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

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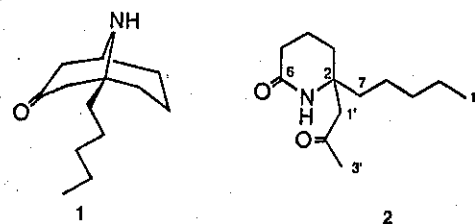
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Adalinine (2), a new piperidine alkaloid, has been isolated from the European two-spotted ladybird beetle *Adalia bipunctata*, and its structure has been determined by spectroscopic methods. This alkaloid was also shown to be present in all the life 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.<sup>1</sup> 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.<sup>2,3</sup> Alkaloids belonging to many different structural families such as pyrrolidines, piperidines, homotropanes, 2-methylperhydro-9b-azaphenalenones, azamacrolides, "dimeric" alkaloids, and long-chain and quinoline derivatives have been characterized from these insects.<sup>4-15</sup> The European two-spotted ladybird *Adalia bipunctata* L. has been found to contain the homotropane alkaloid adaline (1).<sup>6</sup> The deterrent and toxic properties of 1 have been investigated.<sup>16,17</sup> 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  $\text{CHCl}_3$ -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  $\text{C}_{13}\text{H}_{23}\text{NO}_2$ , thus requiring three degrees of unsaturation. The most intense fragment ions were observed at  $m/z$  168.1383 ( $\text{C}_{10}\text{H}_{18}\text{NO}$ ,  $[\text{M}^+ - \text{C}_3\text{H}_5\text{O}]$ ), 154.0873 ( $\text{C}_8\text{H}_{12}\text{NO}_2$ ,  $[\text{M}^+ - \text{C}_5\text{H}_{11}]$ ), and 112.076 ( $\text{C}_6\text{H}_{10}\text{NO}$ ,  $[\text{M}^+ - \text{C}_5\text{H}_{11} - \text{C}_2\text{H}_2\text{O}]$ ), suggesting the presence of a pentyl chain and a  $-\text{CH}_2\text{COCH}_3$  moiety. The  $^{13}\text{C}$ -NMR spectrum displayed 13 carbon atom signals (two  $\text{C}=\text{O}$ , one C, eight  $\text{CH}_2$ , and two  $\text{CH}_3$ ). One of the  $\text{C}=\text{O}$  groups was assigned to a ketone ( $^{13}\text{C}$ -NMR  $\delta$  207.2; IR,  $\nu_{\text{CO}}$  1709  $\text{cm}^{-1}$ ) and the other to an amide ( $^{13}\text{C}$ -NMR  $\delta$  171.4; IR,  $\nu_{\text{CO}}$  1659  $\text{cm}^{-1}$ ). The structure of adalinine was established by a 2D NMR study ( $^1\text{H}$ - $^1\text{H}$  COSY, HMQC, HMBC) and is depicted as 2. The complete assignments of the  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of 2 are

reported in the Experimental Section. Most noteworthy in the  $^1\text{H}$ - $^1\text{H}$  COSY spectrum were the correlations between  $\text{H}_2$ -5 at  $\delta$  2.30 and  $\text{H}_2$ -4 at  $\delta$  1.78 and 1.70, and between the latter and  $\text{H}_2$ -3 at  $\delta$  1.57 and 1.54, as well as the long-range correlation of the  $\text{H}_2$ -1' signal at  $\delta$  2.63 (AB system,  $J = 17.7$  Hz) with the  $\text{H}_3$ -3' singlet at  $\delta$  2.10. Moreover, this  $\text{H}_2$ -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  $^{13}\text{C}$ -NMR signals of 2 are also in good agreement with those reported in the literature for 2-piperidone derivatives.<sup>18</sup> The absolute configuration of 2 was not determined.



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.*,<sup>2</sup> 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  $\beta$ -amino ketone, it could undergo a retro-Mannich reaction to afford the imine 3. Addition of  $\text{H}_2\text{O}$  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  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra were recorded in  $\text{CDCl}_3$  at 600

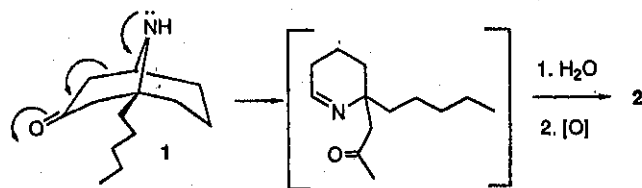
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**Scheme 1.** Hypothetical Pathway for the Formation of 2 from 1

