Synthesis and Absolute Configuration of Hyperaspine, an Alkaloid of the Ladybird *Hyperaspis campestris*

Cédric Dooms,[a] Pascal Laurent,[a] Désiré Daloze,[a] Jacques Pasteels,[b] Oldrich Nedved,[c] and Jean-Claude Braekman*[a]

Keywords: Natural products / Alkaloids / Racemic synthesis / Ladybird / *Hyperaspis campestris* / Coccinellidae

Hyperaspine (**1a**), isolated from the European Coccinellidae *Hyperaspis campestris*, isthe first representative of a new type of ladybird alkaloids having a 3-oxaquinolizidine skeleton. A new total synthesis of (±)-hyperaspine starting from protected piperidin-4-one has been achieved. The absolute configuration of the natural alkaloid was unequivocally established to be 3*S*,4a*S*,6*R*,8*S* by HPLC analyses on a chiral column.

Introduction

In 2001, we reported the isolation of hyperaspine (**1a)** from the European Coccinellidae *Hyperaspis campestris*.[1] Compound **1a** was the first representative of a new type of ladybird alkaloids having a 3-oxaquinolizidine skeleton. The structure of hyperaspine, except its absolute configuration, was established on the basis of its spectral properties on a 400 μg sample.[1] Recently, Ma and Zhu reported the stereospecific total synthesis of (–)-8-epihyperaspine[2] and of (3*S*,4a*S*,6*R*,8*S*)-hyperaspine,[3] confirming the structure of **1a**. Herein we wish to report a new total synthesis of (±)-hyperaspine starting from protected piperidin-4-one as well as the determination of the absolute configuration of the natural alkaloid.

Results and Discussion

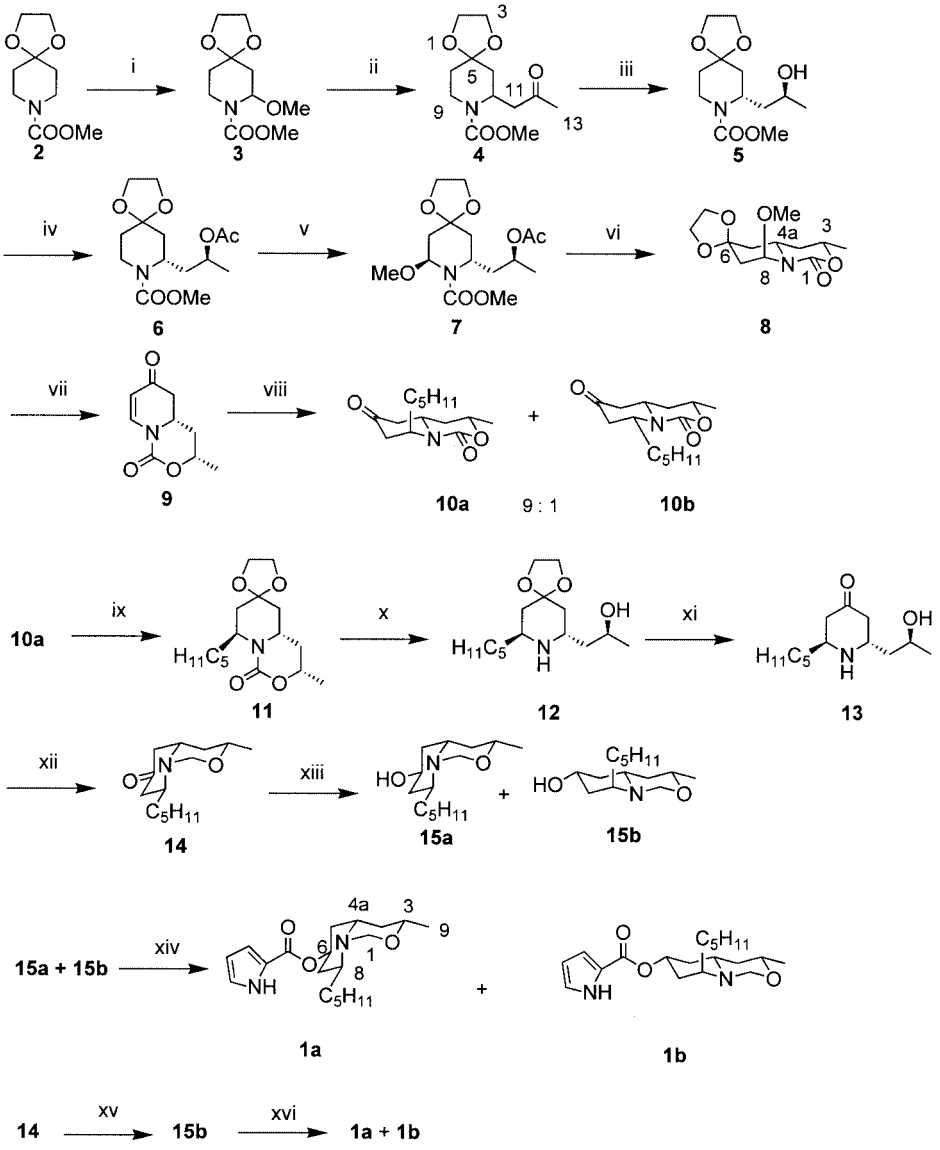
As outlined in Scheme 1, our synthesis started from the protected piperidin-4-one **2** which was submitted to an anodic oxidation–nucleophilic substitution sequence[4] to afford the expected 2-substituted piperidine **4** in 75% yield. Reduction of the ketone in **4** with $LiAlH(OtBu)_3$ furnished the secondary alcohol **5** that was readily protected as the corresponding acetate **6**. Such stereoselective reductions are well described and are known to generate the *syn* derivative as major compound.[4] This relative configuration was confirmed later at the level of the cyclic derivatives **8** and **10a**.

