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ALKALOIDS FROM SEEDS OF *LUPINUS VARIUS* AND *L. HARTWEGII*

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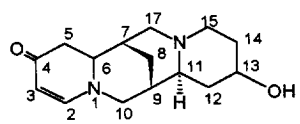
Key Word Index—*Lupinus varius*; *L. hartwegii*; Leguminosae; seeds; quinolizidine alkaloids; lupin alkaloids; (−)-13β-hydroxymultiflorine; (+)-2β-hydroxyaphylline; (+)-13α-hydroxyaphyllidine.

Abstract—A new lupin alkaloid, (−)-13β-hydroxymultiflorine, was isolated from an ethanol extract of the seeds of *Lupinus varius*, together with 13 known lupin alkaloids. In addition, two new lupin alkaloids, (+)-2β-hydroxyaphylline and (+)-13α-hydroxyaphyllidine, were isolated from seeds of *L. hartwegii*, together with another 11 known lupin alkaloids. The identification of all compounds was established by spectral analysis.

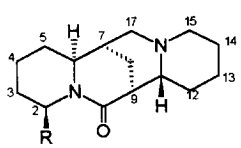
INTRODUCTION

Lupinus varius L. ssp. *orientalis* Franco et Silva (= *L. digitatus* Forssk, *L. pilosus* L., *L. hispanicus* and *L. microanthus*) is an annual herb, growing in the Mediterranean region [1, 2]. Previous work on its basic constituents indicated the presence of four alkaloids [3–8]. *Lupinus hartwegii* (= *L. mexicanus*) is an annual herb grown in gardens as a winter ornamental [9]. Earlier investigations on its basic constituents reported the isolation of eight lupin alkaloids [9–12]. In a recent study, 13 alkaloids from *L. hartwegii* and eight from *L. varius* were detected by GC-mass spectrometry [2].

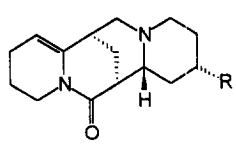
This report deals with the isolation and structural elucidation of the new lupin alkaloid, (−)-13β-hydroxymultiflorine (**1**), together with 13 known lupin alkaloids, nine of which are isolated for the first time, *viz.*, (−)-13α-hydroxymultiflorine (**4**), (+)-tetrahydrorhombifoline, (−)-13α-tigloyloxymultiflorine, (−)-11,12-seco-12,13-didehydromultiflorine, (+)-lupanine, (−)-albine, (+)-ammodendrine, (−)-Δ^5-dehydromultiflorine and (−)-multiflorine *N*-oxide, from viable seeds of *L. varius*. The constituents of the seeds of *L. hartwegii* were also examined and two new alkaloids, (+)-2β-hydroxyaphylline (**2**) and (+)-13α-hydroxyaphyllidine (**3**) were identified. In addition, 11 lupin alkaloids were isolated, five of them for the first time from this species, *viz.*, (+)-3β-hydroxylupanine, (+)-Δ^5-dehydrolupanine, (−)-11,12-seco-12,13-didehydromultiflorine, (+)-aphyllidine (**6**) and (−)-multiflorine.



1 OH$_{(eq)}$
4 OH$_{(ax)}$



2 R = OH
5 R = H



3 R = OH
6 R = H

RESULTS AND DISCUSSION

From a 75% ethanol extract of the seeds of *L. varius*, 13β-hydroxymultiflorine (**1**) was isolated in a yield of 0.007% of fresh weight by silica gel chromatography and preparative HPLC. HR-mass and DEPT spectra indicated the molecular formula $C_{15}H_{22}N_2O_2$. The presence of a hydroxyl group was indicated by the fragments at *m/z* 245 and 244 in the EI-mass spectrum, corresponding with $[M-OH]^+$ and $[M-H_2O]^+$, respectively, and from the IR band at 3300 cm^{-1}. The UV data of **1** (λ_{max} 327 nm) suggests the presence of a γ-pyridone ring [13, 14].

