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Biosynthetic studies on adaline and adalinine, two alkaloids from ladybird beetles (Coleoptera: Coccinellidae)

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Abstract—The biosynthesis of the homotropane alkaloid (−)-adaline in the coccinellid beetle *Adalia bipunctata* has been studied by incorporation experiments with [1-^{14}C] and [2-^{14}C]acetate. A degradation scheme was developed which selectively afforded benzoic acid containing the carbonyl carbon atom of adaline. The labelling pattern thus obtained indicated that the alkaloid is biosynthesized via a polyacetate pathway. Moreover, feeding *A. bipunctata* adults with (−)-[10,10,11,11,12,12,13,13,14,14,14-$^{2}H_{11}$]adaline·HCl afforded (−)-[7,7,8,8,9,9,10,10,11,11,11-$^{2}H_{11}$]adalinine, demonstrating a biogenetic relationship between the two alkaloids. Possible mechanisms for this conversion are discussed. Finally, the synthetic scheme devised to obtain (±)-6-acetonyl-2-pentyl-1-piperideine did not yield the target compound, but afforded instead (±)-adaline in moderate yields. This unexpected result gives support to the hypothesis that this piperideine could be a key intermediate in the biosynthesis of adaline.

1. Introduction

When attacked, many coccinellid beetles emit droplets of hemolymph from the tibio-femoral joints of their legs (reflex bleeding). It has been demonstrated that the deterrency exhibited by many species of coccinellids towards potential predators results from the presence of alkaloids in that fluid.[1,2] Over 50 alkaloids have been characterised from ladybirds up to now, including acyclic amines, piperidines, pyrrolidines, perhydroazaphenalenes, 'dimeric' alkaloids, azamacrolides and homotropanes.[1,2]

Although many synthetic[2] and biological[1] studies of these alkaloids have been performed, the biosynthetic pathways through which they are formed have been the subject of only a handful of investigations.[1] In particular, incorporation experiments with ^{2}H-labelled oleic acid and ^{2}H- and ^{13}C, ^{15}N-labelled L-serine have shown that (+)-epilachnene [(+)-**1**], a member of the azamacrolide group of alkaloids produced by the pupa of the Mexican bean beetle *Epilachna varivestis*, is biosynthesized from oleic acid and L-serine.[3,4] In *Coccinella septempunctata*, the perhydroazaphenalene alkaloid coccinelline (**2**) was shown to be labelled after the beetles had been fed with [1-^{14}C] and [2-^{14}C]acetate. Results of the degradation experiments on the labelled coccinelline were in agreement with a polyacetate origin for that compound.[5] The same observation was made[1] for the homotropane alkaloid (−)-adaline [(−)-(**3**)] in *Adalia bipunctata*, but no degradation experiments were performed to locate the label in the molecule. In *A. bipunctata* and *A. decempunctata*, (−)-adaline is accompanied by ca. 10% of the piperidine alkaloid (−)-adalinine [(−)-(**4**)].[6] Although **3** and **4** are based on different carbon skeletons, a biogenetic hypothesis has been proposed to explain their simultaneous occurrence in *Adalia* beetles.[6a] The aims of the present work were: (i) to bring further proof of the polyacetate origin of (−)-adaline, (ii) to investigate the possible biogenetic relationship between (−)-adaline and (−)-adalinine, and (iii) to develop a synthesis of (±)-6-acetonyl-2-pentyl-1-piperideine [(±)-**5**], which had been tentatively identified in *Calvia* coccinellids,[7] and which is a likely biosynthetic precursor of these two alkaloids.

2. Results and discussion

2.1. Biosynthesis of (−)-adaline [(−)-3]

As shown in Scheme 1, (−)-adaline could be biosynthetically derived from the linear combination of seven acetate units affording a linear C_{14} chain such as **6**. Decarboxylation of the latter followed by reductive amination and intramolecular cyclisation would afford the key intermediate **5**. This imine could then undergo an intramolecular Mannich reaction to furnish (−)-adaline [(−)-**3**]. According to this

Keywords: alkaloids; biogenesis; retro-Mannich; insects; labelling; electrochemistry.

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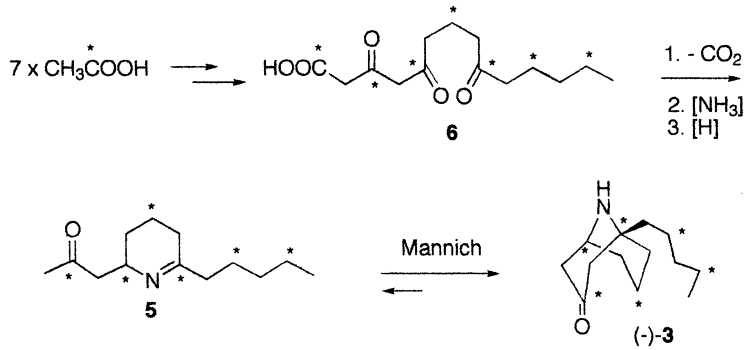


Scheme 1. Biosynthetic hypothesis for the formation of (−)-adaline [(−)-**3**].

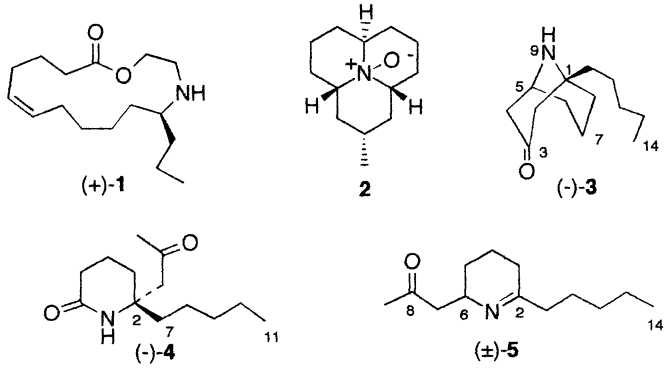
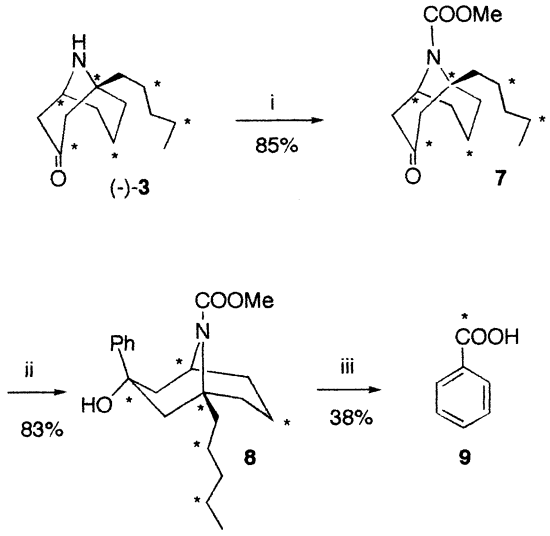


Figure 1. Structures of (+)-epilachnene [(+)-(**1**)], coccinelline (**2**), (−)-adaline [(−)-**3**], (−)-adalinine [(−)-**4**], and (±)-6-acetonyl-2-pentyl-1-piperideine [(±)-**5**].

hypothesis, incorporation of [1-^{14}C]acetate should yield (−)-adaline labelled on the odd numbered carbon atoms (as shown in Scheme 1), whereas incorporation of [2-^{14}C]acetate should afford (−)-adaline labelled on the even numbered carbon atoms (see adaline numbering in Fig. 1).

Exploratory incorporation experiments were performed by feeding *A. bipunctata* with [1-^{13}C]acetate, but the ^{13}C NMR spectrum of the sample of (−)-adaline thus obtained did not show any isotopic enrichment of the expected carbon atom signals. This is presumably due to the low incorporation rate frequently observed in this type of experiments with insects.[5,8] Thus, the incorporation experiments had to be performed with ^{14}C labelled precursors. The latter required a supply of 'cold' adaline to develop a degradation scheme for this alkaloid, and also to serve as carrier for the labelled samples obtained from the feeding experiments. This was achieved by preparing (−)-adaline by the method of Hill and Renbaum.[9] Using this synthetic material, we have developed the degradation scheme shown in Scheme 2. Reaction of the secondary amine of (−)-**3** with methyl chloroformate led to carbamate **7** in an 85% yield. Next, as the 'fork-head' carbonyl group of bicyclo[3.3.1]nonan-3-ones was known to be inert towards organomagnesium and organolithium reagents,[10] or towards Bayer–Villiger oxidation,[11] we treated **7** with phenyllithium in the presence of Ce(III) chloride. Under these conditions we obtained, as expected,[12] the corresponding 3α-alcohol **8** having a chair-boat conformation, in an 83% yield. Finally, oxidation of **8** with $KMnO_4$ furnished 38% of benzoic acid (**9**), containing the C-3 carbonyl carbon atom of (−)-adaline.

