Synthesis of 2,6-disubstituted piperidine alkaloids from ladybird beetles Calvia 10-guttata and Calvia 14-guttata

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Optically pure (+)-calvine, (+)-2-epicalvine, (2S,6S)-(6-pentylpiperidin-2-yl)acetic acid methyl ester and (2R,6S)-(6-pentylpiperidin-2-yl)acetic acid methyl ester, four piperidine alkaloids isolated from ladybird beetles of the genus Calvia (Coccinellidae), were synthesised from a common precursor using cyclisative Pd(II)/Cu(II)-catalysed carboamination-(methoxy)carbonylation tandem reaction of alkylamines as a key step. The first single-crystal X-ray analysis of (+)-calvine confirmed its proposed absolute configuration to be (2S,6S) corresponding to that of natural product.

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1. Introduction

Insects use various toxic molecules as chemical weapons to discourage potential predators.1 It is known that coccinellid beetles often release small droplets of yellow hemolymph at their knee joints, when molested or disturbed (so called ‘reflex bleeding’).2 As a consequence, these insects are only rarely exploited as a food source by other organisms, which is attributed to the presence of deterrent compounds in their blood.3 Among them, piperidine alkaloids represent a prominent class of such natural products displaying defensive properties.4

Recently, four 2,6-disubstituted piperidine alkaloids (+)-calvine 1, (+)-2-epicalvine 2, (2S,6S)-(6-pentylpiperidin-2-yl)acetic acid methyl ester 3 and (2R,6S)-(6-pentylpiperidin-2-yl)acetic acid methyl ester 4, were isolated from two species of ladybird beetles Calvia 10-guttata and Calvia 14-guttata (Coccinellidae)5 (Fig. 1). To the best of our knowledge, no biological activity of these alkaloids has been determined so far.

The structure and relative configuration of 1–4 was established on the basis of NMR spectroscopy and HRMS studies and subsequently confirmed via racemic total synthesis.5 The absolute configuration was determined by enantioselective total synthesis.6 Since then, only one other preparation of 1 has appeared7 along with two formal syntheses.8 All but one approach known so far used alkaloids 3 and/or 4 as key intermediates for the (formal) syntheses of 1 and/or 2.5,6,8 Recently, we have communicated racemic syntheses of calvine and epicalvine.9 In this full account, we wish to report short and efficient stereoselective total syntheses of all four naturally occurring piperidine alkaloids 1–4. Our approach relies on diastereoselective intramolecular Pd(II)/Cu(II)-catalysed tandem aminocyclisation–carbonylation reaction of alkylamines 5 and/or 6 as a key step, while both these substrates were prepared from the common precursor 7 (Scheme 1).
2. Results and discussion

Initial preparation of both substrates 5 and 6 needed for the key Pd(II)-catalysed cyclisation started from the commercially available (R)-epichlorohydrin 8 (Scheme 2). Our intention was to transform it to (R)-undec-1-en-6-ol 7 via double-ring opening of the epoxide using an ‘alkylation first–alkenylation second’ sequence. However, such strategy surprisingly failed to provide the desired alcohol 7 using an ‘alkylation first–alkenylation second’ sequence. However, a reasonable yield even after extensive experimentation. While the Pd(II)-catalysed cyclisation started from the commercially available 7 in ratios ranging from 3:1 (Et$_2$O) to 1:1 (THF)) or complex reaction mixtures only (Scheme 2).

We speculated, that the formation of the undesired bromohydrin 10 from the epoxide 9 is due to the competitive attack of the Br$^{-}$ nucleophile from in situ formed HBr (and/or MgBr$_2$). This may come from the β-hydride elimination of butylmagnesium bromide generating 1,3-butadiene followed by reductive elimination of the magnesium hydride complex. This hypothesis is supported by the experimental observation that grey precipitate (possibly metallic Mg) is gradually deposited on the glassware during the course of reaction (Scheme 3).

Thus, we had to reverse the order of double-ring opening transformation and conducted the ‘alkenylation first–alkylation second’ sequence on the (S)-epichlorohydrin 11. This substrate was initially opened with butenylmagnesium bromide and the resulting chlorohydrin 12 subsequently closed under basic conditions to the unsaturated epoxide 13. Gratifyingly, the following addition of excess butyllithium catalysed by copper(I) iodide afforded the desired alcohol 7 in 70% combined yield over three steps. Activation of the hydroxyl group of 7 using TsCl gave tosylate 14, which was treated either with excess ethanolamine to yield the unsaturated amino alcohol 5 or with excess benzylamine to provide the corresponding alkenylamine 6 (Scheme 4).

