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Chilocorine: Heptacyclic Alkaloid from a Coccinellid Beetle[1]

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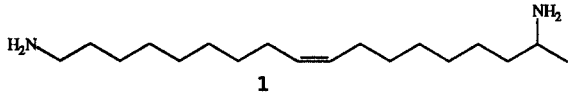
Abstract: A novel heptacyclic alkaloid, for which we coin the name *chilocorine*, was isolated from a ladybird beetle, *Chilocorus cacti*. It has a unique structure made up of two tricyclic substructures, 2-methylperhydro-9*b*-azaphenalene and 3,4-dimethyloctahydro-8*b*-azaacenaphthylene. The proposed structure is based on mass spectrometric, ultraviolet spectroscopic, and NMR evidence.

INTRODUCTION

Coccinellid beetles (Coleoptera, Coccinellidae) synthesize many types of defensive alkaloids, including acyclic[4,5] (**1**) and aromatic amines[6] (**2**), piperidines[6] (**3**), pyrrolidines[6] (**4**), azabicyclo[3.3.1]nonanes[7-9] (**5**, **6**), azaphenalenes[10-12] (**7**, **8**) and azamacrolides[13] (**9**, **10**). Despite this structural diversity, each of the alkaloid skeletons can be seen to consist of a chain of carbon atoms joined at one or more sites to a nitrogen atom. In the case of the azamacrolides (**9**, **10**), an ethanolamine moiety completes the structure.

The first *dimeric* coccinellid alkaloid, exochomine (**11**), was isolated and characterized recently by Timmermans *et al.*[14] from a European ladybird, *Exochomus quadripustulatus*. Its structure is an especially interesting one, since it corresponds to the coupling of a 2-methylperhydro-9*b*-azaphenalene skeleton, frequently encountered among these alkaloids (i.e. **7**, **8**), with a previously unknown partner related to 3,4-dimethyloctahydro-8*b*-azaacenaphthylene (**12**).



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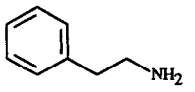

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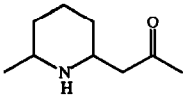

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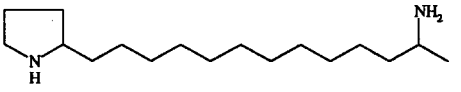

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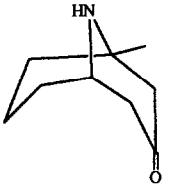

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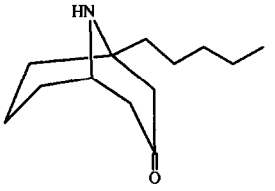

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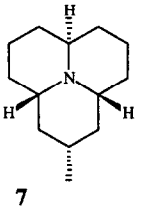

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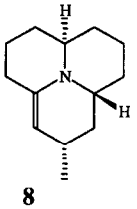

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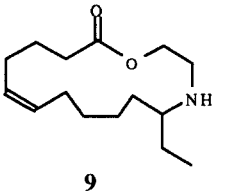

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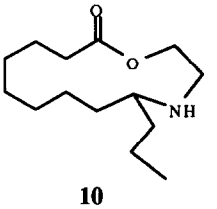

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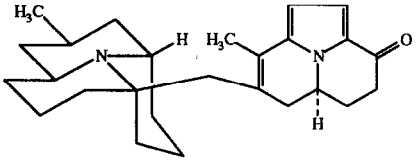

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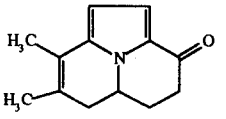

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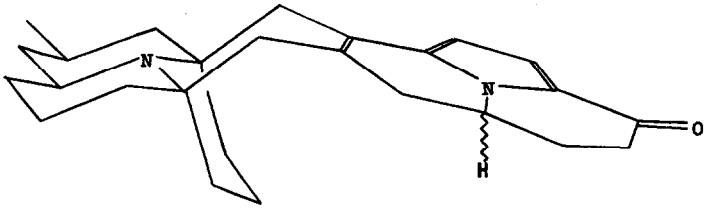
We now report the isolation of a heptacyclic dimeric alkaloid, *chilocorine*, from the coccinellid beetle *Chilocorus cacti*. This beetle, as other members of the genus, is commonly found in association with scale insects upon which it feeds.[15] Like many other coccinellid beetles, *C. cacti* emits droplets of blood when disturbed.[16] Such reflex-bleeding is defensive and protects the beetles against such predators as ants.[17] We assign the structure presented below (**13**) to chilocorine on the basis of mass spectrometric, ultraviolet spectroscopic, and NMR evidence.