Next, 106 *A. bipunctata* beetles were fed with an aqueous solution of sodium [1-^{14}C]acetate (250 μCi, 57 mCi/mmol), whereas 103 beetles were given [2-^{14}C]acetate (250 μCi, 55 mCi/mmol). After 3 weeks, the beetles from each



Scheme 2. Degradation scheme of radioactive (−)-adaline [(−)-**3**]. *Reagents*: (i) ClCOOMe, K_2CO_3, acetone, reflux; (ii) PhLi, $CeCl_3$, THF, −78°C; (iii) $KMnO_4$, Na_2CO_3, H_2O, reflux.

Table 1. Results of the labelling experiments in *A. bipunctata* with [1-^{14}C]- and [2-^{14}C]acetate

Feeding experiment	Precursor	No. of beetles fed	Isolated amount of **3** (mg)	Total activity of **3** (mCi)	Specific activity of **3** (mCi/mmol)	Incorporation rate (%)
1	Sodium [2-^{14}C] acetate (250 μCi, 55 mCi/mmol)	103	1.00	3.26×10^{-5}	6.82×10^{-3}	0.0124
2	Sodium [1-^{14}C] acetate (250 μCi, 57 mCi/mmol)	106	8.31	2.73×10^{-5}	6.86×10^{-4}	0.0012

Table 2. Measured and expected distribution of label within degradation products of (−)-adaline [(−)-**3**] after feeding with sodium [1-^{14}C]acetate (experiments 1a and 1b) and with sodium [2-^{14}C]acetate (experiment 2) (RSA: relative specific activity, adaline=100%)

Experiment No.	Product	Amount (mg)	Total activity (mCi)	Specific activity (mCi/mmol)	C atoms of **3**	RSA (%)	Expected activity (%)
1a	**3**	20.93	1.48×10^{-5}	1.47×10^{-4}	All	100	100
	7	21.46	1.18×10^{-5}	1.47×10^{-4}	All	100	100
	8	22.47	9.60×10^{-6}	1.47×10^{-4}	All	100	100
	9	2.98	5.65×10^{-7}	2.31×10^{-5}	C-3	15.7	16.7
1b	**3**	17.59	1.26×10^{-5}	1.50×10^{-4}	All	100	100
	7	19.90	1.12×10^{-5}	1.50×10^{-4}	All	100	100
	8	21.19	9.20×10^{-6}	1.50×10^{-4}	All	100	100
	9	3.06	6.05×10^{-7}	2.41×10^{-5}	C-3	16.1	16.7
2	**3**	22.01	3.21×10^{-5}	3.05×10^{-4}	All	100	100
	7	22.78	2.60×10^{-5}	3.05×10^{-4}	All	100	100
	8	23.25	2.07×10^{-5}	3.06×10^{-4}	All	100	100
	9	3.05	3.00×10^{-8}	1.20×10^{-6}	C-3	0.4	0

incorporation experiment were killed, (−)-adaline was isolated and its radioactivity measured. The results summarized in Table 1 show that the samples of (−)-adaline from both experiments were labelled.

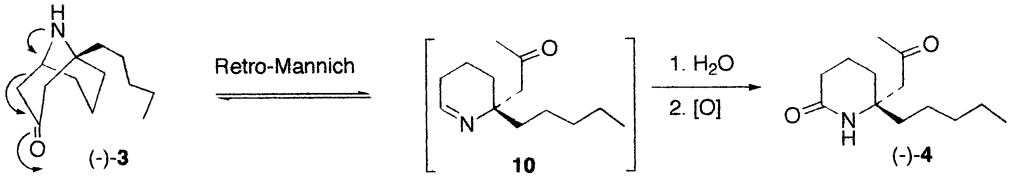
Before applying the degradation procedure, unlabelled (−)-adaline was added as a carrier to each radioactive sample. The quantities obtained from the incorporation experiments (8.3 and 1.0 mg, respectively) allowed us to duplicate the degradation scheme for the sample labelled with [1-^{14}C]acetate, but not for the sample labelled with [2-^{14}C]acetate. The specific activities, and relative specific activities (RSA), measured and expected for compounds **7**, **8** and **9** from the two labelling experiments are reported in Table 2.

If our biosynthetic hypothesis is correct, the benzoic acid obtained from the degradation of (−)-adaline labelled with [1-^{14}C]acetate should contain 16.7% of the radioactivity of the starting material, whereas it should be inactive for the [2-^{14}C]acetate experiment (Scheme 1). Comparison of the measured distribution of radioactivity of compounds **3** and **7**–**9** to that predicted on the basis of our hypothesis (Table 2) fully supports a polyacetate origin of (−)-adaline (Scheme 1).

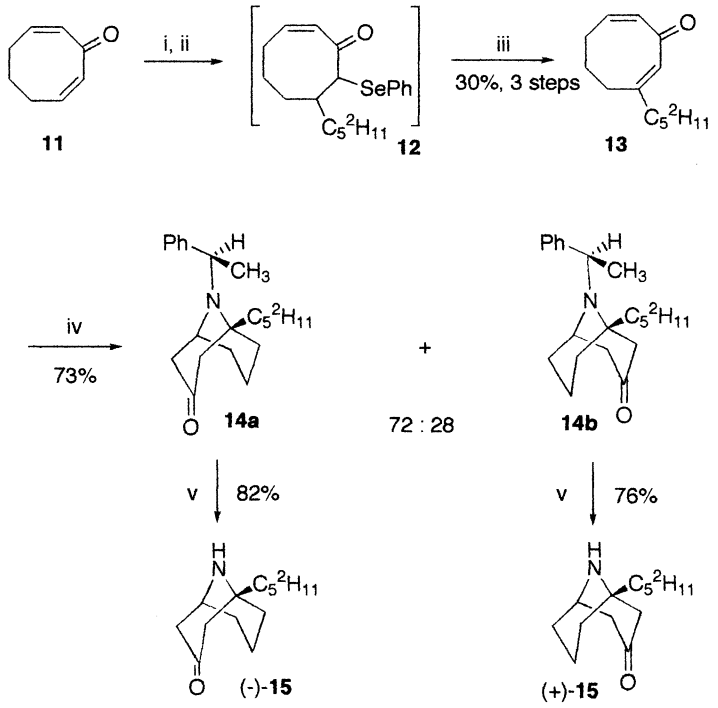
2.2. Biogenetic relationship between (−)-adaline [(−)-3] and (−)-adalinine [(−)-4]

As proposed earlier,[6a] (−)-adaline, being a β-aminoketone, could undergo a retro-Mannich reaction leading to the imine **10** which, after addition of water followed by oxidation, would afford (−)-adalinine (Scheme 3).

We have now tested this hypothesis by feeding *A. bipunctata* beetles with (−)-[10,10,11,11,12,12,13,13,14,14,14-^{2}H$_{11}$]adaline (named hereafter '[^{2}H$_{11}$]adaline'), which was also synthesized by the method of Hill and Renbaum[9] (Scheme 4). Cuprous iodide-catalyzed conjugate addition of C$_5$^{2}H$_{11}$MgBr to 2,7-cyclooctadienone **11**, prepared in four steps from cyclooctanone,[13] followed by trapping the magnesium enolate with phenylselenenyl bromide gave adduct **12**. This was not isolated but directly oxidized to the corresponding selenoxide that furnished the substituted dienone **13** after elimination. The overall yield of this one pot, three steps sequence was 30%, which was significantly lower than that employing C$_5$H$_{11}$MgBr (61%). Addition of (*R*)-(+)-α-methylbenzylamine to **13** gave a mixture of the diastereomeric adducts [**14a**] and [**14b**] in a 73% yield and a 72:28 ratio. This mixture was separated by silica gel chromatography on a Lobar® column.



Scheme 3. Possible biosynthetic relationship between (−)-adaline [(−)-**3**] and (−)-adalinine [(−)-**4**].

