Crinine-type alkaloids from Hippeastrum aulicum and H. calyptratum

Jean Paulo de Andrade [a,b], Ying Guo [a], Mercè Font-Bardia [c,d], Teresa Calvet [c], Jullie Dutilh [e], Francesc Viladomat [a], Carles Codina [a], Jerald J. Nair [a], Jose A. Silveira Zuanazzi [f], Jaume Bastida [a,⇑]

[a] Departament de Productes Naturals, Biologia Vegetal i Edafologia, Facultat de Farmàcia, Universitat de Barcelona, Av. Diagonal 643, 08028 Barcelona, Spain
[b] Facultad de Farmacia, INIFAR and CIPRONA, Universidad de Costa Rica, 2060 San José, Costa Rica
[c] Cristal·lografia, Mineralogia i Dipòsits Minerals, Universitat de Barcelona, Martí i Franquès s/n, 08028 Barcelona, Spain
[d] Unitat de Difracció RX, Centre Cientific i Tecnològic (CCiTUB), Universitat de Barcelona, Sole Sabaris 1-3, 08028 Barcelona, Spain
[e] Departamento de Botânica, Universidade de Campinas, Cidade Universitária, Campinas 13083-970, Brazil
[f] Faculdade de Farmácia, Universidade Federal do Rio Grande do Sul, 2752 Ipiranga Av., Porto Alegre 90610-000, Brazil

⇑ Corresponding author. Tel.: +34 934020268; fax: +34 934029043.

E-mail address: jaumebastida@ub.edu (J. Bastida).

ABSTRACT

An ongoing search for alkaloids in the Amaryllidaceae species using GC–MS resulted in the identification of two crinine-type alkaloids, aulicine (1) and 3-O-methyl-epimacowine, (2) from the indigenous Brazilian species Hippeastrum aulicum and Hippeastrum calyptratum, respectively. In addition, two alkaloids, 11-oxohaemanthamine (3) and 7-methoxy-O-methyllycorenine (4) were both isolated from H. aulicum. Furthermore, we provide here complete NMR spectroscopic data for the homolycorine analogues nerinine (5) and albomaculine (6). The absolute stereochemistry of the 5,10b-ethano bridge in the crinine variants was determined by circular dichroism and X-ray crystallographic analysis, thus presenting the first direct evidence for the presence of crinine-type alkaloids in the genus Hippeastrum.

1. Introduction

GC–MS has proven to be a useful tool in the identification and quantification of Amaryllidaceae alkaloids (Berkov et al., 2011; Torras-Claveria et al., 2013). This spectroscopic technique has been used with success to assist with the isolation of new or unusual structures from alkaloid-rich extracts by comparing their component electron impact-mass fragmentation spectra (EI-MS) with those of known standards (Berkov et al., 2011; Torras-Claveria et al., 2013). For example, candimine from H. morelianum Lem. and 11b-hydroxygalanthamine from H. papilio (Ravenna) Van Scheepen were both isolated based on prior GC–MS screening of these endemic Brazilian species (de Andrade et al., 2012a). Interestingly, both alkaloids have since exhibited promising anti-Trichomonas vaginalis and acetylcholinesterase (AChE) inhibitory activities (de Andrade et al., 2011; Giordani et al., 2010). Therefore, a similar guided approach is attractive in that it circumvents the need for time and labour-intensive chromatographic steps for extracts and alkaloid fractions devoid of new bioactive compounds.

Since the 1970s, X-ray crystallographic and/or circular dichroism (CD) analyses of 5,10b-ethanophenanthridine alkaloids from Hippeastrum have indicated that they belong exclusively to the haemanthamine series, which are enantiomeric to the crinine series. Earlier, a few crinine-type alkaloids were detected in European Hippeastrum cultivars (Boit and Döpke, 1960; Döpke, 1962), but their absolute configurations have been questioned based on the lack of any tangible evidence, such as CD and X-ray crystallography. These two techniques have since become integral to the unambiguous assignment of the orientation of the 5,10b-ethano bridge in the crinine/haemanthamine series of alkaloids (Bastida et al., 2006; De Angelis and Wildman, 1969; Wagner et al., 1996). In the present study, the use of CD and X-ray crystallographic techniques as well as NMR and GC–MS analysis resulted in the identification of the novel crinine-type alkaloids aulicine (1) and 3-O-methyl-epimacowine (2) (Fig. 1) along with two new alkaloids [11-oxohaemanthamine (3) and 7-methoxy-O-methyllycorenine (4)] from the Brazilian species Hippeastrum aulicum Herb. and Hippeastrum calyptratum Herb. Nineteen additional known alkaloids were identified in the process, and a complete NMR data set for nerinine (5) and albomaculine (6) is also reported herein. These findings are significant in that they represent the first direct evidence for the presence of crinine-type alkaloids in Hippeastrum.