A second anodic oxidation carried out on **6** led regio- and stereoselectively to the 6-methoxylated piperidine **7** in 74% yield.[4] Basic treatment of **7** removed the acetate group and the resulting alcohol spontaneously cyclized to provide the cyclic carbamate **8** in 57% yield. In this compound, the methoxy group is axial since 8-H appears as a triplet at δ = 5.48 ppm (J = 2.7 Hz). Moreover, 4a-H (δ = 3.64, $J_{4a,4ax} = J_{4a,5ax}$ = 12.2 Hz and $J_{4a,4eq} = J_{4a,5eq}$ = 2.4 Hz) and 3-H (δ = 4.46, $J_{3,4ax}$ = 13.3 Hz and $J_{3,4eq}$ = 2.7 Hz) are also axial.

At this stage of the synthesis, our efforts were directed toward the introduction of a pentyl chain at C-8. To this end, we first tried to apply the method of Brown et al.[5] Indeed, these authors had shown that 2-(phenylsulphonyl)-piperidines, on reaction with various alkyl nucleophiles, gave the substitution products in good yields and with excellent stereoselectivity. But, when compound **8** was treated with *p*-toluenesulfinic acid to generate the corresponding sulfone, we found that elimination of MeOH, accompanied with conversion of the ketal into the ketone group, took place, leading to the enone **9**. Furthermore, the latter could be obtained in almost quantitative yield on treatment of **8** with *p*TsOH.

Compound **9** was then subjected to a diastereoselective Michael addition with pentylmagnesium bromide in the presence of copper(I) cyanide.[6,7] This provided a 9:1 mixture of bicyclic compounds **10** in 79% yield. The two diastereoisomers **10a** and **10b** could easily be separated by column chromatography on silica gel. The relative configurations at C-3, C-4a and C-8 in **10a** were fully established by NOESY experiments. Most noteworthy were the correlations observed between 3-H, 4a-H, and 10-H_2 (pentyl side chain, α-methylene group).

[a] Department of Organic Chemistry, CP 160/06, Faculty of Sciences, Free University of Brussels,
50 Avenue F. D. Roosevelt, 1050 Brussels, Belgium
Fax: +32-2-650-2961
E-mail: braekman@ulb.ac.be
[b] Laboratory of Cellular and Animal Biology, CP 160/12, Faculty of Sciences, Free University of Brussels,
50 Avenue F. D. Roosevelt, 1050 Brussels, Belgium
[c] Faculty of Biological Sciences, University of South Bohemia, Branisovská 31, 370 05 Ceské Budejovice, Czech Republic



Scheme 1. Reagents: (i) MeOH, K_2CO_3, Et_4NOTs, 6.3 F/mol, room temp., 98%; (ii) CH_2=C(OTMS)CH_3, TMSOTf, CH_2Cl_2, –78 °C, 76%; (iii) LiAlH(O-*t*Bu)$_3$, THF, room temp., 91%; (iv) Ac_2O, pyr., room temp., 82%; (v) MeOH, K_2CO_3, Et_4NOTs, 16 F/mol, room temp., 74%; (vi) K_2CO_3, MeOH, room temp., 57%; (vii) *p*TsOH, CH_2Cl_2, room temp., quant.; (viii) $C_5H_{11}MgBr$, CuCN, THF, –78 °C, 79%; (ix) TMSOCH$_2$CH$_2$OTMS, TMSOTf, CH_2Cl_2, room temp., 90%; (x) KOH, EtOH, reflux, 87%; (xi) MeOH, HCl, 60 °C, 88%; (xii) HCHO, MeOH, room temp., 94%; (xiii) $NaBH_4$, MeOH, room temp., 80%; (xiv) pyrrole-2-carboxylic anhydride, DMAP, CH_2Cl_2, room temp., 76%; (xv) LS-Selectride, THF, –78 °C, 90%; (xvi) pyrrole-2-carboxylic acid, DEAD, PPh_3, C_6H_6, room temp., 33%.

Before to hydrolyse the cyclic carbamate present in **10a**, it was necessary to transform the ketone group into a ketal. Indeed, all the attempts to perform this hydrolysis using basic conditions always furnished complex mixtures of degradation products, while acid hydrolysis conditions let the carbamate unchanged. Thus, conversion of the ketone group of derivative **10a** into the corresponding 1,3-dioxolane followed by basic treatment (EtOH, KOH, reflux) and acid deprotection (MeOH, HCl) provided the 2,6-*trans* disubstituted piperidin-4-one **13**. Treatment of **13** with formaldehyde produced the expected 3-oxaquinolizidine derivative **14** in 94% yield. This compound exists in a *cis*-fused ring conformation as deduced from the lack of Bohlmann bands in the IR spectrum and from the value of the J_{gem} (10.2 Hz) between the two diastereotopic protons at C-1.[8,9] Furthermore, the relative configuration of this molecule was fully proved by NOESY experiments. Most noteworthy were the correlations observed between 1-H_{ax}, 3-H_{ax} and 4a-H, between 4-H_{ax} and 8-H_{ax}, between 8-H_{ax} and H_3C-9, and between 1-H_{eq} and 8-H_{ax}.

The spectroscopic properties (^{1}H and ^{13}C NMR, MS and IR) of compound **14** were identical to those of the equivalent intermediate obtained by Zhu and Ma[3] on their synthesis of (3*S*,4a*S*,6*R*,8*S*)-hyperaspine. As reported by these authors, compound **14** could be stereoselectively transformed into the alcohol **15b** by reduction of the ketone group with LS-Selectride.[3] Interestingly, reaction of **15b** with pyrrole-2-carboxylic acid using Mitsunobu conditions

did not provide, despite several trials, the corresponding ester **1a** in 66% yield as reported by Zhu and Ma,[3] but a 1:1 mixture of **1a** and **1b** in 33% yield. Such partial retention of configuration in the Mitsunobu reaction has already been reported and attributed to steric congestion at the reaction center.[10] When reduction of the ketone group of **14** was performed with $NaBH_4$ rather than with LS-Selectride, a 1:1 mixture of the two epimeric alcohols **15a** and **15b** was obtained. These alcohols could not be separated. Nevertheless, analysis of the ^1H NMR spectrum of the mixture clearly indicated that **15a** exists preferentially in the *cis* conformation (J_{1gem} = 11.2 Hz) and **15b** (J_{1gem} = 8.4 Hz) in the *trans* conformation. This was also the case for the corresponding epimeric pyrrole-2-carboxylic esters **1a** (J_{1gem} = 11.0 Hz) and **1b** (J_{1gem} = 9.3 Hz) obtained by reaction with pyrrole-2-carboxylic anhydride in anhydrous dichloromethane. Contrary to the corresponding alcohols, **1a** and **1b** could be separated by silica gel column chromatography.