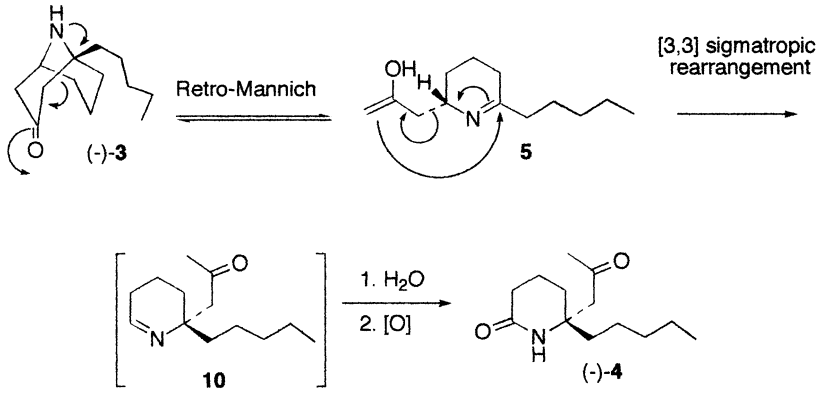


Scheme 4. Synthesis of (−)- and (+)-[$^2H_{11}$]adaline [(−)- and (+)-(**15**)]. *Reagents*: (i) $C_5{}^2H_{11}MgBr$, Et_2O, CuI; (ii) PhSeBr, THF; (iii) H_2O_2, H_2O, pyridine, Δ; (iv) (+)-$PhCH(CH_3)NH_2$, MeOH; (v) H_2, Pd/C, H^+, MeOH.

Hydrogenolysis of the chiral appendage of **14a** gave (−)-[$^2H_{11}$]adaline [(−)-**15**] in an 82% yield, while hydrogenolysis of **14b** afforded its enantiomer (+)-**15** in a 76% yield.

Four adults of *A. bipunctata* were fed with an artificial diet[14] containing 64.1 mg of (−)-**15**·HCl in 5 mL. After 20 days, the four beetles were dipped in acetone and the resulting extract was analysed by GC–MS. Selected-ion retrieval chromatograms, obtained by searching for the molecular ion and some characteristic fragment ions of both deuterium labelled and unlabelled adaline and adalinine, showed that deuteriated adaline and adalinine were both present in these beetles. As expected,[3] the peaks corresponding to the deuteriated products eluted in GC a few seconds before those of unlabelled compounds. However, due to the small difference of retention times between [$^2H_{11}$]adalinine and adalinine there was a partial superimposition of the mass spectra of the two compounds. By substraction of the mass spectrum of adalinine from that of the composite spectrum of both compounds, we obtained the mass spectrum of [$^2H_{11}$]adalinine itself. From this spectrum, it is evident that the eleven deuterium atoms are located, as expected, in the pentyl chain: a peak at *m/z* 179 can be attributed to the loss of 57 mass units from the molecular ion, analogous to the loss of the acetonyl moiety observed in the spectrum of the unlabelled alkaloid.[6a] Assuming that the relative intensities of the ions of unlabelled and [$^2H_{11}$]-labelled alkaloids are similar, integration of the peak areas of selected-ion retrieval chromatograms permitted us to calculate the ratio of labelled to unlabelled adaline and adalinine. This ratio was about 1:6.5 for adaline, and 1:14 for adalinine.

On the other hand, selected-ion retrieval chromatograms from the hemolymph of the ladybirds fed with (+)-[$^2H_{11}$]adaline·HCl [(+)-**15**·HCl], by the same method as above, showed the presence of labelled and unlabelled adaline (in a 1:17 ratio) and of unlabelled adalinine, but



Scheme 5. Possible alternative pathway from (−)-adaline [(−)-**3**] to (−)-adalinine [(−)-**4**].

no deuteriated adalinine could be detected. This result indicates that the transformation of (−)-adaline into (−)-adalinine is stereospecific, and that the enzyme(s) catalysing this transformation recognize(s) only the (−) enantiomer of adaline.

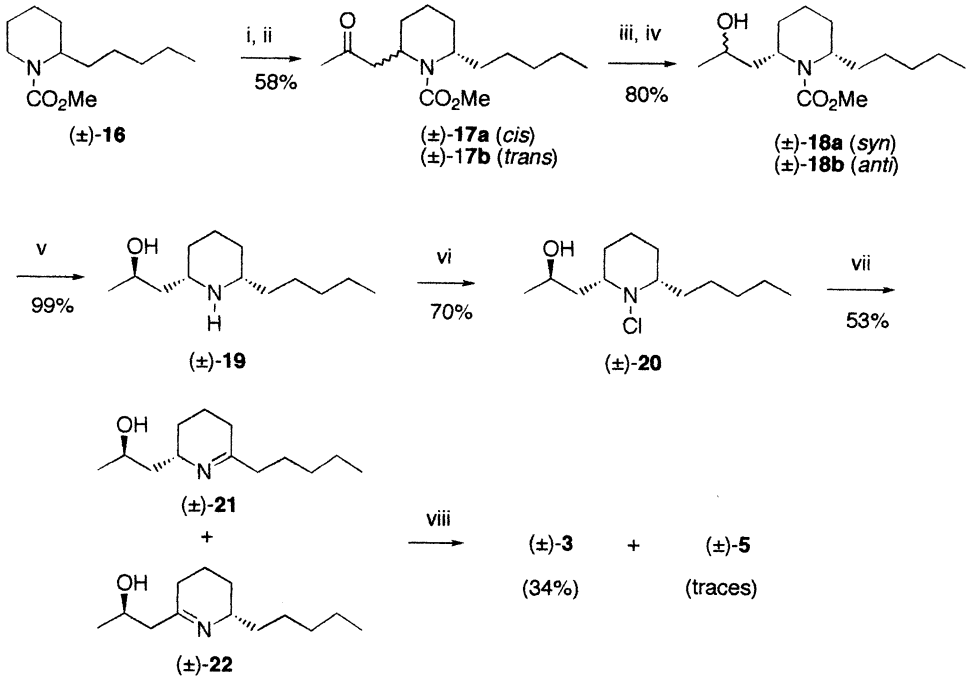
The results presented here demonstrate a biogenetic relationship between (−)-adaline and (−)-adalinine, which was suggested by their absolute configuration. However, even if these results support the mechanism presented in Scheme 3, they do not unambiguously prove it. Indeed, 6-acetonyl-2-pentyl-1-piperideine (**5**), which could also arise from adaline by a retro-Mannich reaction, could undergo a [3,3] sigmatropic rearrangement,[15] affording adalinine after hydration and oxidation (Scheme 5). On the basis of the incorporation experiments presented here, we cannot decide if (−)-adalinine is exclusively formed from (−)-adaline through the pathway of Scheme 3, through that of Scheme 5, or through both. Alternatively, (−)-adalinine could also be formed, at least in part, directly from **5**, which would then be a key intermediate in the biosynthesis of the two alkaloids.

2.3. Attempted synthesis of (±)-6-acetonyl-2-pentyl-1-piperideine [(±)-5]

Since compound **5** could play a pivotal role in the biosynthesis of (−)-adaline and (−)-adalinine, we decided to undertake its total synthesis. This would allow us not only to prepare labelled derivatives of **5** for further incorporation experiments, but also to check its tentative identification in *Calvia 14-guttata* which was made by GC–MS only.[7]

Our synthetic strategy (Scheme 6) is based on the succesive introduction of a pentyl chain at C-2 and of an acetonyl substituent at C-6 of *N*-methoxycarbonylpiperidine using an anodic oxidation-nucleophilic substitution sequence,[16] followed by regioselective introduction of the imine functionality through the method used by Tawara et al.[17] for the synthesis of 6-acetonyl-2-methyl-1-piperideine, the methyl analogue of (±)-**5**.