2. Results and discussion

Of the twenty-three alkaloids identified in H. aulicum and H. calyptratum, thirteen were common to both, while five were unique to either species (Table 1). The major alkaloids detected in H. aulicum were aulicine (1), lycorine (10) and haemanthamine (15), while lycorine (10) was the main constituent present in H. calyptratum. HRESIMS gave a mass of 320.1864 for alkaloid 1, which is expected for the molecular formula $C_{18}H_{26}NO_4$ and the theoretical mass (320.1856) for the parent $[M+H]^+$ ion. Its GC–MS fragmentation pattern was similar to that of the 1,2-dihydroethanophenantridines powellane and deacetylbowdensine (Duffield et al., 1965). As expected, no olefinic proton signals were observed in the ^{1}H-NMR spectrum of 1 and the only low-field resonance signal was assignable to H-7 (δ 6.10, s) due to HSQC correlation with C-7 (δ 101.0, d), spatial NOESY connectivity to the benzylic 2H-6 protons and HMBC contour correlation with C-6 (δ 62.7, t). These data indicated that aulicine (1) possessed a penta-substituted aromatic A-ring and a saturated C-ring moiety. In essence, its 1H-NMR spectrum (Table 2) was similar to that of hippeastidine (Kulhánková et al., 2013; Pacheco et al., 1978; Watson and Zabel, 1982). Although the basic crinane structure of hippeastidine is known with certainty, its absolute stereochemistry still remains unresolved due to its missing CD and X-ray crystallographic data, i.e., it is not clear from the literature whether the compound is of the α- or β-crinane alkaloid series (Kulhánková et al., 2013; Pacheco et al., 1978; Watson and Zabel, 1982).

A comparison of the ^{1}H-NMR data of 1 with that of hippeastidine revealed that the only striking differences pertained to the splitting of the H-3 and H-4 protons, both of which are crucial to the stereochemical relationship between the 3-methoxyl substituent and the 5,10b-ethano bridge. The resonance at δ 1.21 ascribed to H-4β was split into a quartet with an accompanying large coupling constant (J = 12.4 Hz), indicative of two trans-diaxial couplings (with H-3 and H-4a) and the geminal coupling with H-4α (Table 2). Large coupling constants were also observed for H-2β. Thus, the H-4β and H-2β splitting patterns are consistent with a cis relationship between the 3-methoxyl substituent and the 5,10b-ethano bridge. Interestingly, H-1β was shifted to a lower field when compared to H-1α due to its syn-proximity to the hydroxyl group at C-10. The complete NMR data set for aulicine (1) is listed in Table 2. Confirmation of the absolute stereochemistry in 1 was arrived at via CD and X-ray crystallography. The CD spectrum of 1 (Fig. 2A) showed a positive Cotton effect at ca. 250 nm and negative Cotton effect at ca. 290 nm, in agreement with a crinine-type alkaloid (De Angelis and Wildman, 1969; Wagner et al., 1996). X-ray crystallographic data analysis was carried out using a copper source (see Materials and methods), leading to the unambiguous structural assignment of 1 as a crininetype alkaloid (Fig. 2B).

The new crinine alkaloid 3-O-methyl-epimacowine (2) from H. calyptratum exhibited a parent $[M+H]^+$ ion at m/z 288.1595 in its HRESIMS spectrum, thereby suggesting the molecular formula $C_{17}H_{22}NO_3$ (calcd. 288.1594). The NMR data of 2 (Table 3) were similar to those of macowine (Nair et al., 2000), with the only notable difference arising from the differential substitution pattern at C-3. An aliphatic methoxyl group was indicated by the chemical shift and splitting pattern of the resonance at δ 3.42 (3H, s), in accordance with previous studies on 3-substituted alkaloids of the crinine series (Viladomat et al., 1995). A small H-3/H-4β coupling (J = 4.0 Hz) is consistent with the pseudoaxial orientation for the 3-hydroxyl substituent in macowine (Nair et al., 2000). By contrast, in 2, the large coupling constant ($J_{3,4b}$ = 10.5 Hz) suggested a pseudoequatorial disposition for the 3-methoxyl substituent and therefore a cis relationship between this substituent and the 5,10b-ethano bridge. The bridge orientation was confirmed by CD analysis, which showed positive and negative Cotton effects at ca. 250 and ca. 290 nm, respectively (Fig. 2C).