With both substrates in hand, we subjected them to the final key transformation. Under optimal reaction conditions, the Pd(II)/Cu(II)-catalysed aminocyclisation–lactonisation directly provided the desired target alkaloids (+)-calvine 1 and (+)-2-epicalvine 2 in a diastereoselective fashion depending upon the applied catalytic conditions. Thus, using PdCl$_2$ as a catalyst and excess CuCl$_2$ as reoxidant we obtained the diastereomeric product in a ratio of 2.2:1 and 18% yield over six steps. On the other hand, the combination of molecular oxygen (1 atm) with catalytic copper(II) chloride as reoxidant system afforded the diastereomeric (+)-2-epicalvine 2 as a major product in a ratio of 7:3 resulting in the overall yield of 17% in six steps (Scheme 5).
So far, the exact structure of the true catalytic species involved in our Pd(II)/Cu(II)-catalysed aminocyclisation–carbonylation of 5 remains unclear. However, due to the metal composition of optimal reaction conditions it inevitably should be of a heterobimetallic nature. Such Pd/Cu-complexes in analogous transformations involving both palladium and copper salts have been proposed and/or isolated and characterised.18

After the convenient FLC separation of both diastereomers 1 and 2, the enantiomerically pure (+)-calvine 1 turned out to be a crystalline compound.19 The first single-crystal X-ray analysis of 1 confirmed its proposed absolute configuration to be (2R,S)-2-epicalvine (Scheme 6) corresponding to that of the natural product. Moreover, it can be seen that both piperidine and lactone rings adopt chair-like conformation in the solid state (Fig. 2).

Figure 2. An ORTEP view of the crystal structure of natural (+)-calvine 1.

In order to finalise the total synthesis of the other two target alkaloids 3 and 4, we subjected alkenylamine 6 to the Pd(II)/Cu(II)-catalysed aminocyclisation–methoxycarbonylation sequence.17e,21 As the cyclisative preparation of trans-2,6-disubstituted piperidines bearing an ester group in the β-position is more challenging22 in comparison to their cis-counterparts, we have focused particularly on the conditions favouring the formation of the former one.23 Thus, exposure of 6 to catalytic PdCl2 and CuCl2 in MeOH under the CO/O2 atmosphere afforded an inseparable mixture of the desired piperidines 15/16 in the 68% combined yield. The final catalytic debenzylolation of methyl esters 15/16 on Pearlman’s catalyst provided easily separable target alkaloids 3 and 4 in 81% combined yield and in a ratio of 1:3 in favour of the 2,6-trans-configured piperidine 4 (Scheme 6).

Scheme 6. Key step and finalisation of the total synthesis of 3 and 4. Reagents and conditions: (i) CO2Me (ca. 1.1, balloon), 0.1 equiv PdCl2, 0.2 equiv CuCl2, 3 Å molecular sieves, MeOH, rt, 18 h, 68%; (ii) H2 (balloon), 0.2 equiv Pd(OH)2, MeOH, rt, 24 h, 81% (3:4–1:2).

3. Conclusion

We synthesised four optically pure alkaloids (+)-calvine 1, (+)-2-epicalvine 2, (2S,6R)-6-pentylpiperidin-2-ylacetic methyl ester 3 and (2R,6S)-6-pentylpiperidin-2-ylacetic methyl ester 4 using intramolecular Pd(II)/Cu(II)-catalysed aminocyclisation–carbonylation tandem reaction as a key step. Both necessary alkenylamines 5 and 6 required for these crucial transformations were efficiently prepared in five steps starting from the common substrate (S)-epichlorohydrin in 46% and 41% overall yields, respectively. By tuning the reaction parameters we were able to direct the stereoselectivity of the respective Pd(II)/Cu(II)-catalysed cyclisations in order to obtain either diastereomer of target natural compounds 1–4. Thus, using catalytic PdCl2 and excess CuCl2 we obtained 2,6-cis-configured alkaloid 1 as a major product in 18% overall yield in six steps. On the other hand, the combination of molecular oxygen with catalytic PdCl2 and CuCl2 afforded both 2,6-trans-configured alkaloids 2 and 4 as main adducts in 17% and 13% overall yields. Finally, we have performed the first single-crystal X-ray analysis of (+)-calvine 1 and confirmed its proposed absolute configuration to be (2S,6R) corresponding to that of the isolated natural product.