With a sample of racemic hyperaspine in hand, HPLC resolution of **1a** could be achieved successfully on a Chiralcel column. Co-injection with a synthetic sample of (+)-(3*S*,4a*S*,6*R*,8*S*)-hyperaspine, kindly supplied by Dr. Ma, indicated that the fastest-eluting enantiomer is the (+) enantiomer. Moreover, as natural hyperaspine isolated from 35 individuals of *Hyperaspis campestris* collected in the Czech Republic, proved to be chromatographically undistinguishable from the (+) enantiomer, this unequivocally established the absolute configuration of natural hyperaspine to be 3*S*,4a*S*,6*R*,8*S* as represented in formula **1a**.

It is interesting to mention that (+)-hyperaspine has the same absolute configuration at C-8 than the related ladybird alkaloids (+)-calvine and (+)-2-epicalvine[11] suggesting a possible biogenetic relationship between these alkaloids.

Experimental Section

General: EI-MS and HR-EIMS were performed with a Fisons VG Micromass Autospec instrument (70 eV). In all cases, peak intensities are expressed as % relative to the base peak. The ^1H NMR spectra were recorded in $CDCl_3$ at 300 MHz with a Bruker Avance TM-300 or at 600 MHz with a Varian Unity 600 instrument and are reported in ppm from internal TMS on the δ scale. Data are reported as follows: Chemical shift [multiplicity (s: singlet, d: doublet, dd: double doublet, ddd: double double doublet, t: triplet, dt: double triplet, tt: triple triplet, q: quartet, m: multiplet), coupling constants in Hertz, integration]. The ^{13}C NMR spectra were recorded in $CDCl_3$ at 75.4 MHz with a Bruker Avance TM-300 instrument. The IR spectra were recorded with a Bruker IFS 25 instrument as films on a NaCl disk. Thin layer chromatography analyses (TLC) were performed with 0.25 mm Polygram silica gel SILG/UV254 precoated plates (Macherey–Nagel). Chromatographies were performed on silica gel columns (MN Kieselgel 60, 0.04–0.063 mm) using the flash technique or on basic alumina (Macherey–Nagel Aluminiumoxid, basic, Activity 1). HPLC analyses were performed on a Waters LC module 1 apparatus equipped with a ChiralCel OJ column (Daicel) (25 cm, 0.46 cm, hexane/EtOH, 99:1, 1 mL/min.) and a Waters 996 Photodiode Array detector. Water absorbing (WA) filter papers were purchased from Macherey–Nagel (MN, 616 WA 1/4).

Methyl 7-Methoxy-1,4-dioxa-8-azaspiro[4.5]decane-8-carboxylate (3): A solution of **2** (645.4 mg, 3.21 mmol), K_2CO_3 (35 mg, 0.254 mmol) and Et_4NOTs (40 mg, 0.133 mmol) as support electrolyte in MeOH (30 mL) was placed in an electrolysis cell equipped with 4 carbon electrodes. The anodic methoxylation apparatus has been described previously.[4] After 6.3 F/mol had been consumed, the solvent was evaporated under reduced pressure. The residue was diluted in water and the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (hexane/AcOEt, 7:3) to afford **3** (725 mg, 3.14 mmol, 98%) as a colorless oil.EIMS: *m*/*z* (%) = 231 (6) [M^+], 216 (46), 200 (99), 188 (26), 172 (18), 156 (16), 145 (46), 129 (35), 114 (38), 99 (100), 86 (57). IR: $\tilde{\nu}$ = 2956, 1706, 1446, 1411, 1376, 1195, 1113, 1085, 987, 946, 821, 769 cm^{-1}. ^1H NMR (300 MHz): δ = 1.59–2.10 (m, 4 H), 3.21 (m, 1 H), 3.28 (s, 3 H), 3.74 (s, 3 H), 3.88–4.01 (m, 4 H), 4.10 (m, 1 H), 5.49 (br. s, 1 H) ppm. ^{13}C NMR: δ = 34.5, 36.7, 39.4 (C-6, C-9, C-10), 53.1, 55.6 (2 OCH_3), 64.1, 65.4 (C-2, C-3), 82.8 (C-7), 106.6 (C-5), 156.2 (C=O) ppm.

Methyl 7-Acetonyl-1,4-dioxa-8-azaspiro[4.5]decane-8-carboxylate (4): To a solution of **3** (50 mg, 0.216 mmol) in anhydrous CH_2Cl_2 (2 mL) at –78 °C under nitrogen, were added 2-(trimethylsilyloxy) propene (56.3 mg, 0.433 mmol) and TMSOTf (19.2 mg, 0.086 mmol). This mixture was stirred at –78 °C for 1 h. After addition of a 1 M solution of $NaHCO_3$, the mixture was extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a solid residue that was purified by flash chromatography on a silica gel column (hexane/AcOEt, 6:4) to afford **4** (42.3 mg, 0.164 mmol, 76%) as a white solid. EIMS: *m*/*z* (%) = 257 (7) [M^+], 200 (52), 198 (51), 128 (11), 114 (30), 99 (100), 86 (22). IR: $\tilde{\nu}$ = 2957, 2886,1700, 1536, 1450, 1374, 1169, 947, 769, 543 cm^{-1}. ^1H NMR (300 MHz): δ = 1.65–1.87 (m, 4 H), 2.16 (s, 3 H), 2.63 (m, 1 H), 3.10 (m, 2 H), 3.69 (s, 3 H), 3.93 (m, 4 H), 4.10 (m, 1 H), 4.82 (m, 1 H) ppm. ^{13}C NMR: δ = 30.7, 34.7, 36.5, 38.2 (C-6, C-9, C-10, C-11), 45.1 (C-13), 47.6 (C-7), 53.0 (OCH_3), 64.2, 64.9 (C-2, C-3), 107.2 (C-5), 155.9 (C=O), 207.5 (C-12) ppm.

