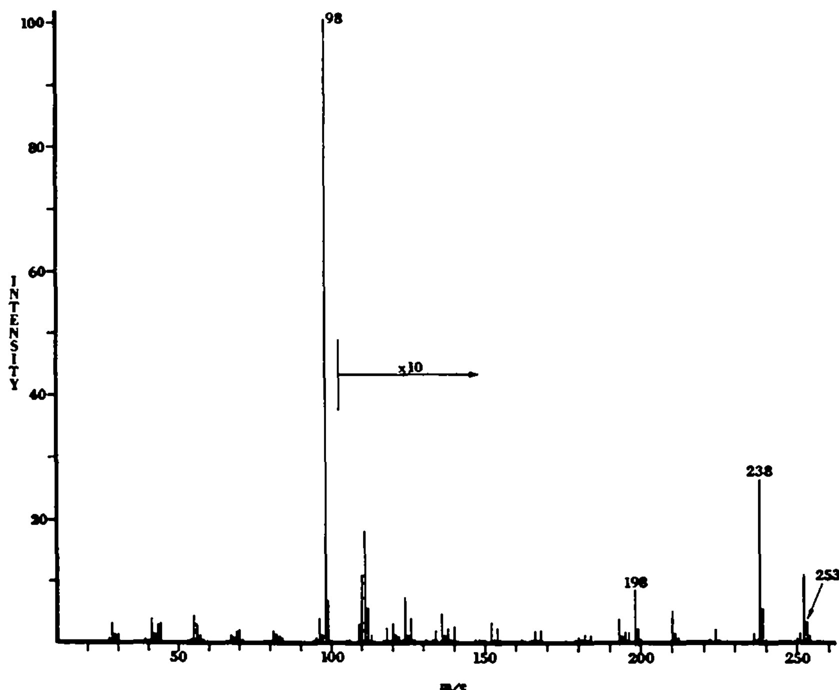


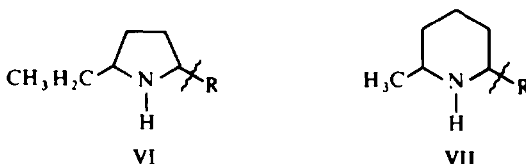
FIG 2. Mass Spectrum of Solenopsin A.

(*m/e* 98); *via* chemionization, however, the intensities are reversed: the signal at *m/e* 98 is about 40% of the P + 1 peak. The gas chromatogram of the venom mixture after hydrogenation exhibits only three peaks. The components of molecular weights 279 and 307 disappeared; those of molecular weights 281 and 309 increased correspondingly. The 253 component was unaffected. Thus, it appears that the 279 component is an unsaturated analogue of the 281 component, and the 307 component is an unsaturated analogue of the 309 component.

A small sample of the total venom was subjected to carbon skeleton chromatography[7]; this hydrogenolytic procedure produced the alkanes *n*-pentadecane, *n*-heptadecane, *n*-nonadecane, and *n*-heneicosane. Thus, the C atoms of each molecule are so situated that all are retained in the hydrogenolysis product. This eliminates from consideration N-Me groups and any Me or other branches.

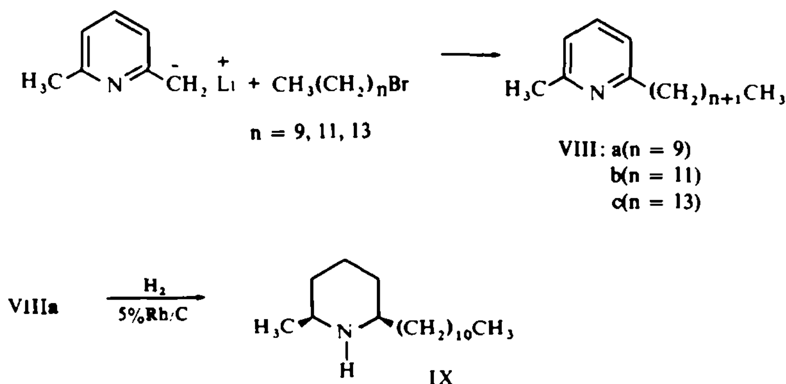
The mass spectral base peak at *m/e* 98 deserves further comment. The formula for this fragment, confirmed by high resolution mass measurement, indicates one unit of unsaturation which can be best accommodated by a ring, most likely 5- or 6-membered.