Thus, *N*-methoxycarbonyl-2-pentylpiperidine[7] [(±)-**16**] was submitted to an anodic oxidation followed by a nucleophilic substitution with 2-trimethylsilyloxypropene to afford a 96:4 mixture of the *cis*- and *trans*-*N*-methoxycarbonyl-2-acetonyl-6-pentylpiperidines [(±)-**17a** and (±)-**17b**]. These two isomers, obtained in a 58% yield from (±)-**16**, could not be separated by column chromatography. The mixture was reduced by $LiAl(O^tBu)_3H$ to afford, as expected,[18] a 91:9 mixture of diastereomeric *syn* and *anti* alcohols (±)-**18a** and (±)-**18b**, which were separated by chromatography on silica gel. The minor *trans* diastereoisomers coming from the reduction of (±)-**17b** were removed during this purification step. Next, treatment of (±)-**18a** with KOH in refluxing EtOH furnished the amino alcohol (±)-**19** in a 99% yield. The introduction of the imine functionality proved troublesome. Indeed, when (±)-**19** was treated under the conditions of Tawahara et al.[17] (TMSCl, then NCS/diethyl ether, and finally NaOH in refluxing EtOH) we obtained only degradation products. Thus, we decided to check the three steps of this procedure and to isolate the intermediate chloroamine [(±)-**20**]. Selective protection of the secondary hydroxyl group of (±)-**19** with TMSCl, followed by NCS treatment in diethyl ether for 3 h yielded the expected chloroamine [(±)-**20**], which was isolated in a 70% yield and fully characterized. Then, several conditions for elimination of HCl from (±)-**20** were tried. All our attempts employing KOH/EtOH at room temperature or at reflux led to poor yields of imino alcohols and afforded complex reaction



Scheme 6. Attempted synthesis of (±)-6-acetonyl-2-pentyl-1-piperideine [(±)-**5**]. *Reagents*: (i) MeOH, Et_4NOTs, 4*F*/mol; (ii) 2-trimethylsilyloxypropene, CH_2Cl_2, −78°C; (iii) $LiAl(O^tBu)_3H$, THF; (iv) silica gel chromatography; (v) KOH/EtOH, reflux; (vi) 1. TMSCl, NEt_3, C_6H_6; 2. NCS, Et_2O, (vii) KO_2, 18-crown-6, Et_2O; (viii) PCC, CH_2Cl_2.

mixtures. Is it to be noted that the same reaction performed on the chloroamine derived from solenopsin B[19] afforded the expected mixture of regioisomeric imines [2-methyl-6-tridecyl-1-piperideine (60%) and 6-methyl-2-tridecyl-1-piperideine (15%)]. The problem could be solved by using a combination of KO_2 and 18-crown-6[20] in Et_2O: under these conditions, we obtained an approximately 35:65 ratio of the Δ^1-imino alcohol (±)-**21** and its Δ^6 isomer (±)-**22**. Treatment of the mixture of these two imino alcohols with Jones reagent, as described by Tawahara et al.[17] for the methyl analogue of (±)-**21**, gave mostly decomposition products. GC–MS analyses of this mixture showed the absence of (±)-**5**, but allowed us to detect the presence of adaline, which was one of the major components. Thus, we turned to less drastic oxidation reagents, namely PCC, PCC buffered with AcONa[21], and finally, IBX[22a] which is reported to permit the oxidation of alcohols into ketones in the presence of amino groups.[22b] In all cases, we obtained one major compound (yield: 34% with PCC), identified as adaline by TLC, GC–MS and ^{1}H NMR. The GC–MS analyses of the oxidation mixtures showed that adaline (t_R=23.30 min) was always accompanied by small amounts (from 1 to 5% yield) of a compound eluting earlier (t_R=21.50 min), and which displayed a mass spectrum nearly identical to that of adaline. The only significant difference between the mass spectra of the two compounds was the relative abundance of the *m/z* 110 fragment which was always higher in the mass spectrum of adaline. These data strongly suggest that the minor compound of the oxidation mixtures is our synthetic target (±)-**5** which, once formed, quickly cyclizes into (±)-adaline through a probably reversible intramolecular Mannich reaction. Several instances of similar Mannich reactions leading to tropane[23] or homotropane[24–26] alkaloids are already reported in the literature.

Finally, a GC–MS co-injection of (±)-**5** (minor compound from the oxidation of (±)-**21**), of authentic (−)-adaline, and of the minor compound of *Calvia 14-guttata* showed that the latter is identical with adaline. Consequently, the previous identification[7] of **5** in *Calvia* beetles should be corrected.

3. Conclusion

The results presented here point to a polyacetate origin for (−)-adaline in *Adalia* sp. Moreover, they also bring experimental support to the hypothesis that the minor piperidine alkaloid (−)-adalinine is formed, at least in part, from the major homotropane (−)-adaline. The exact mechanism of this conversion is not firmly established yet, although it should be emphasized that, whatever pathway is followed, a retro-Mannich reaction must take place.

On the other hand, all our efforts to obtain (±)-**5** in any significant yield failed. It was observed that (±)-adaline was always produced as the major compound during the oxidation of imino alcohol (±)-**21**. This result is interesting on several counts: not only does it demonstrate that (±)-**5** is an unstable compound that spontaneously undergoes a Mannich reaction to afford its bicyclic isomer adaline, but it also reinforces the hypothesis that **5** (or a closely related compound) could be a key biosynthetic intermediate on the way to (−)-adaline and (−)-adalinine. Finally, the results obtained during the attempted synthesis of (±)-**5** permitted us to demonstrate that the minor alkaloid of *Calvia 14-guttata* is not **5**, as tentatively proposed earlier[7], but adaline.

4. Experimental

4.1. General

EI-MS, CI-MS and EI-HRMS were performed with a Fisons VG Micromass Autospec instrument (70 eV) (for the CI-MS analyses, the reagent gas used was ammonia) and GC/EI-MS analyses with a Fisons VG Micromass Autospec apparatus coupled to a gas chromatograph equipped with a 25 m×0.25 mm CP Sil 5 capillary column (Chrompack), at 30°C (2 min), programmed to 100°C at 5°C min^{-1}, then to 270°C at 10°C min^{-1} (hold 5 min); the carrier gas was helium. In all cases, peak intensities are expressed as % relative to the base peak. The ^{1}H NMR spectra were recorded at 250 MHz with a Bruker WM 250, at 300 MHz with a Bruker Avance TM 300, or at 600 MHz using a Varian Unity 600 instruments and are reported in ppm from internal TMS on the δ scale. Data are reported as follows: chemical shift [multiplicity (s: singlet, bs: broad singlet, d: doublet, bd: broad doublet, t: triplet, q: quartet, dd: double doublet, dt: double triplet, quint: quintet, m: multiplet), coupling constants in Hertz, integration]. The ^{13}C NMR spectra were recorded at 75.4 MHz with a Bruker Avance TM 300 instrument. The IR spectra were recorded with a Bruker IFS 25 instrument as a film on a NaCl disk or as KBr pellets, and the UV/Vis spectra with a Philips PU 8700 spectrophotometer in 1 cm cells. GLC analyses were performed with a Varian 3400 apparatus equipped with a capillary column (30 m×0.32 mm fused-silica column coated with OV1701); the carrier gas was N_2. Thin layer chromatography analyses (TLC) were performed with 0.25 mm Polygram silica gel SILG/UV_{254} precoated plates (Macherey–Nagel). Column chromatographies were performed on silica gel (MN Kieselgel 60 0.04–0.063 mm) using the flash technique or on a Lobar® prepacked column (size B, 310-25, Lichroprep® Si 60, 40–63 μm). Melting points are uncorrected. Optical rotations were recorded at 589 nm (sodium D line) in a 1 dm cell at room temperature on a Perkin–Elmer 141 polarimeter. Radioactive compounds were assayed in a Packard Tri-Carb 1600 TR liquid scintillation analyser with methanol as solvent and Packard Insta-Gel® Plus liquid scintillation cocktail (10 mL). Triplicate samples of each compound were counted under comparable conditions of quenching.

4.1.1. 9-Methoxycarbonyl-1-(*R*)-pentyl-9-azabicyclo[3.3.1]-nonan-3-one (7). To a solution of (−)-adaline [(−)-**3**] (0.097 g, 0.464 mmol) in acetone (5 mL) were added methyl chloroformate (0.228 g, 2.42 mmol) and K_2CO_3 (0.440 g, 3.18 mmol). The mixture was refluxed overnight, then cooled to room temperature, filtered and concentrated under reduce pressure to give a residue which was purified by flash chromatography on a silica gel column (hexane/AcOEt 8:2) to afford **7** (0.106 g, 0.397 mmol, 85%) as a yellow oil.