Methyl 7-(2-Hydroxypropyl)-1,4-dioxa-8-azaspiro[4.5]decane-8-carboxylate (5): To compound **4** (37.5 mg, 0.146 mmol) in 3 mL THF was added $LiAlH(O\text{-}tBu)_3$ (74.1 mg, 0.292 mmol) under nitrogen. The mixture was stirred at room temperature for two hours upon which water was added. The aqueous phase was then extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvents evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (hexane/AcOEt, 5:5) to afford **5** (34.6 mg, 0.133 mmol, 91%) as a colorless oil. EIMS: *m*/*z* (%) = 259 (6) [M^+], 200 (66), 128 (10), 114 (24), 99 (100), 59 (15). IR: $\tilde{\nu}$ = 3450, 2962, 1967, 1451, 1374, 1113, 945, 768 cm^{-1}. ^1H NMR (300 MHz): δ = 1.22 (d, *J* = 6.2 Hz, 3 H), 1.63–1.92 (m, 6 H), 3.14 (m, 1 H), 3.70 (s, 3 H), 3.83 (m, 1 H), 3.95 (m, 4 H), 4.07 (m, 1 H), 4.53 (m, 1 H) ppm. ^{13}C NMR: δ = 23.9 (C-13), 34.8, 37.6, 37.8, 40.9 (C-6, C-9, C-10, C-11), 49.3 (C-7), 53.1 (OCH_3), 64.2, 64.9 (C-2, C-3), 66.6 (C-12), 107.4 (C-5), 156.4 (C=O) ppm.

Methyl 7-(2-Acetoxypropyl)-1,4-dioxa-8-azaspiro[4.5]decane-8-carboxylate (6): Alcohol **5** (34.6 mg, 0.133 mmol) was dissolved in 2 mL of a 1:1 mixture of pyridine-acetic anhydride. After stirring for 24 h, 4 mL of ethanol were added and the solvent was removed under reduced pressure. The residue thus obtained was purified by flash chromatography on a silica gel column (hexane/AcOEt, 6:4) to afford **6** (32.9 mg, 0.109 mmol, 82%) as a colorless oil. EIMS:

m/*z* (%) = 301 (1) [M$^{+\cdot}$], 241 (17), 200 (64), 114 (20), 99 (100), 86 (14), 59 (14). IR: $\tilde{\nu}$ = 2957, 1699, 1455, 820, 768, 735, 610, 549 cm^{-1}. ^{1}H NMR (300 MHz): δ = 1.25 (d, J = 6.2 Hz, 3 H), 1.62–1.85 (m, 5 H), 2.02 (s, 3 H), 2.17 (m, 1 H), 3.12 (m, 1 H), 3.69 (s, 3 H), 3.95 (m, 4 H), 4.07 (m, 1 H), 4.46 (m, 1 H), 4.88 (m, 1 H) ppm. ^{13}C NMR: δ = 20.6, 21.6 (2 CH_3), 34.9, 37.0, 37.3, 37.5 (C-6, C-9, C-10, C-11), 48.6 (C-7), 52.9 OCH_3), 64.2, 65.0 (C-2, C-3), 69.6 (C-12), 107.3 (C-5), 156.0 (C=O), 171.0 (C=O) ppm.

Methyl 9-(2-Acetoxypropyl)-7-methoxy-1,4-dioxa-8-azaspiro[4.5]decane-8-carboxylate (7): A solution of **6** (92.3 mg, 0.301 mmol), Et_4NOTs (30 mg, 0.99 mmol) and K_2CO_3 (30 mg, 0.217 mmol) in methanol (30 mL) was submitted to an anodic oxidation as described previously. After 16 F/mol had been consumed, the solvent was evaporated under reduced pressure. Water was then added and the solution was extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (hexane/AcOEt, 5:5) to afford **7** (75 mg, 0.226 mmol, 74%) as a colorless oil. EIMS: *m*/*z* (%) = 331 (1) [M$^{+\cdot}$], 300 (53), 271 (25), 239 (10), 230 (25), 198 (81), 172 (31), 154 (75), 144 (100), 139 (25), 129 (42), 103 (21), 86 (50), 59 (11). IR: $\tilde{\nu}$ = 2954, 1734, 1701, 1444, 1372, 1245, 1113, 947, 772 cm^{-1}. ^{1}H NMR (600 MHz): δ = 1.25 (d, J = 6.1 Hz, 3 H, 13-H), 1.93–2.04 (m, 5 H, 6-H, 10-H, 11-H), 2.06 (s, 3 H, $OCOCH_3$), 2.25 (m, 1 H, 11-H), 3.31 (s, 3 H, OCH_3), 3.75 (s, 3 H, $COOCH_3$), 3.95 (m, 4 H, 2-H, 3-H), 4.37 (m, 1 H, 7-H), 4.98 (m, 1 H, 12-H), 5.58 (m, 1 H, 9-H) ppm. ^{13}C NMR: δ = 20.8, 21.5 (2 CH_3), 34.5, 34.8, 39.6 (C-6, C-10, C-11), 48.2 (C-7), 52.8 (C-9), 53.1 (OCH_3), 64.5, 65.4 (C-2, C-3), 68.8 (C-12), 83.4 (C-9), 106.1 (C-5), 156.6, 171.1 (2 C=O) ppm.

