Journal of Chemical Ecology, Vol. 8, No. 1, 1982

VENOM CHEMISTRY OF ANTS IN THE GENUS *Monomorium*

T.H. JONES,[1,7] M.S. BLUM,[1] R.W. HOWARD,[2] C.A. McDANIEL,[3] H.M. FALES,[4] M.B. DuBOIS,[5] and J. TORRES[6]

[1]*Department of Entomology, University of Georgia Athens, Georgia 30602*
[2]*USDA, Southern Forest Experiment Station Box 2008 GMF, Gulfport, Mississippi 39503*
[3]*National Monitoring and Residue Analysis Laboratory USDA Animal and Plant Inspection Service, Box 989, Gulfport, Mississippi 39501*
[4]*Laboratory of Chemistry, National Heart, Lung and Blood Institute, Bethesda, Maryland 20205*
[5]*Department of Entomology, University of Kansas, Lawrence, Kansas 66045*
[6]*Calle Garcia Ledesma, 945 Villa Navarra, Rio Piedres, Puerto Rico 00924*

(Received March 10, 1981; revised June 1, 1981)

Abstract—A comparative analysis of the venomous alkaloids produced by ant species in the subgenus *Monomorium* of the genus *Monomorium* has been undertaken. All species produce mixtures of unsymmetrical *trans*-2,5-dialkylpyrrolidines, but the proportions of the constituents may vary considerably between species. All alkaloids contain both C_6 and C_9 side chains which are present as C_9-saturated. C_6- monounsaturated, and both C_6-and C_9-monounsaturated dialkylpyrrolidines. The structure of 2-(1-hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine, a previously undescribed alkaloid, was proved by unambiguous synthesis after the location of the double bonds was established by the methoxymercuration–demercuration followed by mass spectrometry. The possible chemotaxonomic significance of the mixtures of venomous alkaloids produced by these species of *Monomorium* is discussed.

Key Words—*Monomorium* spp., Hymenoptera, Formicidae 2,5-dialkyl-pyrrolidines, ant venom alkaloids, methoxymercuration–demercuration, chemotaxonomy.

[7]Present address: Department of Chemistry, U.S. Naval Academy, Annapolis, Maryland 21402.

0098-0331/82 0100-0285$0.300/0

INTRODUCTION

A large variety of novel alkaloids has been identified in the venoms of ant species in the myrmicine genus *Solenopsis*, a taxon particularly well represented in tropical areas. Species in the subgenus *Solenopsis* produce characteristic 2,6-dialkylpiperidines (MacConnell et al., 1971, 1976; Brand et al., 1972), whereas some species in the subgenus *Diplorhoptrum* synthesize 2,5-dialkylpyrrolidines in their poison glands (Pedder et al., 1976; Jones et al., 1979, 1980). However, that alkaloidal venoms are not restricted to the genus *Solenopsis* is demonstrated by the identification of a series of 2,5-dialkylpyrrolidines in the venom of *Monomorium pharaonis* (Ritter and Persoons, 1975; Ritter et al., 1973, 1975; Talman et al., 1974), a "tramp" species that is quite abundant in the Old World tropics. The venom of *M. pharaonis* is particularly distinctive in also containing at least two 3,5-dialkylindolizidines, a class of compounds that has never been encountered as venomous products of *Solenopsis* species.

In order to determine whether the venomous alkaloids produced by *M. pharaonis* are typical of those produced by other species in this genus, we have analyzed the poison gland products of additional species, all of which are in the same subgenus (*Monomorium*) as the former. The results indicate that considerable alkaloidal diversity that may be chemotaxonomically useful characterizes the chemistry of *Monomorium* (*Monomorium*) venoms. These results further demonstrate that the venoms of *Monomorium* species may provide useful characters whenever this rich but taxonomically difficult genus, which has not been analyzed in nearly 60 years (Emery, 1922), is subjected to revisionary studies.

METHODS AND MATERIALS

Ants

Ants were collected in the areas indicated in Table 1 and immediately placed in glass vials containing 1–2 ml of CH_2Cl_2. Since it was demonstrated that the alkaloids produced by *Monomorium* species were poison gland products, analyses were subsequently conducted on extracts of whole ants. For analyses, these solutions were reduced in volume to 0.2 ml with a slow stream of nitrogen.

Chemical Analyses

Gas chromatography was performed on a Tracor model MT-160 chromatograph using a 2.5-m × 2-mm ID column packed with 10% SP-1000 on Gaschrom Q, on a Varian model 90-P chromatograph using a 2-m × 4-mm ID column packed with 10% SP-1000 on Gaschrom Q, or on a Varian model

TABLE 1. ALKALOIDS IDENTIFIED AS VENOM CONSTITUENTS OF *Monomorium* (*Monomorium*) SPECIES

Monomorium species[a] (collection site)	Alkaloids[b]						
	1	2	3	4	5	6	7
M. near *metoecus* (Athens, Georgia)	+	*	○	–	○	○	○
M. viridum (Gainesville, Florida)	*	*	○	○	○	○	○
M. floricola (Vero Beach, Florida)	–	–	–	–	–	–	–
M. floricola[c] (Bahia Honda Key, Florida)	–	–	○	–	–	–	–
M. floricola (San Lorenzo, Puerto Rico)	–	–	–	–	–	–	–
M. minimum (Douglas Co., Kansas)	*	–	–	○	–	–	–
M. minimum (Harvey Co., Kansas)	*	–	–	○	–	–	–
M. minimum (Otero Co., Colorado)	*	–	–	○	–	–	–
M. new sp. near *minimum* (Gainesville, Florida)	*	*	–	–	–	–	–
M. near *emersoni* (Taos Co., New Mexico)	○	*	–	–	○	–	–
M. near *emersoni* (Bernadillo Co., New Mexico)	○	*	–	–	○	–	–
M. near *emersoni* (Lubbock, Texas)	○	*	–	–	○	–	–
M. cyaneum (Hualapai Mt. Park, Arizona)	–	*	–	–	+	–	–
M. ebeninum (San Lorenzo, Puerto Rico)	*	○	○	–	–	*	*

[a] Taxonomy as determined by M.B.D. who is revising the North American species in this genus.