The hydrogenolysis results limit the possibilities to the two partial structures VI and VII. Both would be expected to yield *n*-alkanes on hydrogenolysis. Mass spectral cleavage at the points indicated would yield in each case a reasonably stable ion $C_6H_{12}N^+$, *m/e* 98. The 6-membered ring structure was favored for three reasons. First, the IR spectrum between 2920 and 2980 cm^{-1} is more similar to that of 2,6-dimethylpiperidine than to that of 2,5-dimethylpyrrolidine. Such differences have been



reported by Tallent and Siewers.[8] Secondly, the very intense signal at *m/e* 98 in the mass spectrum is in full accord with a 2-methyl-6-alkylpiperidine structure: the base peak in the mass spectrum of 2,6-dimethylpiperidine occurs at *m/e* 98.[9] Finally, the series of peaks at P-15 involves cleavage on the other side of the ring with loss of Me, whereas pyrrolidine structures such as VI would undoubtedly lose an Et group (P-29). It thus appeared that all five constituents were 2-methyl-6-alkyl (or alkenyl) piperidines.

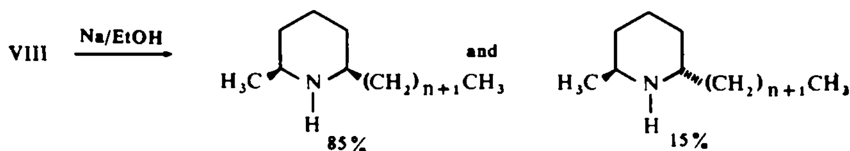
The relative stereochemistry of the two substituents on the ring was determined by comparison with synthetic material of known stereochemistry. The pure *cis* piperidine IX was prepared by the hydrogenation of the corresponding pyridine (VIIIa) catalysed by 5% rhodium on charcoal.[10] The 2-methyl-6-alkylpyridine, in turn, was prepared by the alkylation of the lithium salt of 2,6-dimethylpyridine with the appropriate



Scheme 1

bromoalkane[11] (see Scheme 1). A mixture of the *cis* and *trans* isomers was synthesized by the reduction of VIII with sodium metal in absolute ethanol[12] (see Scheme 2). The *cis* and *trans* isomers are readily separable by column chromatography over alumina; the *cis* isomer elutes first, presumably because of steric hindrance of the nitrogen by

the 2,6-diequatorial substituents. Although the mass spectra of the two isomers are virtually indistinguishable, both by electron impact and chemical ionization, the infrared spectra allow easy differentiation: the pure *cis* isomer exhibits relatively strong absorption near 1320 cm^{-1}, the *trans* isomer absorbs only weakly in this



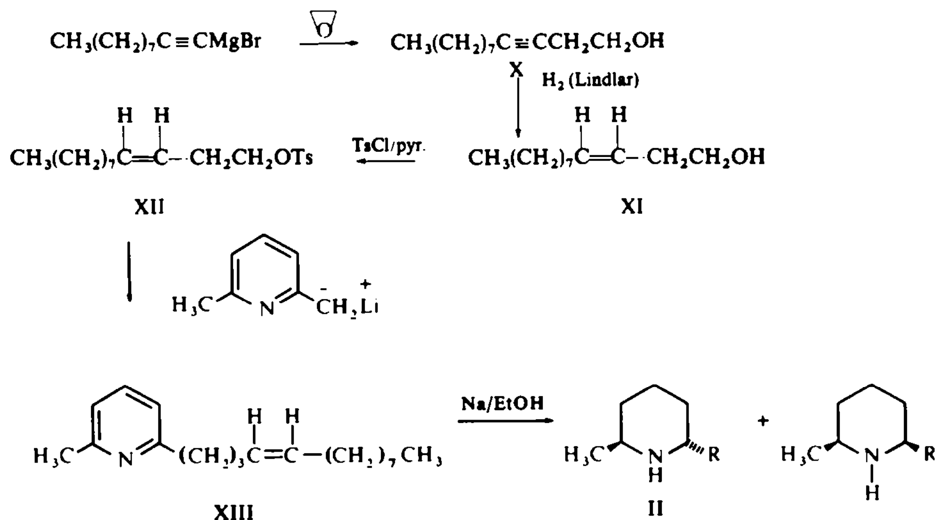
SCHEME 2

region. The *trans* isomer is indistinguishable in gas chromatographic behavior from the corresponding component in the venom on three different stationary phases (Experimental), although the *cis* and *trans* isomers separate easily. The N-acetate of synthetic I is identical to the N-acetate of the venom component 253 (solenopsin A) both in the IR spectrum and in gas chromatographic behavior on the one stationary phase tested. The mass spectra of the synthetic amines (I, III, and V) and corresponding venom components (solenopsins A, B, and C) are identical. Thus, solenopsin A is *trans*-2-methyl-6-*n*-undecylpiperidine (I), solenopsin B is *trans*-2-methyl-6-*n*-tridecylpiperidine (III), and solenopsin C is *trans*-2-methyl-6-*n*-pentadecylpiperidine (V).