6-Ethylenedioxy-8-methoxy-3-methylperhydropyrido[1,2-*c*][1,3]-oxazin-1-one (8): Compound **7** (492 mg, 1.486 mmol) and K_2CO_3 (970 mg, 7.02 mmol) were dissolved in MeOH (10 mL). After stirring at room temperature for 36 h, the methanol was removed under reduced pressure and the crude extract thus obtained washed with H_2O and extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a residue that was purified by chromatography on a basic alumina column (hexane/AcOEt, 6:4) to afford **8** (219.7 mg, 0.855 mmol, 57%) as a white solid. HR-EIMS: *m*/*z* = 226.10712 [M$^{+\cdot}$ − $\cdot$$OCH_3$] calcd. for $C_{11}H_{16}NO_4$ 226.1079. EIMS: *m*/*z* (%) = 242 (10) [M$^{+\cdot}$], 226 (40), 140 (14), 129 (19), 111 (11), 96 (100), 86 (50), 54 (19). IR: $\tilde{\nu}$ = 2633, 1695, 1416, 1294, 1129, 1086, 945 cm^{-1}. ^{1}H NMR (300 MHz): δ = 1.4 (d, J = 6.2 Hz, 3 H, 9-H), 1.63 (br. q, J = 13.3 Hz, 1 H, 4-H_{ax}), 1.88 (dd, J = 13.6 Hz, 1.8, 1 H, 5-H_{eq}), 1.95 (dt, J = 13.3, 2.7 Hz, 1 H, 4-H_{eq}), 2.23 (ABX, J_{AB} = 15.1, J_{AX} = 2.9, J_{BX} = 2.7 Hz, 2 H, 7-H), 2.24 (t, J = 13.3 Hz, 1 H, 5-H_{ax}), 3.43 (s, 3 H, OCH_3), 3.64 (tt, J = 12.2, 2.4 Hz, 1 H, 4a-H), 3.79–3.97 (m, 4 H, 10-H, 11-H), 4.46 (m, 1 H, 3-H), 5.48 (t, J = 2.7 Hz, 1 H, 8-H) ppm. ^{13}C NMR: δ = 21.7 (C-9), 36.7, 39.8, 39.9 (C-4, C-5, C-7), 49.4 (C-4a), 56.5 (OCH_3), 63.7, 65.1 (C-10, C-11), 74.3 (C-8), 82.1 (C-3), 106.3 (C-6), 153.8 (C-1) ppm.

3-Methyl-3,4,4a,5-tetrahydropyrido[1,2-*c*][1,3]oxazin-1,6-dione (9): Compound **8** (14 mg, 0.054 mmol) was treated with *p*-toluenesulfonic acid (51.3 mg, 0.27 mmol) in CH_2Cl_2 (2 mL). After stirring for one hour at room temperature, water was added and the resulting mixture extracted with CH_2Cl_2. The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ and filtered through a WA filter paper. The solvent was then evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (hexane/AcOEt, 1:9) to afford **9** (9.7 mg, 0.054 mmol, 100%) as a colorless oil. HR-EIMS: *m*/*z* = 181.0812 [M$^{+\cdot}$] calcd. for $C_9H_{11}NO_3$ 181.0739. EIMS: *m*/*z* (%) = 181 (50) [M$^{+\cdot}$], 96 (100), 80 (10), 67 (41), 54 (15). IR: $\tilde{\nu}$ = 2923, 2357, 1712, 1668, 1603, 1422, 1317, 1274, 1123, 749 cm^{-1}. ^{1}H NMR (300 MHz): δ = 1.47 (d, J = 6.2 Hz, 3 H, 9-H), 1.88 (br. q, J = 11.6, 1 H, 4-H_{ax}), 2.23 (ddd, J = 14.0, 3.6, 1.9 Hz, 1 H, 4-H_{eq}), 2.51 (m, 2 H, 5-H), 4.14 (m, 1 H, 4a-H), 4.57 (m, 1 H, 3-H), 5.52 (d, J = 8.3 Hz, 1 H, 7-H), 8.01 (d, J = 8.3 Hz, 1 H, 8-H) ppm. ^{13}C NMR: δ = 21.1 (C-9), 35.9, 42.4, 53.7 (C-4, C-4a, C-5), 74.5 (C-3), 110.0 (C-7), 143.8 (C-8), 150.2 (C-1), 192.2 (C-6) ppm.

3-Methyl-8-pentylperhydropyrido[1,2-*c*][1,3]oxazin-1,6-dione (10): In a 25 mL three-necked flask were placed magnesium turnings (516 mg, 21.5 mmol) under nitrogen. A solution of 1-bromopentane (3.76 g, 21.5 mmol) in anhydrous THF (10 mL) was then slowly added. After all the halide was added, the mixture was refluxed for 2 h and added to a solution of CuCN (956 mg, 10.7 mmol) in dry THF (5 mL) at –78 °C. The resulting green mixture was further stirred for 30 min then a solution of **9** (971 mg, 5.37 mmol) in anhydrous THF (1 mL) was added. When the reaction was completed, a saturated solution of NH_4Cl was added and the mixture was extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (hexane/AcOEt, 5:5) to afford the two diastereoisomers **10a** and **10b** in a 9:1 ratio (1.074 mg, 4.24 mmol, 79%).

10a: HR-EIMS: *m*/*z* = 253.1686 [M$^{+\cdot}$] calcd. for $C_{14}H_{23}NO_3$ 253.1678. EIMS: *m*/*z* (%) = 253 (26) [M$^{+\cdot}$], 182 (100), 167 (10), 138 (43), 96 (45), 69 (12), 55 (16). IR: $\tilde{\nu}$ = 2928, 1684, 1428, 1343, 1313, 1276, 1229, 1121, 759 cm^{-1}. ^{1}H NMR (600 MHz): δ = 0.87 (t, J = 6.6 Hz, 3 H, 14-H), 1.27 (m, 6 H, 11-H, 12-H, 13-H), 1.39 (d, J = 6.2 Hz, 3 H, 9-H), 1.48 (m, 2 H, 10-H), 1.69 (br. q, J = 11.1, 1 H, 4-H_{ax}), 2.24 (m, 2 H, 4-H_{eq}, 5-H_{ax}), 2.34 (dt, J = 14.0, 2.0, 1 H, 7-H_{eq}), 2.49 (dt, J = 14.3, 2.1, 1 H, 5-H_{eq}), 2.75 (dd, J = 14.0, 6.0, 1 H, 7-H_{ax}), 3.79 (m, 1 H, 4a-H), 4.35 (m, 1 H, 3-H), 4.99 (m, 1 H, 8-H) ppm. ^{13}C NMR: δ = 13.9 (C-14), 20.7 (C-9), 22.4, 25.4, 31.3 (C-11, C-12, C-13), 31.8 (C-10), 37.6 (C-4), 45.1 (C-7), 47.8 (C-5), 48.8 (C-4a), 53.0 (C-8), 71.9 (C-3), 153.6 (C-1), 206.0 (C-6) ppm.