In the ^1H NMR spectrum of **1**, the most downfield

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protons resonated at δ 6.93 (1H, *d*, J = 7.7, H-2) and δ 4.94 (1H, *d*, J = 7.7, H-3) and this was supported by the downfield methine ^{13}C NMR signals at δ 155.8 and 98.9 for C-2 and C-3, respectively [13]. The IR spectrum of **1**, also showed a band at 1630 cm^{-1} for a conjugated carbonyl group (C=C absorption appeared at 1580 cm^{-1}). This was confirmed by the ^{13}C NMR signal at δ 192.3 [13]. The foregoing results, indicated that **1** is a multiflorine-type alkaloid having OH substitution and close inspection of the mass spectrum showed its similarity to that of 13α-hydroxymultiflorine (**4**) [13, 14].

The chemical shifts of carbon atoms forming rings A, B and C of **1** are similar to those of 13α-hydroxymultiflorine (**4**) [13]. However, the ^{13}C NMR spectrum of **1** showed marked differences in the chemical shift for ring D carbons compared with 13α-hydroxymultiflorine (**4**), especially the chemical shift for C-13 at δ 69.2 (*d*), suggesting that the hydroxyl group in **1** has an equatorial orientation [15, 16]. Moreover, in the ^{1}H NMR spectrum of **1**, the downfield shifted carbinol proton resonated at δ 3.87 (1H, *m*, width 38 Hz, H-13$_{ax}$). The multiplicity, magnitude of coupling, signal width and chemical shift of this proton confirmed that it is axially-oriented; hence, the hydroxyl group is equatorially oriented [13, 17].

Further evidence for the axial orientation of H-13 was shown by the NOE spectra of **1**, where 1-3 diaxial relationships were observed through cross-peaks between H-13$_{ax}$ and both H-11 and H-15$_{ax}$. These data established unequivocally that the skeleton of **1** is 13β-hydroxymultiflorine and that the secondary OH group in ring D has the equatorial β-configuration.

The chemical shift of bridge C-8 in the ^{13}C NMR of **1** indicated a boat-chair conformation of rings C and D; this was supported by the presence of a cross-peak between the C-9 and C-11 protons in the ^{1}H–^{1}H COSY spectrum [15–18].

It is pertinent to mention that the occurrence of 13β-hydroxymultiflorine and its 13α-isomer is reported for the first time in a quinolizidine alkaloid-accumulating species.

Two new lupin alkaloids, (+)-2β-hydroxyaphylline (**2**), (+)-13α-hydroxyaphyllidine (**3**) were also isolated from the basic chloroform extract of the seeds of *L. hartwegii* by silica gel chromatography. The first new alkaloid, (**2**) accounted for 0.0005% of the fresh weight. The IR spectrum revealed the presence of an intense band at 3375 cm^{-1} due to the hydroxyl group and this was supported by peaks assigned to [M—OH]$^+$ and [M—H$_2$O]$^+$ in the mass spectrum. The IR spectrum of **2** also showed a lactam carbonyl band at 1640 cm^{-1} and this was confirmed by a signal at δ 172.3 in the ^{13}C NMR spectrum. The HR-mass and DEPT spectra (Table 1) of **2** indicated the molecular formula $C_{15}H_{24}N_2O_2$.

Preliminary analysis of the spectral data of **2** indicated its close relation to aphylline (**5**). In the ^{13}C NMR spectrum of **2** the chemical shifts of carbon atoms forming rings B, C and D were coincident with those of **5**. The hydroxyl group in **2** was placed at position 2, because the signal corresponding with the carbinol carbon at δ 72.1 (*d*, C-2) was in agreement with those reported [19, 20]. Further evidence for this position was obtained from homonuclear spin decoupling experiments and ^{1}H–^{1}H COSY, where H-2$_{eq}$ collapsed to a singlet upon saturation and was coupled, with the overlapping multiplets at δ 1.67/1.61 assigned to H-3$_{eq}$ and H-3$_{ax}$, respectively.