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- $\mu$ m 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 x 17.5 x 11.5 cm) on damp tissue and a piece of corrugated filter paper (10 x 15 cm). The beetles were fed a mixture of *Acyrtosiphon 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<sub>3</sub> 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<sub>3</sub>-hexane 1:1, to CHCl<sub>3</sub>) 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;  $[\alpha]_{579}^{20}$  -26° (c 0.13, CH<sub>2</sub>Cl<sub>2</sub>); IR (dry film)  $\nu_{\max}$  3380, 3200, 2930, 2860, 1708, 1659, 1453, 1403, 1363 cm<sup>-1</sup>;

<sup>1</sup>H NMR (CDCl<sub>3</sub>, 600 MHz)  $\delta$  6.5 (1H, br s, NH), 2.63 (2H, AB,  $J$  = 17.7 Hz, H<sub>2</sub>-1'), 2.30 (2H, m, H<sub>2</sub>-5), 2.10 (3H, s, Me-3'), 1.78 and 1.70 (2H, m, H<sub>2</sub>-4), 1.73 and 1.60 (2H, m, H<sub>2</sub>-7), 1.57 and 1.54 (2H, m, H<sub>2</sub>-3), 1.28 and 1.22 (2H, m, H<sub>2</sub>-10), 1.22 and 1.20 (2H, m, H<sub>2</sub>-9), 1.22 and 1.10 (2H, m, H<sub>2</sub>-8), 0.85 (3H, t,  $J$  = 7 Hz, Me-11); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 150.87 MHz)  $\delta$  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]<sup>+</sup> 225 (2), 168 (20), 154 (73), 112 (55); HREIMS  $m/z$  225.1728; calcd for C<sub>13</sub>H<sub>23</sub>NO<sub>2</sub>, 225.1729.

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## References and Notes

- (1) Happ, G. M.; Eisner, T. *Science* 1961, 134, 329-331.
- (2) Pasteels, J. M.; Deroe, C.; Tursch, B.; Braekman, J. C.; Daloze, D.; Hootele, C. *J. Insect. Physiol.* 1973, 19, 1771-1784.
- (3) Daloze, D.; Braekman, J. C.; Pasteels, J. M. *Chemoecology*, in press.
- (4) Tursch, B.; Daloze, D.; Braekman, J. C.; Hootele, C.; Pasteels, J. M. *Tetrahedron* 1975, 31, 1541-1543.
- (5) Ayer, W. A.; Bennett, M. J.; Browne, L. M.; Purdham, J. T. *Can. J. Chem.* 1976, 54, 1807-1813.
- (6) Tursch, B.; Braekman, J. C.; Daloze, D.; Hootele, C.; Losman, D.; Karlsson, R.; Pasteels, J. M. *Tetrahedron Lett.* 1973, 201-202.
- (7) Eisner, T.; Goetz, M.; Aneshansley, D.; Ferstandig-Arnold, G.; Meinwald, J. *Experientia* 1986, 42, 204-207.
- (8) Braconnier, M. F.; Braekman, J. C.; Daloze, D.; Pasteels, J. M. *Experientia* 1985, 41, 519-520.
- (9) Timmermans, M.; Braekman, J. C.; Daloze, D.; Pasteels, J. M.; Merlin, J.; Declercq, J. P. *Tetrahedron Lett.* 1992, 33, 1281-1284.
- (10) McCormick, K. D.; Attygalle, A. B.; Xu, S. C.; Svatos, A.; Meinwald, J.; Houck, M. A.; Blankespoor, C. L.; Eisner, T. *Tetrahedron* 1994, 50, 2365-2372.
- (11) Attygalle, A. B.; Xu, S. C.; McCormick, K. D.; Meinwald, J.; Blankespoor, C. L.; Eisner, T. *Tetrahedron* 1993, 49, 9333-9342.
- (12) Brown, W. V.; Moore, B. P. *Aust. J. Chem.* 1982, 35, 1255-1261.
- (13) Attygalle, A. B.; McCormick, K. D.; Blankespoor, C. L.; Eisner, T.; Meinwald, J. *Proc. Natl. Acad. Sci. U.S.A.* 1993, 90, 5204-5208.
- (14) Proksch, P.; Witte, L.; Wray, V.; Hartmann, T. *Entomol. Gener.* 1993, 18, 1-7.
- (15) Wang S. F.; Braekman, J. C.; Daloze, D.; Pasteels, J. M.; Soetens P.; Handjieva N.V.; Kalushkov, P. *Experientia*, in press.
- (16) Marples, N. M.; Brakefield, P. M.; Cowie, R. *J. Ecol. Entomol.* 1989, 14, 79-84.
- (17) de Jong, P. W.; Holloway, G. J.; Brakefield, P. M.; de Vos, H. *Chemoecology* 1991, 2, 15-19.
- (18) See, for example, Amat, M.; Llor, N.; Bosch, J. *Tetrahedron Lett.* 1994, 35, 2223-2226.

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