At this point, the only problem remaining was the determination of the position and geometry of unsaturation in the two unsaturated venom components, dehydrosolenopsin B and dehydrosolenopsin C. The infrared spectrum exhibits little or no absorption which can be attributed to a *trans* double bond; on the basis of this negative evidence, the double bond was assumed to be *cis*. The position of the double bond was determined by both ozonolysis and a potassium permanganate-sodium periodate oxidation[13] of the total venom mixture. In the ozonolysis experiment, the sole volatile product found was nonanal, as determined by gas chromatographic comparison with an authentic sample. The permanganate-periodate oxidation produced nonanoic acid, identified as its methyl ester by gas chromatography. The small amount (about 10%) of octanoic acid detected undoubtedly arises from the further oxidation of the initially-formed nonanoic acid. The formation of only one product during the oxidation of the two unsaturated components requires that both be unsaturated at the same point on the alkyl side chain. This latter conclusion finds support in the mass spectra of these two components, which show slight enhancements in the signals corresponding to allyl ions formed by β-cleavage on either side of the double bond.

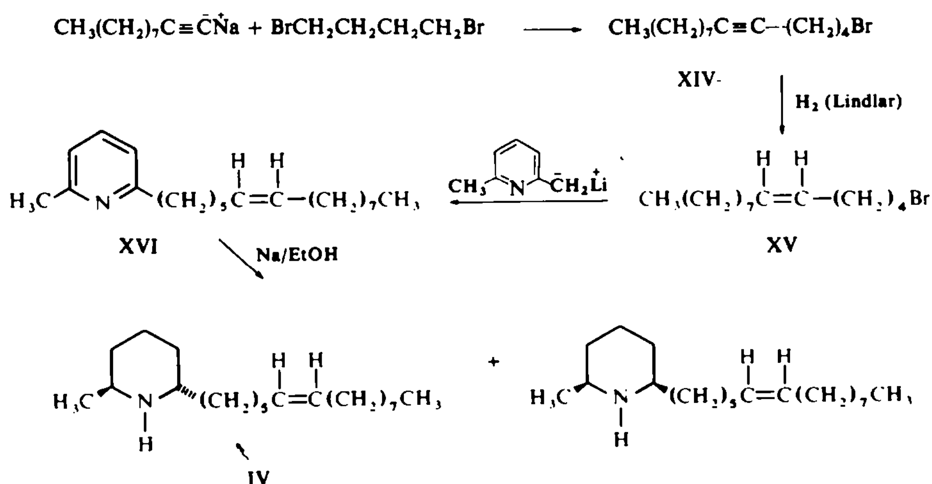
The synthesis of compound II is shown in Scheme 3. The Grignard derivative of 1-decyne was treated with ethylene oxide to produce the acetylenic carbinol, X. Partial reduction of X gave the corresponding *cis* olefinic alcohol, XI. The latter was converted to the tosylate (XII), which was used to alkylate the lithium salt of 2,6-dimethylpyridine. As above, the alkylated pyridine (XIII) was reduced with sodium in ethanol

to a mixture of piperidines; the desired isomer, II, was isolated by column chromatography. IR, mass, and NMR spectral data and gas chromatographic behavior, using three stationary phases, of synthetic II and dehydrosolenopsin B are identical; dehydrosolenopsin B is, therefore, *trans*-2-methyl-6-(*cis*-4-tridecenyl)piperidine.



Scheme 3

The synthesis of compound IV was achieved as indicated in Scheme 4. The acetylenic bromide, XIV, was synthesized (albeit in only 18% yield) directly by the alkylation of the sodium salt of 1-decyne with 1,4-dibromobutane. The product was reduced to the *cis* decenyl bromide, XV. Treatment of the latter with the lithium salt of 2,6-dimethylpyridine gave XVI, which was reduced to a mixture of the *cis* and *trans* piperidines. Separation on an alumina column gave the pure *trans* isomer, IV. Once

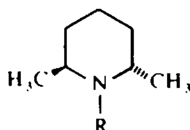


Scheme 4

again all spectra and chromatographic data show the synthetic and natural materials to be identical. Thus, dehydrosolenopsin C, the most abundant component in the ant venom, is *trans*-2-methyl-6-(*cis*-6-pentadecenyl)piperidine.