7. EIMS *m/z* 267 (51, $M^{+\cdot}$), 252 (10), 238 (10), 236 (11), 224 (54), 211 (100), 210 (48), 208 (40), 196 (11), 168 (48), 154 (22), 152 (50), 113 (21), 112 (27), 96 (10), 83 (22), 71 (46), 70 (41), 69 (27), 68 (27), 57 (75), 56 (18), 55 (44), IR (film) 2937, 1714, 1680, 1446, 1379, 1346, 1304, 1253, 1178, 1086 cm^{-1}, ^1H NMR ($CDCl_3$) 0.89 (t, 6.6, 3H); 1.2–1.9 (m, 14H); 2.16 (AB, 7.7, 2H); 2.45 (ABX, 16.4, 6.9, 7.3, 2H); 3.7 (s, 3H); 4.87 (bs, 2H).

4.1.2. 9-Methoxycarbonyl-1-(*R*)-pentyl-3-phenyl-9-azabicyclo[3.3.1]nonan-3-ol (8). Anhydrous cerium(III) chloride (0.783 g, 3.17 mmol) was heated in vacuo at 150°C for 6 h and cooled under a N_2 atmosphere. Dry THF (15 mL) was then added. The resulting suspension was stirred overnight at room temperature and cooled at −78°C. A solution of phenyllithium in cyclohexane/diethyl ether 7:3 (1.38 M, 1.4 mL, 1.93 mmol) was added to the cooled suspension and the mixture was stirred at −78°C for 1 h. A solution of **7** (0.051 g, 0.191 mmol) in dry THF (1.1 mL) was then added to the resulting orange mixture and stirred for 7 h at −78°C. The reaction was quenched with a 10% HCl aqueous solution (10 mL) and extracted with three 10 mL portions of CH_2Cl_2. The organic layers were combined, washed with brine, dried ($MgSO_4$) and evaporated to dryness under reduced pressure to give a residue which was purified by flash chromatography on a silica gel column (CH_2Cl_2/acetone 98:2) to afford **8** (0.055 g, 0.159 mmol, 83%) as a colourless oil.

8. EIMS *m/z* 345 (27, $M^{+\cdot}$), 328 (47), 327 (12), 302 (10), 289 (30), 286 (38), 284 (61), 274 (24), 271 (10), 268 (13), 256 (26), 246 (14), 240 (10), 224 (46), 211 (15), 169 (24), 154 (29), 142 (12), 128 (13), 115 (23), 105 (100), 91 (22), 77 (30), 67 (10), 59 (25), IR (film) 3439, 2937, 2853, 1680, 1437, 1387, 1312, 1195, 1095, 1036, 751, 701 cm^{-1}, ^1H NMR ($CDCl_3$) 600 MHz 0.82 (t, 6.6, 3H); 0.97 (m, 1H); 1.04 (m, 1H); 1.18 (m, 4H); 1.57 (m, 2H); 1.61 (m, 1H); 1.63 (bs, 1H); 1.68 (m, 1H); 1.74 (m, 2H); 1.82 (dd, 14.3, 3.3, 1H); 1.90 (d, 14.3, 1H); 1.92 (m, 1H); 2.41 (dd, 14.3, 2, 1H); 2.46 (m, 1H); 2.61 (ddd, 14.3, 9.1, 2, 1H); 3.54 (s, 3H); 4.79 (m, 1H); 7.23 (t, 7.2, 1H); 7.32 (t, 7.6, 2H); 7.47 (d, 8.2, 2H), UV (MeOH) λ_{max} (ϵ) 206 (5380), 268 nm (1340).

4.1.3. Benzoic acid (9). To a refluxed suspension of **8** (0.020 g, 0.057 mmol) in a 0.16 M Na_2CO_3 aqueous solution (3 mL) was slowly added $KMnO_4$ (0.118 g, 0.747 mmol). The resulting solution was refluxed overnight, cooled to room temperature and acidified with a 10% H_2SO_4 aqueous solution. The mixture was then heated to reflux for a further 30 min and cooled to room temperature. The MnO_2 was reduced by a small amount of sodium metabisulfite, causing the solution to turn colourless. The mixture was then extracted with ten 5 mL portion of CH_2Cl_2. The combined organic layers were filtered on a WA filter paper and evaporated in vacuo to give a white solid which was purified by flash chromatography on a silica gel column (CH_2Cl_2/acetone 98:2) to afford **9** (0.003 g, 0.021 mmol, 38%) as a white solid.

9. Mp 121–122°C, EIMS *m/z* 122 (92, $M^{+\cdot}$), 105 (100), 77 (71), 74 (10), 51 (42), IR (KBr) 3062, 1688, 1421, 1329, 1295, 1186, 1128, 1069, 1028, 935, 810, 710, 668 cm^{-1}, ^1H NMR ($CDCl_3$) 7.48 (m, 2H); 7.62 (m, 1H); 8.13 (m, 2H), UV (MeOH) λ_{max} (ϵ) 205 (5820), 227 (8270), 272 nm (1010).

4.1.4. 3-[$^2H_{11}$]Pentyl-2,7-cyclooctadienone (13). In a 25 mL three-necked flask equipped with a magnetic stirrer and a reflux condenser were placed magnesium turnings (0.032 g, 1.32 mmol) under an argon atmosphere. A solution of *n*-C_5[$^2H_{11}$]Br (0.214 g, 1.32 mmol) in anhydrous diethyl ether (1.4 mL) was then slowly added at a rate sufficient to maintain a gentle reflux (with slight warming to initiate the reaction). After all the halide was added, the mixture was refluxed for 30 min, diluted with dry diethyl ether (1.5 mL) and cooled to 0°C in an ice bath. A small amount of CuI (purified according to Lipshutz's method[27]) was then added, causing the solution to turn dark. A solution of 2,7-cyclooctadienone[13] (**11**) (*Caution*: strong allergen) (0.158 g, 1.30 mmol) in anhydrous diethyl ether (1.5 mL) was added dropwise for 2 h, keeping the temperature below 5°C. When the addition was complete, the mixture was stirred for 60 min at 0°C.

To a solution of diphenyldiselenide (0.224 g, 0.717 mmol) in dry THF (1 mL) was added bromine (0.036 mL, 0.115 g, 0.722 mmol). This mixture was stirred for 30 min and then added to the above stirred Grignard mixture at such a rate that the temperature did not exceed 10°C. The resulting yellow gelatinous mixture was further stirred for 40 min, then poured into water (10 mL) and extracted with five 10 mL portions of diethyl ether. The extracts were washed twice with 10 mL of water, dried and concentrated under reduced pressure to give a dark oily residue containing crude selenide **12**. The latter was taken up in 10 mL CH_2Cl_2 and 1 mL pyridine was added. A solution of H_2O_2 30% (1 mL) in water (1 mL) was then added dropwise, keeping the temperature between 30 and 35°C (warming was necessary to initiate the reaction). When the addition was complete, the mixture was stirred at room temperature for 30 min and poured into a mixture of 10 mL CH_2Cl_2 and 10 mL of a saturated aqueous solution of $NaHCO_3$. The aqueous phase was then extracted twice with CH_2Cl_2 (10 mL). The combined organic layers were dried and evaporated in vacuo to give a residue which was purified by flash chromatography on a silica gel column (hexane/AcOEt 9:1) to afford **13** (0.079 g, 0.390 mmol, 30%) as a yellow oil.

13. HREIMS *m/z* 203.2206 (10, $M^{+\cdot}$, calcd for $C_{13}H_9{}^2H_{11}O$ 203.2205), 175 (5), 161.2100 (4, calcd for $C_{11}H_7{}^2H_{11}$ 161.2099), 137 (10), 121 (27), 109 (18), 93 (34), 81 (100), 67 (22), 53 (31), IR (film) 3021, 2937, 2874, 2205, 2100, 1630, 1605, 1463, 1396, 1337, 1287, 1237, 1161, 1053 cm^{-1}, ^1H NMR ($CDCl_3$) 1.7–1.85 (m, 2H); 2.3–2.45 (m, 4H); 6.18 (s, 1H); 6.28–6.33 (m, 2H).