10b: ^{1}H NMR (300 MHz): δ = 0.86 (t, J = 6.8 Hz, 3 H), 1.26 (m, 6 H), 1.38 (d, J = 6.2 Hz, 3 H), 1.60 (q, J = 11.4 Hz, 1 H), 2.06 (m, 3 H), 2.42 (m, 1 H), 2.60 (m, 2 H), 2.88 (dd, J = 16.6, 5.7, 1 H), 4.07 (m, 1 H), 4.23 (m, 1 H), 4.48 (m, 1 H) ppm. ^{13}C NMR: δ = 13.8 (C-14), 21.1 (C-9), 22.3, 25.6, 31.3 (C-11, C-12, C-13), 36.1 (C-10), 36.3, 41.7, 44.5 (C-4, C-5, C-7), 49.6 (C-4a), 52.7 (C-8), 72.8 (C-3), 152.4 (C-1), 206.2 (C-6) ppm.

6-Ethylenedioxy-3-methyl-8-pentylperhydropyrido[1,2-*c*][1,3]oxazin-1-one (11): Compound **10a** (847.5 mg, 3.35 mmol) was dissolved in anhydrous CH_2Cl_2 (20 mL) then 1,2-bis(trimethylsilyloxy)ethane (4.07 g, 16.75 mmol) and TMSOTf (372 mg, 1.67 mmol) were added. After stirring overnight, water was added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (hexane/AcOEt, 6:4) to afford **11** (896.3 mg, 3.02 mmol, 90%) as a yellow oil. EIMS: *m*/*z* (%) = 297 (3) [M$^{+\cdot}$], 226 (57), 182 (10), 140 (10), 96 (100), 87 (18). IR: $\tilde{\nu}$ = 2926, 1684, 1428, 1377, 1275, 1224, 1121, 1071, 999, 946, 760, 566 cm^{-1}. ^{1}H NMR (300 MHz): δ = 0.73 (t, J = 6.6 Hz, 3 H), 1.14 (m, 6 H), 1.20 (d, J = 6.2 Hz, 3 H), 1.23–1.52 (m, 3 H), 1.61–1.79 (m, 4 H), 1.94 (ddd, J = 13.7, 5.8, 1.5, 1 H), 3.53 (m, 1 H), 3.82 (m, 4 H), 4.14 (m, 1 H), 4.54 (m, 1 H) ppm. ^{13}C NMR: δ = 14.1 (C-17), 20.8 (C-9), 22.6, 26.3, 31.4 (C-14, C-15, C-16), 31.7 (C-13),

35.8, 37.1, 42.4 (C-4, C-5, C-7), 47.2 (C-4a), 51.6 (C-8), 63.9, 64.9 (C-10, C-11), 71.4 (C-3), 106.6 (C-6), 153.8 (C-1) ppm.

7-(2-Hydroxypropyl)-9-pentyl-1,4-dioxa-8-azaspiro[4.5]decane (12): Compound **11** (54.4 mg, 0.183 mmol) was dissolved in an ethanolic KOH solution (5 mL, 2 M) and refluxed for 45 h. The solvent was removed under reduced pressure, water was then added and the resulting mixture was extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (AcOEt/MeOH, 95:5) to afford **12** (43.3 mg, 0.15 mmol, 87%) as a yellow oil. EIMS: *m*/*z* (%) = 271 (1) [M^+], 200 (44), 179 (10), 135 (20), 126 (44), 114 (100), 87 (59), 70 (18). IR: $\tilde{\nu}$ = 3448, 2928, 1645, 1069, 609 cm^{-1}. ^1H NMR (300 MHz): δ = 0.84 (t, J = 6.6 Hz, 3 H), 1.12 (d, J = 6.2 Hz, 3 H), 1.20–1.54 (m, 11 H), 1.65–1.88 (m, 3 H), 3.08 (m, 1 H), 3.30 (m, 1 H), 3.86–3.96 (m, 5 H) ppm. ^{13}C NMR: δ = 14.1 (C-18), 22.6 (C-13), 23.8, 25.9, 31.9 (C-15, C-16, C-17), 35.4 (C-14), 40.5, 40.8, 41.0 (C-6, C-10, C-11), 49.3, 52.8 (C-7, C-9), 64.1, 64.5 (C-2, C-3), 69.7 (C-12), 107.7 (C-5) ppm.

2-(2-Hydroxypropyl)-6-pentylpiperidin-4-one (13): To compound **12** (38 mg, 0.14 mmol), dissolved in a H_2O/MeOH (1:1, 3 mL) solution, were added a few drops of 3 N HCl. After stirring at 60 °C for 43 h, an aqueous solution of $NaHCO_3$ (1 M) was added. The aqueous phase was then extracted with CH_2Cl_2 and the combined organic layers filtered through a WA filter paper. The solvent was evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (AcOEt/MeOH, 95:5) to afford **13** (27.8 mg, 0.12 mmol, 88%) as a colorless oil. EIMS: *m*/*z* (%) = 227 (1) [M^+], 168 (19), 156 (100), 126 (27), 114 (14), 96 (12), 70 (11). IR: $\tilde{\nu}$ = 3299, 2928, 1709, 1461, 1372, 1165 cm^{-1}. ^1H NMR (300 MHz): δ = 0.87 (t, J = 6.6 Hz, 3 H), 1.15 (d, J = 6.2 Hz, 3 H), 1.28–1.56 (m, 10 H), 2.16 (m, 2 H), 2.47 (m, 2 H), 3.33 (m, 1 H), 3.55 (m, 1 H), 3.99 (m, 1 H) ppm. ^{13}C NMR: δ = 14.1 (C-14), 22.5 (C-9), 23.6, 25.7, 31.7 (C-11, C-12, C-13), 35.4 (C-10), 41.6 (C-7), 48.9, 49.4 (C-3, C-5), 53.0, 54.6 (C-2, C-6), 69.0 (C-8), 208.6 (C-4) ppm.