Because of the virtual qualitative and quantitative identity of the total venom and synthetic material, especially in NMR and IR spectral characteristics, and the ninhydrin-negative nature of the total venom, these five components appear to completely account for the chemical properties of the venom. Biological activity of the synthetic compounds is under investigation.

Although similar alkaloids are known from plants, the occurrence of such materials in animals is totally unprecedented. Among the 2,6-disubstituted piperidines occurring in plants, mention might be made of the various *Lobelia* alkaloids, including lobeline, lobelanidine, lobelanine, and lobinine.[14] Pinidine[11,15], *cis*-2-methyl-6-(2-propenyl)piperidine, occurs in some western American pine species. Carpaine[16] and cassine[17] are additional examples. To our knowledge, the only known naturally-occurring *trans*-2,6-dialkylpiperidines are those found[18] in the plant *Nanophyton erinaceum* (XVII).



XVII: R = H, CH_3

EXPERIMENTAL

General. NMR spectra were recorded using either a Varian HA-100 or A-60 instrument. Chemical shifts are reported in τ values, with TMS as internal reference. The solvent was CCl_4. IR spectra were recorded on a Beckman IR-10 instrument, either as a neat film or in CCl_4 soln. B.ps are uncorrected. Gas chromatographic analyses were carried out on a Tracor MT-220 instrument equipped with a flame ionization detector; dry N_2 was used as carrier gas at a flow rate of 60 ml/min. The following columns were used in the analyses (all glass tubular, 1·70 m × 4 mm i.d.): (a) 3% OV-1 (methyl siloxane) on 100/120 mesh Chromosorb W; (b) 10% FFAP (modified polyglycol) on 60/80 mesh Chromosorb W; (c) 3% SE-52 (phenyl methyl silicone) on 100/120 mesh Chromosorb W; (d) 3% Apiezon L (hydrocarbon) on 100/120 mesh Chromosorb W; and (e) 5% SE-30 (methyl silicone) on 60/80 mesh Chromosorb W. Combined gas chromatographic-mass spectrometric analyses were performed using an LKB 9000 instrument. The column used was 1% OV-1 on 80/100 mesh Supelcoport (Supelco, Inc., Bellefonte, Pa.). High resolution and chemical ionization mass spectra were obtained using an AEI MS-9 mass spectrometer; the source was modified by Scientific Research Instruments, Baltimore, Md.

Collection and storage of venom. Several colonies of fire ants were excavated in the vicinity of Griffin, Georgia in April 1969. Pure venom was collected in 1 μl capillary tubes by "milking" worker ants.[2] The colorless, usually clear venom was removed from the capillary tubes with slight pressure from a rubber bulb and stored in spectrograde hexane soln over anhyd Na_2SO_4 at 2–5°. In this way, milligram quantities of pure venom were readily available.

Spot tests on the venom[19]

(a) Into each of four 2·5 ml centrifuge tubes was placed 0·1 ml of a 5% $CuSO_4$ soln. To one was added several mg aniline, to another a small amount 2,5-dimethylpyrrolidine (Aldrich), and to the third was added several μl hexane soln of the venom. To the fourth tube, which served as a blank, nothing was added. Two drops of conc NH_4OH and then two drops of a 3-to-1 mixture of C_6H_6 and CS_2 were added to each of the four tubes. The top layers in the blank and aniline-containing tubes remained colorless, whereas the top layers in the other two tubes became distinctly yellow-brown, indicating the presence of secondary amines.

(b) Into each of three depressions on a porcelain spot plate were placed two drops of an aqueous soln of 1% sodium nitroprusside and 10% acetaldehyde. To each was added about 1 mg Na_2CO_3. To one was added a small amount of 2,5-dimethylpyrrolidine, and to the second, a small amount of venom; the third served as a blank. Both the pyrrolidine and venom tests became slightly blue, again indicating the presence of secondary amines. The blank remained colorless.

