Chilocorine C: A New "Dimeric" Alkaloid from a Coccinellid Beetle, *Chilocorus cacti*[1]

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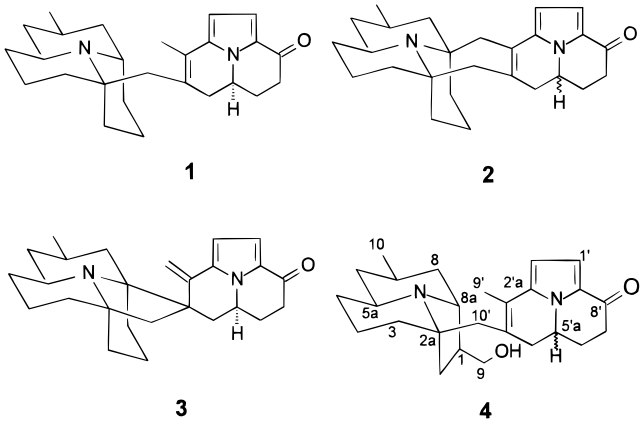
A new hexacyclic alkaloid, chilocorine C (**4**), has been isolated from *Chilocorus cacti* and characterized on the basis of its IR, UV, MS, and NMR data. Although its structure is closely related to that of exochomine (**1**) (isolated from *Exochomus quadripustulatus*) and to chilocorine A (**2**) and B (**3**) (obtained previously from *C. cacti*), the presence of a hydroxymethyl substituent on the saturated tricyclic moiety represents an unexpected structural variation on the dimeric alkaloid theme.

The family Coccinellidae (ladybird beetles) comprises some 5200 species of six subfamilies. The group is diverse in feeding habits and includes both beneficial and pestiferous species.[2] Many coccinellids are aposematically (that is, gaudily) colored, implying that they are chemically protected. Indeed, when disturbed, coccinellids commonly emit droplets of blood from knee joints, a phenomenon known as "reflex bleeding." The blood is bitter to the taste[2,3] and has been shown to be the repository of defensive alkaloids.

Over 30 such alkaloids have been characterized from coccinellids of four subfamilies (Coccinellinae, Chilocorinae, Epilachninae, and Scymninae).[2,3] The compounds include acyclic and aromatic amines, piperidines, pyrrolidines, azabicyclo[3.3.1]nonanes, azaphenalenes, azamacrolides, and "dimeric" alkaloids. Although these alkaloids are structurally quite diverse, their carbon skeletons can be regarded as unbranched chains of carbon atoms linked at one or more sites to a nitrogen atom.

The first dimeric coccinellid alkaloid to be identified, exochomine (**1**), was isolated from an European ladybird, *Exochomus quadripustulatus*.[4] The exochomine structure is derived from a coupling of the well-known 2-methylperhydro-9b-azaphenalene skeleton with a previously unknown 3,4-dimethyloctahydro-8b-azaacenaphthylene moiety. We have investigated[5,6] the chemical defenses of another ladybird species, *Chilocorus cacti* (Coccinellidae), extraction of which yields a mixture of dimeric alkaloids. The two major components of this mixture were characterized as chilocorines A (**2**)[5] and B (**3**),[6] closely related to each other and to exochomine. Here, we report the characterization of chilocorine C (**4**), an alkaloid present in smaller amounts in *C. cacti*. Its hexacyclic structure is proposed on the basis of UV, IR, NMR, and mass spectral evidence. Interestingly, the structure of chilocorine C incorporates a 1-(hydroxymethyl)-7-methylperhydro-8b-azaacenaphthylene skeleton in place of the familiar 2-methylperhydro-9b-azaphenalene moiety.