3-Methyl-8-pentylperhydropyrido[1,2-*c*][1,3]oxazin-6-one (14): To a solution of **13** (27.8 mg, 0.122 mmol) in MeOH (3 mL) was added formaldehyde (4.8 mg, 0.159 mmol). After stirring at room temperature for 2 h, the solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on a silica gel column (hexane/AcOEt, 4:6) to afford **14** (27.4 mg, 0.115 mmol, 94%) as a colorless oil. HR-EIMS: *m*/*z* = 168.1023 [$M^{+\cdot}$ − $\cdot C_5H_{11}$] calcd. for $C_9H_{14}NO_2$ 168.1024. EIMS: *m*/*z* (%) = 239 (1) [M^+], 212 (62), 182 (10), 175 (15), 168 (100), 138 (33), 126 (77), 110 (11), 96 (38), 82 (13), 69 (17), 55 (22). IR: $\tilde{\nu}$ = 2931, 2861, 1721, 1381, 1212, 1098, 998 cm^{-1}. ^1H NMR (600 MHz): δ = 0.87 (t, J = 6.6 Hz, 3 H, 14-H), 1.20 (d, J = 6.2 Hz, 3 H, 9-H), 1.27–1.62 (m, 10 H), 2.18 (ddd, J = 14.1, 4.6, 1.8 Hz, 1 H, 5-H_{eq}), 2.37 (ddd, J = 13.6, 8.9, 1.1 Hz, 1 H, 7-H_{ax}), 2.48 (ddd, J = 13.6, 4.1, 1.8 Hz, 1 H, 7-H_{eq}), 2.69 (ddd, J = 14.1, 6.2, 1.2 Hz, 1 H, 5-H_{ax}), 3.35 (m, 1 H, 8-H), 3.50 (m, 1 H, 4a-H), 3.65 (m, 1 H, 3-H), 4.31 (dd, J = 10.2 Hz, 1 H, 1-H_{ax}), 4.77 (d, J = 10.2 Hz, 1 H, 1-H_{eq}) ppm. ^{13}C NMR: δ = 14.1 (C-14), 21.7 (C-9), 22.7, 24.6, 30.8, 32.1 (C-10, C-11, C-12, C-13), 35.7 (C-4), 45.8 (C-7), 46.9 (C-5), 53.3 (C-8), 56.2 (C-4a), 73.5 (C-3), 81.4 (C-1), 209.0 (C-6) ppm.

6-Hydroxy-3-methyl-8-pentylperhydropyrido[1,2-*c*][1,3]oxazine (15a and 15b). Reduction with LS-Selectride: Ketone **14** (100 mg, 0.418 mmol) was dissolved in dry THF (2 mL) under nitrogen. LS-Selectride (125.2 mg, 0.54 mmol) was then added at –78 °C and this mixture was stirred at –78 °C for 1 h. After addition of MeOH, the solvent was removed under reduced pressure and the crude mixture thus obtained was purified by flash chromatography on a silica gel column (AcOEt/MeOH, 9:1) to afford **15b** (92.2 mg, 0.383 mmol, 90%) as a white solid.

Reduction with $NaBH_4$: To a suspension of $NaBH_4$ (199 mg, 5.23 mmol) in MeOH (2 mL) was added a solution of **14** (54.7 mg, 0.21 mmol) in MeOH (9 mL) and water (1.5 mL). This mixture was stirred for 2 h at room temperature. Water was then added and the mixture extracted with CH_2Cl_2. The combined organic layers were filtered through a WA filter paper and the solvent evaporated in vacuo to give a residue that was purified by flash chromatography on a silica gel column (AcOEt/MeOH, 95:5) to afford a 1:1 mixture of the two epimeric alcohols **15a** and **15b** in a 1:1 ratio (40.5 mg, 0.168 mmol, 80%).

15a: ^1H NMR (600 MHz): δ = 0.87 (t, J = 6.6 Hz, 3 H, 14-H), 1.20 (d, J = 6.1 Hz, 3 H, 9-H), 1.27–1.84 (m, 13 H), 2.02 (m, 1 H, 7-H_{eq}), 3.12 (m, 1 H, 8-H), 3.50 (m, 1 H, 4a-H), 3.65 (m, 1 H, 3-H), 3.88 (m, 1 H, 6-H), 4.27 (d, J = 11.2 Hz, 1 H, 1-H_{ax}), 4.85 (d, J = 11.2 Hz, 1 H, 1-H_{eq}) ppm. ^{13}C NMR: δ = 14.0 (C-14), 21.7 (C-9), 22.6, 26.7, 32.1, 37.6 (C-10, C-11, C-12, C-13), 39.8 (C-4), 39.9 (C-5), 40.8 (C-7), 49.1 (C-8), 56.2 (C-4a), 65.8 (C-6), 74.0 (C-3), 80.6 (C-1) ppm. These values were recorded from the NMR spectra of the mixture of **15a** and **15b**.