[b] * = major component; + = minor component (10–12%); ○ = trace; – = not detected.

[c] Traces of related unidentified alkaloids are also present.

1400 chromatograph equipped with a 2-m × 2-mm ID column packed with 4% OV-1 on Gaschrom Q. Infrared spectra were obtained from neat liquid films with a Perkin-Elmer 297 grating infrared spectrophotometer. NMR spectra were taken on a Varian T-60 instrument. Combustion analyses were performed by Atlantic Microlabs, Atlanta, Georgia.

The initial gas chromatographic–mass spectroscopic (GC/MS) analyses were performed on a LKB-2091 mass spectrometer equipped with a 2-m × 2-mm glass column packed with 10% SP-1000 on Supelcoport. Subsequent mass

spectral analyses of the natural and synthetic alkaloids and their derivatives were performed on a Hewlett-Packard 5710A GC-5983A mass spectrometer interfaced to a Hewlett-Packard 5933 data system. This instrument was equipped with either a 1.2-m × 2-mm ID glass column packed with 2% OV-101 on Chromosorb W-AWS or a 3-m × 2-mm ID nickel column packed with 2% OV-101 on Chromosorb W-AWS. Both mass spectrometers were operated at 70 eV.

Preparation of Heptafluorobutyramodes

Approximately 50 μg of crude pyrrolidine dissolved in CH_2Cl_2 was placed in a 15-ml Teflon-lined screw-cap centrifuge tube, the solvent was removed with nitrogen, and the residue was dissolved in 0.2 ml of neat heptafluorobutryic anhydride. The mixture was warmed for 10 min at 50°C and treated with 2 ml of hexane and 2 ml of saturated $NaHCO_3$. After shaking, the aqueous phase was removed, and the hexane phase was extracted twice with saturated $NaHCO_3$, once with water, and dried over anhydrous sodium sulfate.

Preparation of Methoxyethers

A gentle stream of nitrogen was used to remove the solvent from the crude hexane solutions of the heptafluorobutyramides. The residue was taken up in 2 ml of hexane-methanol (60:40), treated with approximately 50% excess of mercuric acetate, and stirred in the dark for 24 hr. The mixture was then treated with a slight excess of sodium borohydride and after 5 min, with two drops of glacial acetic acid and 2 ml of water. The hexane phase was separated and extracted twice more with water. The combined aqueous phases were extracted with hexane, and then the combined hexane phases were dried over anhydrous sodium sulfate. Following concentration, the hexane solutions were analyzed directly by GC-MS.

Synthesis of 2-(1-Hex-5-enyl)-5-(1-non-8-enyl)pyrrolidine.

9-Decenal(8). A solution containing 10.0 g (64mmol) of 9-decenol-1 in 64 ml of CH_2Cl_2 was added all at once to a rapidly stirred suspension of 21 g of pyridinium chlorochromate (Corey and Suggs, 1975) in 130 ml of Ch_2Cl_2. After 2 hr, 900 ml of ether was added and the mixture was filtered through Florisil, Distillation gave 6.7 g of pure aldehyde (68% yield), at 61–65° C (1.0 mm Hg); IR 3075, 2845, 2710, 1730, 1645, 995, and 905 cm^{-1}; NMR, δ9.7(1H, t, J = 2Hz, C<u>H</u>O), 5.9(1H, d of d of t, J = 18, 10, and 6 Hz, CH_2=C<u>H</u>—), 5.0(1H, br d, J = 18 Hz, CH_2=CH—), 4.9(1 H, br d, J = 10 Hz, CH_2=CH—), 2.38 (2H, br t, C<u>H</u>$_2$CHO), 2.0 (2H, m, C<u>H</u>$_2$CH=CH_2), 1.35[10H, br s, —$(CH_2)_5$—].

Analysis: Calculated for $C_{10}H_{18}O$: C, 77.87; H, 11.76; found: C, 77.09; H, 11.53.

1,11-Dodecadien-3-ol (9). A solution containing 6.7 h (43.5 mmol) of aldehyde **8** in 10 ml of tetrahydrofuran was added dropwise to a well-stirred solution of 1.5 equivalents of vinyl magnesium bromide in tetrahydrofuran under a nitrogen atmosphere. The mixture was stirred at room temperature for 45 min, heated to reflux for 2 hr, cooled, and quenched with saturated NH_4Cl solution. The mixture was extracted with ether (3 × 50 ml), and the ether extracts were dried over anhydrous magnesium sulfate. After filtration, distillation gave 5.8 g of pure diene alcohol **9** (73% yield), bp 72–76° C (0.2 mm Hg): IR 3330 (br), 3070, 1640, 990, and 905 cm^{-1}; NMR, δ5.71(2H, complex m, CH_2=C<u>H</u>—), 5.0(4H, complex m, C<u>H</u>$_2$=CH—), 4.0(1H, br m, C<u>H</u>—OH), 4.03(1H, s, —OH), 1.95(4H, m, CH_2=CH—C<u>H</u>$_2$— and C<u>H</u>$_2$CH—OH), 1.3 [10H, br s, —$(CH_2)_5$—].

Analysis: Calculated for $C_{12}H_{22}O$: C, 79.06; H, 12.16; found: C, 79.11; H, 12.17.