Results and Discussion

HPLC analysis of the acid-soluble (basic) components extracted from *C. cacti* revealed the presence of several minor compounds, which show UV spectra similar to those of chilocorine A (**2**) and B (**3**), the two major components. The minor components were separated by HPLC and analyzed by LC−MS. The positive-ion electrospray mass spectrum of one of the peaks showed an $[M + 1]^+$ ion at *m/z* 409 and a major fragment ion at *m/z* 208. The molecular formula of this alkaloid, which we named chilocorine C, was determined to be $C_{26}H_{36}N_2O_2$ by high-resolution CI mass spectrometry (*m/z* of $[M + 1]^+$, 409.2844, calcd for $C_{26}H_{37}N_2O_2$ 409.2855). The infrared spectrum obtained by an IR microscope showed absorptions for a hydroxy group (3381−3281 cm^{-1}) and a conjugated carbonyl group (1652 cm^{-1}). The ultraviolet spectrum of this alkaloid (λ_{max} 346 nm) indicated the presence of an extended conjugated system, similar to that found in **2** (λ_{max} 356 nm) and **3** (λ_{max} 338 nm). By several cycles of preparative thin-layer chromatography, about 0.6 mg of **4** was obtained from 460 beetles, along with 11 mg of **2** and 12 mg of **3**.

The molecular formula of **4** indicated the presence of one oxygen atom and two hydrogen atoms more than

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Table 1. ^{1}H (500 MHz) and ^{13}C (100 MHz) Data of Chilocorine C (**4**) as Its Hydrochloride

position	group	^{13}C data δ (ppm)	^{1}H data δ (ppm)	mult	int	J (Hz)	gHMBC correlations (C-position)
1	CH	39.3	2.48	m	1 H		
2	CH_2	31.9	2.17	m	1 H		C-2a, 8a
			2.23	m	1 H		C-2a
2a	C	70.5					
3	CH_2	29.6	1.57	m	1 H		
			1.86	m	1 H		C-2a
4	CH_2	19.9	1.51	m	1 H		
			1.77	m	1 H		
5	CH_2	28.6	1.67	m	1 H		
			2.02	m	1 H		
5a	CH	58.2	2.85	m	1 H		
6	CH_2	37.3	1.66	m	1 H		
			1.92	m	1 H		
7	CH	26.0	1.84	m	1 H		
8	CH_2	31.9	1.83	m	1 H		
			2.12	m	1 H		
8a	CH	59.3	4.55	m	1 H		
9	CH_2	58.3	3.69	dd	1 H	12.2, 4.0	C-8a
			3.94	dd	1 H	12.2, 3.3	C-2, 8a
10	CH_3	21.1	1.00	d	3 H	5.7	C-6, 7, 8
1′	CH	113.7	6.86	d	1 H	4.2	C-2′, 8′a
2′	CH	107.3	6.12	d	1 H	4.2	
2′a	C	135.1					
3′	C	125.2					
4′	C	125.1					
5′	CH_2	36.6	2.10	dd	1 H	16.0, 10.6	C-3′ (4′)
			2.53	dd	1 H	16.0, 5.8	C-3′ (4′)
5′a	CH	49.1	3.18	m	1 H		
6′	CH_2	30.6	1.74	m	1 H		
			2.13	m	1 H		
7′	CH_2	35.9	2.13	m	1 H		C-8′
			2.44	dd	1 H	17.5, 4.4	C-8′
8′	C	186.1					
8′a	C	128.7					
9′	CH_3	15.5	2.24	s	3 H		C-2′a, 3′ (4′)
10′	CH_2	40.2	3.23	d	1 H	12.4	C-2a, 3, 3′ (4′)
			3.54	d	1 H	12.4	C-2, 2a, 3′(4′), 5′
8b	NH^+		10.87	bs	1 H		

its congeners. Since **4** readily forms a hydrochloride, and a signal for the corresponding ammonium proton (R_3N^+H) is observed at 10.87 ppm in its ^{1}H NMR spectrum, it was evident that this alkaloid is not an *N*-oxide, although this functionality is common among coccinellid alkaloids. ^{1}H NMR data also revealed the presence of two aromatic protons at chemical shifts that are in good agreement with those observed in the previously characterized dimeric alkaloids. ^{13}C NMR data indicated the presence of 26 carbon atoms, as expected on the basis of the mass spectrometrically determined molecular formula (Table 1), although only 25 signals were observed directly. ^{13}C NMR data also established the presence of one carbonyl carbon (δ 186.1) and six other sp^2 carbons (δ 107.3−135.1). A subsequent DEPT experiment revealed that the compound contains seven CH, 10 CH_2, and two CH_3 groups. The unusually high intensity of the CH_2 carbon signal at δ 31.9 suggested that the missing carbon signal may be due to a superposition of two methylene signals. This was later confirmed by a gHMQC experiment, in which the carbon signal at δ 31.9 could be correlated with four protons (δ 2.17, 2.23 and 1.83, 2.12). Furthermore, the gHMQC experiment allowed the assignment of all protons to the appropriate carbon atoms (Table 1).