The remaining two new alkaloids, 11-oxohaemanthamine (3) and 7-methoxy-O-methyllycorenine (4), were identified in H. aulicum. The HRESIMS of 3 suggested the molecular formula $C_{17}H_{18}NO_4$ for the parent $[M+H]^+$ ion at m/z 300.1239 (calcd. 300.1230). Its GC–MS fragmentation pattern was similar to that of an alkaloid tentatively assigned to 11-oxohaemanthamine by Kreh et al. (1995). The CD data determined for 3 (see Experimental) were in agreement with those of a crinane-type alkaloid of the α-series (Wagner et al., 1996). Characteristic ^{1}H-NMR signals included the following: (1) two para-oriented aryl protons (δ 6.83 and 6.52, for H-10 and H-7, respectively), (2) two AB doublets at δ 4.58 and 3.83, correspondent with the C-6 benzylic proton system in which H-6β was assigned to a lower field due to its cis relationship with the nitrogen lone pair, and (3) two vicinal olefinic proton resonances (δ 6.54 and δ 6.21, $J_{1,2}$ = 10.0 Hz), the more shielded of which was assigned to H-2 due to its COSY correlation with H-3 resonant at δ 3.84. The magnitude of the coupling constant between H-2 and H-3 ($J_{2,3}$ = 5.5 Hz) and the small coupling constants between H-3 and both H-4 protons ($J_{3,4\alpha}$ ~ 4.0 and $J_{3,4\beta}$ = 2.0 Hz) are in agreement with a pseudoequatorial orientation for H-3, thus suggesting a trans relationship between the 3-methoxyl substituent and the 5,10b-ethano bridge (Pabuççuogˇlu et al., 1989). The NMR data for 3 (Table 4) are consistent with 11-oxohaemanthamine, which was recently synthesised by Cedrón et al. (2012). The isolation of 3 from a natural source is reported here for the first time.

Homolycorine-type alkaloids bearing trimethoxyaryl substituents were originally reported during the 1950s (Boit and Döpke, 1957; Briggs et al., 1956). The mass fragmentation pattern of 7-methoxy-O-methyllycorenine (4) was in agreement with patterns typical for homolycorine-type alkaloids (Kreh et al., 1995; Schnoes et al., 1962). The HRESIMS data for 4 was consistent with the molecular formula $C_{20}H_{28}NO_5$ for the parent ion $[M+H]^+$ at m/z 362.1964 (calcd. 362.1962). The ^{1}H-NMR data of 4 (Table 5) were similar to of the data for O-methyllycorenine, originally reported by Codina et al. (1993) and differing only by the presence of a third aromatic methoxyl group resonance at δ 3.89 (3H, s). Thus, the 7,8,9-trimethoxyaryl substitution in 4 was confirmed by the NOESY correlation evident between H-10 and the N-methyl group. The C-7 and C-8 methoxyl carbon resonances (δ 61.5 and δ 61.2, respectively) were diagnostically downfield shifted from that of C-9 (δ 56.6), as previously indicated (Bastida et al., 1992). The large coupling constant $J_{4a,10b}$ = 10.0 Hz confirmed a trans-diaxial relationship between H-4a and H-10b. A cis B/C ring junction was suggested based on the small value of the coupling constant measured between H-1 and H-10b (J = 2.0 Hz). NOESY correlation between 6-OMe and H-1 confirmed the β-orientation for H-6, a feature characteristic of hemiacetal functionalised homolycorine alkaloids (Bastida et al., 2006; Codina et al., 1992). The complete NMR data of 4 are provided in Table 5.

The structures of nerinine (5) and albomaculine (6) were confirmed by comparing their respective physical and spectroscopic data with the data available in the literature (Berkov et al., 2011; Codina et al., 1992; Jeffs and Hawksworth, 1963; Kreh et al., 1995; Schnoes et al., 1962). However, in both instances, these were found to be incomplete and are therefore comprehensively presented here in the Experimental section as well as in Tables 6 and 7.

Aulicine (1)[1]: white crystals; $[\alpha]_D^{24}$ -2,3 (c 0.38, CHCl3); CD $[\Theta]^{20}\lambda$: $[\Theta]_{255}$+ 1043, $[\Theta]_{279}$ -768; UV (MeOH) λ_{max}(log ε) 233 (3.50), 273 (2.70) nm; IR ($CHCl_3$) ν_{max} 3291, 2931, 2858, 1605, 1577, 1495, 1455, 1424, 1126, 1103 cm^{-1}; 1H-NMR (CDCl3, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) see Table 2; EIMS data shown in Table 1; HRESIMS of $[M+H]^+$ m/z 320.1864 (calcd for $C_{18}H_{26}NO_4$, 320.1856).

3-O-Methyl-epimacowine (2): white needles; $[\alpha]_D^{22}$ -47 (c 0.42, $CHCl_3$); CD $[\Theta]^{20}_1$: $[\Theta]_{254}$ +2528, $[\Theta]_{290}$ -2215; UV (MeOH) λ_{max}(log ε) 230 (3.31), 288 (3.23) nm; IR ($CHCl_3$) ν_{max} 2925, 2854, 1507, 1461, 1312, 1277, 1219, 1098, 754 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 3; EIMS data shown in Table 1; HRESIMS of $[M+H]^+$ m/z 288.1595 (calcd for $C_{17}H_{22}NO_3$, 288.1594).