Carbon-skeleton chromatography[7]. The National Instruments Laboratories-Beroza Carbon-Skeleton Determinator was used in these experiments; it was attached to the injection port of the gas chromatograph. The Pd catalyst supplied was washed with 10% Na_2CO_3aq, rinsed and dried at 120° for 5 hr. The catalyst tube was charged with 2 ml Chromsorb W (to act as an inert filler) and 2 ml base-washed catalyst. The precolumn was held at 275°. H_2 was used as carrier gas. Using column b with temp programing from 70° to 200° at 10°/min, *n*-pentadecane, *n*-heptadecane, *n*-nonadecane, and *n*-heneicosane were identified among the hydrogenolysis products by comparison with authentic samples.

Spectral data on the venom. The IR spectrum (CCl_4, about 10% solution) exhibits absorption at 3010 cm^{-1} (C—H stretch for double bond); weak and broad absorption at about 1650 cm^{-1} (C=C stretch, *cis*); 1460 and 1380 cm^{-1} (C—H bending); weak absorption at 1185 cm^{-1}, 1065 cm^{-1}, and quite broad and strong absorption at 710 cm^{-1} (C—H bend for *cis* alkene, skeletal vibration for —$(CH_2)_4$—). Little or no N-H stretching absorption is apparent in the spectrum. The NMR spectrum of the total venom is as follows: τ 4·75 (distorted triplet, vinyl protons), 6·4 (broad and weak), 6·9 to 7·3, approximately 8·0 (broad), 8·46, 8·72 (methylene protons), 8·95 and 9·01 (doublet, Me protons), and 9·11 (triplet, Me protons). No absorption was found that could be attributed to acidic, aldehydic, aromatic, or N-Me protons. The mass spectrum of each component in the venom exhibits a base peak at *m/e* 98 and a very weak parent peak by normal electron impact. Compared to the parent peak, the P-1 and P-15 peaks are quite strong in each case. The loss of 15, 29, 43, etc, in the three saturated components is highly indicative of an alkyl chain.

Acetylation of the venom. Several mgs of venom was acetylated with Ac_2O-pyridine reagent (Applied Science, State College, Pa.). The acetylation mixture was stirred for 24 hr at room temp and worked up as usual.

Syntheses

2-Methyl-6-n-undecylpyridine (VIIIa). To a soln of 5·35 g (50 mmol) 2,6-dimethylpyridine (Eastman, dried over KOH) in about 50 ml anhyd ether was added 22 ml (50 mmol) 2·28M benzene-ether solution of phenyllithium (Alfa Inorganics, Inc., Beverly, Mass). The reaction was conducted in a helium atmosphere with precautions taken to prevent the introduction of air or moisture. After 15 min of stirring at room temp, the contents of the flask were brought to gentle reflux for 15 min. With the ether refluxing, 11·06 g (50 mmol) of 1-bromodecane (Eastman) was added over a 30 min period. Reflux was continued with stirring for 1 hr after the addition was complete. The reaction mixture was hydrolyzed with the addition of 20 ml MeOH and several volumes water. The organic material was extracted with ether; the ether layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure to give about 9 g of a light yellow oil. Vacuum distillation gave a 2 g forerun of 1-bromodecane, and 6·08 g (49%) 2-methyl-6-*n*-undecylpyridine, VIIIa, b.p. 96–98°/0·015 mm. The IR spectrum is as follows: 3040 cm^{-1} (aromatic C—H stretch), 1570, 1445, 1370, 1210, 1150, and broad absorption 700–800 cm^{-1}. The NMR spectrum is as follows: τ 2·72 (triplet, J = 8·0 Hz, 1H, proton in position 4 on ring), 3·23 (doublet, 2H, protons in positions 3 and 5 on ring), 7·35 (triplet, J = 7·2 Hz, 2H, methylene adjacent to ring), 7·55 (singlet, 3H, Me group on ring), 8·71 (singlet, 18H, methylene protons), and 9·12 (distorted triplet, 3H, terminal Me group). The low resolution mass spectrum exhibits a base peak at *m/e* 107, a parent peak at *m/e* 247, and prominent signals corresponding to cleavage at different points on the alkyl side chain. The molecular weight, determined by high resolution mass spectrometry, was 237·2300. $C_{17}H_{29}N$ requires 247·2299.

Using the above procedure, VIIIb and VIIIc were synthesized in yields of 44% and 61%, respectively, using 1-bromododecane and 1-bromotetradecane. VIIIb distilled at 114–117°/0·03 mm and VIIIc at 124–127°/0·02 mm. The IR and NMR spectra were identical to those of VIIIa, except for integration in the NMR spectra. The molecular weights were found to be 275·2601 and 303·2921. $C_{19}H_{33}N$ requires: 275·2612; $C_{21}H_{37}N$ requires: 303·2925.