The observed HMBC correlations for **4** are listed in Table 1. The methyl doublet at δ 1.00 correlated with two CH_2 carbons (δ 37.3 and 31.9) and one CH carbon (δ 26.0), indicating the connection between C-10 and

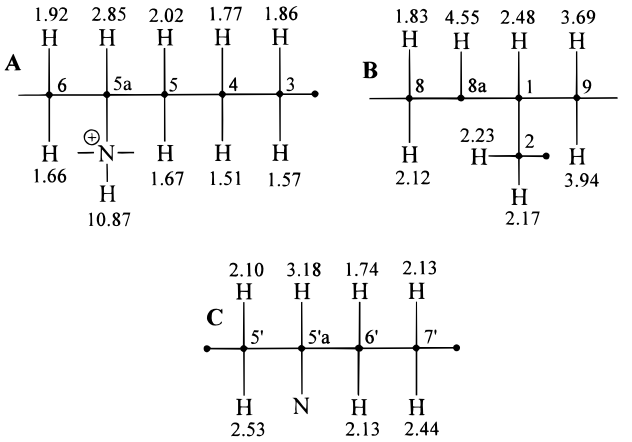

Figure 1. Three major substructures identified from the dqCOSY spectrum of chilocorine C (**4**).

C-7, while both CH_2 groups are connected to the C-7 CH group. Analysis of the dqCOSY spectrum revealed the existence of three major substructures (Figure 1). In substructure A, the observed coupling of a methine proton (δ 2.85) with the NH^+ proton (δ 10.87) and two pairs of CH_2 protons (δ 1.92, 1.66 and 2.02, 1.66) placed it in the H-5a position. In conjunction with HMQC and HMBC data, position 6 was assigned to δ 1.92, 1.66/37.3 signals. Subsequently, C-3−C-5 protons were assigned by successive correlations observed in the dqCOSY spectrum. In substructure B, the methine

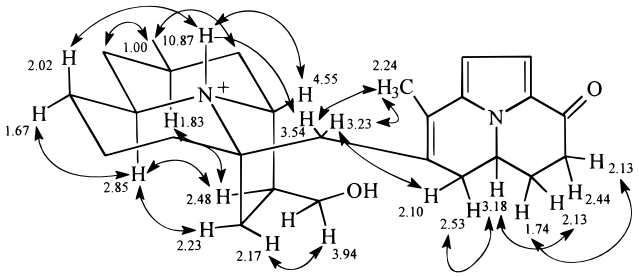

Figure 2. Some key ^{1}H−H correlations observed in the NOESY spectrum of chilocorine C (**4**).

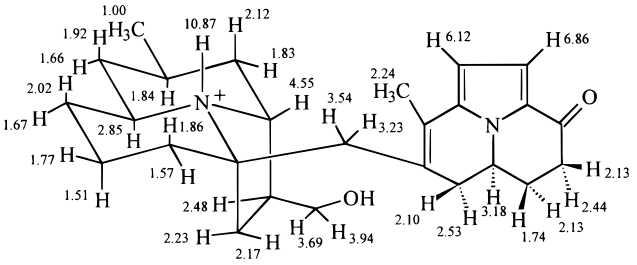

Figure 3. Proposed stereochemistry of protonated chilocorine C (**4**), based on the X-ray structures of exochomine (**1**) and chilocorine B (**3**).

proton observed at δ 2.48 was identified as that at the H-1 position because of its vicinal couplings to both methylene proton pairs at H_2-9 (δ 3.69, 3.94), H_2-2 (δ 2.17, 2.23), and a methine proton at H-8a (δ 4.55). This assignment agreed with HMBC observations of long-range correlations of H_2-2 to C-2a and C-8a and of H_2-9 to C-2 and C-8a.