15b: EIMS: *m*/*z* (%) = 241 (2) [M^+], 170 (100), 140 (12), 96 (11). IR: $\tilde{\nu}$ = 3430, 2861, 1458, 1381, 1168, 1065, 825, 758 cm^{-1}. ^1H NMR (600 MHz): δ = 0.87 (t, J = 6.6 Hz, 3 H, 14-H), 1.19 (d, J = 6.0 Hz, 3 H, 9-H), 1.20–2.00 (m, 14 H), 2.84 (m, 1 H, 4a-H), 3.13 (m, 1 H, 8-H), 3.51 (m, 1 H, 3-H), 3.94 (m, 1 H, 6-H), 4.15 (d, J = 8.4 Hz, 1 H, 1-H_{ax}), 4.52 (d, J = 8.4 Hz, 1 H, 1-H_{eq}) ppm. ^{13}C NMR: δ = 14.0 (C-14), 21.7 (C-9), 22.6, 26.4, 26.7, 32.1 (C-10, C-11, C-12, C-13), 37.6 (C-7), 39.4 (C-4), 41.0 (C-5), 51.8 (C-4a), 53.6 (C-8), 64.3 (C-6), 73.6 (C-3), 83.1 (C-1) ppm.

(±)-Hyperaspine (1a) and (±)-6-Epihyperaspine (1b): Alcohol **15b** (63.7 mg, 0.264 mmol) was dissolved in benzene (8 mL), then PPh_3 (67.6 mg, 0.528 mmol), DEAD (91.9 mg, 0.528 mmol) and pyrrole-2-carboxylic acid (117 mg, 1.05 mmol) were added under nitrogen. After stirring at room temperature for 16 h, the solvent was removed under reduced pressure and the residue thus obtained was purified by flash chromatography on a silica gel column (hexane/acetone, 9:1) to afford a mixture of (±)-hyperaspine (**1a**) and (±)-6-epihyperaspine (**1b**) (29.1 mg, 0.087 mmol, 33%).

A 1:1 mixture of the alcohols **15a** and **15b** (40.5 mg, 0.168 mmol) was dissolved in dry CH_2Cl_2 (8 mL). Then DMAP (12.3 mg, 0.101 mmol) and pyrrole-2-carboxylic anhydride (376 mg, 1.68 mmol) were added under nitrogen. After stirringat room temperature for 48 h, the solvent was removed under reduced pressure to afford (±)-hyperaspine (**1a**)and (±)-6-epihyperaspine(**1b**) in a 1:1 ratio (43.2 mg, 0.129 mmol, 76%) that could be separated by chromatography on a silica gel column (hexane/acetone, 9:1). For the spectral properties of **1a** see ref.[1].

1b: HR-EIMS: *m*/*z* = 263.1413 [M^+ − C_5H_{11}] calcd. for $C_{14}H_{19}N_2O_3$ 263.1395. EIMS: *m*/*z* (%) = 335 (7) [$M^{+\cdot}$+ H^+], 263 (20), 152 (100). IR: $\tilde{\nu}$ = 3305, 2931, 2860, 1799, 1697, 1554, 1412, 1380, 1313, 1263, 1167, 1120, 1030, 984, 748, 606 cm^{-1}. ^1H NMR (600 MHz): δ = 0.88 (t, J = 6.6 Hz, 3 H, 14-H), 1.21 (d, J = 6.1 Hz, 3 H, 9-H), 1.30–1.84 (m, 11 H), 1.89 (m, 2 H, 7-H), 2.08 (m, 1 H, 4-H_{eq}), 3.03 (m, 1 H, 4a-H), 3.22 (m, 1 H, 8-H), 3.55 (m, 1 H, 3-H), 4.19 (d, J = 9.3 Hz, 1 H, 1-H_{ax}), 4.63 (d, J = 9.3 Hz, 1 H, 1-H_{eq}), 5.19 (m, 1 H), 6.27 (m, 1 H), 6.92 (m, 1 H), 6.96 (m, 1 H) ppm. ^{13}C NMR: δ = 14.1 (C-14), 21.9 (C-9), 22.7, 25.1, 32.3, 34.1 (C-10, C-11, C-12, C-13), 34.5 (C-7), 36.7 (C-5), 38.1 (C-4), 52.3 (C-8), 52.5 (C-4a), 68.0 (C-6), 73.8 (C-3), 82.7 (C-1), 110.6, 115.3, 123.0, 123.8 (C of the pyrrole ring), 160.7 (C=O) ppm.

Acknowledgments

C. D. would like to thank the Fonds pour la Formation à la Recherche dans l′Industrie et l'Agriculture (FRIA) for financial support. The authors would also like to thank Dr. M. Luhmer and Mrs. R. d′Orazio for the NMR spectra, Mr. M. Pamart for the mass spectra and Dr. D. Ma (Shanghai Institute of Organic Chemistry) for the kind supply of a sample of synthetic (+)-hyperaspine. The financial support of Entomed S.A. (http://www.entomed.com) is also gratefully acknowledged.

[1] B. Lebrun, J. C. Braekman, D. Daloze, P. Kalushkov, J. M. Pasteels, *Tetrahedron Lett.* **2001**, *42*, 4621–4623.
[2] D. Ma, W. Zhu, *Tetrahedron Lett.* **2003**, *44*, 8609–8612.
[3] W. Zhu, D. Ma, *Org. Lett.* **2003**, *5*, 5063–5066.
[4] F. Driessens, C. Hootelé, *Can. J. Chem.* **1991**, *69*, 211–217.
[5] D. S. Brown, P. Charreau, T. Hansson, S. V. Ley, *Tetrahedron* **1991**, *47*, 1311–1328.
[6] B. H. Lipshutz, R. S. Wilhem, J. A. Kozlowski, *J. Org. Chem.* **1984**, *49*, 3938–3942.
[7] D. L. Comins, H. Hong, *J. Am. Chem. Soc.* **1993**, *115*, 8851–8852.
[8] F. Halin, P. Slosse, C. Hootelé, *Tetrahedron* **1985**, *41*, 2891–2897.
[9] P. Merlin, J. C. Braekman, D. Daloze, *Tetrahedron Lett.* **1988**, *29*, 1691–1694.
[10] C. Ahn, P. DeShong, *J. Org. Chem.* **2002**, *67*, 1754–1759.
[11] P. Laurent, J. C. Braekman, D. Daloze, *Eur. J. Org. Chem.* **2000**, 2057–2062.

Received: November 10, 2004