1,18-Nonadecadien-7,10-dione (10). A solution containing 5.8 g (32 mmol) of dienol **9** in 10 ml of CH_2Cl_2, was added to a suspension of pyridinium dichromate (Corey and Schmidt, 1979), in 50 ml of CH_2Cl_2, and the mixture was stirred 10 hr. After the usual work-up, distillation gave 3.0 g of the unstable 1,11-docecadien-3-one (53% yield, bp 68–73° C (0.3 mm Hg): IR 3070, 1695, 1685, 1645, 1615, 990, 960, and 904 cm^{-1}. This vinyl ketone was immediately mixed with 1.87 g (16.6 mmol) of 6-heptenal (Jones et al., 1980) and 0.5 g of 5-(2′-hydroxyethyl)-4-methyl-3-benzylthiazolium chloride (Stetter and Kuhlmann, 1974). After purging with nitrogen, 2.3 ml of triethylamine were added, and the mixture was refluxed for 20 hr. After cooling, the mixture was taken up in ether and filtered through a short Florisil column. The solvent was removed, and kugelrohr distillation at 0.2 mm Hg gave 3.3 g of diketone **10** as a waxy solid (68% yield), mp 41–44° C. Sublimation of the pot residue produced another 0.65 g of **10** (13% yield), mp 42–43° C; NMR, δ5.8(2H, d of d of t, J = 18, 10, and 6 Hz, CH_2=C<u>H</u>—), 5.0 (2H, br d, J = 18 Hz, trans C<u>H</u>$_2$=CH—), 4.9(2H, br d, J = 10 Hz, cis C<u>H</u>$_2$=CH—), 2.55(4H s, CO<u>CH</u>$_2$<u>CH</u>$_2$CO), 2.41(4H, m, C<u>H</u>$_2$CO), 2.0(4H, m C<u>H</u>$_2$C=C), 1.3(14H, br s, alkyl —CH_2—); MS m/z(rel. intensity) 292 (0.5, M+), 237(2), 224(2), 209(2), 195(2), 182(2), 181(4), 173(1), 167(2), 154(10), 153(4), 149(5), 138(8), 136(15), 135(5), 127(4), 125(3), 122(2), 121(5), 114(12), 112(1), 111(20), 110(8), 109(5), 107(13), 99(10), 98(8), 95(11), 93(9), 83(28), 81(10), 79(9), 71(20), 69(31), 68(5), 67(25), 57(10), 56(11), 55(100), 53(9).

Analysis: Calculated for $C_{19}H_{32}O_2$: C, 78.03; H, 11.03; found: C, 77.78; H, 11.06.

trans-*2-(1-Hex-5-enyl)-5-(1-non-8-enyl) pyrrolidine (1) and its cis Isomer.* A solution containing 2.0 g of diketone **10** (6.85 mmol), 0.1 g of KOH, 0.54 g of ammonium acetate, and 0.5 g of sodium cyanoborohydride in 15 ml of methanol was stirred for 15 hr. An excess of sodium borohydride was added, and the mixture was stirred an additional hour. The usual work up gave 1.8 g of pyrrolidine **1** as a 50:50 mixture of *cis* and *trans* isomers that was greater

than 95% pure by GLC analysis (OV-1, SP—1000) (approx. 90% yield): IR 3260 (w), 3070, 1640, 990, and 905 cm^{-1}; NMR δ of the mixture 5.8(2H, d of d of t, J = 18, 10, and 6 Hz, CH_2=C<u>H</u>—), 5.0(2H, br d, J = 18 Hz, trans C<u>H</u>$_2$=CH—), 4.9 (2H, br d, J = 10 Hz, cis C<u>H</u>$_2$=CH—), 3.0 (3H, br m, C<u>H</u>—N and N—H), 2.1(4H, m, CH_2—CH=CH_2), 1.3(22H, br s, alkyl —CH_2—); MS m/z (rel. intensity), 277(3, M+), 276(2), 234(12), 220(14), 195(15), 194(100), 178(10), 153(9), 152(83), 150(4), 136(3), 124(2), 122(2), 110(2), 109(1), 108(2), 96(5), 95(3), 94(2), 83(7), 82(30), 81(5), 70(2), 69(4), 68(10), 67(18), 57(1), 56(4), 55(20), 54(3), 53(2).

Analysis: Calculated for $C_{19}H_{35}N$: C, 82.24; H, 12.71; N, 5.05; found: C, 82.10; H, 12.72; N, 5.02.

Treatment of a small sample of the mixture of pyrrolidine **1** and its *cis* isomer with heptafluorobutyric anhydride as described above produced the *N*-heptafluorobutyramide derivative, MS m/z (rel. intensity) 473(1, M+), 416(3), 391(10), 390(45), 350(1), 349(18), 348(100), 305(2), 304(21), 280(2), 267(3), 266(40), 240(5), 169(3), 135(3), 135(12), 121(1), 109(8), 107(4), 95(12), 93(8), 81(18), 79(10), 69(15), 67(33), 55(22). Methoxymercuration–demercuration of this derivative as described above gave 2-(5-methoxyhex-1-yl)-5-(8-methoxynon-1-yl)-*N*-heptafluorobutyrylpyrrolidine as a major product whose mass spectrum is identical with that shown in Figure 1, which was obtained from the natural compound.

2-(1-Hex-5-enyl)-5-nonylpyrrolidine (2)

The preparation of the monounsaturated pyrrolidine **2** has already been described (Jones et al., 1980). A small sample of **2** was treated with heptafluorobutyric anhydride to give the *N*-heptafluorobutyryl derivative, MS m/z (rel. intensity) 475(0.5 M+), 446(1), 393(18), 392(100), 369(3), 368(8), 351(7), 350(62), 349(5), 348(50), 306(10), 280(3), 267(11), 266(60), 264(6), 240(15), 197(2), 180(1), 169(7), 145(2), 135(18), 123(12), 111(10), 110(7), 109(28), 107(10), 97(35), 95(47), 93(18), 91(5), 85(10), 83(44), 81(57), 79(20), 71(20), 70(15), 69(84), 68(22), 67(82), 57(33), 55(98). Methoxymercuration-demercuration of this derivative as described above gave 2-(5-methoxyhex-10-yl)-5-nonyl-*N*-heptafluorobutyryl-pyrrolidine as the major product. MS m/z (rel. intensity) 507(0.5, M+), 492(3), 407(18), 393(10), 392(44), 380(1), 368(12), 353(2), 349(8), 348(45), 306(3), 294(3), 281(2), 280(4), 266(28), 264(40), 197(2), 182(8), 169(5), 152(3), 147(2), 145(2), 135(18), 123(9), 121(4), 119(2), 111(7), 109(21), 107(10), 105(6), 97(22), 95(30), 93(17), 91(8), 85(13), 83(23), 81(30), 79(20), 71(16), 69(40), 67(38), 59(100), 57(20), 55(48).