4.1.5. *N*-[(*R*)-1-phenylethyl]-1-[$^2H_{11}$]pentyl-9-azabicyclo[3.3.1]nonan-3-one (14a) and *N*-[(*S*)-1-phenylethyl]-1-[$^2H_{11}$]pentyl-9-azabicyclo[3.3.1]nonan-3-one (14b). In a 10 mL flask were placed **13** (0.073 g, 0.360 mmol), (*R*)-(+)-α-methylbenzylamine (0.118 g, 0.976 mmol) and methanol (5 mL). The mixture was stirred at room temperature for 12 h and concentrated under reduced pressure to give a residue which was purified by flash chromatography on a silica gel column (hexane/AcOEt 9:1) to afford a

mixture of **14a** and **14b** (0.085 g, 0.262 mmol, 73%). The diastereomers were then separated by chromatography on a silica gel Lobar® (Merck) pre-packed column (hexane/AcOEt 9:1). This yielded **14a** (0.109 g) as a white solid and **14b** (0.041 g) as a yellow oil (de 45%).

14a. Mp 55–58°C, HREIMS *m/z* 324.3096 (52, $M^{+\cdot}$, calcd for $C_{21}H_{20}{}^{2}H_{11}NO$ 324.3096), 309 (15), 281 (27), 274 (6), 260.1966 (23, calcd for $C_{17}H_{20}{}^{2}H_{3}NO$ 260.1968), 239 (8), 225 (6), 219 (21), 205 (5), 191 (6), 177 (29), 170 (6), 163 (17), 156 (20), 105 (100), 91 (7), 79 (26), 69 (12), IR (KBr) 2937, 2205, 2100, 1697, 1638, 1605, 1463, 1404, 1287, 1178, 1095, 751, 693 cm^{-1}, ^{1}H NMR ($CDCl_3$) 1.4–1.8 (m, 6H); 1.5 (d, 6.9, 3H); 2.32 (AB, 16.8, 2H); 2.4 (ABX, 16.8, 7.3, 0, 2H); 3.35 (m, 1H); 4.52 (q, 6.9, 1H); 7.2–7.5 (m, 5H).

14b. EIMS *m/z* 324.3096 (52, $M^{+\cdot}$, calcd for $C_{21}H_{20}{}^{2}H_{11}NO$ 324.3096), 309 (12), 281 (20), 274 (5), 260.1967 (17, calcd for $C_{17}H_{20}{}^{2}H_{3}NO$ 260.1968), 242 (3), 239 (6), 219 (14), 214 (5), 191 (6), 177 (15), 170 (5), 163 (11), 156 (16), 105 (100), 79 (20), 77 (16), IR (film) 2937, 2205, 2100, 1705, 1454, 1362, 1295, 1237, 1186, 1028, 760, 701 cm^{-1}, ^{1}H NMR ($CDCl_3$) 1.4–1.7 (m, 6H); 1.55 (d, 6.9, 3H); 1.96 (m, 2H); 2.3 (s, 2H); 3.4 (ms, 1H); 4.52 (q, 6.9, 1H); 7.2–7.5 (m, 5H).

4.1.6. (−)-[$^{2}H_{11}$]Adaline [(−)-15]. Compound **14a** (0.107 g, 0.330 mmol) in methanol (10 mL) was hydrogenated in the presence of 10% Pd–C and one drop of 60% $HClO_4$ at atmospheric pressure and room temperature for 48 h. The mixture was filtered through Celite and the filtrate was concentrated in vacuo. The residue was taken up in a 5 mL of 20% aqueous NaOH and extracted with five 10 mL portions of diethyl ether. The combined organic layers were dried ($MgSO_4$) and evaporated in vacuo to give a residue which was purified by flash chromatography on a silica gel column (AcOEt/MeOH/NH_4OH 95:5:1) to afford (−)-**15** (0.059 g, 0.270 mmol, 82%) as a yellow oil.

(−)-**15**. GC (OV 1701, injector temperature 220°C, isothermal 200°C, detector temperature 230°C): t_R=8.13 min, KI=1949, $[\alpha]_D{}^{20}$=−9.45 (*c* 1.99, $CHCl_3$). HREIMS *m/z* 220.2468 (50, $C_{13}H_{12}{}^{2}H_{11}NO$) [calcd for $C_{13}H_{12}{}^{2}H_{11}NO$ 220.2470], 219.2396 (17, $C_{13}H_{11}{}^{2}H_{11}NO$) [calcd for $C_{13}H_{11}{}^{2}H_{11}NO$ 219.2392], 191.2077 (17, $C_{11}H_{7}{}^{2}H_{11}NO$) [calcd for $C_{11}H_{7}{}^{2}H_{11}NO$ 191.2079], 186 (10), 178.1984 (27, $C_{10}H_{6}{}^{2}H_{11}NO$) [calcd for $C_{10}H_{6}{}^{2}H_{11}NO$ 178.2000], 177 (66), 176 (12), 171 (12), 170 (36), 163.2130 (62, $C_{10}H_{7}{}^{2}H_{11}N$) [calcd for $C_{10}H_{7}{}^{2}H_{11}N$ 163.2130], 162 (13), 161 (11), 157 (49), 156.1343 (100, $C_{9}H_{12}{}^{2}H_{3}NO$) [calcd for $C_{9}H_{12}{}^{2}H_{3}NO$ 156.1342], 154 (21), 149 (11), 138 (14), 128 (13), 115 (10), 114 (18), 113 (67), 112 (10), 100 (15), IR (film) 3314, 2937, 2205, 2100, 1705, 1463, 1404, 1354, 1287, 1237, 1170, 1061 cm^{-1}, ^{1}H NMR ($CDCl_3$) 1.4–1.75 (m, 6H); 2.19 (d, 16.5, 2H); 2.47 (ABX, 16.5, 6.9, 0, 2H); 3.68 (bs, 1H).

(−)-**15**·HCl: ^{1}H NMR ($CDCl_3$) 1.5–2.1 (m, 6H); 2.57 (B part of ABX, 17.0, <1.0, 1H); 2.64 (B part of AB, 17.0, 1H); 2.78 (A part of AB, 17.0, 1H); 3.10 (A part of ABX, 17.0, 6.0, 1H); 4.1 (X part of ABX, bs, 1H), ^{13}C NMR ($CDCl_3$) 17.2, 29.1, 34.6, 43.5, 48.7, 51.6, 60.2, 205.3 (deuterium-bearing carbons were not detected in this experiment).

4.1.7. (+)-[$^{2}H_{11}$]Adaline [(+)-15]. Compound **14b** (0.040 g, 0.124 mmol) in methanol (10 mL) was hydrogenated in the presence of 10% Pd–C as for **14a** to afford (+)-**15** (0.021 g, 0.094 mmol, 76%) as a yellow oil.

(+)-**15**. $[\alpha]_D{}^{20}$=+9.3 (*c* 1.3, $CHCl_3$). Otherwise, the spectroscopic properties of (+)-**15** were identical to those of its enantiomer.

4.1.8. (±)-*cis*- and (±)-*trans*-*N*-Methoxycarbonyl-2-acetonyl-6-pentylpiperidines [(±)-17a) and (±)-17b]. *N*-Methoxycarbonyl-2-pentylpiperidine [(±)-**16**] (0.200 g, 0.94 mmol) and tetraethylammonium *p*-toluenesulfonate (0.200 g, 0.66 mmol) in MeOH (14.8 mL) were submitted to an anodic oxidation as described previously.[7] After 4 Faraday/mol had been consumed, a few drops of NH_4OH were added, and the solvent was evaporated under reduced pressure. Water was then added and the mixture extracted three times with $CHCl_3$. The organic extracts were pooled, dried over $MgSO_4$ and the solvent removed in vacuo. Flash chromatography of the residue on a silica gel column (hexane–AcOEt, 95:5) afforded 0.180 g of *N*-methoxycarbonyl-2-methoxy-6-pentylpiperidine (78%). To a solution of the latter compound (0.040 g, 0.16 mmol) in CH_2Cl_2 (3.5 mL) at −78°C under a nitrogen atmosphere were added trimethylsilyloxypropene (0.025 g, 0.181 mmol) and TMSOTf (3 μL, 0.016 mmol). This mixture was then stirred at −78°C for 1 h. After addition of a saturated aqueous solution of $NaHCO_3$, the mixture was extracted with $CHCl_3$ (3×10 mL) and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography on a silica gel column (hexane/AcOEt, 8:2), to afford a 96:4 mixture of (±)-**17a** and (±)-**17b**, in a 74% yield.