Catalytic reduction of VIIIa. A small amount (2·59 g, 10·5 mmol) of VIIIa was reduced in EtOH soln using 0·50 g 5% Rh/C and a pressure of 50 lbs H_2. After 36 hr, the mixture was filtered through Celite and concentrated under reduced pressure to give a light yellow oil, b.p. 91–96°/0·02 mm, 2·24 g (84%). The IR spectrum indicated that no starting material remained. A low resolution mass spectrum gave a molecular

weight of 253. The hydrochloride of *cis*-2-methyl-6-*n*-undecylpiperidine was prepared and recrystallized from hexane–EtOH, m.p. (uncorr) 154–155°.

Sodium-in-ethanol reduction of VIII.[12] In a typical experiment, 0·56 g (2·3 mmol) of VIIIa was added to 100 ml "superdry" ethanol[20] in a flask equipped with a mechanical stirrer and a reflux condenser protected with a $CaCl_2$ drying tube. With the EtOH gently refluxing, 5·0 g Na metal was added in small chunks over a 45 min period. After all the Na had dissolved, the mixture was refluxed an additional 2·5 hr. The mixture was hydrolyzed by the addition of several volumes water. The organic layer was removed by extraction with light petroleum (40–60°), dried ($MgSO_4$), and concentrated to a yellow oil. Gas chromatographic examination (column a, 180°) revealed two components; the first, about 85% of the mixture, was identical to the *cis* material prepared above; the second was presumably the desired *trans* isomer. The IR spectrum of the crude mixture of isomers displays no absorption attributable to C=C or C=N linkages. The isomers were separated on a 50 g column of alumina (Merck Reagent Grade). To the column, packed in light petroleum, was added 0·38 g of the mixture. The *cis* isomer, a total of 300 mg, was eluted in solvents from 10% ether/petroleum to pure ether. The *trans* isomer, 105 mg, was eluted in 20% EtOH in ether. The latter material was identical to solenopsin A in gas chromatographic behavior on columns a (180°), b (200°), c (180°), and d (210°). The *trans*-2-methyl-6-*n*-undecylpiperidine (I) exhibited a parent peak in the mass spectrum at *m/e* 253 (high resolution indicated this to be primarily the ^{13}C satellite of the P–1 peak), and a base peak at *m/e* 98, and in all respects was identical to the mass spectrum of the venom material, solenopsin A. The IR spectrum of the synthetic *trans* isomer was identical to that of the total venom, except for double bond absorption in the latter. The NMR spectrum is as follows: τ 6·9 to 7·3 (2H, broad and unresolved, protons in positions 2 and 6 on ring), 8·4 to 8·6 (6H, broad), 8·72 (singlet, 20H, methylene protons), 8·96 and 9·02 (doublet, 3H, Me group on ring), and 9·11 (distorted triplet, 3H, terminal Me group).

Compounds VIIIb and c were likewise reduced to the corresponding mixture of piperidines. Column chromatographic separation gave the isomers in about 95% isomeric purity. In each case, the *trans* isomers are identical to the venom components, solenopsins B and C, in gas chromatographic behavior (columns a at 190°, b at 200°, and d at 210°), and in mass, NMR, and IR spectral properties. High resolution mass spectrometry gave the following exact masses for the P–1 fragments: 280·3300 and 308·3306. $C_{19}H_{38}N$ requires: 280·3004; $C_{21}H_{42}N$ requires: 308·3317.

trans-2-*Methyl*-6-(cis-4-*tridecenyl*)*piperidine* (II)