Table 1. ^{13}C NMR data of compounds **2**, **3**, **5**, and **6**

C	**2**	**5**	**3**	**6**
2	72.1*d*	41.1*t*	40.7*t*	40.1*t*
3	30.3*t*	24.3*t*	21.1*t*	21.3*t*
4	25.0*t*	23.7*t*	21.4*t*	21.1*t*
5	28.1*t*	28.3*t*	101.8*d*	102.6*d*
6	58.3*d*	59.1*d*	138.6*s*	139.2*s*
7	31.7*d*	32.0*d*	35.2*d*	34.6*d*
8	22.2*t*	22.3*t*	21.6*t*	21.4*t*
9	43.3*d*	42.8*d*	43.6*d*	44.0*d*
10	172.3*s*	172.2*s*	171.2*s*	171.0*s*
11	59.0*d*	59.3*d*	52.6*d*	59.1*d*
12	23.9*t*	22.6*t*	29.8*t*	23.6*t*
13	25.0*t*	24.9*t*	64.1*d*	25.0*t*
14	19.1*t*	19.2*t*	26.7*t*	19.2*t*
15	54.1*t*	54.4*t*	48.6*t*	54.1*t*
17	53.3*t*	53.1*t*	53.7*t*	53.0*t*

Concerning the stereochemistry of the hydroxyl group, the ^{1}H NMR spectrum of **2** showed a downfield-shifted carbinol proton at δ 6.13 (1H, *dd*, J = 2.9, 1.8 Hz, H-2$_{eq}$) [19, 20]. The small coupling constants between H-2 and both H-3 protons (no diaxial coupling) indicated its equatorial orientation and, hence, the hydroxyl group must be axial. The β-configuration of the hydroxyl group was established by comparison of the NMR data of **2** with those of a series of lupin alkaloids [19, 20] and; the compound is thus 2β-hydroxyaphylline.

The second new alkaloid (+)-13α-hydroxyaphyllidine (**3**) accounted for 0.001% of the fresh weight of *L. hartwegii* seeds. The IR spectrum showed a band at 3270 cm^{-1} (OH), which was confirmed by inspection of other spectral data (see Experimental). The IR spectrum of **3** also showed C=C absorption at 1560 cm^{-1} and a lactam carbonyl at 1635 cm^{-1}. The latter was confirmed by the presence of a signal at δ 171.2 in the ^{13}C NMR spectrum. HR-mass and DEPT ^{13}C NMR spectra of **3** indicated the molecular formula $C_{15}H_{22}N_2O_2$. The UV spectrum showed (λ_{max} 239 nm) indicating a vinyl amide group, as found in aphyllidine (**6**) [20]. From the above data, it was concluded that **3** is an aphyllidine type alkaloid having OH substitution.

The chemical shifts of carbon atoms forming rings A, B and C of **3** were similar to those of aphyllidine (**6**), except those for ring D (Table 1) which can be explained by assuming that there is hydroxyl sub-

stitution in it. This hydroxyl group was located at C-13, having an α-axial orientation by comparison of the ^{13}C NMR signal at δ 64.1 (*d*, C-13) with those reported [15, 16, 21]. This was also established from the ^1H NMR spectrum of **1**, where the H-13 signal appeared at δ 4.11 (1H, *t*, *J* = 2.7, H-13β). The small coupling constant, multiplicity and chemical shift of H-13 confirmed its β-equatorial nature [17, 18, 22]. The NOE spectral data of **3** further confirmed this conclusion, where irradiation at H-13 gave negative effects on H-11 and on both the two protons at C-15. These data established unambiguously that the skeleton of **3** is 13α-hydroxyaphyllidine.

The complete assignments of the protons and carbons of the above mentioned compounds were confirmed by ^1H–^1H COSY and ^{13}C–^1H COSY. All the other known alkaloids were identified by comparison of their physical and spectral parameters with published data and available authentic samples [3–12, 13, 14, 23].

EXPERIMENTAL

General. Mps: uncorr. IR: thin films of KBr or $CHCl_3$. High and low resolution EI-MS: 70 eV. ^1H and ^{13}C NMR; 500 and 125 Hz, respectively, with TMS as int. standard in CD_3OD and $CDCl_3$. TLC: silica gel (Kieselgel 60, F 254) in CH_2Cl_2–MeOH–28% NH_4OH (90:9:1 and 43:6:1). Prep. HPLC: LiChrosorb Si-60 5 μm (φ4.6 × 250 mm) column with 30% MeOH in Et_2O–5% NH_4OH. Analytical HPLC and GC: as described in ref. [24].