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RESULTS AND DISCUSSION

GC-MS analysis of the acid-soluble (basic) components extracted from *C. cacti* revealed the presence of several alkaloids. The mass spectra obtained from two minor GC peaks which appeared early in the chromatogram, showed molecular ions at *m/z* 191 ($C_{13}H_{21}N$) and resemble the mass spectra of propylein[18,19] and hippocasine,[11,19] both of which are monounsaturated derivatives of precoccinellin (**7**), a common ladybug alkaloid based on the 2-methylperhydro-9*b*-azaphenalene skeleton. The major components of the extract, however, appeared much later in the chromatogram as three peaks (ratio 2:1:3). From the mass spectra corresponding to these peaks, it was evident that these three compounds must be novel. The alkaloid corresponding to the last-eluting peak, chilocorine, was isolated by flash chromatography. Its molecular formula was determined to be $C_{26}H_{34}N_2O$ by high resolution mass spectrometry (*m/z* of M^+, 390.2638; calculated for $C_{26}H_{34}N_2O$, 390.2671), requiring eleven unsaturation equivalents. The ultraviolet spectrum of chilocorine (λ_{max} 337 nm) indicated the presence of an extended conjugated system. The exact nature of this chromophore as an α-keto-α'-vinylpyrrole became apparent when the ultraviolet spectrum of exochomine (**11**) became available.[14] Although the ^1H- and ^{13}C-NMR spectra of chilocorine are complex, a variety of NMR techniques described below allowed us to assign the observed signals in considerable detail (Table 1). The ^{13}C-NMR spectrum of chilocorine shows all 26 signals expected on the basis of the mass spectrometrically determined molecular formula. Seven of these signals have chemical shift values greater than 100 ppm, indicating that they represent sp^2 hybridized carbon atoms. Six of these seven can be attributed to carbon atoms participating in carbon-carbon double bonds (106.6, 114.2, 126.1, 130.0, 130.2, and 134.6 ppm), while the seventh, at 186.5 ppm, can be assigned to a carbonyl group. Since these assignments account for four double bonds, the structure of chilocorine must be heptacyclic.

In a DEPT experiment, the 26 carbon signals were observed as 1 quartet (CH_3), 13 triplets (CH_2), 5 doublets (CH), and 7 singlets (quaternary C). From an HMQC spectrum, it was possible to locate all of the individual proton signals and to determine to which carbon atoms these protons are bonded. All of the chilocorine proton signals were found to belong to one of six different substructures, **A** - **F**, shown in Figure 1. From the DQ-COSY and TOCSY spectra, it was evident that these partial structures are not J-coupled to each other. They must therefore be linked through the quaternary carbons.

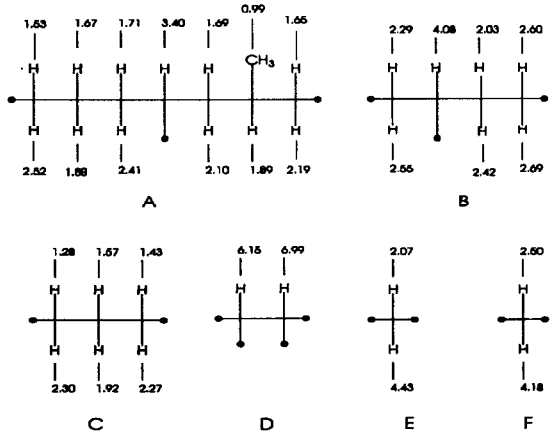
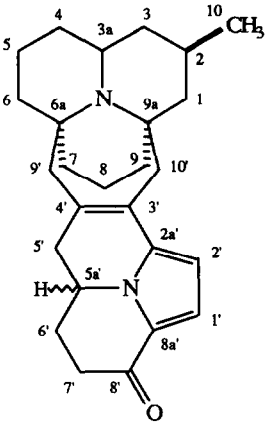


Fig. 1. The six substructures identified in chilocorine from the DQ-COSY and TOCSY spectra.

Table 1. The ^{13}C NMR (125 MHz) and ^{1}H NMR (500 MHz) assignments of chilocorine, **13**. Chemical shifts are given in ppm relative to the $CDCl_3$ peak at 77.0 and 7.24 ppm respectively.

