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Alkaloids of the Mexican Bean Beetle, *Epilachna varivestis* (Coccinellidae)†[1]

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Abstract: Two novel alkaloids, 2-(12-aminotridecyl)-pyrrolidine and its 1-(2-hydroxyethyl) derivative, are characterized from extracts of adult Mexican bean beetles. These pyrrolidines, together with a previously identified homotropane alkaloid, euphococcinine, account for 90% of the alkaloids present in this beetle. A number of piperidine derivatives are also identified as minor components. The total mixture represents the most complex bouquet of alkaloids reported hitherto from any coccinellid beetle.

INTRODUCTION

Ladybird beetles are virtually untouched by predators. A variety of alkaloids, which apparently confer this protection, have been characterized from these beetles.[3,4] As an example, the homotropane, 9-aza-1-methyl-bicyclo-[3.3.1]nonan-3-one (euphococcinine), distinctly deterrent to ants and spiders, was identified in the hemolymph of the Mexican bean beetle, *Epilachna varivestis*.[5] In the present study, we examined the alkaloids obtained by extraction of whole bodies of this coccinellid beetle and found the composition of the extract to be considerably more complex than was reported previously.

RESULTS AND DISCUSSION

Gas chromatographic examination of the alkaloid mixture obtained from 14-day-old adult beetles showed that the compounds fall into two groups, based on their volatilities. The molecular weights of the more volatile compounds (peaks A-F in Figure 1) are odd numbered (in agreement with expectations for molecules

† This publication is dedicated to Prof. Carl Djerassi on the ocassion of his seventieth birthday.

containing a single nitrogen atom) and range from 121 to 157 (Table 1). The *m/z* values of the parent ions of the less volatile compounds (peaks G-I) are even numbered (corresponding to the presence of two nitrogen atoms), and extend from 268 to 312. Moreover, a comparison of the mass spectra of compounds G-I with those of previously described alkaloids[6] indicated that these higher molecular weight components possess novel structures.

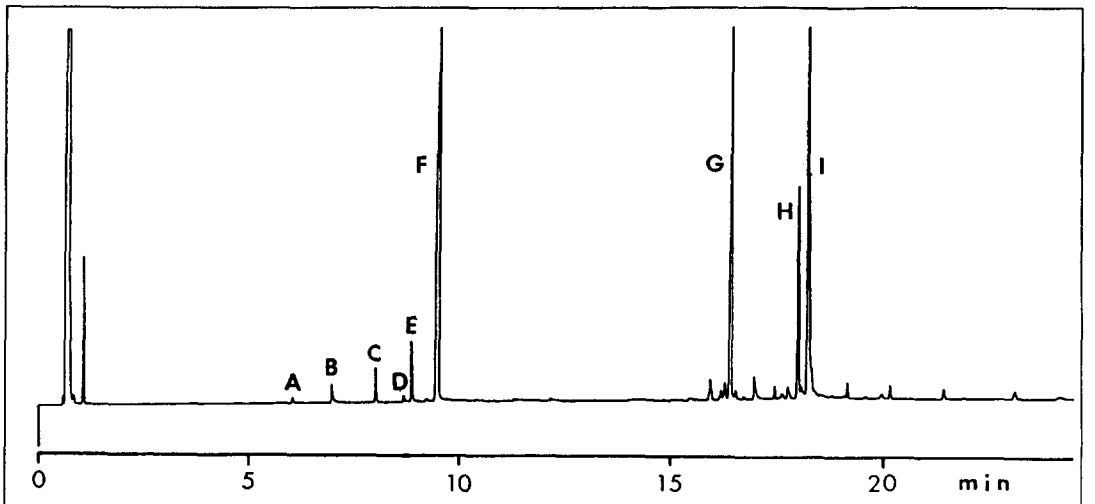
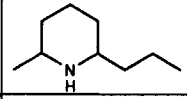
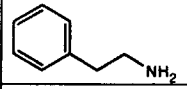
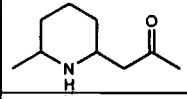
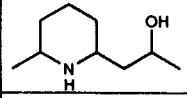
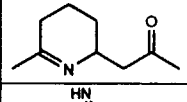
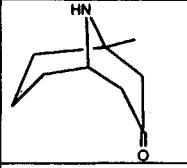
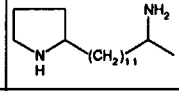
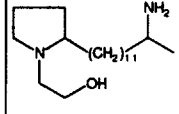


Figure 1. A typical gas chromatogram obtained from the alkaloids of 14-day-old adult *E. varivestis*. A fused-silica column (12 m x 0.22 mm) coated with methyl silicone (0.33 µm, HP-1) was used. The oven temperature was kept at 60° C for 4 min and programmed to 280° C at 15° C/min.