Plant materials. Seeds of *L. varius* were collected from plants growing in the Sinai region near El Arish, Egypt, in April 1992. Seeds of *L. hartwegii* were supplied by Prof. N. El-Keltawy (Dept. of Horticulture, Faculty of Agriculture, Assiut University, Assiut, Egypt). Both sources of seeds were cultivated at the Medicinal Plant Experimental Station at Al-Azhar University, Assiut, in October 1992 and collected in April 1993. Species were identified by Prof. A. Fayed (Dept. of Systematic Botany and Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt).

Extraction and isolation of L. varius. The total alkaloid fr. from 75% EtOH extracts of air-dried finely ground seeds (1 kg) was obtained in yield of 1.9% of the fr. wt using the method described in refs [13, 14, 18]. The mixt. of bases (19 g) was chromatographed on a silica gel column (Merck, type 60, 230–400 mesh, 1 kg, 7 × 150 cm) and gradient elution using MeOH in $CHCl_3$–28% NH_4OH to yield the following alkaloids.

(+)-*Tetrahydrorhombifoline.* Colourless oil (9 mg). $[\alpha]_D^{25}$ +83° (EtOH; *c* 0.09) eluted by 1% MeOH in CH_2Cl_2. (−)-13α-Tigloyloxymultiflorine and (−)-11,12-*seco*-12,13-didehydromultiflorine were obtained as a mixt. (110 mg). This was rechromatographed on another silica gel column using cyclohexane–diethylamine as eluting solvent.

(−)-13α-*Tigloyloxymultiflorine.* Oil (14 mg). $[\alpha]_D^{25}$ −289° (EtOH; *c* 0.014) eluted by 8% diethylamine in cyclohexane.

(−)-11,12-Seco-12,13-*didehydromultiflorine.* Oil (32 mg). $[\alpha]_D^{25}$ −532° (MeOH; *c* 0.1) eluted by 13% diethylamine in cyclohexane.

(+)-*Lupanine.* Yellow oil (79 mg). $[\alpha]_D^{25}$ +52° (MeOH; *c* 0.1) eluted by 4% MeOH in CH_2Cl_2 from the main column.

(−)-*Multiflorine.* Oil (220 mg). $[\alpha]_D^{25}$ −229° (CH_2Cl_2; *c* 0.1) eluted by 5% MeOH in CH_2Cl_2.

(−)-*Albine.* Yellow oil (80 mg). $[\alpha]_D^{25}$ −103° (CH_2Cl_2; *c* 0.1) eluted by 6% MeOH in CH_2Cl_2.

(+)-*Ammodendrine.* Yellow oil (32 mg). $[\alpha]_D^{25}$ +7.1° (MeOH; *c* 0.08) eluted by 8% MeOH in CH_2Cl_2.

(−)-Δ^5-*Dehydromultiflorine.* Yellow oil (12 mg). $[\alpha]_D^{25}$ −94° (CH_2Cl_2; *c* 0.017) eluted by 9% MeOH in CH_2Cl_2.

(+)-*Epilupinine.* Needles (370 mg). Mp 79°. $[\alpha]_D^{25}$ +31° (EtOH; *c* 0.017) eluted by 9% MeOH in CH_2Cl_2.

13β-*Hydroxymultiflorine* (**1**) *and* (−)-13α-*hydroxymultiflorine* (**4**). Eluted as mixt. by 11% MeOH in CH_2Cl_2. The mixt. was isolated by prep. HPLC. HPLC and GC indicated the presence of these two alkaloids in a ratio of 3:1, respectively.

(+)-*Epilupinine* N-*oxide.* Needles (370 mg). Mp 210°. $[\alpha]_D^{25}$ 31° (EtOH; *c* 0.01) eluted by 13% MeOH in CH_2Cl_2.