Dodec-3-*yn*-1-*ol* (X). Into a flask equipped with mechanical stirrer, dry-ice condenser, and pressure-equalized addition funnel, were placed 2·88 g (120 mg-atoms) Mg metal turnings and about 400 ml anhydrous ether. MeI (Eastman, 17·0 g, 120 mmol) was added from the funnel over a 15 min period. After the addition was complete, the mixture was heated to reflux for 1 hr. 1-Decyne (99%, Chemical Samples Co), 13·8 g, (100 mmol) was added dropwise to the Grignard mixture. Methane evolution began immediately and ceased after about 3 hr stirring. Ethylene oxide (Matheson, 20 g, 500 mmol) was added in small portions through a glass tube, until the reaction mixture gelled. After refluxing for 2 hr, the mixture was hydrolyzed by the addition of 50 ml of 0·5N HCl. The reaction mixture was worked up as usual. After a large forerun of ethylene iodohydrin, b.p. 30–40° at aspirator pressure, the desired alcohol, 4·58 g (25% yield), was obtained, b.p. 75–80°/0·005–0·01 mm. No attempt was made to improve the yield. The IR spectrum exhibits O–H absorption at 3300–3600 cm^{-1} and C–O stretch appropriate for primary alcohols at 1050 cm^{-1}. The NMR spectrum is as follows: τ 6·41 (distorted triplet, 3H, carbinol methylene plus (?) proton on oxygen), 7·66 (multiplet, 2H, methylene next to triple bond, on alcohol side), 7·88 (multiplet, 2H, methylene next to triple bond, on hydrocarbon side), 8·70 (broad singlet, 12H, 6 methylenes closest to Me group), and 9·10 (distorted triplet, 3H, Me group). The mass spectrum exhibits a parent peak at *m/e* 182, and prominent signals at *m/e* 164 and 151, corresponding to the loss of H_2O and $-CH_2OH$, respectively. Also present is a series of peaks at *m/e* 149, 135, 121, 107, etc., corresponding to the loss of H_2O plus Me, Et, Pr, Bu, etc., respectively. High resolution mass measurement gave a molecular weight of 182·1670; $C_{12}H_{22}O$ requires: 182·1670.

cis-*Dodec*-3-*en*-1-*ol* (XI). The acetylene X (7·93 g, 43·6 mmol) was reduced in 100 ml MeOH containing 5 drops synthetic quinoline[21] and 100 mg catalyst (5% Pd/$BaSO_4$) prepared according to Augustine.[22] The progress of the reduction was followed with gas chromatography (column b, 170°). The product elutes between 1-dodecanol and the starting material, as expected. Short-path distillation afforded 5·52 g (69%) of the *cis* alcohol, b.p. about 65°/0·04 mm. The IR spectrum exhibits *cis* double bond absorption (3020, 690 cm^{-1}) and primary alcohol absorption (3640, 3300–3500, and 1050 cm^{-1}). The NMR spectrum is as follows: τ 4·56 (complex, 2H, vinyl protons), 6·45 (triplet, 2H, carbinol methylene), 7·6–7·9 (broad, complex

multiplet, 4H, allytic protons), 8·70 (singlet, 12H, six methylene groups), and 9·11 (distorted triplet, 3H, methyl protons). The mass spectrum exhibits a parent peak at *m/e* 184 and a prominent signal at *m/e* 166, representing the loss of water from the molecule. Molecular weight 184·1850; calculated for $C_{12}H_{24}O$: 184·1827.

2-*Methyl*-6-(cis-4-*tridecenyl*)*pyridine* (XIII). The tosylate XII was prepared[23] by treating 1·0 g of XI with 2·5 g *p*-toluenesulfonyl chloride (Eastman White Label) in anhyd pyridine. After standing 20 hr at 2–5°, the mixture was poured onto 400 ml water-crushed ice mixture. The suspension was extracted 3 times with ether. The ether extracts were combined and washed with one volume of ice-cold HCl (1:1). After washing again with water, the organic layer was dried over $MgSO_4$ and concentrated to a yellow oil. The IR spectrum of this material exhibits two bands characteristic of tosylates[23] at 1180 and 1190 cm^{-1}. No OH absorption was present.

A 3-necked flask was charged with 5·35 g (50 mmol) 2,6-dimethylpyridine and 150 ml anhyd ether. PhLi (50 mmol, 22 ml of a 2·28M soln) was added from a dropping funnel. The tosylate prepared above was added to the mixture, which was heated to reflux during the addition and maintained at reflux for an additional 3 hr. The mixture was hydrolyzed by the addition of 50 ml MeOH and two volumes water. After the usual work-up, the desired material was obtained in a reasonable state of purity by two short-path distillations (about 100°/0·01 mm). The IR spectrum is almost identical to that of the side-chain saturated analogue, VIIIb. The mass spectrum exhibits a parent peak at *m/e* 273, and signals corresponding to the loss of Me, Et, Pr, etc, fragments. The base peak appears at *m/e* 107. The spectrum is quite similar to that of VIIIb.

trans-2-*Methyl*-6-(cis-4-*tridecenyl*)*piperidine* (II). About 300 mg of XIII was reduced as described above with Na in EtOH. Work-up and chromatography gave 200 mg of the *cis* isomer and 61 mg of the desired *trans* isomer. The latter was identical in gas chromatographic behavior to the venom component dehydrosolenopsin B on columns a (190°), b (200°), and d (210°). The mass spectrum of the synthetic material exhibits a parent peak at *m/e* 279 and a base peak at *m/e* 98. Also present is a somewhat enhanced signal at *m/e* 180, representing favored cleavage at an allylic position in the side chain.