Mass Spectral Analysis of M. ebeninum *Alkaloids*

The GC/MS from the extracts of *M. ebeninum* showed one broad peak whose mass spectrum had ions at m/z = 277 (M+), 194, and 152, indicative of pyrrolidine **1**. In addition there were ions at m/z = 275, 274, 207, 206, 192,

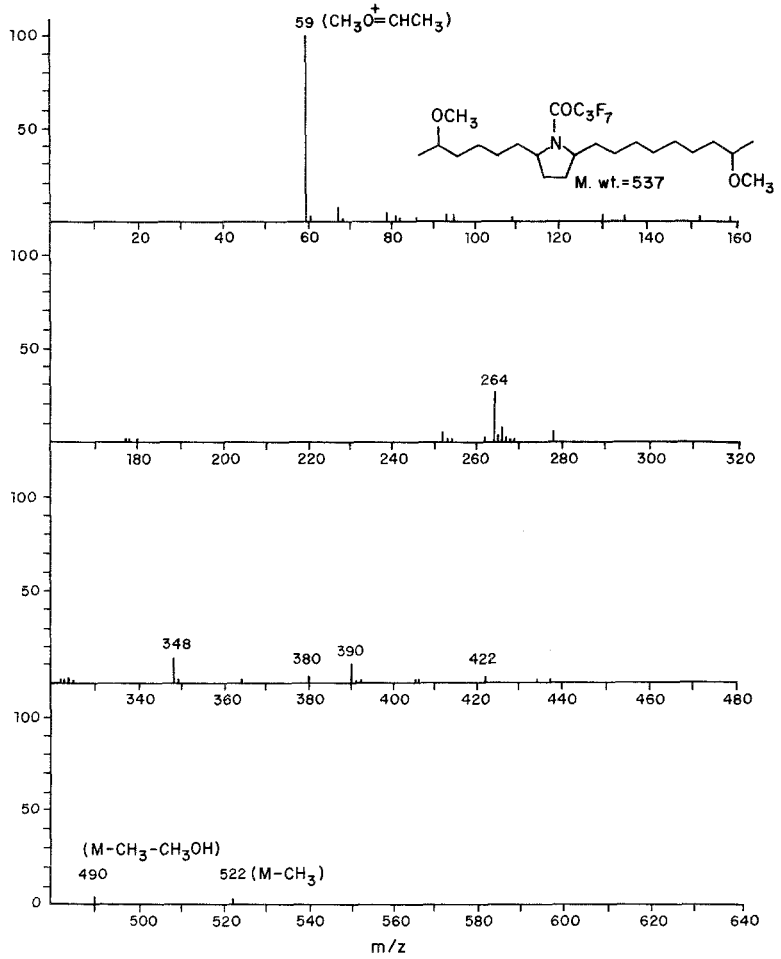


FIG. 1. MS of the dimethoxyheptafluorobutyryl derivative of **1**.

165, 164, 150, and 82, indicative of the pyrrolines **6** and **7**. Treatment of a portion of the extract with sodium borohydride converted the mixture to a single component with a mass spectrum identical to that of **1**. Treatment of another portion of the extract with sodium borodeuteride gave an inseparable mixture of isotopomers with a mass spectrum containing fragment ions at $m/z = 278$, 195, and 153 as well as those at $m/z = 277$, 194, and 152. The intensities of these ions indicated that there were essentially equivalent amounts of deuterated and undeuterated pyrrolidines. Traces of pyrrolidines **2** and **3** were also present in these extracts as well separated GC peaks. Compound **2** mass spectrum identical with that previously reported (Jones et al., 1980), while **3** showed the following important ions; $m/z = 281$(1, M+), 196(85), and 154(100), indicative of its saturated side chains.

Quantitation of Alkaloids in M. *near* metoecus

Five or ten ant workers were taken from a group of ants freshly killed by freezing and macerated in 10 μl of either CH_2Cl_2 or ethanol. A 1.0-μl sample of the supernatant liquid was immediately analyzed by gas chromatography. This was repeated seven times using a total of 60 ants. The results were calibrated with the gas chromatogram from 1.0 μl of a solution containing 2.3 mg/ml of pyrrolidine **2** in CH_2Cl_2, and they indicate that each ant worker contains between 0.5 μg and 2.0 μg of alkaloid.

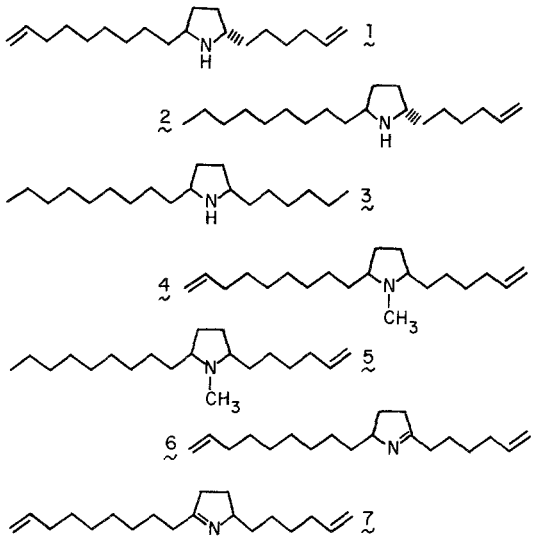
Methylation of Alkaloids in M. *near* metoecus

A CH_2Cl_2 extract of a sample of *M.* near *metoecus* containing pyrrolidines **1**, **2**, and **3** was taken to dryness with a gentle stream of nitrogen and treated with one drop of 28% formaldehyde and one drop of formic acid. The resulting mixture was heated in a closed vial overnight. The solution was made basic with 10% KOH and extracted with chloroform to provide a mixture containing only *N*-methyl-pyrrolidines. GC-MS (2-m × 2-mm column packed with 10% SP-1000 on Supelcoport) showed **4** and **5** eluting at about the same temperature and characterized by their mass spectral fragmentation patterns: **4**, m/z = 291(M+), 208(M—C_6H_{11}), and 166(M—C_9H_{17}); **5**, m/z = 293(M+), 210(M—C_6H_{11}), and 166(M—C_6H_{19}). The methylated derivative of **3** was well separated under these conditions and its mass spectrum had important ions at m/z = 295(M+), 210, 168. The mass spectrum of the nearly inseparable mixture of **4** and **5** prepared this way was almost identical to that of these two alkaloids and served to identify them when they were found in fresh venom extracts of the various species listed in Table 1.