4. Experimental section

4.1. General

All solvents were distilled before use: diethylether, THF and dioxane from Na/benzophenone, MeOH from MeONa and CH2Cl2 from P2O5. Thin layer chromatography (TLC) was performed on aluminium plates pre-coated with 0.2 mm silica gel 60 F254 (Merck). Flash column liquid chromatography (FCL) was performed on Kieselgel 60 (40–63 μm). GC was performed on HP-5 column (30 m, ID 0.25 mm, film thickness 0.12 μm) equipped with split/splitless injector and FID detector. Optical rotations were measured with a Perkin–Elmer 241 polarimeter with a 1.000 cm cell at λ=589 nm. Elemental analyses were performed by the Microanalytical Service of Slovak Academy of Sciences. Infrared (IR) spectra were recorded on a Nicolet 5700 FTIR spectrometer. X-ray analysis was performed on Oxford Diffraction GEMINI R diffractometer. Melting point was determined on Büchi B-540 apparatus. NMR spectra were recorded on Varian VXR-300 (300 MHz) and Inova 600 (600 MHz) spectrometers, respectively. Chemical shifts (δ) are quoted in parts per million and the residual protic solvent was used as internal reference. The COSY and NOESY techniques were used in assignment of 1H–1H relationships and the determination of relative configuration. The multiplicities of carbons were assigned from a broadband decoupled analysis used in conjunction with APT. The HMBC technique was used throughout for the assignment of 1H–13C relationships.

4.1.1. (S)-1-Chlorohept-6-en-2-ol (12). To a cooled freshly prepared THF solution (0.96 M) of butylenimagnesium bromide (1.5 equiv) [made of Mg turnings (2.339 g, 96.23 mmol) and butenyl bromide (13.392 g, 10.07 mL, 96.23 mmol) in dry THF (100 mL)] was added anhydrous Cul (1.833 g, 0.15 equiv) at −50 °C in one portion under Ar. After 5 min stirring, while the colour of the mixture changed from grey to deep turquoise, the solution of (S)-epichlorohydrin 11 (5.936 g, 5.03 mL, 64.15 mmol) in dry THF (50 mL) was added dropwise via syringe at −50 °C over 20 min under Ar. The resulting deep-blue mixture was left to stir for 16 h while the temperature gradually rose to 25 °C over the indicated time. The reaction was then quenched by addition of satd aq NH4Cl solution (350 mL) and water (200 mL). After 30 min stirring, the mixture was extracted with Et2O (2×150 mL), combined organic extracts were dried over
anhydrous Na2SO4, filtered and concentrated in vacuo. The resulting brownish oil (9.54 g) was subjected to TLC (390 g SiO2, 6.28 cm, ethyl acetate/hexanes=1/5) yielding pure chlorodiricyl 12 (8.196 g, 96%) as a pale-yellow oil.

**Compound 12:** Rp=0.35 (ethyl acetate/hexanes=1/6); [z]D+2.4 (c 0.502, CH2Cl2); IR (film): ν/cm−1=737, 911, 994, 1038, 1064, 1433, 1640, 2861, 2932, 3361 (br); 1H NMR (300 MHz, CDCl3): 1.40–1.64 (m, 4H, 5-H, 6-H), 2.04–2.13 (m, 2H, 2-H, 3-H), 2.47 (dd, 1H, J2,3=2.8, Jgem=5.1 Hz, 3-Ha), 2.68–2.84 (m, 1H, 1-H, 2-H), 3.58 (2H, 4-H), 3.73–3.87 (br s, 1H, 2-H), 4.94–5.05 (m, 2H, 7-H), 5.79 (tdd, 1H, J=4.94–5.05, J=6.6, 10.2, 16.8 Hz, 6-H); 13C NMR (75 MHz, CDCl3): 24.8 (CH2, 4-C), 33.6 (2CH2, 1-C), 71.3 (CH, 2-C), 115.0 (CH2, 7-C), 138.3 (CH, 6-C). C7H13ClO2 (148.63): calcd C 56.57, H 8.82, Cl 23.85, O 10.7; found: C 56.38, H 8.92, Cl 23.67.