1-*Bromotetradec*-5-*yne* (XIV). A 3-necked flask equipped with mechanical stirrer, dry-ice condenser, and gas inlet tube was charged with about 200 ml liquid ammonia. Into the ammonia was placed 4·0 g (102·6 mmol) $NaNH_2$. The gas inlet tube was replaced by a pressure-equalized dropping funnel containing a mixture of 14·2 g (102 mmol) 1-decyne and 20·6 g (95·4 mmol) 1,4-dibromobutane (Eastman). The contents of the funnel were added to the stirred ammonia soln over a 30 min period, after which stirring was continued for an additional 2 hr. The ammonia was allowed to evaporate; the reaction was worked-up as usual. Distillation through a short-path apparatus, gave 4·85 g (18%) colorless oil, b.p. about 80°/0·015 mm. The mass spectrum of this material exhibits parent peaks of approximately equal intensity at *m/e* 272 and 274, due to the two bromine isotopes of equal abundance. Molecular weight: 272·1134; $C_{14}H_{25}{}^{79}Br$ requires: 272·1140.

cis-1-*Bromotetradec*-5-*ene* (XV). The acetylene (3·00 g, 10·9 mmol) was reduced as described for X. In gas chromatographic behavior (column b, 200°), the reduced compound is eluted between 1-bromotetradecane and the starting material, as expected. The IR spectrum displays C–Br absorption at 640 and 560 cm^{-1}, and *cis* double bond absorption at 3000, 1650, and 690 cm^{-1}. A small amount of *trans* absorption also appears at 970 cm^{-1}. The mass spectrum exhibits parent peaks at *m/e* 274 and 276. Molecular weight: 274·1281; $C_{14}H_{27}{}^{79}Br$ requires: 274·1296.

2-*Methyl*-6-(cis-6-*pentadecenyl*)*pyridine* (XVI). This material was prepared by the treatment of the Li salt of 2,6-dimethylpyridine with the bromide XV. The yield of crude product was 70%. Distillation through a short path apparatus afforded pure XVI, b.p. about 100°/0·02 mm. The IR spectrum exhibits aromatic absorption at 3060, 1580, and 1560 cm^{-1} and *cis* double bond absorption at 3000, 1650, and 690 cm^{-1}. The mass spectrum exhibits a base peak at *m/e* 107 and a parent peak at *m/e* 301. Molecular weight 301·2747 $C_{21}H_{35}N$ requires 301·2769.

trans-2-*Methyl*-6-(cis-6-*pentadecenyl*)*piperidine* (IV). The pyridine XVI, 1·82 g, was reduced with Na in EtOH. Work-up and chromatography of the product afforded 1·21 g of the *cis* isomer and 0·39 g of the *trans* isomer. Short-path distillation of the latter (80°/0·010 mm) gave a slightly yellow oil. The material could not be distinguished gas chromatographically from dehydrosolenopsin C on columns a (200°), b (200°), and d (210°). The IR spectrum exhibits double bond absorption at 3000, 1650, and 690 cm^{-1}, and absorption in the fingerprint region characteristic of a *trans* 2,6-dialkylpiperidine. The NMR spectrum is as follows: τ 4·75 (distorted triplet, 2H, vinyl protons), 6·9 to 7·3 (2H, protons in positions 2 and 6 on ring), 7·9 to 8·1 (4H, allylic protons), about 8·48 (broad, 6H), 8·70 singlet, 20H, methylene protons), 8·96 and 9·02 (doublet, 3H, Me group on ring), and 9·11 (distorted triplet, 3H, terminal Me group). The N–H proton

absorption is probably obscured by the other absorption. The mass spectrum of the synthetic dehydrosolenopsin C exhibits a parent peak at *m/e* 307 and strong signals at *m/e* 98 (base peak), 111, 124, 152, 154, 206, 208, and 292 (loss of Me). Except for a few minor differences, the mass spectra of the synthetic and natural dehydrosolenopsins C are identical. Molecular weight: (P-1) 306·3102; $C_{21}H_{40}N$ requires: 306·3132.

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