Notes

2-Dehydrococcinelline, a New Defensive Alkaloid from the Ladybird Beetle *Anatis ocellata* (Coccinellidae)

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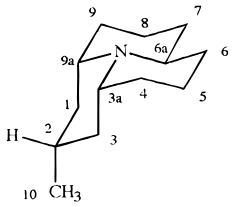
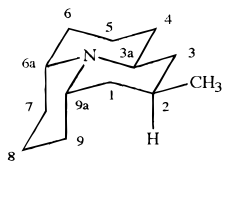
2-Dehydrococcinelline (**6**), a novel coccinellid defensive alkaloid, has been isolated from the European ladybird *Anatis ocellata*. Its structure was established by spectroscopic methods and confirmed by chemical correlation with precoccinelline (**1**).

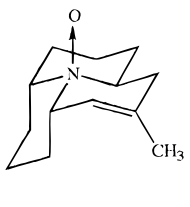
Ladybird beetles (Coleoptera; Coccinellidae) are well protected against predation and a variety of alkaloids, which contribute to this protection, have been characterized from these beetles.[1,2] Several of these alkaloids belong structurally to the 2-methylperhydro-9b-azaphenalene ring system [e.g. precoccinelline (**1**), coccinelline (**2**), hippodamine (**3**), and convergine (**4**)] which appears to be specific to these insects. In a preliminary chemical survey of a variety of coccinellid beetles,[3] we reported the presence in the European species *Anatis ocellata* (L.) of two alkaloids [AO_1 (M^+, 191) and AO_2 (M^+, 207)], the mass spectra of which suggested that they are the *N*-oxide/free base pair of an alkaloid belonging to the 2-methyldecahydro-9b-azaphenalene ring system. At that time, because of lack of biological material, the structure elucidation of AO_1 and AO_2 could not be achieved. In the present study we report the isolation and structure determination of the major alkaloid AO_2 obtained by extraction of whole bodies of *A. ocellata*.



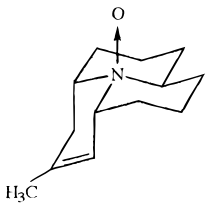
(**1**) Precoccinelline
(**2**) Coccinelline (N-oxide)



(**3**) Hippodamine
(**4**) Convergine (N-oxide)



(**5**) Hippocasine N-oxide



(**6**) 2-Dehydrococcinelline

The insects (35 adults) were exhaustively extracted with CH_3OH, and successive chromatographic separations of the extract (352 mg) on alumina and silica gel led to the isolation of the main Dragendorff positive compound AO_2, 1.4 mg. HREIMS measurements coupled to the presence of 13 signals in the proton decoupled ^{13}C NMR spectrum showed the molecular formula to be $C_{13}H_{21}NO$. Characteristic fragment ions were observed at *m/z* 191.1667 (46; $C_{13}H_{21}N$), 190.1599 (93; $C_{13}H_{20}N$), 188.1448 (27; $C_{13}H_{18}N$), 176.1438 (100; $C_{12}H_{18}N$), 162 (20) and 148 (16) reminiscent of the mass spectrum of hippocasine *N*-oxide (**5**),[4] a defensive alkaloid isolated 20 years ago by Ayer *et al.*[4] from *Hippodamia caseyi*, a ladybird indigenous to Western Canada. Loss of O and OH from the molecular ion is distinctive of a *N*-oxide group. The ^1H and ^{13}C NMR spectra of AO_2 contained signals for one trisubstituted double bond, one vinylic methyl group, three methines α to the nitrogen atom, and seven methylenes. All of these data were suggestive of formula **6**, the connectivities of which were further corroborated by a 2D NMR study (^1H-^1H COSY, HMQC, HMBC). The complete assignments of the ^1H and ^{13}C NMR signals are reported in the Experimental Section. Most noteworthy in the ^1H-^1H COSY spectrum were the correlations between H-3 (δ 5.19) and H-3a (δ 3.97) and H_2-1 (δ 2.17 and 2.56), and between the latter and H-9a (δ 3.51).

AO_2 thus has the same connectivity as hippocasine *N*-oxide (**5**), the structure of which has been determined by X-ray diffraction analysis.[4] At the time, neither ^{13}C nor high-field ^1H NMR spectra of **5** had been reported so that we could not make an accurate comparison between **5** and AO_2, but the reported chemical shift for the vinylic proton of **5** (δ 5.42, instead of 5.19 for AO_2) suggested that the two compounds were not identical and could thus be stereoisomeric. The establishment of the relative configuration of AO_2 was based on the following NMR arguments. The ^{13}C chemical shifts of the carbon atoms adjacent to the nitrogen atom for coccinelline (**2**), convergine (**4**), and AO_2 are reported in Table 1. It appears from the examination of this table that C-3a is shielded in convergine while C-6a is shielded in coccinelline. These shieldings can be attributed to gauche interactions between H-3a/H7ax and H-3a/H-9ax in convergine and between H-6a/H-3ax and H-6a/H-1ax in coccinelline. In this respect, AO_2 behaves as coccinelline, suggesting that they have the same

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Table 1. Comparison of the ^{13}C NMR Chemical Shifts (δ) of the Carbon Atoms Adjacent to the Nitrogen Atom in **2**, **4**, and **6**

carbon atom	coccinelline (**2**)	2-dehydrococcinelline (**6**)	convergine (**4**)
C-9a	72.7	69.2	74.2
C-6a	59.0	59.4	73.5
C-3a	72.7	71.6	58.0

relative configuration. Catalytic hydrogenation of AO_2 led to precoccinelline (**1**), thus confirming the configuration attributions. The hydrogenation proceeds selectively from the less hindered face (*si*−*si* face) of the double bond to give the compound with an equatorial methyl group. From all these results it can be deduced that AO_2 is 2-dehydrococcinelline (**6**).