11-Oxohaemanthamine (3): white needles; $[\alpha]_D^{20}$ +44 (c 0.12, $CHCl_3$); CD $[\Theta]^{20}_\lambda$: $[\Theta]_{255}$ -3429, $[\Theta]_{320}$ +3298; UV (MeOH) λ_{max}(log ε) 250 (2.94), 295 (2.92), 313 (2.82) nm; IR ($CHCl_3$) λ_{max} 2924, 2854, 1744, 1503, 1481, 1463, 1377, 1238, 1086, 1038 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) see Table 4; EIMS data shown in Table 1; HRESIMS of $[M+H]^+$ m/z 300.1239 (calcd for $C_{17}H_{18}NO_4$, 300.1230).

7-Methoxy-O-methyllycorenine (4): amorphous solid; $[\alpha]_D^{23}$ +31 (c 0.33, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 230 (3.55), 270 (2.75) nm; IR ($CHCl_3$) ν_{max} 2924, 2853, 2783, 1601, 1460, 1336, 1128, 1053, 1025; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 5; EIMS data shown in Table 1; HRESIMS of $[M+H]^+$ m/z 362.1964 (calcd for $C_{20}H_{28}NO_5$, 362.1962).

Nerinine (5): amorphous solid; $[\alpha]_D^{23}$ +40 (c 0.33, $CHCl_3$); UV (MeOH) λ_{max}(log ε) 232 (3.59), 273 (2.89) nm; IR ($CHCl_3$) ν_{max} 3145, 2918, 2849, 1587, 1460, 1410, 1336, 1243, 1122, 1018 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) see Table 6; EIMS data shown in Table 1; HRESIMS of $[M+H]^+$ m/z 348.1807 (calcd for $C_{19}H_{26}NO_5$, 348.1805).

Albomaculine (6): amorphous solid; $[\alpha]_D^{23}$ +25 (c 0.95, $CHCl_3$); UV (MeOH) λ_{max}(logε) 222 (4.26), 266 (3.86), 298 (3.34) nm; IR (CHCl3) ν_{max} 2929, 2849, 2783, 1725, 1592, 1334, 1254, 1111, 1022 cm^{-1}; ^{1}H-NMR (CDCl3, 400 MHz) and ^{13}C-NMR ($CDCl_3$, 100 MHz) see Table 7; EIMS data shown in Table 1; HRESIMS of $[M+H]^+$ m/z 346.1651 (calcd for $C_{19}H_{24}NO_5$, 346.1649).

3. Conclusions

In summary, phytochemical investigation of H. aulicum and H. calyptratum led to the identification of 23 Amaryllidaceae alkaloids. Of these alkaloids, aulicine, 3-O-methyl-epimacowine, 11-oxohaemanthamine and 7-methoxy-O-methyllycorenine are reported here for the first time. The structures of these alkaloids were determined by physical and spectroscopic methods, including GC–MS, NMR, CD and X-ray crystallography. The identification of the b-crinane alkaloids aulicine and 3-O-methyl-epimacowine in Hippeastrum is of considerable biosystematic significance because previous findings have revealed that all crinane compounds from this genus are reminiscent of the a-series. Efforts to further delineate this anomaly via targeted studies of other species of Hippeastrum are presently underway in our laboratories.

4. Materials and methods

4.1. General procedure

NMR spectra were recorded on a Mercury 400 MHz (Palo Alto, CA, USA) or a Varian 500 MHz (Palo Alto, CA, USA) instrument using CDCl3 (CD3OD for 4 and 10) as the solvent and TMS as the internal standard. Chemical shifts are reported in d units (ppm) and coupling constants (J) in Hz. The GC–MS spectra were obtained on an Agilent 6890N GC 5975 inert MSD operating in the EI mode at 70 eV (Agilent Technologies, Santa Clara, CA, USA) using a DB5 MS column (30 m x 0.25 mm x 0.25 lm, Agilent Technologies). The temperature program was as follows: 100–180 °C at 15 °C min^{-1}, 1 min hold at 180 °C and 180–300 °C at 5°C min^{-1} and 40 min hold at 300 °C. The injector temperature was 280 °C. The flow rate of carrier gas (helium) was 0.8 ml min^{-1}, and the split ratio was 1:20. HRESIMS spectra were obtained on a LC/MSD-TOF (2006) mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) by direct injection of the compounds dissolved in H2O-MeCN (1:1). Optical rotations were carried out on a Perkin-Elmer 241 polarimeter (Waltham, MA, USA). A Jasco-J-810 Spectrophotometer (Easton, MD, USA) was used to run CD spectra, all recorded in MeOH. UV spectra were obtained on a DINKO UV2310 instrument (Barcelona, Spain) and IR spectra were recorded on a Nicolet Avatar 320 FT-IR spectrophotometer (Waltham, MA, USA). Silica gel (Kieselgel – mesh 0.15/0.30, Val-de-Reuil, France) was used for all vacuum liquid chromatography procedures (VLC). For thin layer chromatography (TLC), silica gel F254 was used as the stationary phase with a plate dimension of 20 cm x 20 cm x 0.20 mm for analytical TLC and 20 cm x 20 cm x 0.25 mm for semi-preparative TLC (SPTLC) (Val-de-Reuil, France). Exclusion chromatography was carried out using a Sephadex LH-20 (Uppsala, Sweden).