Position	^{13}C Data	^{1}H Data			
	δ (ppm)	δ (ppm)	mult.	int.	J in Hz
1	47.8	1.65	dd	1H	J = 13.9, 3.0
		2.19	dd	1H	J = 14.2, 12.7
2	24.9	1.89	m	1H	
3	39.3	1.69	m	1H	
		2.10	m	1H	
3a	56.8	3.40	br d	1H	J = 10.9
4	31.0	1.71	m	1H	
		2.41	m	1H	
5	18.7	1.67	m	1H	
		1.69	m	1H	
6	39.2	1.53	m	1H	
		2.52	m	1H	
6a	61.5			1H	
7	24.7	1.28	dd	1H	J = 15.7, 7.4
		2.30	ddd	1H	J = 15.2, 14.6, 6.5
8	16.5	1.57	m	1H	
		1.92	m	1H	
9	25.2	1.43	dd	1H	J = 15.4, 7.1
		2.27	ddd	1H	J = 15.0, 14.5, 8.1
9a	61.2			1H	
10	20.9	0.99	d	3H	J = 6.3
1'	114.2	6.99	d	1H	J = 4.2
2'	106.6	6.15	d	1H	J = 4.2
2a'	134.6				
3'	130.2				
4'	126.1				
5'	39.0	2.29	dd	1H	J = 17.2, 5.5
		2.55	m	1H	
5a'	50.1	4.08	d	1H	J = 11.1
6'	30.5	2.03	dddd	1H	J = 13.7, 13.3, 11.5, 4.5
		2.42	dddd	1H	J = 13.3, 4.9, 2.7, 2.6
7'	36.2	2.60	ddd	1H	J = 17.3, 13.7, 4.9
		2.69	ddd	1H	J = 17.3, 4.3, 2.6
8'	186.5				
8a'	130.0				
9'	47.1	2.07	d	1H	J = 15.6
		4.43	br d	1H	J = 15.3
10'	41.0	2.50	d	1H	J = 16.6
		4.18	br d	1H	J = 16.6
NH^+		11.57	br s	1H	



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The HMBC spectrum of chilocorine contains several long range ^{1}H-^{13}C correlations that link substructures **A** and **C** through the quaternary carbons whose ^{13}C signals appear at 61.5 and 61.2 ppm, to form a 12-membered carbon macrocycle (Fig. 2a). Relatively high chemical shift values of signals corresponding to carbons 3a, 6a and 9a indicated that these atoms are bonded to nitrogen. Irradiation of protonated chilocorine's ammonium proton at 11.57 ppm gave NOE enhancements of signals appearing at 2.19, 2.10, 2.41, and 2.52 ppm for the axial protons on carbon atoms 1, 3, 4, and 6, respectively. While there were no enhancements for any proton signals of fragment **C**, enhancements were observed at the 4.43 and 4.18 ppm signals of fragments **E** and **F**. In addition, when the signals at 4.43 and 4.18 ppm for protons on C-9' and C-10' of fragments **E** and **F**, respectively, were irradiated, NOE enhancements were observed in proton signals at 2.52 and 2.19 ppm. This information is consistent with chilocorine containing the well-known 2-methylperhydro-9*a*-azaphenalene ring system shown in Fig. 2b.

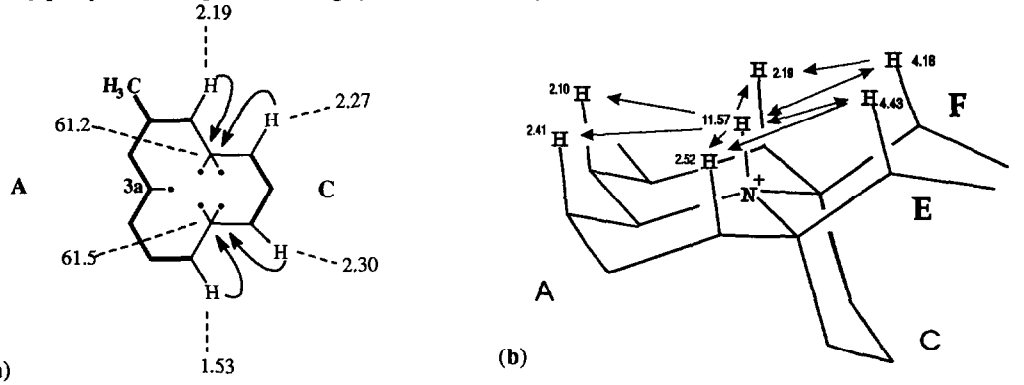


Figure 2. (**a**) Long range ^{1}H-^{13}C correlations observed in the HMBC spectra linking substructures **A** and **C**. (**b**) The observed NOE signals that define the 2-methylperhydro-9*a*-azaphenalene ring system and its stereochemistry.

