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Isopsylloborine A, a New Dimeric Azaphenalene Alkaloid from Ladybird Beetles (Coleoptera: Coccinellidae)

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Abstract

A new dimeric azaphenalene alkaloid, isopsylloborine A (**2**), has been isolated from the ladybird beetles *Halyzia 16-guttata* and *Vibidia 12-guttata*, and its structure determined by 2D NMR methods. It is a double bond isomer of the recently described psylloborine A (**1**), which is also present in these beetles as a minor alkaloid. © 1999 Published by Elsevier Science Ltd. All rights reserved.

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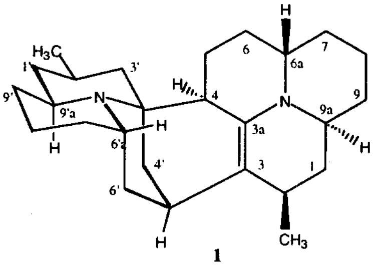
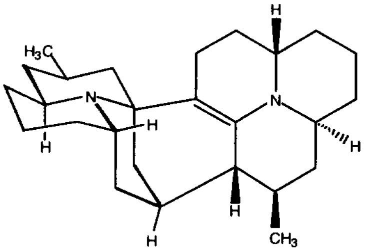
Ladybird beetles are protected against predators and competitors by bitter alkaloids which are present in their hemolymph [1,2]. Amongst the great variety of structural types exhibited by these alkaloids, the 2-methylperhydro-9b-azaphenalene skeleton is surely the most characteristic [1,2]. Recently, Schröder and Tolasch have described the first true azaphenalene dimer, psylloborine A (**1**), isolated from *Psyllobora 22-punctata* (L.) [3].



1



2

We report here on the isolation and structure determination of a related azaphenalene dimer, isopsylloborine A (**2**), isolated together with **1** from two species of European ladybird beetles, *Halyzia 16-guttata* and *Vibidia 12- guttata.* The MeOH extract of adults of the two species were identical in TLC and HPLC. Accordingly, the extract of 954 beetles (1.755 g) was submitted to a series of chromatographies on silica gel, using mixtures of CH_2Cl_2-MeOH-NH_4OH, from 100:0:0.5 to 97:3:0.5 as eluent. This procedure yielded 22 mg of the major alkaloid (**2**) and 4.5 mg of the minor one, which was identified as psylloborine A (**1**) [3] by NMR, MS, and direct TLC and HPLC comparison with an authentic sample isolated from *Psyllobora 22-punctata.*

Compound **2** [oil; $[\alpha]_{579}$ +17 (c= 0.53, CH_2Cl_2); $C_{26}H_{40}N_2$ by HR-EIMS: *m/z* 380.3190 Daltons; calc. 380.3191)] was isomeric with **1**, and displayed the same base peak at *m/z* 188.1436 (calc. for $C_{13}H_{18}N$: 188.1439). The ^1H and ^{13}C NMR spectra of **2** measured in CD_2Cl_2 showed only broadened peaks precluding a

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detailed analysis. This problem was solved by running the NMR spectra in the presence of 0.2 µl of TFA. Under these conditions, the ^1H NMR and ^{13}C NMR spectra (Table 1) showed well-resolved signals. The structure of **2** was solved by a detailed analysis of 1D and 2D NMR spectra (^1H, ^{13}C, COSY, HMQC, HMBC and NOESY). The assignment of all the H and C atoms of the molecule is reported in Table 1. Compound **2** is thus a double bond isomer of psylloborine A (**1**), named isopsylloborine A, which is also present as a minor derivative in *P. 22-punctata*.

Table 1. NMR data of **2** (600 and 150.85 MHz, CD_2Cl_2 + TFA, δ, *J* in Hz,)

Position	δ_C	δ_H	HMBC (H to C)[a]	NOESY
1ax	39.8	1.80, m	C-2, 2-Me, C-9a	
1eq		2.04, m	C-3, C-9a	H-2ax, 2-Me, H-9a,
2ax	35.0	1.77, m	C-1, C-9a	H-9a, H-1eq, H-4'eq, H-5'eq
3ax	49.7	2.17, m[b]	C-2, 2-Me, C-3a, C-4, C-4', C-6'	2-Me, H-6'a, H-5'eq, H-6'eq
3a	145.1	-	-	-
4	127.3	-	-	-
5ax	22.3	2.67, bm	C-3a, C-4, C-6	H_2-6, H-6a
5eq		2.46, bdd, 17.5, 5.6	C-3a, C-4, C-6, C-6a	H-6a, H_2-3'
6ax	20.4	2.10, m	C-6a	H-9a
6eq		1.83, m		
6a	56.0	3.80,bd, 12.0	C-5, C-6, C-7, C-8, C-9a	H_2-5, H_2-7, H-6'a
7ax	28.2	1.83, m[c]		H-6a
7eq		2.12, m[c]	C-6a, C-8	H-6a
8ax	17.0	1.60, m	C-6a	
8eq		1.70, m		
9ax	31.4	1.70, m		
9eq		1.87, m		H-9a
9a	63.6	3.34, tt, 12.0, 3.0		H-1eq, H-2ax, H-6ax, H-9eq
2-Me	19.6	1.03, d, 6.6	C-1, C-2, C-3, C-9a	H-1eq, H-2ax, H-3ax, H-5'eq
1'ax	39.0	1.49, m	C-2', 2'-Me, C-3', C-9'a	2'-Me
1'eq		1.80, m		2'-Me
2'ax	24.9	1.98, m		2'-Me, H-4'ax, H-9'a,
3'ax	41.8	2.04, m	C-4, C-2', C-3'a, C-4'	H-5eq
3'eq		1.60, m		2'-Me, H-5eq
3'a	63.5	-	-	-
4'ax	25.6	2.44, dd, 15.0, 3.5	C-3, C-4, C-3'a, C-5'	H-2'ax, H-6'ax, H-9'a
4'eq		1.55, m		H-2ax, H-5'eq
5'eq	26.5	2.40, bs	C-6'	H-2ax, H-3ax, H-4'eq, H-6'eq
6'ax	30.0	2.28, td, 14.5, 4.5	C-3, C-5', C-6'a, C-7'	H-4'ax, H-7'ax, H-9'a
6'eq		1.48, bd, 14.5		H-3ax, H-5'eq, H-6'a, H-7'eq
6'a	52.6	4.17, dq, 13.5, 3.0	C-7', C-8', C-9'a	H-3ax, H-6a, H-6'eq, H_2-7'
7'ax	28.7	2.00, m	C-6', C-6'a, C-8'	H-6'a, H-6'ax
7'eq		1.70, m		H-6'eq, H-6'a
8'ax	17.7	1.60, m		H-9'a
8'eq		1.60, m		
9'ax	31.8	1.70, m[d]		
9'eq		1.78, m[d]		
9'a	56.1	3.57, bm		H-2'ax, H-4'ax, H-6'ax, H-8'ax
2'-Me	21.6	0.99, d, 6.6	C-1', C-2', C-3', C-3'a, C-9'a	H_2-1', H-2'ax, H-3'eq

[a]Optimized for $^nJ_{CH}$ = 5 and 10 Hz; [b]Homoallylic coupling with H-5ax; [cd]these assignments may be interchanged;

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