The composition of the alkaloid mixture from adult beetles varied both qualitatively and quantitatively with the age of the beetles. The chromatogram presented in Figure 1 is typical of 10-14 day old beetles. Two of the major components, G and I, together with the more volatile constituent F, which was readily recognized by its mass spectrum as the homotropane alkaloid previously identified from *E. varivestis* (Table 1), constitute over 90% of the defensive alkaloid mixture. Interestingly, the compounds A-F were absent in eggs and larvae, although G-I were present in eggs and larvae as well as in adults.

Component I was the most abundant constituent in extracts of eggs, larvae, and adults (41-84 %). The mass spectrum of this component suggested a molecular weight of 312. In addition, the peaks at *m/z* 297(M-15), 294(M-18) and 281(M-31) indicated the presence of a methyl group and a primary OH or an OCH_3 group. A strong peak at *m/z* 44 implied that a $-CH(NH_2)CH_3$ moiety might be present in the molecule. The CI mass spectrum of I, which showed an intense peak at *m/z* 313 (M^++1, 56%) confirmed the molecular weight assignment. The high-resolution EI mass spectrum established the molecular formula of I to be $C_{19}H_{40}N_2O$ (M^+-1 at 311 daltons; calculated mass for $C_{19}H_{39}N_2O$: 311.3062; found: 311.3051). Similarly, the composition of the peak at *m/z* 114 was found to be $C_6H_{12}NO$ (calculated mass: 114.0919; found: 114.0918). This suggested that the base peak might contain a heterocyclic ring system. Furthermore, the compound was resistant to catalytic hydrogenation, which established I as a monocyclic compound. Nevertheless, I could be reduced to its basic carbon skeleton by high temperature hydrogenolysis[7] with Pt and $LiAlH_4$. The most abundant hydrocarbon in the resulting mixture was identified as *n*-heptadecane by GC-MS. This indicated that I contains an uninterrupted skeleton of seventeen carbon atoms.

Table 1. Defensive Alkaloids of Adult *Epilachna varivestis*.

