Pergamon

Tetrahedron Letters 43 (2002) 7465–7467

TETRAHEDRON
LETTERS

Chilocorine D, a novel heptacyclic alkaloid from a coccinellid beetle (*Chilocorus renipustulatus*)

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Accepted 27 August 2002

Abstract—Chilocorine D, a new heptacyclic 'dimeric' alkaloid, has been isolated from the coccinellid beetle *Chilocorus renipustulatus* and its structure determined by 1D and 2D NMR analyses. It is constituted of a hippodamine moiety linked to a modified octahydro-azaacenaphtylene skeleton encompassing a seven-membered ring. © 2002 Elsevier Science Ltd. All rights reserved.

Ladybird beetles are protected against predators and competitors by the presence in their hemolymph of bitter alkaloids.[1,2] The first alkaloids isolated from these beetles, such as precoccinelline, coccinelline, hippodamine and convergine, were based on the characteristic 2-methyl-perhydro-9b-azaphenalene ring system.[1,2] Subsequently, dimers of this ring system, such as psylloborine A[3] and isopsylloborine A,[4] have been reported, whereas four other alkaloids isolated from the genera *Exochomus* and *Chilocorus* (exochomine,[5] and chilocorines A,[6] B[7] and C[8]) are not dimers stricto sensu, since they are made up of a 2-methyl-perhydro-9b-azaphenalene unit (modified in the case of chilocorine C[8]) diversely linked to a dimethyl-octahydro-azaacenaphtylene skeleton.

In our continuing search for new alkaloids from ladybirds, we have now examined *Chilocorus renipustulatus*, a European member of the *Chilocorus* genus (subfamily Chilocorinae, tribe Chilocorini). As a result, we report here on the isolation and structure determination of a new heptacyclic alkaloid, chilocorine D (**1**), which is characterized by a modified octahydro-azaacenaphtylene skeleton possessing a seven-membered ring.

The MeOH extract (96.7 mg) of 292 specimens of *C. renipustulatus* collected near Draguignan (France) was first submitted to a flash chromatography on silica gel (eluent: from AcOEt to AcOEt/MeOH/NH_4OH, 80:20:1) to afford two alkaloid-containing fractions (10.7 and 6.0 mg, resp.). The first of these fractions was further chromatographied on a column of basic alumina (eluent: CH_2Cl_2), affording 1.76 mg of a new alkaloid, chilocorine D (**1**), as a colorless oil, $[\alpha]_D^{25}$ 43 (CH_2Cl_2, *c* 0.139). The EI mass spectrum of **1** (HR-EI MS: molecular ion at m/z 406.2617; calcd for $C_{26}H_{34}N_2O_2$: 406.2620) had a base peak at m/z 389.2592 (calcd for $C_{26}H_{33}N_2O$: 389.2593), indicating the loss of an OH radical from the molecular ion, and a fragment ion at m/z 190.1589 (calcd for $C_{13}H_{20}N$: 190.1596), suggesting the presence of the 2-methyl-perhydro-9b-azaphenalene ring system commonly found in coccinellid beetles. The UV spectrum [λ_{max} (MeOH) 204 nm (ε 15750) and 340 nm (ε 29000)] indicated the presence of an α-keto-α′-vinylpyrrole chromophore, characteristic of the octahydro-azaacenaphtylene substructure present in exochomine[5] and in the chilocorines.[6–8] The connectivity of chilocorine D (**1**) was deduced from its 1D and 2D NMR data (C_6D_6, 600 and 75.4 MHz, $^1H/^1H$ COSY, HMQC, HMBC, Table 1). The ^{13}C NMR spectrum displayed 26 carbon atom signals, as expected from the MS data. Four spin systems (H_2C-1 to H_2C-6, H_2C-7 to H_2C-9, HC-1′ to HC-2′ and H_2C-6′ to H_2C-8′) were disclosed from the $^1H/^1H$ COSY spectrum. One isolated vinyl hydrogen (δ_H 5.71; δ_C 136.7) and one isolated AB system (CH_2, δ_H 1.98 and 1.33, $J_{AB} = 14.0$ Hz; δ_C 52.8) were also present. Comparison of the δ_C of C-1 to C-10 of **1** (Table 1) with those of hippodamine[9] and chilocorines

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Keywords: insects; alkaloids; NMR.