(−)-*Multiflorine* N-*oxide.* Oil (27 mg). $[\alpha]_D^{25}$ −147° (MeOH; *c* 0.027) eluted by 14% MeOH in CH_2Cl_2.

(−)-*Sparteine.* Oil (132 mg). $[\alpha]_D^{25}$ −17° (MeOH; *c* 0.01) eluted by 16% MeOH in CH_2Cl_2.

13β-*Hydroxymultiflorine* (**1**). Yellow oil (13 mg). $[\alpha]_D^{25}$ −304° (CH_2Cl_2; *c* 0.13). HRMS *m/z* (rel int.): 262.1687 $[M]^+$ (63), (calc. for $C_{15}H_{22}N_2O_2$, 262.1682). EI-MS *m/z* (rel int.): 262 (63) 245 (11), 244 (14), 164 (19), 152 (17), 150 (100), 134 (11). IR ν_{max} cm^{-1} 3330 (OH), 2950–2800 (Bohlmann bonds), 1630 (pyridone C=O), 1580 (C=C). UV λ_{max}^{MeOH} 327 ($\log \varepsilon = 4.14$). ^1H NMR ($CDCl_3$, 500 MHz): δ 6.93 (1H, *d*, *J* = 7.7, H-2), 4.94 (1H, *d*, *J* = 7.7, H-3), 3.87 (1H, *m*, H-13), 3.27 (1H, *ddd*, *J* = 16.2, 5.3, 2.7 Hz, H-6), 3.07 (1H, *d*, $J = 13.2$, H-10_{ax}), 2.99 (1H, *dd*, *J* = 13.1, 3.2 Hz, H-10_{eq}), 2.81 (1H, *dd*, *J* = 11.4, 3.2 Hz, H-17_{eq}), 2.41 (1H, *br d*, *J* = 11.9 Hz, H-15_{eq}), 2.32 (1H, *dd*, *J* = 13.7, 2.9 Hz, H-17_{ax}), 2.11 (1H, *m*, H-15_{ax}), 2.07 (1H, *ddd*, *J* = 13.2, 3.9, 3.4 Hz, H-11), 1.99 (1H, *m*, H-7), 1.83 (1H, *m*, H-5_{eq}), 1.77 (2H, *m*, H-8_{ax}, H-5_{ax}), 1.60 (1H, *m*, H-9), 1.47–1.22 (5H, *m*, H-8_{eq}, 2 × H-14, 2 × H-12). ^{13}C NMR ($CDCl_3$, 500 Mz): δ 155.8 (*d*, C-2), 98.9 (*d*, C-3), 192.3 (*s*, C-4), 39.2 (*t*, C-5), 58.0 (*d*, C-6), 31.6 (*d*, C-7), 25.3 (*t*, C-8), 32.4 (*d*, C-9), 57.1 (*t*, C-10), 61.2 (*d*, C-11), 38.9 (*t*, C-12), 69.2 (*d*, C-13), 31.2 (*t*, C-14), 51.2 (*t*, C-15), 52.3 (*t*, C-17).

13α-*Hydroxymultiflorine* (**4**). Oil (51 mg). $[\alpha]_D^{25}$ −330° (CH_2Cl_2; *c* 0.1). IR ν_{max} cm^{-1} 3350 (OH), 2950–2800 (Bohlmann bands), 1620 (pyridone C=O), 1580 (C=C). EI-MS and UV see ref. [13]. ^1H NMR ($CDCl_3$, 500 MHz): δ 6.85 (1H, *d*, *J* = 7.7 Hz, H-2), 4.96 (1H, *d*, *J* = 7.7, H-3), 4.13 (1H, *br s*, H-13), 3.49 (1H, *ddd*, *J* = 14.7, 5.0, and 2.5 Hz, H-6), 3.27 (1H, *d*, *J* = 12.1,