Peak[a]	Structure	%[b]	Mass specrum (m/z, %)	Gasphase infrared spectrum
A		0.2	141 (M^+, 2), 126(7), 112(3), 99(9), 98(100), 84(5), 83(2), 82(4), 81(7), .70(11), 56(16), 55(14), 44(20), 43(11), 42(14), 41(16), 30(14)	
B		0.6	121(M^+, 4), 92(6), 91(15), 77(3), 65(9), 63(4), 51(6), 50(3), 49(3), 42(2), 39(7), 32(2), 30(100)	
C		1.2	155(M^+, 9), 140(21), 126(1), 112(20), 99(12), 98(100), 97(7), 96(10), 84(8), 82(46), 70(24), 69(9), 56(15), 55(15), 44(23), 43(54), 42(18), 41(18)	2955(s), 2867(w), 1728(s), 1424(w), 1366(m), 1157(m)
D		0.2	157(M^+, 3), 156(1), 142(12), 124(4), 99(7), 98(100), 96(4), 84(8), 82(9), 81(7), 70(15), 56(11), 55(10), 44(19), 43(14)	3425(w), 2934(s), 2865(w), 1446(w), 1397(w), 1321(m)
E		2.2	153(M^+, 3), 111(8), 110(100), 98(6), 97(3), 96(11), 95(5), 94(5), 84(3), 83(12), 82(19), 69(6), 68(12), 58(4), 55(9), 43(57), 42(22), 41(16), 39(13)	2946(s), 1728(s), 1664(s), 1431(m), 1362(s), 1225(w), 1169(m)
F		28.4	153(M^+, 42), 138(3), 125(4), 124(10), 112(3), 111(36), 110(100), 98(14), 97(12), 96(82), 95(21), 94(16), , 83(26), 82(46), 81(9), 80(5), 79(3), 70(11), 69(6), 68(20), 55(14), 43(27), 42(46), 41(30)	2935(s), 1727(s), 1415(w), 1355(w), 1291(w), 1234(m), 1175(w)
G		18.4	268(M^+, 0.4), 267(M-1, 0.6), 254(0.6), 253(3), 224(1), 200(1) , 199(2), 182(2), 168(1), 154(1), 126(1), 98(1), 96(3), 84(2), 83(3), 70(100), 56(7), 55(5), 44(57), 43(10), 42(4), 41(8).	2993(s), 2864(m), 1624(w), 1460(w), 1369(w), 789(w).
H	Unknown	7.8	310(M^+,2), 309(3), 295(8), 292(3), 281(5), 279(9), 267(12), 250(2), 238(5), 236(10),198(6), 140(7), 114(15) 113(14), 112(86), 98(9), 84(12), 55(15), 44(100).	2993(s), 2865(m), 1462(w), 1364(w), 1040(w)
I		40.9	312(M^+,0.5), 311(M-1, 3), 297(M^+-CH_3,10), 295(3), 294(8), 282(12), 281(58), 268(3), 267(2), 250(3), 238(17), 226(7), 224(3), 212(2), 210(3), 198(2), 154(3), 140(4), 124(5), 115(13), 114(100), 112(5), 98(10), 96(7), 84(35), 70(21), 56(6), 55(9), 45(5), 44(48), 43(6), 42(6), 41(8)	2993(s), 2864(m), 2804(w), 1461(w), 1354(w), 1061(w), 790(w)

[a]Letters refer to gas chromatographic peaks in Figure 1; [b]The values given are typical, although the amounts varied significantly from sample to sample.

Further structural evidence was obtained by preparing a series of derivatives of I (Table 2). For example, I readily formed a diacetyl derivative with acetic anhydride and pyridine. The EI mass spectrum of the derivative showed only a weak molecular ion at *m/z* 396; however, the CI mass spectrum showed a peak at *m/z* 397 (M^++1, 100%), in accord with this formulation. A significant peak at *m/z* 337 (22%), attributable to a loss of acetic acid, was also observed. The gas phase infrared spectrum of this diacetyl derivative was particularly informative. It showed two strong absorptions in the carbonyl region; one peak at 1710 cm^{-1} corresponds to a secondary carboxamide, while the other at 1760 cm^{-1} represents an acetate ester. A strong peak at 1231 cm^{-1} [C-O-C] supports the ester assignment. Thus, we can conclude that I has one primary amino group and one hydroxy group. Although these were not themselves evident from the infrared spectrum of I, this is not uncommon for gas phase spectra.[8]

Table 2. Mass Spectral Data of Derivatives of Alkaloids G and I.

Derivative	Mass Spectrum of Component [*m/z*(%)]	
	G	I
Acetyl	352(M^+, 3), 337(2), 310(8), 309(36), 266(7), 252(5), 240(3), 238(2), 224(3), 210(2), 196(2), 182(2), 168(2), 154(2), 140(5), 127(5), 126(4), 113(35), 112(56), 87(3), 86(10), 71(5), 70(100), 55(9), 44(40) .	396(M^+, 0.3), 395(1), 381(2), 324(4), 323(21), 310(5), 296(1), 268(2), 238(2), 157(9), 156(100), 140(1), 124(2), 116(2), 110(2), 98(2), 97(3), 96(7), 88(2), 87(47), 86(10), 84(10), 70(9), 44(34).
Trifluoroacetyl	460(M^+, 1), 392(6), 391(28), 363(2), 361(1), 321(1), 320(1), 278(1), 252(3), 210(3), 195(2), 194(4), 181(13), 167(17), 166(100), 141(1), 140(11), 96(4), 70(4), 69(8).	504 (M^+, 0.2), 503(0.6), 489 (1), 435(0.5), 391(1), 377(2), 364(2), 211(9), 210(100), 170(1),141(6), 140(7), 96(5), 84(2), 82(1), 70(2), 69(4).
Pentafluorobenzyl	447(M^+-181, 6), 433 (3), 251(13), 250(100), 224(17), 181(32), 96(1), 44(5).	492 (M^+, 1), 491(2), 477(3), 475(1), 474(3), 463(2), 462(12), 461(45), 447(1), 311(2), 294(3), 293(10), 281(3), 238(3), 225(3), 224(27), 182(4), 181(41), 114(100), 84(22), 70(16).