RESULTS

The *trans*-2,5-dialkylpyrrolidines **1–5** are characteristic venomous components of all species of *Monomorium* analyzed. While quantitative and qualitative variations are evident, the venoms of the five native species, and those of *M. ebeninum* and one population of *M. floricola* as well, are all characterized by the presence of 2,5-dialkyl-pyrrolidines with the same C_{19} carbon skeleton (Scheme 1).

The novel 2-(1-hex-5-enyl)-5-(1-non-8-enyl) pyrrolidine (**1**) and the previously reported 2-(1-hex-5-enyl)-5-nonylpyrrolidine (**2**) (Jones et al., 1980), like the major alkaloidal components in every species studied, are easily recognizable from their mass spectra (Pedder et al., 1976). The mass spectra of these compounds show a pair of intense peaks resulting from α-cleavage of side chains and a weak molecular ion of odd mass: M+ 277(3%), 194(100), 152(83), and M+ 279(4%), 196(83), 152(100). These data indicate a 6-carbon monounsaturated side chain and either a 9-carbon monounsaturated or a



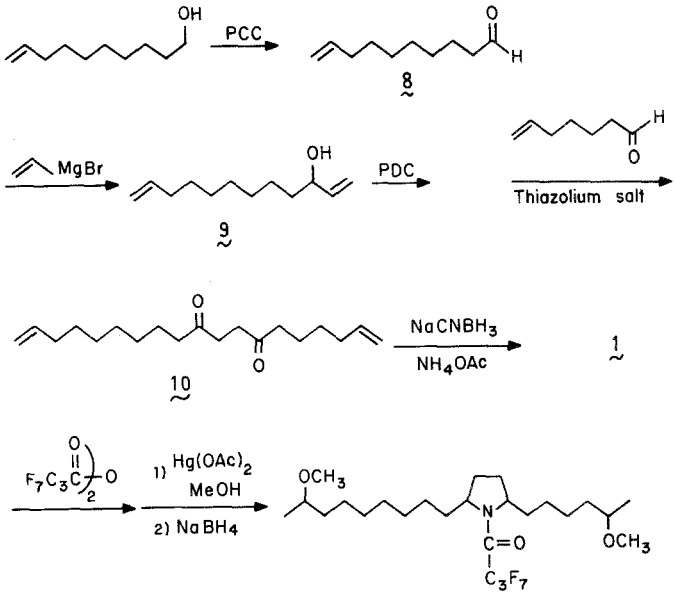
SCHEME 1

saturated side chain, but do not reveal the positions of the double bonds.

The technique of methoxymercuration–demercuration was utilized to locate the double bonds in the side chains (Howard et al., 1978; Blomquist et al., 1980). Since this technique was not successful with the free amines, the pyrrolidines were converted to the corresponding *N*-heptafluorobutyramides. These derivatives, upon treatment with mercuric acetate and methanol, followed by reduction with sodium borohydride, yielded the methoxylated compounds, albeit in rather modest yields. When the starting alkaloid was diunsaturated, both the di- and monomethoxylated derivatives were formed. The mass spectra of these compounds, via the exclusive formation of ions at $m/z = 59$, showed the incorporation of methoxy ether groups on the penultimate carbon of the side chains, indicating terminal double bonds in every case (Figure 1).

Pyrrolidine **1**, prepared by previously described methodology (Scheme 2) (Jones et al., 1980), was identical by GC/MS to the natural alkaloid; the derivatives of the natural alkaloid and synthetic compound were also identical. The monomethoxy-*N*-heptafluorobutyramide derivative of pyrrolidine **2** was also prepared, and again its mass spectrum as well as the mass spectrum of the free base matched the corresponding spectra from the ant extracts.

The synthetic alkaloids **1** and **2** are a 1:1 mixture of *cis* and *trans*-2,5-dialkylpyrrolidine isomers separable by gas chromatography (Jones et al., 1980). As shown previously, the *cis* configuration can be assigned to the isomer that elutes first. Comparison by retention time and coinjection showed that



SCHEME 2

the naturally occurring pyrrolidines **1** and **2** were of the *trans* configuration in every sample of *Monomorium* extract available. In *M. viridum* the ratio of pyrrolidine **1** to pyrrolidine **2** was found to be approximately 3:2, while in *M.* new sp. near *minimum* this ratio was about 1:2.

The 1-pyrrolines **6** and **7**, major components in the alkaloidal mixture from *M. ebeninum*, were characterized by their parent ion at $m/z = 275$ as well as the M-1 ion at 274 and the McLafferty rearrangement ions at 207 and 165 in **6** and **7**, respectively. They also showed characteristic allylic cleavage ions at $m/z = 206$ and 150 from **6** and $m/z = 192$ and from **7**. An ion at $m/z = 82$ arises from both pyrrolines. The intensities of these ions indicated that there was slightly more of **6** and **7**.

These Schiff bases were further characterized by their conversion to pyrrolidine **1** upon treatment with sodium borohydride. In *M. ebeninum*, their incorporation of deuterium upon treatment with sodium borodeuteride was evident because of the appearance of ions at m/z =278, 195, and 153; the intensity of these ions relative to the undeuterated ions showed that the total amount of **6** and **7** was roughly equal to the amount of **1** in the alkaloid mixture from this ant.

Three minor alkaloidal components were also found in the *Monomorium* species included in this investigation. In extracts of *M.* near *metoecus*, *M. viridum*, and *M. ebeninum*, a minor component (approximately 1–2% of the mixture) had a characteristic mass spectrum [281(1%), 196(85), 154(100)] that

could be attributed to 2-hexyl-5-nonylpyrrolidine (**3**). In addition, small amounts of the *N*-methyl and analogs of pyrrolidines **1**, and **2** (**4** and **5**) were detected. Their structures were suggested by their gas chromatographic behavior and their mass spectra. Confirmation of the structures of **4** and **5** was obtained by *N*-methylation of the extracts of a sample of *M.* near *metoecus* with formaldehyde and formic acid, which converted all of the N-H alkaloids present into their *N*-methyl analogs.