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0040-4039/02/$ - see front matter
PII: S0040-4039(02)01794-X

Table 1. NMR data of **1** (600.0 and 75.4 MHz, C_6D_6, δ, J in Hz)

Position	δ_C	δ_H	HMBC (H to C)[a]	Key NOESY correlations
1ax	50.2	1.14, m	C-2, C-3, C-9a, C-10	
1eq		1.28, m	C-2, C-9a, C-10	
2ax	26.2	1.30, m		H-3a, H-9ax
3ax	44.3	0.63, q, 12.0	C-3a, C-4, C-10	
3eq		1.18, m		
3a	52.5	2.40, m		H-2ax, H-5ax, H-7ax, H-9ax
4ax	35.4	1.14, m		
4eq		1.24, m		
5ax	21.4	1.32, m		H-3a, H-7ax
5eq		1.32, m		
6ax	45.4	2.37, m	C-5, C-6a, C-10′	H-4-ax
6eq		1.28, m	C-5	
6a	58.8	–		
7ax	27.0	1.82, td, 10.0, 3.6	C-6a, C-8, C-9, C-10′	H-3a
7eq		0.47, m	C-6a, C-9	H-10eq
8ax	20.3	1.72, qt, 13.0, 3.5	C-9	H-10′eq
8eq		1.50, dt, 12.5, 3.5		
9ax	26.3	1.78, m	C-1, C-8, C-9a	H-3a
9eq		0.84, bd, 13.0	C-7, C-8, C-9a	H-1eq
9a	63.3	–		
10-CH_3	21.9	0.65, d, 6.0	C-1, C-2, C-3	H-2ax, H-3eq
1′	114.4	7.39, d, 4.2	C-2′, C-2′a, C-9′, C-9′a	H-2′
2′	105.5	6.40, d, 4.2	C-1′, C-2′a, C-6′a, C-9′, C-9′a	H-1′
2′a	134.0	–		
3′	136.7	5.71, s	C-2′a, C-4′, C-5′, C-6′, C-1, C-7, C-9a	H-8ax, H-10′eq
4′	133.3	–		
5′	69.0	–		
6′ax	46.0	1.08, dd, 13.0, 11.3	C-6′a, C-7′, C-10′	H-7′ax, H-10′eq
6′eq		1.79, m	C-5′	
6′a	47.5	4.10, tt, 11.3, 3.0		H-6′eq, H-7′eq, H-8′ax
7′ax	30.1	1.28, m		
7′eq		1.38, m		
8′ax	36.4	2.05, ddd, 17.5, 14.0, 5.0	C-6′a, C-7′, C-9′	H-6′a, H-7′ax, H-7′eq
8′eq		2.39, m	C-7′, C-9′	H-7′ax
9′	185.3	–		
9′a	129.5	–		
10′ax	52.8	1.33, d, 14.0		
10′eq		1.98, d, 14.0	C-5, C-6, C-6a, C-7, C-4′, C-5′	H-7eq, H-8ax, H-6′ax
OH[b]	–	6.97, bs	C-5′a, C-6′	H-1ax, H-6ax, H-6′eq, H-6′a, H-10′ax

[a] Optimized for $^{n}J_{CH}=5$ and 10 Hz.

[b] In DMSO.

A[6] and B[7] suggested the presence in chilocorine D of a hippodamine substructure containing two quaternary ring junction carbon atoms (C-6a and C-9a) (Fig. 1A). This assignment was confirmed by the HMBC spectrum and by the observation of strong correlations between H-3a, H-2ax, H-5ax, H-7ax and H-9ax in the NOESY spectrum (Table 1).

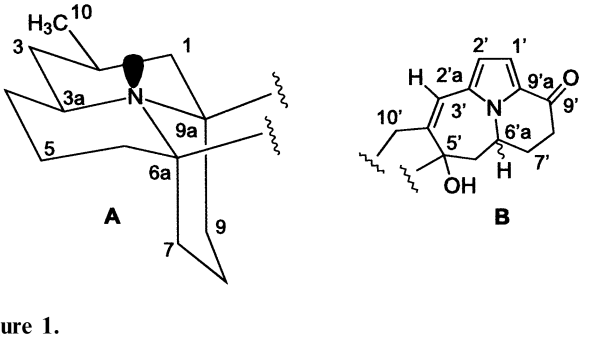

Figure 1.

The structural assignment of the other moiety (C-1′ to C-10′) proved less straightforward. The ^{1}H NMR data mentioned above together with a comparison of the δ_C of C-1′, C-2′, C-2′a, C-6′a, and C-7′ to C-9′a with the ^{13}C NMR data of exochomine[5] and the three chilocorines[6–8] demonstrated the presence of the pyrrole/cyclohexanone moiety present in all these alkaloids (Fig. 1B). Moreover, since H_2C-6′ could be connected to HC-6′a by ^{1}H/^{1}H COSY, there remained only four carbon atoms to locate: one CH_2 (C-10′, δ_C 52.8), two sp^2 carbon atoms (CH-3′ at δ_C 136.7, δ_H 5.71; C-4′ at δ_C 133.3), and one quaternary C (C-5′, δ_C 69.0) which, according to its chemical shift, must bear the hydroxyl group. This was confirmed by the observation in the NMR spectrum measured in DMSO of an HMBC correlation between the hydroxylic hydrogen at δ_H 6.97 and the C-5′ carbon at δ_C 69.0. The connectivity C-2′a-HC-3′-C-4′-C-5′-H_2C-6′, leading to a seven-membered ring as shown in Fig. 1B, was established by key HMBC correlations, most noteworthy between H-3′ at δ_H 5.71 and C-2′a (δ_C 134.0), C-4′ (δ_C 133.3), C-5′ (δ_C 69.0), and between H-6′eq at δ_H 1.79 and C-5′ (δ_C 69.0). The only carbon atom left (H_2C-10′, δ_C 52.8) could be located at C-4′ through HMBC correlations between H-10′eq at δ_H 1.98 and C-4′ and C-5′.