4.2. Plant material

Bulbs of H. aulicum Herb. and H. calyptratum Herb. were collected in October 2011 during the flowering period from a population located in Cunha City, Sao Paulo Province (Brazil). Both species were identified by Mr. Mauro Peixoto and Dr. Jullie Dutilh (University of Campinas, Unicamp, Brazil). The voucher specimens of H. aulicum were deposited in the herbarium at the Plantarum Institute under the reference number HPL 13043. The voucher specimens of H. calyptratum were deposited in the Herbarium of the University of Campinas (Unicamp, Brazil) under the reference number UEC 59648.

4.3. Extraction and isolation of alkaloids

Dried bulbs (370 g) of H. aulicum were crushed and thrice extracted for 48 h with MeOH at room temperature, and the combined macerate was filtered and evaporated under reduced pressure. The crude extract (90 g) was acidified with sulphuric acid (2%) to pH 2 and extracted with Et2O (4x 250 ml) and EtOAc (4 x 250 ml) to remove neutral material. The aqueous solution was basified with ammonia (25%) up to pH 10 and extracted with n-hexane (8 x 250 ml) to give extract IA (0.86 g). Another extraction using EtOAc (8 x 250 ml) produced extract IIA (2.0 g), wherein lycorine (10) precipitated spontaneously. A final extraction using EtOAc–MeOH (3:1, 3 x 250 ml) showed negative results for alkaloids as confirmed by Dragendorff's reagent stain and GC–MS.

Extract IA was subjected to VLC (2.5 x 6 cm) on silica gel (10 g), starting with n-hexane (100%), gradually enriching with EtOAc (0 → 100%), and finally with MeOH (0 → 30%). A total of 150, 50 ml fractions were collected, monitored by analytical TLC (Dragendorff's reagent, UV light k 254 nm) and combined after TLC analysis. Nerinine (5, 15 mg) was isolated by precipitation of fractions 65–86, and the supernatant was submitted to SPTLC (EtOAc–Me2- CO–n-hexane–MeOH – 6:2:1:1, in NH3 atmosphere), which allowed for the isolation of 7-methoxy-O-methyllycorenine (4, 6.5 mg) and galanthamine (13, 10 mg). Fractions 87–118 gave haemanthamine (15) and aulicine (1) again by precipitation and further purification by SPTLC (n-hexane–EtOAc–Me2CO–MeOH–n-BuOH – 4:3:3:2:1, in NH3 atmosphere). The supernatant was oaded onto a VLC column (1.5 x 4 cm) of silica gel (3 g), using nhexane (100%) as the starting solvent, gradually enriched with EtOAc (0?100%), and finally with MeOH (0?30%), ultimately yielding 250 fractions (each 10 ml). After combining the fractions according to the TLC profiles, 11-oxohaemanthamine (3, 5.3 mg) was isolated from pooled fractions 93–113 using SPTLC (n-hexane–Me2CO–EtOAc–MeOH – 15:10:5:2, in NH3 atmosphere). Fractions 222–250 were combined and subjected to SPTLC (n-hexane–EtOAc–Me2CO–MeOH–n-BuOH – 4:3:3:2:1, in NH3 atmosphere), after which 1 and 15 were again isolated.

Alkaloid 15 precipitated spontaneously from extract IIA after resuspension in MeOH. The supernatant (700 mg) was purified by silica gel VLC (2 x 6 cm column, 10 g), starting with n-hexane (100%), gradually enriching with EtOAc (0 → 100%) and finally with MeOH (0 → 30%), ultimately yielding 200 fractions (50 ml each) that were then pooled according to TLC profile analysis. SPTLC (n-hexane–EtOAc–Me2CO–MeOH–n-BuOH – 4:3:3:2:1, in NH3 atmosphere) of fractions 134–190 gave 1 (152 mg), 15 (161.1 mg) and 10 (135 mg), while a small quantity of tazettine (19, 3.2 mg) precipitated from fractions 191–205. GC–MS spectra of the remaining fractions indicated the presence of only known compounds (Table 1), which therefore precluded the need for further chromatographic analyses.

Dried bulbs (135 g) of H. calyptratum were crushed and extracted by stirring with MeOH at room temperature for 48 h (repeating three times), and the combined macerate was filtered and evaporated under reduced pressure. The crude extract (50 g) was acidified with sulphuric acid (2%) to pH 2 and extracted with Et2O (4 x 250 ml) and EtOAc (4 x 250 ml) to remove neutral material. The aqueous solution was then basified with ammonia (25%) up to pH 10 and extracted with n-hexane (8 x 250 ml) to give extract IC (100 mg). Extraction with EtOAc (8 x 250 ml) gave extract IIC (300 mg). A final extraction using EtOAc-MeOH (3:1) showed negative results for alkaloids as confirmed by Dragendorff's reagent and GC–MS analysis.

