Adalinine, a New Piperidine Alkaloid from the Ladybird Beetles *Adalia bipunctata* and *Adalia decempunctata*

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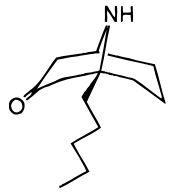
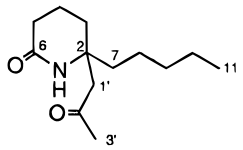
Adalinine (**2**), a new piperidine alkaloid, has been isolated from the European two-spotted ladybird beetle *Adalia bipunctata*, and its structure has been determined by spectroscopic methods. This alkaloid was also shown to be present in all the life cycle stages of *Adalia bipunctata* as well as in the adults of a related species, *A. decempunctata*.

Many ladybird beetles (Coleoptera; Coccinellidae) exhibit a defense mechanism known as reflex bleeding.[1] When disturbed or molested, they emit droplets of an yellowish fluid at the joints of their legs. The presence of defensive alkaloids in this fluid is well documented.[2,3] Alkaloids belonging to many different structural families such as pyrrolidines, piperidines, homotropanes, 2-methylperhydro-9b-azaphenalenes, azamacrolides, "dimeric" alkaloids, and long-chain and quinoline derivatives have been characterized from these insects.[4−15] The European two-spotted ladybird *Adalia bipunctata* L. has been found to contain the homotropane alkaloid adaline (**1**).[6] The deterrent and toxic properties of **1** have been investigated.[16,17] We recently decided to reexamine the chemical defense secretion of *A. bipunctata*, in the context of a research project aimed at understanding the relationships between different species of predators competing for the same aphid population. Analysis of a $CHCl_3$-soluble extract of *A. bipunctata* adults by GC−MS showed the presence, in addition to adaline (**1**), of a minor, nitrogen-containing compound, amounting to about 10% of the concentration level of **1**. We report here the isolation and structure determination of this new alkaloid **2**, for which the name adalinine has been coined.

The HREIMS of adalinine (**2**) showed its molecular formula to be $C_{13}H_{23}NO_2$, thus requiring three degrees of unsaturation. The most intense fragment ions were observed at *m*/*z* 168.1383 ($C_{10}H_{18}NO$, $[M^+ - C_3H_5O]$), 154.0873 ($C_8H_{12}NO_2$, $[M^+ - C_5H_{11}]$), and 112.076 ($C_6H_{10}NO$, $[M^+ - C_5H_{11} - C_2H_2O]$), suggesting the presence of a pentyl chain and a $-CH_2COCH_3$ moiety. The ^{13}C-NMR spectrum displayed 13 carbon atom signals (two C=O, one C, eight CH_2, and two CH_3). One of the C=O groups was assigned to a ketone (^{13}C-NMR δ 207.2; IR, ν_{CO} 1709 cm^{-1}) and the other to an amide (^{13}C-NMR δ 171.4; IR, ν_{CO} 1659 cm^{-1}). The structure of adalinine was established by a 2D NMR study (^{1}H−^{1}H COSY, HMQC, HMBC) and is depicted as **2**. The complete assignments of the ^{1}H- and ^{13}C-NMR spectra of **2** are reported in the Experimental Section. Most noteworthy in the ^{1}H−^{1}H COSY spectrum were the correlations between H_2-5 at δ 2.30 and H_2-4 at δ 1.78 and 1.70, and between the latter and H_2-3 at δ 1.57 and 1.54, as well as the long-range correlation of the H_2-1′ signal at δ 2.63 (AB system, J = 17.7 Hz) with the H_3-3′ singlet at δ 2.10. Moreover, this H_2-1′ signal showed strong correlations with C-2, C-3, and C-7 in both the 5 and 10 Hz HMBC spectra. On the other hand, the chemical shifts of the ^{13}C-NMR signals of **2** are also in good agreement with those reported in the literature for 2-piperidone derivatives.[18] The absolute configuration of **2** was not determined.



1



2

Both adaline (**1**) and adalinine (**2**) have been detected in adults, eggs and the first- to fourth-instar larvae of *A. bipunctata.* A GC−MS study of *Adalia decempunctata* adult extracts showed the presence of both adaline (**1**), already reported in this species by Pasteels *et al.*,[2] and adalinine (**2**). The detection of **2** by GC and TLC in fresh ladybird extracts indicates that this compound is not an artifact of the isolation procedure. Despite being a piperidone derivative, **2** is obviously structurally related to the major alkaloid adaline (**1**). As the latter is a β-amino ketone, it could undergo a retro-Mannich reaction to afford the imine **3**. Addition of H_2O followed by oxidation would afford **2** (Scheme 1). However, because the biosynthetic pathway leading to **1** is as yet unknown, the precise relationship existing between the two compounds is still a matter of speculation. The study of adalinine (**2**) biosynthesis is currently underway in our laboratories.