Trifluoroacetylation of I with trifluoroacetic anhydride gave corroborative data. The EI mass spectrum of the bis-trifluoroacetyl derivative showed a weak molecular ion at *m/z* 504 and a base peak at *m/z* 210. The latter in fact is the monotrifluoroacetylated derivative of the ion responsible for the base peak at *m/z* 114 in the spectrum of I. The analogous ion in the spectrum of the acetyl derivative appears at *m/z* 156 (Table 2).

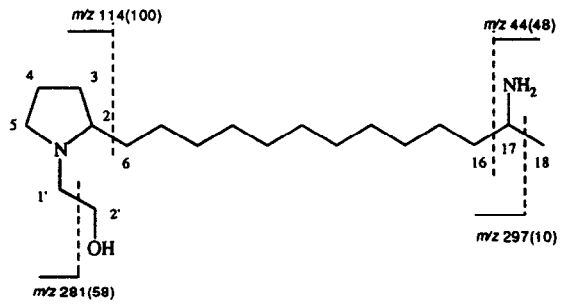
Treatment of I with pentafluorobenzyl bromide and triethylamine resulted in the formation of a monopentafluorobenzyl (PFB) derivative [M^+, *m/z* 492]. Pentafluorobenzyl bromide reacts only with the amino group of bifunctional compounds containing both OH and NH_2 groups. For example, under identical reaction conditions, ethanolamine ($HOCH_2CH_2NH_2$) produced only a mono-derivative. The mass spectrum of the ethanolamine derivative [*m/z*(%): 241(M^+,1), 210(48), 181(100), 161(8)] has a diagnostic peak at *m/z* 210.

This fragmentation, which represents the ion $[CH_2{=}NHCH_2C_6F_5]^+$, allows determination of branching at the α carbon. The corresponding ion in the spectrum of the PFB derivative of **I** was observed at *m/z* 224. A $-CH(NH_2)CH_3$ moiety must therefore be present in **I**. Finally, it follows that the second nitrogen atom is present as a tertiary amino group.

We can also conclude that the hydroxy group in **I** is attached to a methylene group, since a facile loss of 31 mass units (CH_2OH) from the parent ion is evident in the mass spectrum of **I**. This is corroborated by the strong ions observed at *m/z* 323 (M^+-73) and 461 (M^+-31) in the mass spectra of the acetyl and PFB derivatives of **I**, respectively.

Repeated recrystallization of the extract from 130 beetles afforded a sample of **I** of sufficient quantity and purity for NMR investigations. The ^{13}C NMR data identified five signals as hetero-atom-attached carbons (δ 66.5, 58.7, 56.5, 54.1, and 47.5). The HMQC spectrum revealed that the carbon atom whose signal appears at δ 47.5 exists as a CH group; the proton signal of this methine group appears at δ 2.92 in the ^1H NMR spectrum. From the COSY spectrum, it was evident that this CH group is coupled to a CH_3 group (δ 1.09, d). This confirmed the presence of the $-CH(NH_2)CH_3$ structural fragment that was indicated from the mass spectrum. From the HMQC spectrum, it was also clear that the two carbons atoms which show signals at δ 56.5 and 58.7 are directly linked to proton pairs that appear at δ 3.06/2.40 and 3.69/3.62, respectively. The COSY spectrum established that these two CH_2 groups are coupled to each other, but isolated from all other protons. Since mass spectral evidence identified a primary OH group, we conclude that a $-CH_2-CH_2-OH$ group is attached to a ring nitrogen in **I**. It follows that the other two hetero-atom-attached carbons must be the ring carbons adjacent to the ring nitrogen. From the HMQC spectrum, we could see that these two carbons (δ 66.5 and 54.1) exist as a CH group (δ 2.48) and a CH_2 group (δ 3.30 and 2.28), respectively. The COSY spectrum further showed that these two groups are connected by a CH_2-CH_2 fragment (δ 1.96/1.51 and 1.82/1.75). Thus, we could establish a pyrrolidine ring with a *N*-(2-hydroxyethyl) group, and a long-chain substituent on the methine α to the nitrogen atom in the structure of **I**. The overlapping signals between δ 1.2-1.4 in the ^1H NMR spectrum, and those between δ 26.0 and 29.7 in ^{13}C NMR spectrum, indicated the presence of a long chain of CH_2 groups.