4.1.2. (R)-Undec-1-en-6-ol (7). Chlorodiricyl 12 (1 g, 6.73 mmol) was dissolved in THF (10 mL) and 0.3 M solution of NaOH (3 g, 75 mmol, 1 equiv) in H2O (10 mL) was added. The mixture was stirred at 25 °C for six days to ensure the complete conversion. Then, mixture was diluted with Et2O (5 mL) and phases were separated. The water layer was extracted with Et2O (10 mL) and combined organic extracts were dried over anhydrous Na2SO4, filtered and carefully concentrated in vacuo (300 mbar, 35 °C) to ca. 1/10-th of volume. The obtained THF solution (1.187 g) was subjected to FLC (52 g SiO2, 4 cm, ethyl acetate/hexanes=1/5) yielding pure chlorodiricyl 7 (727 mg, 96%) as a pale-yellow oil. C13H27NO (213.36): calcd C 73.18, H 12.76, N 6.56; found: C 73.23, H 12.70, N 6.52, O 7.55%. All other physico-chemical data are identical with those obtained for its racemate.

**Compound 5:** Rp=1.57 (ethyl acetate) and ethanolamine (4.343 g, 4.3 mL, 71.1 mmol, 15 equiv) in dry THF (20 mL) was refluxed at 80 °C under condenser over 40 h. The reaction mixture was cooled, diluted with Et2O (100 mL) and washed with satd aq NaHCO3 solution (50 mL). Separated water layer was extracted with Et2O (2×50 mL), combined organic extracts were dried over anhydrous Na2SO4, filtered and concentrated in vacuo. The resulting oil (1.437 g) was subjected to TLC (58 g SiO2, 4×11 cm, ethyl acetate containing 2% aq NH4OH) yielding pure amino alcohol 5 (917 mg, 91%) as a pale-yellow oil.

4.1.5. (S)-Benzy1-(1-pentyl-5-ethyl)-amine (6). The solution of tosylate 14 (2 g, 6.17 mmol) and benzylamine (2 g, 2 mL, 18.5 mmol, 3 equiv) in dry THF (25 mL) was refluxed at 85 °C under condenser over eight days. The reaction mixture was cooled, diluted with H2O (50 mL) and extracted with Et2O (2×20 mL). Combined organic extracts were dried over anhydrous Na2SO4, filtered and concentrated in vacuo. The resulting oil (2.136 g) was subjected to TLC (85 g SiO2, 6×12 cm, gradient elution: ethyl acetate/hexanes=1/10 containing 1% aq NH4OH–ethyl acetate/hexanes=1/1 containing 1% aq NH4OH) yielding pure aminooalcohol 6 (1.3 g, 81%) as a pale-yellow oil.

**Compound 6:** Rp=0.45 (ethyl acetate/hexanes=1/4); [z]D+1.5 (c 0.74, CH2Cl2); IR (film): ν/cm−1=698, 733, 910, 993, 1028, 1073, 1099, 1147, 1377, 1455, 1495, 1641, 2857, 2928, 3390 (br); 1H NMR (300 MHz, CDCl3) of 0.89 (t, 3H, 5-Ha), 1.24–1.48 (m, 12H, 1-H, 2-H), 2.8–3.0 (m, 2H, 3-H, 4-H), 4.94–5.05 (m, 2H, 1-H, 1-H), 5.79 (tdd, 1H, J=6.6, 10.2, 16.8 Hz, 6-H); 13C NMR (75 MHz, CDCl3): 14.1 (3CH, 5-C), 22.7, 24.9, 25.3, 32.1, 33.3, 33.8, 4.0 (all CH2, 1′-C, 2′-C, 3′-C, 4′-C, 4′-C), 51.1 (CH2, 2′-Ph), 56.8 (CH, 1′-C), 114.4 (CH2, 2′-C, 6′-C, 138.3 (CH, 4′-C). C13H27NO (213.36): calcd C 73.18, H 12.76, N 6.52, O 7.55%.

4.1.6. (-)-Calvin (1) and (+)-2-epicadin (2). PdCl2 (83 mg, 0.469 mmol, 0.1 equiv), CuCl2 (130 mg, 0.938 mmol, 0.2 equiv) and activated 3 Å molecular sieves (1 g) were placed in a dry, argon filled flask containing stirring bar and equipped with side-arm stopcock. Balloon with CO2 mixture (ca. 1:1) was attached and the gases were exchanged by repeated evacuation (20 Torr) and filling (three times). Solids were left to stand as such for 20 min and then anhydrous dioxane (80 mL) was added. The brown suspension was stirred under CO2 atmosphere for 1 h at 25 °C. The solution of aminooalcohol 5 (1 g, 4.69 mmol) in anhydrous dioxane (14 mL) was then added and the resulting deep-green reaction mixture was stirred under CO2 balloon for 45 h at 50 °C. After evaporation of volatiles in vacuo, ethyl acetate (150 mL) was added and the mixture was washed with 3% aq NH4OH solution (150 mL). The water layer was extracted with ethyl acetate (3×100 mL), combined organic extracts were dried over anhydrous Na2SO4, filtered and concentrated in vacuo. The resulting oil (1.299 g) was subjected to TLC (52 g SiO2, 4×10 cm, ethyl acetate/hexanes=1/4) yielding pure tosylate 14 (8.508 g, 73%) as a pale-yellow oil.