Extracts IC and IIC (400 mg) were combined after GC–MS showed them to be similar. Alkaloid 10 precipitated after re-suspension in MeOH and the supernatant was purified by VLC (2.5 x 4 cmcolumn, 10 g of silica gel) using the same solvent systemas that for H. aulicum. Alkaloid 10 (115 mg) precipitated directly from fractions 93–140. Fractions 71–170 were combined (250mg) and subjected to VLC (1.5 x 4 cm column) in silica gel (3 g) using n-hexane (100%) followed by EtOAc (0 → 100%) and finally with MeOH (0 → 30%), ultimately yielding 250 fractions (10ml each). Only fractions 145–200 (110 mg) showed alkaloids with unknown GC–MS fragmentation patterns and were therefore selected for further VLC, which was carried out on silica gel (3 g) using a 1.5 4 cmcolumn, starting with nhexane (100%) and increasing solvent polarity with EtOAc (0 → 50%). Thereafter, $CHCl_3$ and EtOAc were gradually added until a $CHCl_3$-EtOAc ration of 1:1 was reached. Finally, the system was gradually enriched with MeOH (0?30%), ultimately yielding 200 fractions (10ml each). Albomaculine (6, 19.3mg) was isolated from fractions 69–88 by SPTLC (Me_2CO-CH_2C_{l2} – 3:10, in NH_3 atmosphere) together with 2a,7-dimethoxyhomolycorine (9, 3.2 mg). Likewise, 3-Omethyl-epimacowine (2, 18.3mg) and alkaloid 13 (7.7 mg) were isolated from fractions 89–148 using SPTLC (EtOAc-Me_2CO-CH_2Cl_2-MeOH – 3:1:1:0.5, in NH3 atmosphere).

291 *4.4. Identification of alkaloids by GC–MS*

292

293 The alkaloids were identified by comparing their GC–MS spectra and Kovats retention indices (RI) with
294 our library database. This library has been regularly updated with alkaloids isolated and unequivocally
295 identified via physical and spectroscopic means (Berkov et al., 2008; de Andrade et al., 2011, 2012b;
296 Giordani et al., 2011; Llabrés et al., 1986). NMR data for the known alkaloids described here closely
297 matched those reported elsewhere (Bastida et al., 2006; Kobayashi et al., 1980). Mass spectra were
298 deconvoluted using the AMDIS 2.64 software (NIST) (WA, USA), and RIs were recorded using a
299 standard n-hydrocarbon calibration mixture (C9-C36). The proportion of individual components in the
300 alkaloid fractions are expressed as a percentage of total alkaloid content. GC–MS peak areas are
301 dependent on the concentration of the injected alkaloid as well as the intensity of its mass spectral
302 fragmentation. Although the data given in Table 1 are not representative of a validated alkaloid
303 quantification method, these data can be used for relative comparison purposes.

304

305 *4.5. Crystals of aulicine (1)*

306

307 Compound 1 was dissolved in a MeOH-$CHCl_3$ (1:1) mixture under a pentane atmosphere. After 14 days
308 standing at ~5°C, small crystals of 1 formed and were selected for X-ray crystallography.

309

310 *4.6. X-ray analysis*

311

312 A prismatic crystal (0.1 0.1 0.2 mm) was selected and mounted on a Bruker D8 Venture four-circle
313 diffractometer (Karlsruhe, Germany). Intensities were collected with a multilayer monochromator and
314 a Cu high brilliance microfocus sealed tube using the / and x scan-technique. A total of 24158 reflections
315 were measured in the range of 2.93 6 h 6 74.32, with 6377 of the reflections non-equivalent by symmetry
316 (Rint(on I) = 0.031). Overall, 6028 reflections were assumed to be as observed by applying the condition
317 I > 2r(I). Lorentz-polarisation and absorption corrections were performed.

318 The structure was solved by direct methods, using the SHELXS computer program (and refined by a
319 full-matrix least-squares method with the SHELX97 computer program (Sheldrick, 2008)) and 24158
320 reflections, (very negative intensities were not assumed). The function minimised was $\sum w$ $||Fo|^2$ $|Fc|^2$
321 $|^2$, where w = $[\rho^2(I) + (0.0343P)2 + 0.8335P]^{-1}$, and P = $(|Fo|^2 + 2\ |Fc|^2)/3$, f, f and f" were taken from
322 the International Tables of X-ray Crystallography (1974). All H atoms were computed and refined using
323 a riding model, with an isotropic temperature factor equal to 1.2 times the equivalent temperature factor
324 of the atoms that are linked. The final R(on F) factor was 0.0298, wR(on $|F|^2$) = 0.074 and goodness of
325 fit = 1.042 for all observed reflections. The number of refined parameters was 423. Max. shift/esd =
326 0.00, Mean shift/esd = 0.00. Max. and min. peaks in the final difference synthesis were 0.215 and 0.164
327 e$Å^{-3}$, respectively.