All the evidence presented above points to the following structure:



It is a pyrrolidine alkaloid with a 2-hydroxyethyl group attached to the ring nitrogen and a thirteen carbon chain terminating in a $-CH(NH_2)CH_3$ group linked to C-2 . The ^{13}C and ^1H NMR peak assignments reported in Table 3 are based on one and two dimensional methods (DQ-COSY, TOCSY, HMQC).

Table 3. ^{13}C (100 MHz) and ^{1}H (500 MHz) NMR data for compound I in $CDCl_3$. Chemical shifts are given in ppm relative to $CDCl_3$ peak at 77.0 and 7.24 ppm, respectively.

Position	^{13}C DATA		^{1}H Data			
	δ (ppm)	mult.	δ (ppm)	mult.	int.	J in Hz
2	66.5	d	2.48	dtd	1H	8.6, 7.8, 3.0
3	30.0	t	1.96	dddd	1H	12.5, 9.1, 7.6, 5.9
			1.51	dddd	1H	12.5, 9.8, 8.2, 5.8
4	22.2	t	1.82	ddddd	1H	12.5, 9.1, 8.6, 7.5, 5.8
			1.75	ddddd	1H	12.5, 9.8, 8.6, 5.9, 3.6
5	54.1	t	3.30	ddd	1H	9.6, 7.5, 3.6
			2.28	dt	1H	9.6, 8.6
6	32.8	t	1.66	m	1H	
			1.32	m	1H	
7-15	29.7	t	1.2-1.4	m	16 H	
	29.3-29.5 (6C)	t				
	26.5	t				
	26.0	t				
16	37.7	t	1.36	m	2H	
17	47.5	d	2.92	br q	1H	5.8
18	21.6	q	1.09	d	3H	6.3
1'	56.5	t	3.06	ddd	1H	12.5, 9.6, 4.8
			2.40	ddd	1H	12.5, 3.5, 3.4
2'	58.7	t	3.69	ddd	1H	11.1, 9.6, 3.4
			3.62	ddd	1H	11.1, 4.8, 3.5

A comparison of mass spectral and infrared data of component G with those of I revealed that these two compounds are closely related. Although the quantity of G available was not sufficient for NMR spectroscopy, the mass spectral and infrared data obtained from G and its derivatives sufficed to establish its structure. The mass spectrum of G suggested a molecular weight of 268, confirmed by the presence of a strong peak at *m/z* 269 (M^++1, 51%) in its CI spectrum. The infrared spectrum of the diacetyl derivative of G showed two carbonyl bands at 1677 and 1709 cm^{-1} (tertiary and secondary carboxamide absorptions, respectively), establishing the presence of a primary and a secondary amino group. Since the base peak at *m/z* 70 in the mass spectrum of G strongly suggests a substituted pyrrolidine ring system,[9] we can propose the structure given for G in Table 1. The mass spectrum of G, similar to that of I, shows an intense peak at *m/z* 44 for the -$CH(NH_2)CH_3$ group. The mass spectral properties of the derivatives of G (Table 2) are also congruent with the proposed structure. For example, the mass spectrum of the diacetyl derivative shows an intense peak at *m/z* 112 characteristic of an *N*-acetylpyrrolidine. The analogous ion in the bistrifluoroacetyl derivative spectrum appears at *m/z* 166, which is also the base peak; in addition, a peak at *m/z* 140 is observed for the ion

$[CH_3CH{=}NHCOCF_3]^+$. In contrast to the behavior of **I**, the reaction of **G** with pentafluorobenzyl bromide produced a bis-PFB-derivative. Although the mass spectrum of this product lacked a molecular ion, the base peak at *m/z* 250 for the *N*-PFB-pyrrolidine fragment, and the peak at *m/z* 224 for the $[CH_3CH{=}NH\text{-}PFB]^+$ ion, confirm the proposed structure.