With the exception of *M. floricola*, each species appeared to produce a characteristic venom fingerprint with no significant differences in alkaloidal composition being associated with different populations of the same species (e.g., *M. minimum*) (Table 1). No alkaloids were detected in extracts of two populations of *M. floricola* whereas that from Bahia Honda Key, Florida (Table 1) contained a very small quantity of pyrrolidine **3**, along with traces of several unidentified alkaloids. The venom of *M. viridum* was found to be especially distinctive in being the only one of the venoms studied that contained all of the dialkylpyrrolidines detected in these secretions. In addition, it and *M.* new sp. near *minimum* produce the only venom in which both pyrrolidines **1** and **2** are major constituents. Among the *Monomorium* species, the venom of *M. ebeninum* was especially characteristic in containing two pyrrolines as major constituents; that of *M. cyaneum* was exceptional in producing the *N*-methylpyrrolidine **5** as a major venomous alkaloid.

Since *M.* near *metoecus* was readily available, samples of these ants were analyzed in order to determine the approximate amount of alkaloid produced by each ant. The extracts from five or ten individuals macerated in a known volume of solvent were compared to a standard solution of pyrrolidine **2** by gas chromatography. Seven replicates of this comparison indicated that each *M.* near *metoecus* worker contained between 0.5 μg and 2.0 μg of alkaloids.

DISCUSSION

M. ebeninum and the native North American species of *Monomorium* appear to have limited biosynthetic capacities in that they produce only *trans* isomers of 2,5-dialkylpyrrolidines possessing C_6 and C_9 side chains. The most characteristic compound produced by these ants, pyrrolidine **1**, which has not been previously detected as a natural product, appears to be the only known example of a diunsaturated pyrrolidine synthesized by animals. While terminally unsaturated side chains on 2,5-dialkylpyrrolidines have been previously reported as being produced by *Monomorium* species (Jones et al., 1980; Ritter and Persoons, 1975; Ritter et al., 1975), and the synthesis of one of these compounds has been described (Ritter and Stein, 1978), this is the first report of experimental evidence for this assignment. The technique of methoxymercuration-demercuration produced the dimethoxy-*N*-heptafluoro-

butyramide derivative of natural and synthetic alkaloid **1** on a microscale. Figure 1 depicts the mass spectrum of this compound from the major venom component of *M. minimum* (Douglas Co., Kansas), and Figure 2 shows the molecular fragments that can be assigned to this spectrum. The base peak at $m/z = 59 (CH_3CHOCH_3)^+$ indicates incorporation of the methoxyl groups at the penultimate carbon atoms of the side chains. There is no evidence for the presence of methoxyl groups at any other site on the molecule. An analogous fragmentation pattern was also observed for the monomethoxy-*N*-heptafluorobutyramide derivative of pyrrolidine **2**, which again indicated a terminal double bond.

Since oxymercuration reactions proceed to give mostly Markovnikov addition of the hydroxyl species (Brown and Geoghegan, 1970), it is not surprising that no observable terminal methoxylation occurred. The *Monomorium* unsaturated pyrrolidines thus contain only terminal double bonds, similar to the unsaturated pyrrolidines reported in *M. pharaonis* (Ritter et al., 1975, 1980).

Synthesis by an unambiguous route confirms the above structural assignments for pyrrolidines **1** and **2** (Scheme 1). This synthetic methodology also permits assignment of stereochemistry of the alkyl groups about the

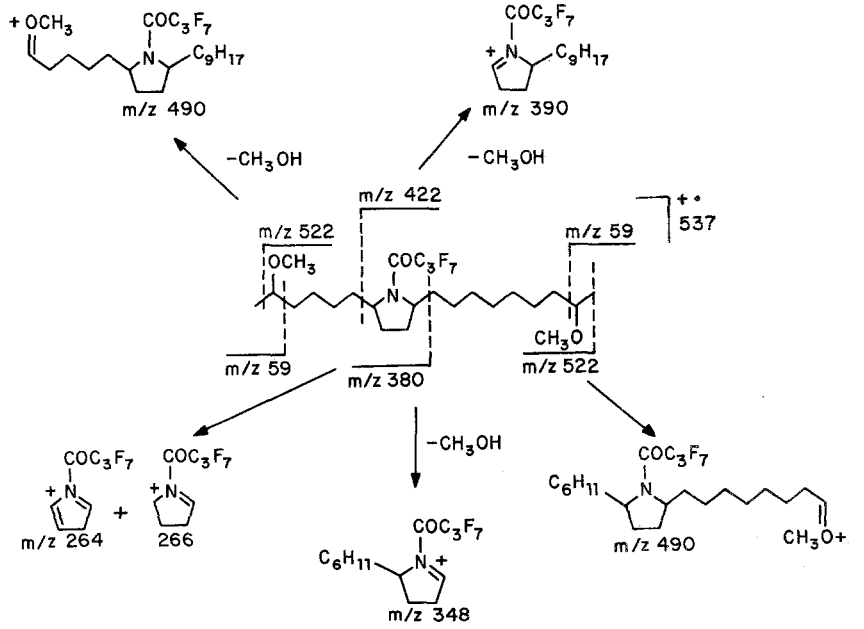


FIG. 2. Suggested interpretation of the MS of the dimethoxyheptafluorobutyryl derivative of **1**.

pyrolidine ring since both *cis* and *trans* isomers are formed (Jones et al., 1980) having different gas chromatographic retention times but identical mass spectra. Comparison of the natural and synthetic alkaloids shows that in every case, the later-eluting *trans* isomer is produced by the *Monomorium* species listed in Table 1.

Since it has been shown that 1-pyrrolines such as **6** and **7** can be formed as artifacts in GC-MS instruments (Fales et al., 1980), their appearance in trace amounts is always somewhat suspect. On the other hand, reduction of the extract of *M. ebeninum* with sodium borodeuteride shows that the 1-pyrrolines **6** and **7** make up at least half of the alkaloid mixture in this species. The allylic cleavage ions and McLafferty rearrangement ions that characterize these compounds arise from previously described fragmentation pathways (Pedder et al., 1976; Fales et al., 1980).