328

329 **ACKNOWLEDGEMENTS**

330

331 The authors are grateful to the Generalitat de Catalunya (2009 – SGR1060) for the financial support of
332 this research and to the SCT-UB personnel for technical assistance. Special thanks is given to Mr. Mauro
333 Peixoto for the collection of plant material. J.A.S.Z. acknowledges CNPq (Brazil) for a research
334 fellowship. J.P.A. thanks the Agencia Española de Cooperación Internacional para el Desarollo
335 (BECAS-MAEC-AECID) for a doctoral fellowship.

336

References

Bastida, J., Codina, C., Viladomat, F., Rubiralta, M., Quirion, J.C., Weniger, B., 1992. Narcissus alkaloids, XV: Roserine from Narcissus pallidulus. J. Nat. Prod. 55, 134–136.

Bastida, J., Lavilla, R., Viladomat, F., 2006. Chemical and biological aspects of Narcissus alkaloids. In: Cordell, G.A. (Ed.), The Alkaloids, vol. 63. Elsevier Inc., Amsterdam, pp. 87–179.

Berkov, S., Codina, C., Viladomat, F., Bastida, J., 2008. N-Alkylated galanthamine derivatives: potent acetylcholinesterase inhibitors from Leucojum aestivum. Bioorg. Med. Chem. Lett. 18, 2263–2266.

Berkov, S., Bastida, J., Sidjimova, B., Viladomat, F., Codina, C., 2011. Alkaloid diversity in Galanthus elwesii and Galanthus nivalis. Chem. Biodivers. 8, 115–130. Boit, H.G., Döpke, W., 1957. Alkaloids of the Amaryllidaceae. XVIII. Alkaloids from Urceolina, Hymenocallis, Elisena, Calostemma, Eustephia, and Hippeastrum. Chem. Ber. 90, 1827–1830.

Boit, H.G., Döpke, W., 1960. New alkaloids from Hippeastrum hybrids and Nerine flexuosa. Naturwissenschaften 47, 470–471.

Briggs, C.K., Highet, P.F., Highet, R.J., Wildman, W.C., 1956. Alkaloids of the Amaryllidaceae. VII. Alkaloids containing the hemiacetal or lactone group. J. Am. Chem. Soc. 78, 2899–2904.

Cedrón, J.C., Gutiérrez, D., Flores, N., Ravelo, Á.G., Estévez-Braun, A., 2012. Synthesis and antimalarial activity of new haemanthamine-type derivatives. Bioorg. Med. Chem. 20, 5464–5472.

Codina, C., Viladomat, F., Bastida, J., Rubiralta, M., Quirion, J.C., 1992. 2D NMR studies of lycorenine as a model for the structural assignment of lycoreninetype alkaloids. Nat. Prod. Lett. 1, 85–92.

Codina, C., Bastida, J., Viladomat, F., Fernández, J.M., Bergoñón, S., Rubiralta, M., Quirion, J.C., 1993. Alkaloids from Narcissus muñozii-garmendiae. Phytochemistry 32, 1354–1356.

de Andrade, J.P., Berkov, S., Viladomat, F., Codina, C., Zuanazzi, J.A.S., Bastida, J., 2011. Alkaloids from Hippeastrum papilio. Molecules 16, 7097–7104.

de Andrade, J.P., Pigni, N.B., Torras-Claveria, L., Guo, Y., Berkov, S., Reyes-Chilpa, R., El Amrani, A., Zuanazzi, J.A.S., Codina, C., Viladomat, F., Bastida, J., 2012a. Alkaloids from the Hippeastrum genus: chemistry and biological activity. Rev. Latinoam. Quim. 40, 83–98.

de Andrade, J.P., Pigni, N.B., Torras-Claveria, L., Berkov, S., Codina, C., Viladomat, F., Bastida, J., 2012b. Bioactive alkaloids from Narcissus broussonetii: mass spectral studies. J. Pharm. Biomed. Anal. 70, 13–25.

De Angelis, G.G., Wildman, W.C., 1969. Circular dichroism studies – I. A quadrant rule for the optically active aromatic chromophore in rigid polycyclic systems. Tetrahedron 25, 5099–5112.

Döpke, W., 1962. Alkaloids of the Hippeastrum type. Arch. Pharm. Ber. Dtsch. Pharm. Ges. 295, 920–924.

Duffield, A.M., Aplin, R.T., Budzikiewicz, H., Djerassi, C., Murphy, C.F., Wildman, W.C., 1965. Mass spectrometry in structural and stereochemical problems. LXXXII. A study of the fragmentation of some Amaryllidaceae alkaloids. J. Am. Chem. Soc. 87, 4902–4912.

