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Hyperaspine, a new 3-oxaquinolizidine alkaloid from *Hyperaspis campestris* (Coleoptera: Coccinellidae)

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Abstract—A new 3-oxaquinolizidine alkaloid (3*R**,4a*R**,6*S**,8*R**)-6-hydroxy-3-methyl-8-pentylperhydropyrido[1,2-*c*][1,3]oxazine 6-(pyrrole-2-carboxylate) (hyperaspine) has been isolated from the ladybird beetle *Hyperaspis campestris* and its structure determined by 2D NMR and MS methods.

Ladybird beetles are protected against predators and competitors by the presence in their hemolymph of bitter alkaloids.[1,2] Some of these alkaloids, e.g. coccinelline,[3] epilachnene,[4,5] adaline and adalinine,[6] have been shown to be biosynthesised de novo by the beetles and to be derived from a C_{14} fatty acid or polyketide chain, which undergoes various oxidation, decarboxylation and cyclisation reactions to afford the different alkaloids.

In our continuing search for new alkaloids from ladybirds, we have now examined *Hyperaspis campestris*, a member of an as yet uninvestigated tribe (subfamily Scymninae, tribe Hyperaspini). As a result, we report here on the isolation and structure determination of hyperaspine (**1**), which is the first representative of a new type of ladybird alkaloids having a 3-oxaquinolizidine skeleton.

The MeOH extract (20 mg) of 203 specimens of *H. campestris* collected in Bulgaria was submitted to two successive flash chromatographies on silica gel (eluent: AcOEt/hexane/NH_4OH, 8:2:0.1) to afford 400 μg of a new alkaloid for which the name hyperaspine was coined. The connectivity of hyperaspine (**1**) (Fig. 1) (HR-EIMS: m/z 334.2236; calcd for $C_{19}H_{30}N_2O_3$: 334.2256) was deduced from its MS and 1D and 2D NMR data (Shigemi tube, CD_2Cl_2, 600 MHz, $^1H/^1H$ COSY, HMQC, HMBC). The EI mass spectrum[7] of **1** showed, besides a small molecular ion at m/z 334, two diagnostic fragment ions at m/z 263.1399 (calcd for $C_{14}H_{19}N_2O_3$: 263.1396) and at m/z 152.1070 (base peak; calcd for $C_9H_{14}NO$: 152.1070) arising, respectively, from the loss of a pentyl chain and of a pentyl chain and a 2-pyrrolecarboxylic acid moiety (confirmed by the UV spectrum:[8] λ_{max} 263 nm, ε 3650).

The $^1H/^1H$ COSY, HMQC and HMBC spectra permitted us to assign all the H and C atoms of the molecule (Table 1). These assignments, in comparison with literature data, disclosed the presence in the molecule of a perhydropyrido[1,2-*c*][1,3]oxazine (3-oxaquinolizidine) skeleton[9,10] (C-1 to C-8) and of a secondary methyl group (H_3C-9, d at δ_H 1.15, $J=6.6$ Hz, δ_C 22.0), and confirmed the presence of a 2-pyrrolecarboxylate moiety[11] (C-1′ to C-5′), and of a *n*-pentyl side chain (C-10 to C-14). The positions of the methyl, *n*-pentyl

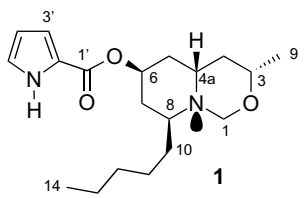

Figure 1. Structure and relative configuration of **1**.

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and pyrrolecarboxylate substituents at C-3, C-8 and C-6, respectively, of the perhydropyrido[1,2-*c*][1,3]-oxazine skeleton were deduced from the $^1H/^1H$ COSY spectrum and from HMBC correlations (see Table 1). With the connectivity of the molecule being firmly established, we could investigate its conformation and relative configuration. That hyperaspine (**1**) exists predominantly in a *cis*-fused ring conformation was shown by the lack of Bohlmann bands[12] in the IR spectrum and by the value of the J_{gem} (−10.8 Hz) between the two diastereotopic H at C-1. Indeed, it has been repeatedly shown that in *trans*-fused 3-oxaquinolizidines, the J_{gem} of the H_2C-1 has a value of ca. −8.0 Hz, whereas it is approximately −10 Hz for the corresponding *cis*-fused systems.[9,12,13]