The minor constituents of these alkaloidal mixtures, pyrrolidines **3**, **4**, and **5**, serve to further characterize the venom mixtures from each *Monomorium* species. 2-Hexyl-5-nonylpyrrolidine **3** is immediately identifiable from its mass spectral fragmentation pattern which is characteristic of other 2,5-dialkylpyrrolidines (Jones et al., 1980). The *N*-methylpyrrolidines, on the other hand, have mass spectral fragmentation patterns identical with their parent pyrrolidines **1** and **2**, with the α-cleavage and parent ions increased by 14 mass units (Figure 3). Computer-constructed, selected ion chromatographs of these compounds show symmetrical peaks, whereas the parent pyrrolidines

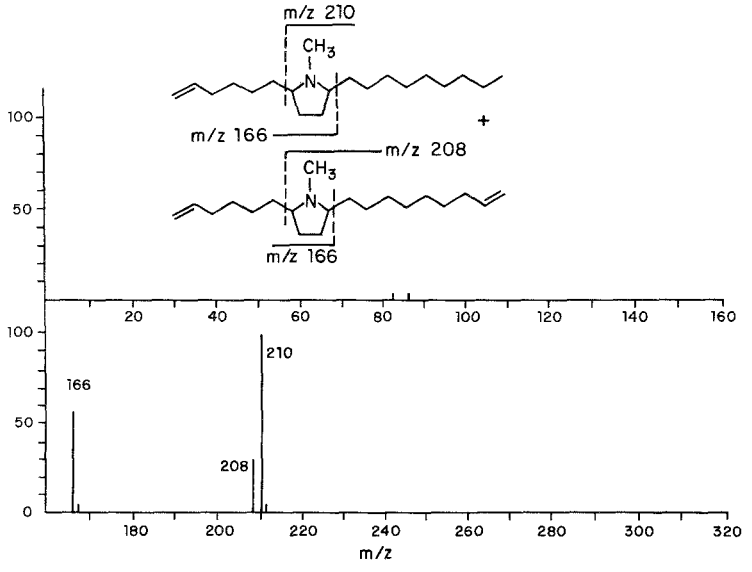


FIG. 3. *N*-Methylpyrrolidines **4** and **5** from *M. viridum*.

show distinctly unsymmetrical peaks (OV-101), presumably because of interactions of the active hydrogen on the nitrogen with the column substrate. The symmetrical shape of the peaks for these compounds tends to exclude the possibility that the compounds are dialkyl or dialkenyl piperidines. In addition, their mass spectra are essentially identical to those of the *N-methylpyrrolidines prepared from a sample of natural Monomorium* pyrrolidines by *N*-methylation. Since there is so little of **4** and **5** present, there is no direct evidence that the position of their double bonds is terminal. However, the fact that several species of *Monomorium* that were examined contain these *N*-methyl analogs in exactly the same relative proportions as pyrrolidines **1** and **2** in their venoms, suggests that perhaps *N*-methylation is a terminal biosynthetic process, so it would be unlikely that the compounds differ in the location of their double bonds.

From a chemotaxonomic standpoint, these *Monomorium* venoms would appear to possess at least a modicum of value. All apparent species that were analyzed produce distinctive venoms in terms of their qualitative and quantitative compositions. For example, *M. viridum* is the only species that produces all the dialkylpyrrolidines detected in these venoms, being further distinguished by synthesizing both the diunsaturated and monounsaturated compounds, **1** and **2**, as major constituents. The venom of the closely related species *M.* near *metoecus* is almost as qualitatively rich as that of *M. viridum*, differing only in the absence of the *N*-methyl compound **4**. The only pyrrolidine-producing species lacking the diunsaturated pyrrolidine **1**, *M. cyaneum*, is especially distinctive in producing significant amounts of the *N*-methyl pyrrolidine **5** (Table 1).

Even though relatively few *Monomorium* (*Monomorium*) species have been analyzed, there appears to be a considerable amount of variation in the chemistry of the venoms synthesized by different species. Although the venoms of native North American species in this subgenus contain only variations on 2-hexyl-5-nonylpyrrolidine, that of *M. pharaonis* contains a diversity of other nitrogen heterocycles which include pyrrolidine **2**, a major component in the venoms of the *Monomorium* species analyzed in this investigation. In addition, the venom of *M. pharaonis* contains 2,5-dialkylpyrrolidines with different side-chain lengths, as concomitants of dialkylindolizidines (Ritter et al., 1975).

The dialkylpyrrolidines are probably utilized as repellents for ants in interspecific contexts. Hölldobler (1973) demonstrated that *M. pharaonis* can utilize its venom as a powerful repellent while plundering brood from the nests of other species. This plundering *modus vivendi* is characteristic of thief ants in the genus *Solenopsis*, many of which produce dialkylpyrrolidines in their venoms (Jones et al., 1979, 1980). It has been recently demonstrated that the thief ant *S. fugax* produces 2-butyl-5-heptylpyrrolidine in its poison gland,

utilizing this compound as a venom mace while stealing brood from the nests of foreign ant species (Blum et al., 1980). It appears that the dialkylpyrrolidines synthesized by *Monomorium* species are also utilized to repel other species of ants in competitive situations involving the acquisition of food. One of us (J.T.) has observed workers of *M. ebeninum* and *M. floricola* effectively repelling other species of ants by utilizing their venomous secretions. The biochemical evolution of alkaloidal repellents may have enabled these diminutive species to exploit sociality in a way that would not have been otherwise possible.

Note Added in Proof—As this manuscript was going to press, we had the opportunity to examine two other *Monomorium* species. *M. minutum* (San Sebastian, Spain) was found to contain only pyrrolidines **1** and **2** in approximately a 20:1 ratio. *M. carbonarium* (Key Largo, Florida) was found to contain only pyrrolidine **1**. None of the other alkaloids listed in Table 1 were detected in these species.