The remainder of the alkaloid molecule consists of substructure **B**, the seven sp^2 carbon atoms, and the second nitrogen atom. The relatively low chemical shift value of the carbonyl carbon (186.5 ppm) indicated that this group is conjugated to a carbon-carbon double bond. The chemical shift value of the 5a' carbon (50.1 ppm) suggested that this atom in substructure **B** is adjacent to a nitrogen atom . With this information and the observation of several long range ^{1}H-^{13}C correlations in the HMBC spectrum, chilocorine was shown to have a 3,4-dimethyloctahydro-8*b*-azaacenaphthylene ring system (Fig. 3).

In summary, the NMR evidence leads to structure **13** for chilocorine, in which the two tricyclic moieties are joined to give two diastereoisomers, shown as (a) and (b) in Figure 4. Unfortunately, neither NOE difference spectroscopy nor a ROESY experiment led to an unambiguous choice of one of these possibilities, and no additional information was obtained by changing from $CDCl_3$ to C_6D_6 as the NMR solvent. If we assume that chilocorine and exochomine stem from common biosythetic precursors, then the formula shown as (b) in Figure 4 seems more likely, since this diastereomer has the same relative configuration of the two chiral tricyclic moieties as exochomine. An x-ray crystallographic study of a chilocorine derivative would demonstrate whether these two dimeric alkaloids are in fact as closely related as they appear to be.

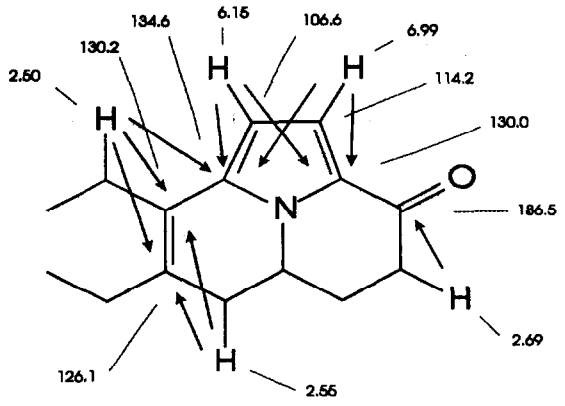
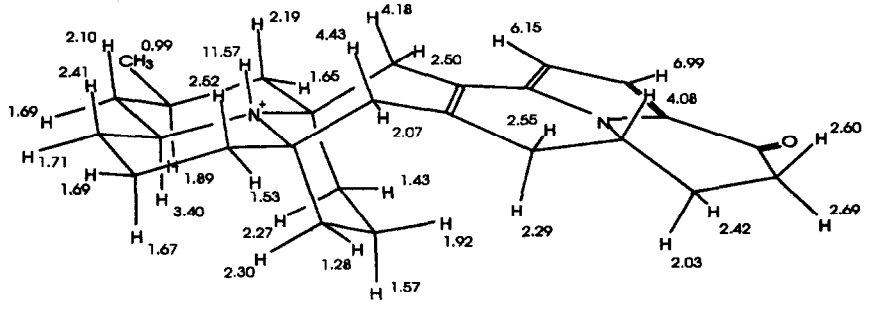


Figure 3. The long range ^{1}H-^{13}C correlations that define the 3,4-dimethyloctahydro-8*b*-azaacenaphthylene ring system.

(a)



(b)

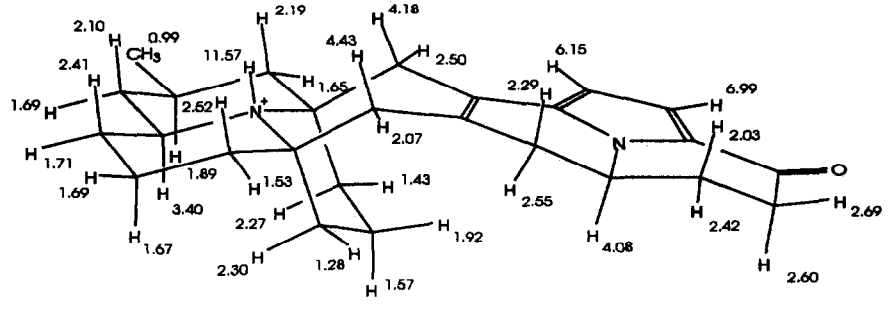


Figure 4. The two diastereomeric candidate structures for protonated chilocorine. The structures are MM2 energy minimized using the computer program PC Model (Serena Software, Bloomington, IN). The ^{1}H-NMR signals were assigned on the basis of coupling constants and NOE observations.