From the mass spectra, it appears that the component **H** is also closely related to **I** (Table 1). The accurate mass of the base peak at *m/z* 112 indicates a difference of two hydrogen atoms from the ion at 114 in the spectrum of **I**. However, the data do not yet allow the assignment of an unambiguous structure for this alkaloid.

Of the more volatile compounds which elute earlier in the chromatogram, **A** and **B** were identified as 2-methyl-6-propylpiperidine and 2-phenylethylamine by their mass spectra, which are congruent with the corresponding literature spectra.[6] 2,6-Dialkylpiperidines are common in ant venoms,[10,11] although, 2-methyl-6-propylpiperidine itself has not been identified hitherto from any arthropod source.

The mass spectrum of compound **C** shows a molecular ion at *m/z* 155, a base peak at 98 and a prominent ion at 43. Furthermore, its infrared spectrum, with a strong absorption at 1728 cm,$^{-1}$ establishes the presence of a carbonyl group. Upon acetylation, **C** produced a monoacetyl derivative whose infrared spectrum shows a tertiary carboxamide group (1671 cm^{-1}). The mass spectrum of this derivative is congruent with that published for the acetyl derivative of *cis*-1-(6-methyl-2-piperidyl)propan-2-one[12] [197(M^+, 4), 154(62), 140(24), 112(33), 98(100), 82(37), 43(76)]. Thus, component **C** was identified as 1-(6-methyl-2-piperidyl)propan-2-one, an alkaloid previously identified from an Australian coccinellid, *Cryptolaemus montrouzieri*.[12]

The infrared spectrum of component **D** shows that the compound is an alcohol (3425 cm^{-1}), and its mass spectrum indicates a close structural relationship to **C**. The mass spectrum of **D** agrees well with that reported for the $LiAlH_4$ reduction product of *cis*-1-(6-methyl-2-piperidyl)propan-2-one.[12] In addition, component **D** formed a diacetyl derivative [MS: 241(M^+, 2), 166(34), 140(35), 98(100), 43(49)] whose infrared spectrum indicates the presence of an ester group (1755 cm^{-1}) and a tertiary carboxamide group (1670 cm^{-1}). Furthermore, the peak for **C** was absent, and the intensity of that of **D** was enhanced, in the gas chromatograms obtained from the $NaBH_4$ reduction product of the alkaloid mixture. Thus, component **D** is identified as 1-(6-methyl-2-piperidyl)propan-2-ol. The relatively low frequency of the absorption observed for the hydroxyl group in the infrared spectrum of **D** is compatible with this structure, since the hydroxyl group can form a 6-membered intramolecularly hydrogen bonded ring system. Although this compound has been reported as a reduction product of 1-(6-methyl-2-piperidyl)propan-2-one,[12] the alcohol **D** has not been identified previously from nature.

In addition to a carbonyl absorption at 1728 cm^{-1}, the infrared spectrum of compound **E** shows a strong absorption at 1664 cm^{-1}, which can be attributed to a C=N stretch. The mass spectrum of **E** was clearly not that of a piperidine, however it agrees well with the lower mass region of that of 2-methyl-6-undecyl-$\Delta^{1,2}$-piperideine.[13] Brand et al.[13] have discussed the mass spectral fragmentation pathways of $\Delta^{1,2}$ and $\Delta^{1,6}$ double bond isomers of 2-methyl-6-undecylpiperideine. According to these authors, a base peak at *m/z* 110 is characteristic of the $\Delta^{1,2}$ isomer. On this basis, **E** was identified as 2-methyl-6-(2-oxopropyl)-$\Delta^{1,2}$-piperideine. Treatment of the alkaloid mixture with $NaBH_4$ followed by GC examination revealed that the peak for **E** had also disappeared, in accord with the proposed structure. Interestingly, the mass spectrum of an unidentified compound reported from *C. montrouzieri*[12] agreed well with our mass spectrum for **E**. The defensive alkaloids of this Australian ladybug also include euphococcinine (**F**) and cis-1-(6-methyl-2-piperidyl)propan-2-one (**C**).