To complete the planar structure of chilocorine D (**1**), it remained only to link the two tricyclic substructures A and B. This is only possible between C-6a (δ_C 58.8) and C-9a (δ_C 63.3) of substructure A on one hand, and H_2C-10′ (δ_C 52.8) and C-5′ (δ_C 69.0) of substructure B on the other hand. Again, several key HMBC correlations (e.g. H_2C-10′ to C-6, C-6a, and C-7) (Table 1) permitted us to attach C-6a to H_2C-10′, and C-9a to C-5′. This conclusion was strengthened by the observation that C-9a is deshielded by 4.5 ppm in comparison with C-6a. This deshielding is expected since C-9a is β to the C-5′ hydroxyl group. All these arguments point to structure **1** for chilocorine D (Fig. 2).

The relative configuration of chilocorine D was determined on the basis of NOESY data. Indeed, the NOESY spectrum in C_6D_6 showed that H-6′eq, H-6′a, H-7′eq and H-8′ax were all located on the same face of the molecule. Moreover, a correlation between H-10′eq at δ_H 1.98 and H-6′ax at δ_H 1.08 suggested a *cis*-relationship between these hydrogen atoms. The relative configuration between the OH group at C-5′ and HC-6′a could not be deduced from this spectrum since the OH at C-5′ did not correlate with any other hydrogen. In the NOESY spectrum taken in DMSO, however, the hydroxylic hydrogen exhibited correlations with H-6′a, showing that they are in a *cis* relationship, and with H-1ax, H-6ax and H-10′ax, thus establishing the relative configuration of chilocorine D as shown in Fig. 3.

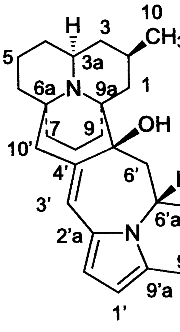

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Figure 2.

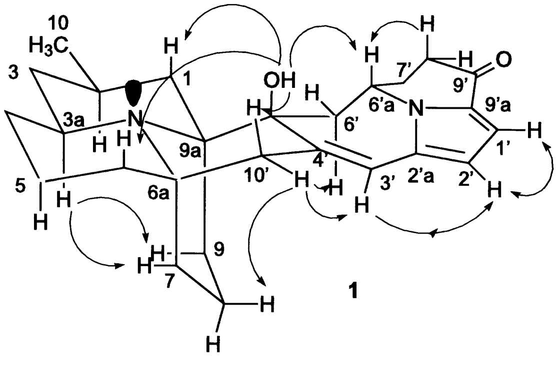

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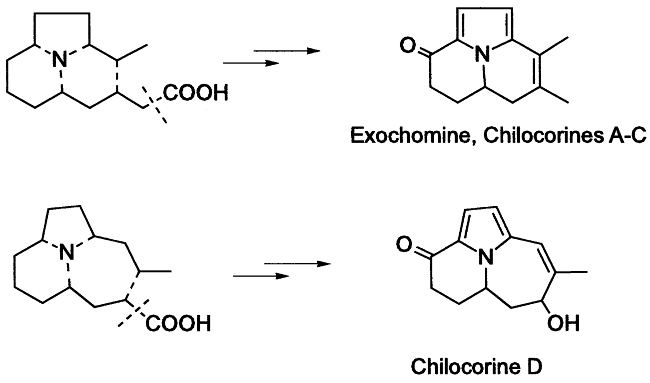
Figure 3. Key NOE correlations in C_6D_6 and in DMSO.



Scheme 1.

Chilocorine D joins to the growing list of complex dimeric coccinellid alkaloids. It is characterized by the presence of a modified octahydro-azaacenaphtylene ring system encompassing a seven-membered ring. The biosynthetic origin of this new ring system is probably similar to that of the dimethyl-octahydro-azaacenaphtylene system present in exochomine[5] and the chilocorines A–C.[6–8] Its formation could be envisioned through a different folding pattern of the open chain precursor, as shown for example in Scheme 1.

Acknowledgements

The financial support of Entomed (http://www.entomed.com) is gratefully acknowledged. We thank Dr. M. Luhmer and Mrs. R. d'Orazio for the NMR spectra and Mr. C. Moulard for the mass spectra.

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