Acknowledgments—We thank J. Moody for collecting *M.* near *emersoni* (Lubbock, Texas) and James Trager for providing *M. floricola* (Vero Beach, Florida) and *M.* new sp. near *minimum* (Gainesville, Florida), Dennis Howard and Mike Tomalski for collecting *M. floricola* (Bahia Honda Key, Florida) and *M. carbonarium*, and C. Collingwood for collecting and identifying *M. minutum*.

REFERENCES

BLOMQUIST, G.J., HOWARD, R.W., MCDANIEL, C.A., REMALEY, S., DWYER, L.A., and NELSON, D.R. 1980. Application of methoxymercuration–demercuration followed by mass spectrometry as a convenient microanalytical technique for double-bond location in insect-derived alkenes. *J. Chem. Ecol.* 6:257–269.

BLUM, M.S., JONES, T.H., HÖLLDOBLER, B., FALES, H.M., and JAOUNI, T. 1980. Alkaloidal venom mace: Offensive use by a thief ant. *Naturwissenschaften* 67:144–145.

BRAND, J.M., BLUM, M.S., FALES, H.M., and MACCONNELL, J.G. 1972. Fire ant venoms: Comparative analysis of alkaloidal constituents. *Toxicon* 10:259–273.

BROWN, H.C., and GEOGHEGAN, P.J. 1970. Solvomercuration–demercuration. I. The oxymercuration of representative olefins in aqueous solution. *J. Org. Chem.* 35:1844–1850.

COREY, E.J., and SCHMIDT, G. 1979. Useful procedures for the oxidation of alcohols involving pyridinium dichromate in aprotic media. *Tetrahedron Lett.* 1979:399–402.

COREY, E.J., and SUGGS, W.J. 1975. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* 1975:2647–2650.

EMERY, C. 1922. Fam. Formicidae, Subfam. Myrmicinae. Genus *Monomorium*. *Genera Insectorum*. Fasc. 174:166–185.

FALES, H.M., COMSTOCK, W., and JONES, T.H. 1980. Test for dehydrogenation in gas chromatography–mass spectrometry systems. *Anal. Chem.* 52:980–982.

HÖLLDOBLER, B. 1973. Chemische Strategie beim Nahrungserwerb der Diebsameise (*Solenopsis fugax* Latr.) und der Pharaoameise (*Monomorium pharaonis* L.). *Oecologia* 11:371–380.

HOWARD, R.W., MCDANIEL, C.A., and BLOMQUIST, G.J. 1978. Cuticular hydrocarbons of the eastern subterranean termite, *Reticulitermes flavipes* (Xollay) (Isoptera: Rhinotermitidae). *J. Chem. Ecol.* 4:233–245.

JONES, T.H., BLUM, M.S., and FALES, H.M. 1979. Synthesis of unsymmetrical 2,5-di-*n*-alkylpyrrolidines: 2-Hexyl-5-pentylpyrrolidine from the thief ants *Solenopsis molesta*, *S. texanas*, and its homologues. *Tetrahedron Lett.* 1979:1031–1034.

JONES, T.H., FRANKO, J.B., BLUM, M.S., and FALES, H.M. 1980. Unsymmetrical 2,5-dialkl-pyrrolidines via reductive amination of 1,4-diketones. *Tetrahedron Lett.* 21:789–792.

MACCONNELL, J.G., BLUM, M.S., and FALES, H.M. 1971. The chemistry of fire ant venom. *Tetrahedron* 26:1129–1139.

MACCONNELL, J.G., BLUM, M.S., BUREN, W.F., WILLIAMS, R.N., and FALES, H.M. 1976. Fire ant venoms: Chemotaxonomic correlations with alkaloidal compositions. *Toxicon* 14:69–78.

PEDDER, D.J., FALES, H.M., JAOUNI, T., BLUM, M.S., MACCONNELL, J., and CREWE, R.M. 1976. Constituents of the venom of a South African fire ant (*Solenopsis punctaticeps*). *Tetrahedron* 32:2275–2279.

RITTER, F.J., and PERSOONS, C.J. 1975. Recent developments in insect pheromone research, particularly in the Netherlands. *Neth. J. Zool.* 25:261–275.

RITTER, F.J., and STEIN, F. 1978. Attractant for Ants. U.S. Patent 4075320 (21 Feb. 1978).

RITTER, F.J., ROTGANS, I.E.M., TALMAN, E., VERWIEL, P.E.J., and STEIN, F. 1973. 5-Methyl-3-butyloctrahydriondolizidine, a novel type of pheromone attractive to Pharaoh's ants (*Monomorium pharaonis* (L.)). *Experientia* 29:530.

RITTER, F.J., ROTGANS, I.E.M., VERKUIL, E., and PERSOONS, C.J. 1975. The trail pheromone of the Pharaoh's ant *Monomorium pharaonis:* Components in the odour trail and their origin, pp. 99–103, *in* Ch. Noirot, P.E. Howse, and G. le' Masne, (eds.). Pheromones and Defensive Secretions in Social Insects, A symposium of the International Union for the Study of Social Insects, University of Dijon Press, Dijon, France.

RITTER, F.J., BRÜGGEMANN, T.E.M., VERWIEL, P.E.J., TALMAN, E., STEIN, F., and PERSOONS, C.J. 1980. Faranal and monomorines, pheromones of the Pharaoh's ant, *Monomorium pharaonis* (L.) Proc. Conf. on Regulation of Insect Development and Behavior, Karpacs, Poland, June 23–28, 1980.

STETTER, H., and KUHLMANN, H. 1974. Addition von aldehyden an Aktivierte Doppelbindugen, VI. Uber Additionen Aliphatischer Aldehyde an Methylvinlketon. *Tetrahedron Lett.* 1974:4505–4508.

TALMAN, E., RITTER, F.J., and VERWIEL, P.E.J. 1974. Structure elucidation of pheromones produced by the Pharaoh's ant, *Monomorium pharaonis* L., pp. 197–217. *in* A. Frigerio and N. Castagnoli, (eds.). Mass Spectrometry in Biochemistry and Medicine, Raven Press, New York.