H-10$_{ax}$), 3.13 (1H, *dd*, J = 12.7, 3.0 Hz, H-10$_{eq}$), 2.96 (1H, *dd*, J = 11.2, 3.3 Hz, H-17$_{eq}$), 2.73 (1H, *br d*, J = 11.7 Hz, H-15$_{eq}$), 2.62 (1H, *dd*, J = 12.3, 2.7 Hz, H-17$_{ax}$), 2.42 (1H, *br d*, J = 14.7 Hz, H-15$_{ax}$), 2.26 (1H, *ddd*, J = 12.9, 3.7 and 3.0 Hz, H-11), 2.11 (1H, *ddd*, J = 16.7, 5.6, 5.2 Hz, H-5$_{eq}$), 1.93 (3H, *m*, H-5$_{ax}$, H-7, H-8$_{ax}$), 1.71 (1H, *d like*, H-9), 1.65–1.53 (3H, *m*, 2 × H-14, H-12$_{eq}$) 1.47–1.32 (2H, *m*, H-8$_{eq}$, H-12$_{ax}$), ^{13}C NMR: see ref. [13].

Extraction and isolation of L. hartwegii. The total alkaloid fr. from 75% EtOH extracts of air-dried finely ground seeds (1.23 kg) was obtained in yield of 1.8% of the fr. wt using the method of refs [13. 14, 19]. The mixt. of bases (22 g) was chromatographed on silica gel column (Merck, type 60, 230–400 mesh, 1 kg, 7 × 150 cm) and gradient elution using MeOH in $CHCl_3$–28% NH_4OH to yield the following alkaloids.

(+)-Δ^5-*Dehydrolupanine*. Oil (23 mg). $[\alpha]_D^{25}$ 35° ($CHCl_3$; *c* 0.01) eluted by 1% MeOH in $CHCl_3$.

(+)-*Aphylline* (**5**). Rosettes (110 mg). Mp 57°. $[\alpha]_D^{25}$ 11° (EtOH; *c* 0.1) eluted by 2% MeOH in $CHCl_3$.

(−)-11,12-Seco-12,13-*didehydromultiflorine*. Oil (27 mg) eluted by 2% MeOH in $CHCl_3$.

(+)-*Aphyllidine* (**6**). Fine needles (51 mg). Mp 113°. $[\alpha]_D^{25}$ 7.3° ($CHCl_3$; *c* 0.1) eluted by 3% MeOH in $CHCl_3$.

(+)-α-*Isolupanine*. Fine needles (50 mg). Mp 178°. $[\alpha]_D^{25}$ 66° (EtOH; *c* 0.1) eluted by 4% MeOH in $CHCl_3$.

(+)-*Lupanine*. Oil (44 mg), eluted by 5% MeOH in $CHCl_3$. Pure **2** eluted by 5% MeOH in $CHCl_3$.

(−)-*Multiflorine*. Yellow oil (63 mg) eluted by 7% MeOH in $CHCl_3$.

(−)-*Virgiline*. Needles (82 mg). Mp 249°. $[\alpha]_D^{25}$ −46° (EtOH; *c* 0.1) eluted by 7% MeOH in $CHCl_3$.

(+)-3β-*Hydroxylupanine*. Needles (51 mg). Mp 108°. $[\alpha]_D^{25}$ 7.3° ($CHCl_3$; *c* 0.1) eluted by 8% MeOH in $CHCl_3$. Pure **3** eluted by 9% MeOH in $CHCl_3$.

(+)-13α-*Hydroxylupanine*. Needles (80 mg). Mp 172°. $[\alpha]_D^{25}$ 45° ($CHCl_3$; *c* 0.1) eluted by 11% MeOH in $CHCl_3$.

(±)-*Gramine*. Needles (23 mg). Mp 134°. Eluted by 17% MeOH in $CHCl_3$. Epiaphylline and 10,17-dioxosparteine were detected in some frs eluted by MeOH containing 1 and 8%, respectively, of $CHCl_3$ and detected only by GC-Ms.