On the basis of the HMBC correlations of H-3 (δ 1.86), H_2-2 (δ 2.17, 2.23), and H_2-10′ (δ 3.23, 3.54) protons to the C-2a carbon (δ 70.5), it was confirmed that substructures A and B are connected through a quaternary carbon (δ 70.5) at C-2a. From all these observations, it can be concluded that chilocorine C has a 1-(hydroxymethyl)-7-methylperhydro-8b-azaacenaphthylene subunit, rather than the familiar 2-methylperhydro-9b-azaphenalene substructure.

The two coupled aromatic protons (δ 6.86 and 6.12) were assigned to the 1′ and 2′ positions of the other tricyclic subunit, based on the HMBC correlations of H-1′ (δ 6.86) to C-2′ (δ 107.3), C-2′a (δ 135.1), and C-8′a (δ 128.7) and H-2′ (δ 6.12) to C-1′ (δ 113.7), C-2′a, and C-8′a. The singlet methyl protons (δ 2.24) at H_3-9′ gave ^{1}H−^{13}C correlations to C-3′ (δ 125.2) and C-2′a (δ 135.1) in the HMBC spectrum. The dqCOSY spectrum showed an ^{1}H−^{1}H homoallylic coupling with one of the protons at H_2-10′ (δ 3.54). In substructure C, the methine proton (δ 3.18) was placed at 5′a because of chemical shift considerations and its couplings to vicinal H_2-5′ (δ 2.10, 2.53) and H-6′ (δ 1.73) protons. Connections between C-4′−C-5′ and C-7′−C-8′ were confirmed by HMBC data. The skeleton of the aromatic tricyclic subunit was thus determined to be the same as that in exochomine (**1**). In addition, the connection between the two subunits via the carbons 4′ and 10′ was established by HMBC correlations of protons attached to C-10′ (δ 3.23, 3.54) with the carbon atoms at 4′ and 5′ positions (Table 1). On the basis of all these data, we propose structure **4** for chilocorine C.

The stereochemistry of **4** is proposed mainly on the analysis of NOESY and dqCOSY spectra and also the previous knowledge of the stereochemistry of **1**[4] and **3**.[6] The most significant correlations observed in the NOESY experiment are shown in Figure 2. The correlation of the NH^+ proton (δ 10.87) with the H-8a proton (δ 4.55) and one of the H_2-5 protons (δ 2.02) indicated they are on the same face of the molecule. Therefore, H-8a should occupy an equatorial position while H-5 should be in an axial position if we assume that the NH^+ proton is in an axial position.[5] This assumption was supported by the axial−axial coupling observed for the NH^+ proton with the H-5a methine proton (δ 2.85) in the dqCOSY spectrum. The correlations between the H_3-10 methyl protons (δ 1.00) and two methylene pairs at H_2-6 (δ 1.66, 1.92) and H_2-8 (δ 1.83, 2.12) placed the methyl group in an equatorial position. Consequently, the methine proton of H-7 (δ 1.83) should be in an axial position. The H-1 proton (δ 2.48) and one of the H_2-2 protons (δ 2.23) must be in endo positions because of their correlations with the two axial methine protons, H-7 (δ 1.83) and H-5a (δ 2.85). This places the 1-(hydroxymethyl) group in an *exo* position, which is on the same face as the bridge carbon C-10′. The strong axial−axial homonuclear $^3J_{H-H}$ couplings observed in the dqCOSY spectrum suggested that protons δ 1.86, 1.51, 2.02, 1.96, and 2.12 occupy axial positions at C-3, C-4, C-5, C-6, and C-8, respectively. In the aromatic subunit, NOESY correlations indicate protons at δ 2.53 of H_2-5′, δ 3.18 of H-5′a, δ 2.13 of H_2-6′, and δ 2.44 of H_2-7′ are present on the same face of the molecule. Single-crystal X-ray crystallographic analysis of **1** and **3** has revealed that the methine proton at 5′a possessed the *S* configuration in both molecules. Assuming a similar configuration for chilocorine C, it can be represented as shown in Figure 3.

In summary, a new hexacyclic alkaloid, chilocorine C (**4**), has been isolated from *C. cacti* and is shown to bear a structure closely related to that of exochomine (**1**). An unusual feature of this structure, however, is its branched carbon skeleton, which in fact requires an unusual step in its biosynthesis.