EXPERIMENTAL

The Beetle. *Chilocorus cacti* specimens used in this study were taken near Welasco, Texas. We extracted both whole beetles and droplets of blood emitted by the beetles in response to prodding or mild pinching with forceps. Such blood could be readily taken up in microcapillary tubes.

Extraction and Isolation of the Alkaloid. Male and female beetles (50 specimens) were soaked in 2% sulfuric acid in methanol (20 mL), crushed, and left for 3-4 hr at room temperature. The supernatant liquid was removed and the residue was washed with methanol. The combined methanol extract was concentrated and diluted with water. This 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 beetle contains about 30 μg of alkaloids).

The alkaloidal extract was subjected to flash chromatography on silica gel (60 μm, EM Science, Gibbstown, NJ). The column was eluted with CH_2Cl_2 / CH_3OH mixtures of increasing polarity ranging from 9.5:0.5 to 7.0:3.0. The fractions collected with 9:1 CH_2Cl_2 / CH_3OH showed virtually one spot by TLC on silica (Baker-flex IB2-F, $CHCl_3$/MeOH/NH_4OH 4:10:1). These fractions were combined and evaporated, and the residue, after checking the purity by gas chromatography, was used to obtain analytical data of chilocorine.

Gas Chromatography. Gas chromatography was performed on a Hewlett-Packard (HP) 5890 instrument equipped with a splitless injector and a flame ionization detector (FID). Analyses were performed using a 12 m x 0.22 mm fused-silica capillary coated with methyl silicone (0.33 μm, HP-1). The oven temperature was kept at 60 °C for 4 min, increased at a rate of 15 °C /min to 280 °C, and held at 280 °C for 40 min. From the crude extract, five alkaloid peaks were recognized at retention times 11.5, 11.6, 30.0, 31.7, and 34.4 min. The first two peaks represent about 2% of the alkaloid fraction (ratio of 3:7), and the last three peaks about 98% (peak area ratios 2:1:3) of the total alkaloids. The most abundant constituent, represented by the peak at 34.4 min, is chilocorine.

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. Of the five peaks in the chromatogram obtained from the crude alkaloid mixture, the earlier-eluting minor peaks represent compounds of molecular weight 191 [EI-MS *m/z*(%) first-eluting peak, 191(75), 190(100), 176(89), 162(11), 148(33), 134(19), 120(11); second-eluting peak, 191(70), 190(100), 176(62), 162(14), 148(34), 134(19), 120(12)]. The last three peaks in the chromatogram represented isomeric compounds of molecular mass 390, $C_{26}H_{34}N_2O$ [EI-MS *m/z*(%) first peak, 390(69), 348(42), 347(61), 237(17), 191(100), 190(65); second peak, EI-MS *m/z*(%), 391(M^++1,3), 390.2647(M^+, 6), 191(15), 190.1626(100), 188(6), 174(4), 148(5), 120(2); third peak represents chilocorine, see below]. High-resolution GC-MS was performed on a VG 70-VSE instrument (resolution=5000).

Ultraviolet Spectroscopy. Ultraviolet spectra were obtained either from CH_2Cl_2solutions or using a diode-array detector (HP) linked to a HP 1090 HPLC instrument. A 25 cm x 4.6 mm ID Supelcosil LC-Si (5 micron)(Supelco, Bellefonte, PA) column was eluted with 75% CH_2Cl_2 and 25% methanol containing 0.15% ammonia as the mobile phase.

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.

Chilocorine (13): R_f=0.63 (Baker-flex IB2-F, $CHCl_3$/MeOH/NH_4OH 4:10:1); UV (CH_2Cl_2) λ_{max} 337 nm; EI-MS *m/z*(%), 391(M^++1,4), 390.2638(M^+, 12; calc. for $C_{26}H_{34}N_2O$ 390.2671), 192(14), 191.1651(100; calc. for $C_{13}H_{21}N$ 191.1674), 190(37), 189(4), 188(6), 176(19), 149(5), 148(5), 134(3); ^{13}C NMR and ^1H NMR data, Table 1.

ACKNOWLEDGMENTS

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