The stereochemistry of **A**, **C**, and **D**, was assigned by the comparison of their GC retention times with those of synthetic standards (Table 4), which were prepared by the reduction of 2,6-disubstituted pyridines by successive treatment with Na/ethanol and $NaBH_4$. This reduction is known to produce a *cis* and *trans* mixture with the former isomer predominating[11]. These stereoisomers are separable by gas chromatography; the *cis*

isomers, in which the nitrogen atom is less exposed due to the diequatorial disposition of the substituents, elute first on methyl silicone columns.[11,14] Since the retention times of **A**, **C**, and **D** were congruent with those of the earlier-eluting isomers, we can conclude that they all have *cis* configurations. The mass spectra of these earlier-eluting isomers of the synthetic products were also in excellent agreement with those of the components **A**, **C**, and **D** respectively.

Table 4. GC Retention Times of Stereoisomeric Piperidines, and Components **A**, **C**, and **D**.

Synthetic Compound	Retention time (min)	
	cis	*trans*
2-methyl-6-propylpiperidine	7.6[a]	8.1[a]
1-(6-methyl-2-piperidyl)propan-2-one	13.2[b]	13.9[b]
1-(6-methyl-2-piperidyl)propan-2-ol	14.1[b]	14.6[b]

Beetle Compound	Retention time (min)
Component **A**	7.7[a]
Component **C**	13.2[b]
Component **D**	14.1[b]

A 25 m x 0.32 mm fused-silica column coated with DB-5 stationary phase was used. The oven temperature was kept at [a]60° C (or [b]40° C) for 4 min and programmed to 260°C at a rate of [a]15° C (or [b]10° C)/min.

Synthetic efforts are now in progress to establish the absolute configurations of these alkaloids. A diamino compound, (Z)-1,17-diaminooctadec-9-ene, which appears biosynthetically related to **G** and **I** has been identified from two coccinellid beetles.[15] The absolute configuration at C-17 of this compound has been established as R.[16]

Experimental

Extraction of Alkaloids. Eggs, larvae (1-19 day old), and adult beetles (1-14 day old) were immersed in 2% sulfuric acid in methanol, crushed and left for 3-4 hr at room temperature. The supernatant liquid was removed and the residue was re-extracted. The combined methanol extract was concentrated and diluted with water. The aqueous solution was extracted (6 x) with ether, made alkaline with 2M NaOH and extracted with CH_2Cl_2 (4 x). The combined CH_2Cl_2 extract was washed with water, concentrated, and used for alkaloid analysis (a 10-14 day-old beetle contains about 50 μg of alkaloids).

Gas Chromatography. Gas chromatography was performed on a Hewlett-Packard (HP) 5890 instrument equipped with a splitless injector and a flame ionization detector (FID).

Gas Chromatography-FTIR. Infrared spectra were obtained on a Hewlett-Packard gas chromatograph linked to a HP 5965A IR detector. Analyses were performed using a 25 m x 0.32 mm fused-silica column coated with DB-5 (J&W Scientific).

Gas Chromatography-MS. The EI mass spectra were obtained on an HP 5890 gas chromatograph linked to a HP 5970 mass selective detector (MSD). Analyses were performed using a 25 m x 0.32 mm fused-silica column coated with DB-5. CI mass spectra were obtained on a HP gas chromatograph linked to a Finnigan ion trap detector (ITD), using methane as reactant gas. High-resolution GC-MS was performed on a VG 70-VSE instrument.

NMR Spectroscopy. The 500 MHz ^1H NMR and 125 MHz ^{13}C NMR spectra were recorded on a Varian Unity 500 spectrometer. Additional ^{13}C data were obtained using a Varian XL 400 instrument at 100 MHz. For NMR examination, an extract obtained from 10-14 day old beetles (about 130) was dried and the residue was recrystallised several times from 9:1 hexane/CH_2Cl_2.

Derivatizations. 1) *Micro-hydrogenation.* A small sample of the alkaloid extract in ether (50 µl) was placed in a glass vial and about 0.5 mg of 10% Pd on activated charcoal was added. A balloon filled with hydrogen was attached to the vial. After 10 hr, 25 µl of ether was added and the supernatant was withdrawn and examined by GC-MS.