Experimental Section

General Experimental Procedures. HPLC analysis was carried out using an HP 1090 HPLC instrument linked to a diode-array detector (HP). A Supelcosil LC-Si column (25 cm × 4.6 mm i.d., 5 μm) (Supelco, Bellefonte, PA) was used for the separation. LC−MS data were obtained by linking the above HPLC system with a Quattro I mass spectrometer (Micromass Inc.). High-resolution mass spectra were obtained using a VG 70-VSE instrument at the University of Illinois Mass Spectrometry Laboratory.

Insect Material. Insects were collected near Weslaco, Texas (voucher specimens are kept in the Natural Sciences Research Laboratory, The Museum, Texas Tech University, ref no. MAH-031098-01).

Extraction and Isolation of the Alkaloid. *C. cacti* adults (50−60 specimens) were soaked in 2% sulfuric acid in methanol (10 mL), crushed, and left at room temperature for 1 h. The supernatant was removed, and the residue was crushed again with an 8 mL portion of the acid−methanol solution. The combined extract was concentrated to ca. 2 mL and diluted with water (20 mL). The aqueous solution was extracted with ether

(8 × 20 mL), made alkaline with 2 M NaOH, and extracted with CH_2Cl_2 (5 × 10 mL). The combined CH_2Cl_2 extract was washed with water, concentrated, and used for HPLC analysis and preparative thin-layer chromatography.

A small portion of the crude alkaloid mixture was dissolved in CH_2Cl_2 containing 1% NH_3, filtered through an Acrodisc CR PTFE syringe filter, and injected into the HPLC system. The column was eluted with 15% MeOH in CH_2Cl_2 containing 0.2% NH_4OH, at a flow rate of 1 mL/min. Eight fractions were collected while monitoring the absorption at 330 nm. LC−MS analysis of each fraction was carried out using the same LC system coupled to an electrospray mass spectrometer operating in the positive ion mode. The column was eluted using 25% MeOH in CH_2Cl_2 containing 0.2% NH_4OH at a flow rate of 0.4 mL/min. The sixth peak (**4**), which eluted at 26.7 min, gave an $[M + 1]^+$ ion at *m*/*z* 409. Its high-resolution mass spectrum obtained on a VG 70-VSE instrument (resolution 5000) gave $[M + 1]^+$ at *m*/*z* 409.2844 (calcd for $C_{26}H_{37}N_2O_2$ 409.2855).

The remainder of the crude extract was dissolved in CH_2Cl_2 containing 1% NH_4OH, loaded on Baker Si254F TLC plates (20 × 20 cm, 250 μm thickness), and developed with a 100 mL of a mixture of solvents: CH_2Cl_2/MeOH/NH_3 = 9:1:0.1. The bands at R_f 0.25 and 0.70 were identified as chilocorines A (**2**) and B (**3**), respectively, by a comparison of their mass spectra and GC retention times with those of authentic samples. The UV-active bands that eluted after the chilocorine A band were isolated and reeluted with the same solvent mixture. After the solvents were removed, the residue was purified once again using the same procedure. The band that corresponds to the TLC spot of peak 6 collected by HPLC separation, was cut off and extracted as before. After most of the solvent was removed, the residue gave virtually one spot when analyzed by TLC. The residue was dried under high vacuum overnight, dissolved in 350 μL of $CDCl_3$, and transferred to a Shigemi tube for NMR studies.

NMR Studies. ^{1}H, gHMQC, gHMBC, dqCOSY, and NOESY spectra were recorded on a Varian Unity 500 spectrometer. ^{13}C and DEPT spectra were obtained using a Varian XL 400 instrument. Spectra were obtained in $CDCl_3$, using a CMS-500V Shigemi NMR microtube (Shigemi Inc., Allison Park, PA), and referenced to residual $CHCl_3$ (7.26 ppm) for proton spectra and $CDCl_3$ (77.0 ppm) for carbon-13 spectra.

IR Studies. The IR spectrum of chilocorine C (4) was obtained by acquiring 128 scans using a SpectraTech IRμs FTIR microscope at McCrone Associates, Inc. After the background was subtracted (resolution = 8 cm^{-1}), major absorptions were observed at 3381−3281, 2980, 2949, 2625, 2501, 1652, 1478, 1401, 1355, and 1038 cm^{-1}.

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