(+)-2β-*Hydroxyaphylline* (**2**). Amorphous (13 mg). $[\alpha]_D^{25}$ +30.4° ($CHCl_3$; *c* 0.13). HREI-MS *m/z* (rel int.): 264.1844 $[M]^+$ (9), (calc. for $C_{15}H_{24}N_2O_2$, 264.1839). EI-MS *m/z* (rel int.): 264 (8), 247 (36), 246 (22), 220 (17), 149 (9), 136 (100), 110 (49), 98 (71). IR ν_{max}^{KBr} cm^{-1} 3375 (OH), 2950–2850 (Bohlmann bands), 1640 (amide C=O). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.13 (1H, *dd*, J = 2.9 and 1.8 Hz, H-2$_{eq}$), 3.27 (1H, *ddd*, J = 12.1, 5.1, 2.0 Hz, H-6), 2.87 (1H, *dd*, J = 11.9. 8.9 Hz, H-17$_{eq}$), 2.63 (1H, *br d*, J = 14.7, H-15$_{eq}$), 2.31 (1H, *dd*, J = 11.9, 3.2 Hz, H-17$_{ax}$), 2.19 (1H, *m*, H-9), 2.11 (1H, *dd*, J = 12.1, 3.0 H-8$_{eq}$), 1.89 (1H, *d like*, Hz, H-15$_{ax}$), 1.88–1.81 (3H, *m*, H-4$_{eq}$, H-11, H-13$_{eq}$), 1.74, (1H, *m*, H-8$_{ax}$), 1.70 (2H, *m*, H-4$_{ax}$, H-7), 1.67 (1H, *m*, H-3$_{eq}$), 1.61 (1H, *m*, H-3$_{ax}$), 1.58–1.53 (2H, *m*, 2 × H-14) 1.47–1.41 (2H, *m*, H-5$_{ax}$, H-12$_{eq}$), 1.38–1.24 (3H, *m*, H-5$_{eq}$, H-12$_{ax}$, H-13$_{ax}$). ^{13}C NMR ($CDCl_3$, 125 Mz): Table 1.

(+)-13α-*Hydroxyaphyllidine* (**3**). Oil (24 mg). $[\alpha]_D^{25}$ 44° ($CHCH_3$; *c* 0.1). HREI-MS *m/z* (rel int.): 262.1687 $[M]^+$ (63), (calc. for $C_{15}H_{22}N_2O_2$, 262.1680). EI-MS *m/z* (rel int.): 262 (63), 245 (9), 244 (13), 220 (66), 163 (22), 150 (100), 110 (11), 83 (55). IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3270 (OH), 2850–2700 (Bohlmann bands), 1635 (amide C=O), 1560 (C=C). UV λ_{max}^{MeOH} 239, (log ε = 3.90). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.77 (1H, *dd*, J = 5.7, 1.8 Hz, H-5), 4.11 (1H, *t*, J = 2.7 Hz, H-13$_{eq}$), 3.77 (1H, *ddd*, J = 12.9, 4.3 and 1.8 Hz, H-2$_{eq}$). 3.66 (1H, *ddd*, J = 13.1, 8.1, 3.9 Hz, H-2$_{ax}$), 3.36 (1H, *dd*, J = 11.3, 2.6 Hz, H-17$_{eq}$), 3.09 (1H, *m*, H-11), 2.82 (1H, *ddd*, J = 12.9, 12.9. 1.9 Hz, H-15$_{ax}$), 2.78 (1H, *ddd*, J = 12.9, 2.1, 2.1, H-15$_{eq}$), 2.56 (1H, *d-like*, H-7), 2.43 (1H, *br s*, H-9), 2.33 (1H, *d*, J = 11.3 Hz, H-17$_{ax}$), 2.11 (1H, *ddd*, J = 14.1, 5.0, 1.8 Hz-H-4$_{ax}$), 2.07 (1H, *dd*, J = 13.9, 4.9 Hz, H-4$_{eq}$), 1.87 (1H, *m*, H-12$_{ec}$), 1.83 (1H, *dd*, J = 11.8, 3.6 Hz, H-8$_{eq}$), 1.77–1.63 (4H, *m*, 2 × H3, H-8$_{ax}$, H-14$_{eq}$), 1.47–1.22 (2H, *m*, H-12$_{ax}$, H-14$_{ax}$). ^{13}C NMR ($CDCl_3$, 125 Mz): Table 1.

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