Experimental Section

General Experimental Procedures. HREIMS were performed on a Fisons Autospec instrument. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ at 600

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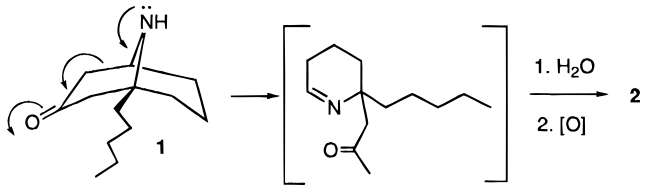
Scheme 1. Hypothetical Pathway for the Formation of **2** from **1**



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 rotation was measured on a Perkin-Elmer 141 polarimeter at 579 nm in a 10-cm cell at 20 °C.

The GC−MS analyses were carried out in the EI mode (70 eV) on a Hewlett-Packard HP 5972A mass spectrometer coupled to a HP 5890 series II gas chromatograph fitted with a cold "on-column" injector and using a 30 m x 0.25 mm polydimethylsiloxane (0.25-μm film thickness) HP-5MS column. Other analytical conditions were as follows: carrier gas: He at a constant flow of 1.3 mL/min; temperature program, injection at 50 °C and programmed to 140 °C at 20 °C/min, then from 140 °C to 280 °C at 5 °C/min. Under such conditions, adaline (**1**) and adalinine (**2**) were eluted after 14.2 and 15.5 min, respectively.

Insect Material. The stock cultures of *A. bipunctata* and *A. decempunctata* were kept in an environmental chamber at 16 °C and a 16 L:8 D photoperiod. Groups of 40 ladybirds (sex ratio 1:1) were kept in containers (6 × 17.5 × 11.5 cm) on damp tissue and a piece of corrugated filter paper (10 × 15 cm). The beetles were fed a mixture of *Acyrthosiphon pisum* (Harris) and *Aphis fabae* Scopoli three times a week. All ladybirds used for the extraction of alkaloids were approximately 1 month old.

Extraction and Isolation. A total of 545 adult specimens of *A. bipunctata* was ground and exhaustively extracted with $CHCl_3$ affording 640 mg of an orange oil which was further fractionated. The GC−MS analysis of this material showed the presence, besides adaline (**1**), of a minor compound (about 10% of **1**) having a mol wt of 225 Da. Two column chromatography separations of the extract on neutral alumina (eluent: $CHCl_3$−hexane 1:1, to $CHCl_3$) afforded 3.1 mg of adalinine (**2**), 95% pure by GC and TLC (Si gel, eluent: hexane−EtOAc, 7:3, visualized with 2% phosphomolybdic acid in EtOH). Compound **2** exhibited: oil; $[\alpha]^{20}_{579}$ −26° (*c* 0.13, CH_2Cl_2); IR (dry film) ν_{max} 3380, 3200, 2930, 2860, 1708, 1659, 1453, 1403, 1363 cm^{-1}; ^1H NMR ($CDCl_3$, 600 MHz) δ 6.5 (1H, br s, NH), 2.63 (2H, AB, J = 17.7 Hz, H_2-1′), 2.30 (2H, m, H_2-5), 2.10 (3H, s, Me-3′), 1.78 and 1.70 (2H, m, H_2-4), 1.73 and 1.60 (2H, m, H_2-7), 1.57 and 1.54 (2H, m, H_2-3), 1.28 and 1.22 (2H, m, H_2-10), 1.22 and 1.20 (2H, m, H_2-9), 1.22 and 1.10 (2H, m, H_2-8), 0.85 (3H, t, J = 7 Hz, Me-11); ^{13}C NMR ($CDCl_3$, 150.87 MHz) δ 207.2 (s, C-2′), 171.4 (s, C-6), 56.1 (s, C-2), 51.3 (t, C-1′), 39.2 (t, C-7), 31.94 (t, C-9), 31.9 (t, C-3), 31.4 (q, C-3′), 31.3 (t, C-5), 23.9 (t, C-8), 22.5 (t, C-10), 17.2 (t, C-4), 13.9 (q, C-11); EIMS (70 eV) m/z $[M]^+$ 225 (2), 168 (20), 154 (73), 112 (55); HREIMS m/z 225.1728; calcd for $C_{13}H_{23}NO_2$, 225.1729.

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