(±)-**17a**. HREIMS *m/z* 269.1991 (2, $M^{+\cdot}$, calcd for $C_{15}H_{27}NO_3$ 269.1992), 212.1652 (8, calcd for $C_{12}H_{22}NO_2$ 212.1652), 210 (10), 198 (22), 166 (11), 140 (100), 95 (9), 81 (15), 67 (8), 55 (14), IR (film) 2955, 2870, 1717, 1694, 1448 cm^{-1}, ^{1}H NMR ($CDCl_3$) 0.89 (t, 6.6, 3H); 1.2–1.7 (m, 14H); 2.18 (s, 3H); 2.55 (dd, 18.0, 4.0, 1H); 2.75 (dd, 18.0, 11.0, 1H); 3.69 (s, 3H); 4.10 (m, 1H); 4.65 (m, 1H).

4.1.9. (±)-*cis*- and (±)-*trans*-*N*-Methoxycarbonyl-2-[1-(2-hydroxypropyl)]-6-pentylpiperidine [(±)-18a) and (±)-18b]. To the 96:4 mixture of (±)-**17a** and (±)-**17b** (0.143 g, 0.53 mmol) in THF (9 mL) was added $LiAl(O^{t}Bu)_3H$ (0.271 g, 1.06 mmol). The mixture was stirred at room temperature for 9 h upon which a few droplets of water were added. The solvent was then evaporated in vacuo, diluted NH_4OH (10 mL) was added and the solution was extracted with $CHCl_3$ (3×10 mL). The $CHCl_3$ extracts were pooled, dried over $MgSO_4$ and evaporated to afford a residue which was purified by flash chromatography on a silica gel column (hexane/AcOEt, 65:35) to afford (±)-**18a** (0.117 g, 80%) and (±)-**18b** (0.011 g, 8%).

(±)-**18a**. HREIMS *m/z* 271.2147 (3, $M^{+\cdot}$, calcd for $C_{15}H_{29}NO_3$ 271.2147), 213 (18), 212 (99), 200 (100), 182 (28), 168 (8), 156 (14), 142 (100), 140 (88), 126 (13), 114 (10), 102 (12), 95 (15), 88 (13), 81 (30), 69 (15), 67 (15), 59 (14), 55 (34), IR (film) 3452, 2944, 2860, 1665, 1453, 1105 cm^{-1}, ^{1}H NMR ($CDCl_3$) 0.89 (t, 6.5, 3H); 1.19 (d,

6.5, 3H); 1.2–1.7 (m, 14H); 1.80 (m, 2H); 3.70 (s, 3H); 3.73 (m, 1H); 4.10 (m, 1H); 4.22 (m, 1H), ^{13}C NMR 66.2, 52.6, 52.2, 50.7, 49.8, 34.3, 31.7, 27.3, 26.9, 26.6, 22.6, 23.9, 14.0, 13.9.

(±)-**18b**. HREIMS *m/z* 271.2147 (3, $M^{+\cdot}$, calcd for $C_{15}H_{29}NO_3$ 271.2147), 212 (100), 200 (90), 182 (27), 168 (8), 156 (30), 142 (90), 140 (91), 126 (11), 114 (9), 102 (13), 95 (15), 88 (14), 81 (39), 69 (18), 67 (16), 59 (17), 55 (36), IR (film) 3470, 2947, 2860, 1668, 1450, 1408, 1366, 1107 cm^{-1}, ^{1}H NMR ($CDCl_3$) 0.89 (t, 6.5, 3H); 1.18 (d, 6.5, 3H); 1.2–1.7 (m, 15H); 1.90 (m, 1H); 3.65 (m, 1H); 3.72 (s, 3H); 4.12 (m, 1H); 4.45 (m, 1H).

4.1.10. (±)-*cis*-2-[1-(2-Hydroxypropyl)]-6-pentylpiperidine [(±)-19]. Compound (±)-**18a** (0.046 g, 0.169 mmol) was dissolved in a 10% ethanolic KOH solution (5.7 mL) and refluxed for 8 h. The solvent was then evaporated, water was added and the resulting mixture extracted with $CHCl_3$ (3×10 mL). Evaporation of the organic extracts yielded pure (±)-**19** (0.036 g, 99%).

(±)-**19**. HREIMS *m/z* 213.2083 (2, $M^{+\cdot}$, calcd for $C_{13}H_{27}NO$ 213.2092), 212.2017 (4, calcd for $C_{13}H_{26}NO$ 212.2014), 198 (5), 168 (5), 154 (60), 142 (100), 124 (28), 98 (14), 82 (33), 81 (15), 69 (15), 57 (16), 55 (29), IR (film) 3317, 2927, 2850, 1458, 1127 cm^{-1}, ^{1}H NMR ($CDCl_3$) 0.87 (t, 6.5, 3H); 1.15 (d, 6.5, 3H); 1.2–1.4 (m, 9H); 1.50–1.90 (m, 5H); 2.47 (m, 1H); 2.75 (m, 1H); 4.0 (m, 1H), ^{13}C NMR ($CDCl_3$) 69.8, 59.2, 57.2, 44.7, 37.5, 34.2, 33.5, 32.5, 26.4, 25.2, 24.6, 23.2, 14.7.

4.1.11. (±)-*cis*-*N*-Chloro-2-[1-(2-hydroxypropyl)]-6-pentylpiperidine [(±)-20]. As described,[17] a solution of TMSCl (11 μL, 0.09 mmol) in anhydrous benzene (0.3 mL) was slowly added to a stirred solution of (±)-**19** (0.019 g, 0.09 mmol) and Et_3N (39 μL, 0.31 mmol) in benzene (1.4 mL). The mixture was stirred for 2 h at room temperature. The solvent and the excess Et_3N were then removed in vacuo, the residue was dissolved in benzene and quickly filtered through Celite. After evaporation of the solvent, the residue was dissolved in anhydrous diethyl ether (1 mL), NCS (0.026 g, 0.195 mmol) was added at once and the solution was stirred for 3 h. The reaction mixture was then filtered and the solvent evaporated in vacuo. Addition of diluted NH_4OH and extraction with CH_2Cl_2 (4×5 mL) furnished (±)-**20** (0.016 g, 70%).

(±)-**20**. EIMS *m/z* 249/247 ($M^{+\cdot}$, not detected), 234 (3), 232 (1), 212 (20), 190 (18), 188 (55), 178 (17), 176 (51), 154 (44), 142 (100), 124 (17), 120 (18), 118 (57), 96 (33), 82 (33), 81 (43), 69 (25), 55 (48), CIMS (NH_3) *m/z* 250/248 [40, 86 $(M+H)^+$], 214 (65), 212 (100), 188 (13), 176 (16), 154 (16), 142 (44), 96 (11), IR (film) 2968, 2929, 2857 cm^{-1}, ^{1}H NMR ($CDCl_3$) 0.88 (t, 6.5, 3H); 1.18 (d, 6.5, 3H); 1.2–1.7 (m, 14H); 1.78 (m, 1H); 1.98 (m, 1H); 2.88 (m, 1H); 3.10 (m, 1H), 4.0 (m, 1H).

4.1.12. (±)-6-[1-(2-Hydroxypropyl)]-2-pentyl-1-piperideine [(±)-21] and (±)-2-[1-(2-hydroxypropyl)]-6-pentyl-1(6)-piperideine [(±)-22]. To compound (±)-**20** (0.017 g, 0.068 mmol) in Et_2O (0.24 mL) were added 2.2 equiv. of KO_2 (0.010 g, 0.15 mmol) and 18-crown-6 (0.3 mg, 0.001 mmol). The reaction mixture was stirred for 20 h. The mixture was then filtered, the solvent was evaporated, and a mixture of CH_2Cl_2-diluted NH_4OH was added. Extraction with CH_2Cl_2 (3×10 mL) and evaporation yielded an oil which was purified by chromatography on a silica gel column (hexane/AcOEt/NH_3, 70:30:1) to afford a 40:60 mixture (0.008 g) of (±)-**21** and (±)-**22** in a 53% yield.

(±)-**21**. GC-EIMS (t_R=22.17 min), *m/z* 211 (0.5, $M^{+\cdot}$), 196 (6), 182 (3), 168 (5), 166 (5), 155 (20), 153 (9), 137 (5), 124 (6), 122 (6), 111 (9), 96 (100), 82 (10), 67 (9), 55 (14), ^{1}H NMR (CD_2Cl_2) 0.80 (t, 6.5, 3H, H-14); 1.20 (d, 6.5, 3H, H-9); 1.15 and 1.74 (m, 2H, H-5); 1.32 and 1.65 (m, 2H, H-7); 3.50 (m, 1H, H-6), 4.20 (m, 1H, H-8).