**Compound 14:** Rp=0.59 (ethyl acetate/hexanes=1/4); [z]D+4.3 (c 1, CH2Cl2), C18H20O5S (324.48): calcd C 66.63, H 8.70, O 14.79, S 9.88; found: C 66.70, H 8.44, O 14.92, S 10.06%.
triethylamine–14/86/1) yielding (+)-calvine 1 (177 mg, 16%) as colourless foam and (+)-2-epicalvine 2 (412 mg, 37%) as a pale-yellow oil. (+)-Calvine 1 was subsequently crystallized from heptane to obtain single-crystal suitable for X-ray analysis.

**Compound 1:** mp=59 °C; [α]D218 +16.6 (c 0.451, CH2Cl2) {Ref. 6 [α]D218 +18 (c 0.66, CH2Cl2), Ref. 7 [α]D218 +18.3 (c 0.35, CH2Cl2). All physico-chemical data were in perfect accordance with those previously published, see Ref. 6, 7.

**Compound 2:** [α]D218 +8.7 (c 0.584, CH2Cl2) {Ref. 6 [α]D218 +8 (c 0.58, CH2Cl2). All physico-chemical data were in perfect accordance with those previously published, see Ref. 6.

4.1.7. (2S,6S)-(6-Pentylpiperidin-2-yl)acetic acid methyl ester (3)

To a dry, argon filled flask containing stirring bar and equipped with a short-path coupling, 5% aq Mg(OH)2 was added. Then 1,3-dioxane (200 mL) was added. Combined organic extracts (632 mg) was subjected to FLC (38 g SiO2, 418°C). The deep-brown suspension was stirred under CO/O2 atmosphere for 15 min at 25 °C. Then anhydrous MeOH (30 mL) was added. In the course of overnight, solids were stirred for 10 min and then anhydrous MeOH (30 mL) was added. The mixture was diluted with CH2Cl2 (5 mL), filtered through Celite pad and rinsed with CH2Cl2 (2×15 mL). The residue after evaporation (1.362 g) was redissolved in ethyl acetate (100 mL), washed with 2% aq NH4OH solution (2×70 mL) and brine (50 mL). The water phase was extracted with ethyl acetate (100 mL). Combined organic extracts were dried over anhydrous Na2SO4, filtered and concentrated in vacuo. The resulting oil (1.225 g) was subjected to FLC (30 g SiO2, 4×7 cm, diethylther/hexanes=4/1) yielding the mixture of mytylesters 15/16 (827 mg, 68%) as a pale-yellow oil. This was dissolved in MeOH (45 mL) and Pd(OH)2 (73 mg, 0.521 mmol, 0.2 equiv) was added. The resulting suspension was stirred under H2 atmosphere (balloon) for 24 h at 25 °C. The reaction mixture was filtered through Celite pad and rinsed with CH2Cl2 (3×10 mL). The residue after evaporation (632 mg) was subjected to FLC (38 g SiO2, 4×8 cm, 2-propanol/chloroform=1/25 containing 1% aq NH4OH) yielding 3 (127 mg, 21%) and 4 (350 mg, 60%) as pale-yellow oils.

**Compound 3:** Rf=0.8 (2-propanol/chloroform=1/25 containing 1% aq NH4OH); [α]D218 +22 (c0.45, CH2Cl2) {Ref. 6 [α]D218 +23 (c0.52, CHCl3)}.

**Compound 4:** Rf=0.63 (2-propanol/chloroform=1/25 containing 1% aq NH4OH); [α]D218 +5.5 (c 0.58, CHCl3) {Ref. 6 [α]D218 +5 (c 0.53, CHCl3)}. All physico-chemical data were in perfect accordance with those previously published, see Ref. 6.

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**Supplementary data**

1H and 13C NMR spectra of 5-7, 12-14: X-ray structure refinement details of 1. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.01.106.

**References and notes**


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