2) *Acetylation.* A small sample of the CH_2Cl_2 extract (50 µl) was evaporated to dryness; the residue was dissolved in ether (50 µl) and treated with a mixture of acetic anhydride and pyridine (60:40, 20 µl).[17] After 3 hr at room temperature, the reaction mixture was examined by GC-MS and GC-IR.

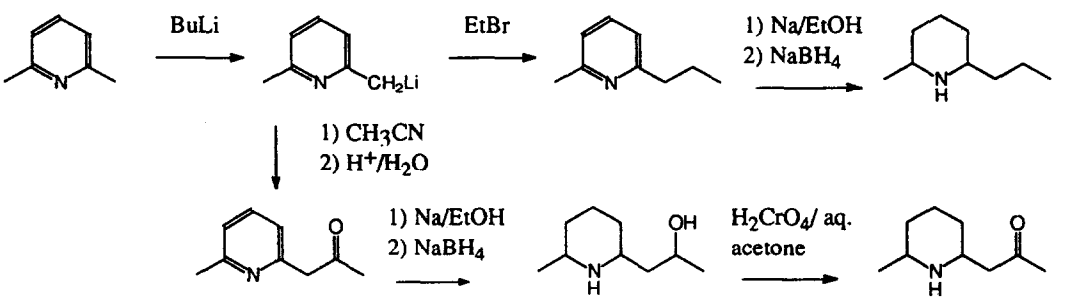
3) *Trifluoroacetylation.* To an aliquot of the extract in CH_2Cl_2 (10 µl), trifluoroacetic anhydride (5 µl) was added. After 3 hr, the mixture was evaporated with a slow stream of nitrogen, the residue was dissolved in CH_2Cl_2 and examined by GC-MS.

4) *Pentafluorobenzylation.* To a CH_2Cl_2 extract (10 µl), α-bromo-2,3,4,5,6-pentafluorotoluene (5 µl) and triethylamine (5 µl) were added.[18] After 3-4 hr, the solution was evaporated to dryness and the residue was examined as above.

5) *Sodium borohydride reduction.* A small quantity of the CH_2Cl_2 (20 µl) extract was evaporated with a steam of nitrogen. Methanol (20 µl) and sodium borohydride (1-2 mg) were added,[12] and the mixture was allowed to stand for 4 hr. The solvent was removed in vacuo, water was added, and the mixture was extracted with CH_2Cl_2 for GC-MS.

6) *Hydrogenolysis.* A small amount of the sample used for NMR spectroscopy (20 µl) was evaporated and the residue was dissolved in hexane (20 µl). This was added to a mixture of 2% Pt on GasChrom P and $LiAlH_4$ (1:1) (2-3 mg) in a one-end-sealed glass tube.[7] The solvent was removed by a stream of nitrogen, the open end was sealed, and the tube was heated at 280° C for 1 hr. After cooling, the contents were extracted with hexane (3 x) and analyzed by GC-MS.

Synthesis. Small samples of three 2,6-disubstituted piperidines, as *cis* and *trans* mixtures, were prepared from 2,6-lutidine by standard procedures as outlined in Scheme 1.



Scheme 1.

Alkylation of the lithium salt[11] of 2,6-lutidine with ethyl bromide gave 2-methyl-6-propylpyridine [135(M^+, 2), 134(M^+-1,10), 120(24), 107(100)], which upon reduction[11] with Na/EtOH followed by $NaBH_4$ yielded a mixture of *cis* and *trans*-2-methyl-6-propylpiperidine in 10:1 ratio (GC). Similarly, the reaction of CH_3CN with lutidyllithium, according to the method of Wibaut et al.,[19] gave 1-(6-methyl-2-pyridyl)propan-2-one [149(M^+,10), 107(100), 43(50)], which was reduced with sodium/ethanol and $NaBH_4$ to give 1-(6-methyl-2-piperidyl)propan-2-ol.[12] The oxidation[20] of this alcohol with H_2CrO_4 gave 1-(6-methyl-2-piperidyl)propan-2-one.[12]

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