(±)-**22**. GC-EIMS (t_R=20.49 min), *m/z* 193 (30, $M^{+\cdot}-H_2O$), 178 (19), 164 (9), 150 (11), 136 (100), 124 (75), 122 (92), 108 (30), 94 (18), 80 (10), 68 (40), 55 (25), ^{1}H NMR (CD_2Cl_2) 0.80 (t, 6.5, 3H, H-14); 1.20 (d, 6.5, 3H, H-9); 1.18 and 1.75 (m, 2H, H-3); 1.45 and 1.58 (m, 2H, H-10); 2.10 (m, 2H, H-5); 2.18 (m, 2H, H-7); 3.20 (m, 1H, H-2), 4.20 (m, 1H, H-8).

4.1.13. (±)-Adaline [(±)-3] and (±)-6-acetonyl-2-pentyl-1-piperideine [(±)-5]. Only oxidation with PCC is described as it gave the highest yields.

PCC (0.010 g, 0.046 mmol) was suspended in anhydrous CH_2Cl_2 (1 mL) and the mixture of (±)-**21** and (±)-**22** (0.006 g, 0.028 mmol) in CH_2Cl_2 was quickly added. The reaction was monitored by TLC. After all the starting compounds had disappeared, Et_2O was added and the mixture was filtered on Celite. After evaporation of the solvent the residue was quickly filtered on silica gel (AcOEt/MeOH 95:5, +0.1% NH_4OH). GC–MS analysis showed the presence of one major compound (t_R=23.30 min) identified as adaline by its mass spectrum (HREIMS: *m/z* 209.1778, calcd for $C_{13}H_{23}NO$ 209.1779) and by co-injection with a reference sample. A minor compound (t_R=21.50 min) (±15% of adaline) having a mass spectrum indistinguishable from that of adaline (only the *m/z* 110 peak was of lower intensity) was tentatively identified as (±)-**5**. The major compound from the PCC oxidation reaction had an ^{1}H NMR identical to that of authentic adaline.

4.2. Incorporation experiments

With [1-^{14}C]- and [2-^{14}C]acetate. Adult *A. bipunctata* beetles were field collected and complemented by beetles purchased from Horpi Systems (Verlaine—Belgium). The beetles were placed by groups of five in Petri dishes (5.5 cm ∅), the bottom of which was covered with a humidified Whatman® filter paper. About 100 beetles were used for each experiment. A small piece of banana, to which 10 μL of either a sodium [1-^{14}C]acetate (250 μCi, 57 mCi/mmol, Amersham), or a sodium [2-^{14}C]acetate (250 μCi, 55 mCi/mmol, Amersham) solution was mixed, was placed on the cover of each dish. Food was replaced every 2 or 3 days and the filter paper re-humidified. The experiments were performed at room temperature (around 22°C) with 10L/14D photoperiod for 3 weeks, after which

the beetles were killed. Usual extraction and purification led to 1.0 mg of radioactive adaline from the sodium [2-^{14}C]acetate experiment and 8.31 mg from the sodium [1-^{14}C]acetate experiment (see Table 1).

With (−)- and (+)-[$^{2}H_{11}$]adaline. Four field collected *A. bipunctata* beetles were fed as described above, except that they were offered pieces of agar-based artificial diet,[14] mixed with a few drops of a solution of (−)-[$^{2}H_{11}$]-adaline·HCl or (+)-[$^{2}H_{11}$]adaline·HCl (both 5×10^{-2} M) in 10^{-1} M aqueous sucrose. Additional solution (as above) was offered on pieces of cotton wool. After 20 days the beetles were killed, quickly dipped into acetone and the resulting extract analyzed by GC–MS for the presence of unlabelled and deuterium labelled adaline and adalinine using selected-ion retrieval chromatograms.

[$^{2}H_{11}$]adalinine. EIMS $M^{+\cdot}$ not detected; *m/z* 179 (23, $M^{+\cdot}-C_3H_5O$), 154 (90, $M^{+\cdot}-C_5{}^{2}H_{11}$), 112 (100), 96 (30), 82 (27), 70 (36), 56 (35).

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References

1. Daloze, D.; Braekman, J. C.; Pasteels, J. M. *Chemoecology* **1995**, *5/6*, 173–183.
2. King, G. A.; Meinwald, J. *Chem. Rev.* **1996**, *96*, 1105–1122.
3. Attygalle, A. B.; Blankespoor, C. L.; Eisner, T.; Meinwald, J. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 12790–12793.
4. Attygalle, A. B.; Svatos, A.; Veith, M.; Farmer, J. J.; Meinwald, J.; Smedley, S.; Gonzales, A.; Eisner, T. *Tetrahedron* **1999**, *55*, 955–966.
5. Tursch, B.; Daloze, D.; Braekman, J. C.; Hootelé, C.; Pasteels, J. M. *Tetrahedron* **1975**, *31*, 1541–1543.
6. (a) Lognay, G.; Hemptinne, J. L.; Chan, F. Y.; Gaspar, C. H.; Marlier, M.; Braekman, J. C.; Daloze, D.; Pasteels, J. M. *J. Nat. Prod.* **1996**, *59*, 510–511. (b) Yamazaki, N.; Ito, T.; Kibayashi, C. *Tetrahedron Lett.* **1999**, *40*, 739–742.
7. Braekman, J. C.; Charlier, A.; Daloze, D.; Heilporn, S.; Pasteels, J. M.; Plasman, V.; Wang, S. F. *Eur. J. Org. Chem.* **1999**, 1749–1755.
8. Renson, B.; Merlin, P.; Daloze, D.; Braekman, J. C.; Roisin, Y.; Pasteels, J. M. *Can. J. Chem.* **1994**, *72*, 105–109.
9. Hill, R. K.; Renbaum, L. A. *Tetrahedron* **1982**, *38*, 1959–1963.
10. Paquette, L. A.; Underiner, T. L.; Gallucci, J. C. *J. Org. Chem.* **1992**, *57*, 86–96.
11. Momose, T.; Muraoka, O.; Atarashi, S.; Horita, T. *Chem. Pharm. Bull.* **1979**, *27*, 222–229.
12. Momose, T.; Takizawa, S.; Kirihara, M. *Synth. Commun.* **1997**, *27*, 3313–3320.
13. Krabbenhoft, H. O. *J. Org. Chem.* **1978**, *43*, 4556–4558.
14. Majerus, M.; Kearns, P. *Ladybirds*; Richmond Publishing: Slough, 1989; pp. 85–86.
15. We thank Prof. F. R. Stermitz, Colorado State University, for bringing this possibility to our attention.
16. Shono, T. *Tetrahedron* **1984**, *40*, 811–850.
17. Tawara, J. N.; Blokhin, A.; Foderaro, T. A.; Stermitz, F. R. *J. Org. Chem.* **1993**, *58*, 4813–4818.
18. Mill, S.; Hootelé, C. *J. Nat. Prod.* **2000**, *63*, 762–764.
19. Leclercq, S.; Thirionet, I.; Broeders, F.; Daloze, D.; Vander Meer, R.; Braekman, J. C. *Tetrahedron* **1994**, *50*, 8465–8478.
20. Scully, F. *J. Org. Chem.* **1980**, *45*, 1515–1517.
21. Corey, E. J.; Suggs, J. W. *Tetrahedron Lett.* **1975**, 2647–2650.
22. (a) Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277–7287. (b) Frigerio, M.; Santagostino, S.; Sputore, S.; Palmisano, G. *J. Org. Chem.* **1995**, *60*, 7272–7276.
23. Leete, E.; Kim, S. H. *Chem. Commun.* **1989**, 1899–1900.
24. Tursch, B.; Chome, C.; Braekman, J. C.; Daloze, D. *Bull. Soc. Chim. Belges* **1973**, *82*, 699–703.
25. Yue, Ch.; Royer, J.; Husson, H. P. *J. Org. Chem.* **1992**, *57*, 4211–4214.
26. Mechelke, M. F.; Meyers, A. I. *Tetrahedron Lett.* **2000**, *41*, 4339–4342.
27. Lipshutz, B. H. In *Organometallics in Synthesis: A Manual*, Schlosser, M., Ed.; Wiley: New York, 1994; pp 286.