A minor Dragendorff positive derivative was also isolated from the CH_3OH extract during the separation procedures. Its mass spectrum (EIMS) presented a molecular ion at *m*/*z* 191, indicating that traces of the corresponding free base (AO_1) may be present in the beetle.

A. ocellata belongs to the tribe Coccinellini. Until now members of this tribe were found to produce alkaloids with either 2-methylperhydro-9b-azaphenalene, homotropane or long-chain skeletons, and these alkaloids were never found in members of other subtribes.[1] The identification of 2-dehydrococcinelline in *A. ocellata* thus confirms this pattern.

Experimental Section

General Experimental Procedures. HREIMS were performed on a Fisons Autospec instrument. The ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 600 and 150.87 MHz, respectively, using a Varian Unity 600 instrument. The IR spectrum was obtained on a Bruker IFS 48 FT instrument as a film on a NaCl disk. The optical rotations were measured on a Perkin-Elmer 141 polarimeter (Hg vapor lamp) in a 10 cm cell at 20 °C. Thin layer chromatography analyses (TLC) were performed on 0.25 mm Polygram silica gel SILG/UV_{254} precoated plates (Macherey Nagel) or on 0.2 mm neutral alumina 60 F_{254} precoated plates (Merck, type E). Column chromatographies were performed over silica gel (MN Kieselgel 0.04−0.063 mm), using the flash technique or over MN neutral alumina. GC analyses were performed on a Varian 3700 apparatus equipped with an OV-1701 capillary column (Rescom, 25 m, 0.32 mm i.d.).

Extraction and Isolation. A total of 35 adult specimens of *Anatis ocellata* collected near Brussels were ground and exhaustively extracted with MeOH affording 352 mg of an orange oil which was chromatographed over alumina (eluent: CH_2Cl_2/MeOH, gradient from 100:0 to 80:20 and then MeOH 1% NH_4OH). The fractions showing Dragendorff positive spots by TLC were combined and flash chromatographed on silica gel (eluent: CH_2Cl_2/MeOH 0.1% NH_4OH, gradient from 98:2 to 90:10). This afforded 1.4 mg of compound **6**, homogeneous by TLC, exhibiting the following properties: oil; [α] + 8 at 579 nm and + 19 at 407 nm (CH_2Cl_2, *c* = 0.16); IR (film) ν_{max} 2926, 1660, 1442, 1382, 956 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 5.19 (1H, br s, H-3), 3.97 (1H, br s, H-3a), 3.51 (1H, br s, H-9a), 3.14 (1H, t, *J* = 12 Hz, H-6a), 2.90 (1H, tt, *J* = 13.5 and 5 Hz, H-9ax), 2.70 (1H, tt, *J* = 13.5 and 4.5 Hz, H-4ax), 2.56 (1H, br t, *J* = 14 Hz, H-1ax), 2.17 and 2.20 (2H, m, H-1eq and H-7ax), 1.98 (1H, dq, *J* = 12 and 4 Hz, H-6ax), 1.74 (3H, s, H_3-10), 1.64 (1H, dt, *J* = 13 and 5 Hz, H-8ax), 1.58 (1H, m, H-8eq), 1.52 and 1.43 (3H, m, H_2-5 and H-4eq), 1.26 (2H, m, H-6eq and H-7eq); ^{13}C NMR ($CDCl_3$, 150.87 MHz) δ 133.4 (C-2), 121.7 (C-3), 71.6 (C-3a), 69.2 (C-9a), 59.4 (C-6a), 33.2 (C-1), 27.2 (C-6), 26.6 (C-7), 26.3 (C-4), 23.6 (C-9), 22.2 (C-10), 18.7 (C-5), 17.2 (C-8); HREIMS M^+ at *m*/*z* 207.1615 (calcd for $C_{13}H_{21}NO$, 207.1623; 8). Characteristic fragment ions were observed at *m*/*z* 191.1667 (46; $C_{13}H_{21}N$), 190.1599 (93; $C_{13}H_{20}N$), 188.1448 (27; $C_{13}H_{18}N$), 176.1438 (100; $C_{12}H_{18}N$), 162 (20) and 148 (16).

Catalytic Hydrogenation of 2-Dehydrococcinelline. A solution of 2-dehydrococcinelline (1.4 mg) in CH_3OH containing Pd/C was hydrogenated under 3 atm of hydrogen overnight. The solution was filtered over silica gel and flash chromatographed (silica gel; CH_2Cl_2/MeOH 0.1% NH_4OH, 98:2). This afforded 0.5 mg of a compound showing chromatographic and spectroscopic properties (GC, TLC, MS and ^1H NMR) identical to those of precoccinelline (**1**).

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References and Notes

(1) Daloze, D.; Braekman, J. C.; Pasteels, J. M. *Chemoecology* **1995**, *5/6*, 173−183.
(2) King, A. G.; Meinwald, J. *Chem. Rev.* **1996**, *96*, 1105−1122.
(3) Pasteels, J. M.; Deroe, C.; Tursch, B.; Braekman, J. C.; Daloze, D.; Hootele, C. *J. Insect Physiol.* **1973**, *19*, 1771−1784.
(4) Ayer, W. A.; Bennett, M. J.; Browne, L. M.; Purdham, J. T. *Can. J. Chem.* **1976**, *54*, 1807−1813.

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