With this information in hand, we could now determine the relative configuration of **1**. The coupling constants of the three methines linked to substituent-bearing carbons (C-3, C-6 and C-8) (Table 1) clearly showed that they had an axial position on a six-membered chair ring. This conclusion was strengthened by the values of the coupling constants of H-4ax ($J=11.0$, 11.0 and 11.0 Hz) and H-7ax ($J=12.0$, 12.0 and 12.0 Hz). All these arguments led us to deduce that **1** has the relative configuration shown in Fig. 1 (we have arbitrarily represented the enantiomer having the same absolute configuration at C-8 as the related ladybird alkaloid (+)-calvine and (+)-epicalvine).[14] These structural assignments were fully confirmed by NOESY experiments, the most telling results of which are reported in Table 1. Most noteworthy were the correlations observed between H-1ax, H-3ax and H-4a, between H-4ax, H-6ax and H-8ax, and between H-1eq, H-8ax and H_2-10. Fig. 2 represents the preferred conformation of **1**.

It is worth noting that the *cis*-fused conformation deduced for **1** is not unexpected. Indeed, whereas (4a*R**,8*R**)-8-methylperhydropyrido[1,2-*c*][1,3]oxazine exists in a *trans*-conformation, its diastereoisomer, (4a*R**,8*S**)-8-methylperhydropyrido[1,2-*c*][1,3]oxazine, exists predominantly in a *cis*-fused conformation.[13] This conformational preference of the latter compound was ascribed to the *trans* conformation being disfavoured by an axial methyl group at C-8 and by a destabilising dipole–dipole interaction between the N and O atoms which does not exist in the *cis* conformation.[13]

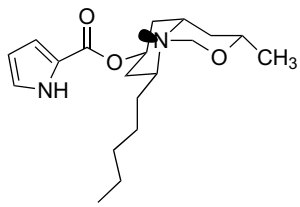

Figure 2. Preferred conformation of **1**.

Table 1. NMR data of **1** (600.0 and 150.85 MHz, Shigemi tube, CD_2Cl_2, δ, J in Hz)

Position	δ_C[a]	δ_H	HMBC (H to C)[b]	NOESY
1ax	81.0	4.22, d, 10.8	C-3, C-4a, C-8	H-3ax, H-4a
1eq		4.77, d, 10.8	C-3, C-4a, C-8, C-5	H-8ax, CH_2-10
3ax	74.0	3.60, ddq, 12.0, 4.2, 6.6		H-1ax, H-4a
4ax	33.8	1.76, ddd, 11.0, 11.0, 11.0	C-3, C-4a, C-9	H-6ax, H-8ax
4eq		1.23, m		
4a	56.0	3.38, m		H-1ax, H-3ax
5ax	36.2	1.86, m	C-4, C-4a, C-6, C-7	
5eq		1.86, m	C-4, C-4a, C-6, C-7	
6ax	69.0	5.10, dddd, 11.0, 11.0, 5.0, 5.0	C-1′, C-4a	H-4ax, H-8ax
7ax	37.0	1.44, ddd, 12.0, 12.0, 12.0	C-5, C-6, C-8, C-10	
7eq		2.06, bd, 12.0		
8ax	49.0	3.16, dddd, 11.0, 11.0, 3.0, 3.0		H-1eq, H-4ax, H-6ax
9-Me	22.0	1.15, d, 6.6	C-3, C-4, C-4a	
10a	32.5	1.44, m		H-1eq
10b		1.57, m		H-1eq
11a	24.5	1.25, m		
11b		1.35, m		
12a	33.0	1.27, m		
12b		1.27, m		
13a	23.0	1.25, m		
13b		1.25, m		
14-Me	14.5	0.85, t, 6.6	C-12, C-13	
1′	160.0	–		
2′	123.0	–		
3′	115.0	6.86, bs		
4′	110.0	6.24, t, 3.0		
5′	123.0	6.96, bs	C-2′, C-3′, C-4′	

[a] Assignments by HMQC and HMBC
[b] Optimised for $^nJ_{CH}=5$ and 10 Hz

Hyperaspine (**1**) is the first 3-oxaquinolizidine alkaloid reported so far from ladybird beetles. Its skeleton, however, which is based on a chain of 13 carbon atoms (C-3 to C-14), is biosynthetically related to those of the numerous piperidine, homotropane and perhydroazaphenalene alkaloids already isolated from these beetles.[1,2,14]

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