376 Giordani, R.B., Vieira, P.B., Weizenmann, M., Rosember, D.B., Souza, A.P., Bonorino, C., de Carli,
377 G.A., Bogo, M.R., Zuanazzi, J.A.S., Tasca, T., 2010. Candimine-induced cell death of the
378 amitochondriate parasite Trychomonas vaginalis. J. Nat. Prod. 73, 2019–2023.

379 Giordani, R.B., de Andrade, J.P., Verli, H., Dutilh, J.H., Henriques, A.T., Berkov, S., Bastida, J.,
380 Zuanazzi, J.A.S., 2011. Alkaloids from Hippeastrum morelianum Lem. (Amaryllidaceae).
381 Magn. Reson. Chem. 49, 668–672.

382 International Tables of X-ray Crystallography, 1974. Ed. Kynoch Press, Birmingham, vol. IV, pp 99–
383 100, 149.

384 Jeffs, P.W., Hawksworth, W.A., 1963. Aromatic oxygenation patterns of some trioxyaryl
385 Amaryllidaceae alkaloids belonging to the hemi-acetal and lactone group. Tetrahedron Lett. 4,
386 217–223.

387 Kobayashi, S., Kihara, M., Shingu, T., Shingu, K., 1980. Transformation of tazettine to pretazettine.
388 Chem. Pharm. Bull. 80, 2924–2932.

389 Kreh, M., Matusch, R., Witte, L., 1995. Capillary gas chromatography-mass spectrometry of
390 Amaryllidaceae alkaloids. Phytochemistry 38, 773–776.

391 Kulhánková, A., Cahlíková, L., Novák, Z., Macáková, K., Kunes, J., Opletal, L., 2013 Alkaloids from
392 Zephyranthes robusta Baker and their acetylcholinesterase- and butyrylcholinesterase-
393 inhibitory activity. Chem. Biodivers. 10, 1120–1127.

394 Llabrés, J.M., Viladomat, F., Bastida, J., Codina, C., Serrano, M., Rubiralta, M., Feliz, M., 1986. Two
395 alkaloids from Narcissus requienii. Phytochemistry 25, 1453–1459.

396 Nair, J.J., Machocho, A.K., Campbell, W.E., Brun, R., Viladomat, F., Codina, C., Bastida, J., 2000.
397 Alkaloids from Crinum macowanii. Phytochemistry 54, 945–950.

398 Pabuççuog˘lu, V., Richomme, P., Gözler, T., Kivçak, B., Freyer, A.J., Shamma, M., 1989. Four new
399 crinine-type alkaloids from Sternbergia species. J. Nat. Prod. 52, 785–791.

400 Pacheco, P., Silva, M., Steglich, W., 1978. Alkaloids of Chilean Amaryllidaceae I. Hippeastidine and
401 epi-homolycorine two novel alkaloids. Rev. Latinoam. Quim. 9, 28–32.

402 Schnoes, H.K., Smith, D.H., Burlingame, A.L., Jeffs, P.W., Döpke, W., 1962. Mass spectra of
403 Amaryllidaceae alkaloids – the lycorenine series. Tetrahedron 24, 2825–2837.

404 Sheldrick, G.M., 2008. A program for automatic solution of crystal structure refinement. Acta
405 Crystallogr. A64, 112–221.

406 Torras-Claveria, L., Berkov, S., Codina, C., Viladomat, F., Bastida, J., 2013. Daffodils as potential crops
407 of galanthamine. Assessment of more than 100 ornamental varieties for their alkaloid content
408 and acetylcholinesterase inhibitory activity. Ind. Crops Prod. 43, 237–244.

409 Viladomat, F., Codina, C., Bastida, J., Mathee, S., Campbell, W.E., 1995. Further alkaloids from
410 Brunsvigia josephinae. Phytochemistry 40, 961–965.

411 Wagner, J., Pham, H.L., Döpke, W., 1996. Alkaloids from Hippeastrum equestre Herb. -5. Circular
412 dichroism studies. Tetrahedron 52, 6591–6600.

413 Watson, W.H., Zabel, V., 1982. Hippeastidine C17H23O4N. Cryst. Struct. Commun. 11, 157–162.

414

415 **Legends to figures**

416

417 **Figure 1.** Alkaloids identified in H. aulicum ([a]) and H. calyptratum ([b]).

418

419 **Figure 2.** CD spectrum (A) and ORTEP projection (B) of alkaloid 1. CD spectrum (C) of alkaloid 2.

420

421

Table 1. GC–MS data for H. aulicum and H. calyptratum alkaloids. Values are expressed as a relative percentage of TIC.

Alkaloid	RI	*H. aulicum*[a] (%)		*H. calyptratum*[b] (%)		M+	MS
		IA	IIA	IC	IIC		
Ismine (**21**)*	2280	–	tr[c]	–	0.45	257(35)	238(100), 211(6), 196(8), 168(6), 154(3), 106(4), 77(3)
Trisphaeridine (**22**)*	2282	tr	0.61	–	tr	223(100)	222(38), 167(8), 165(9), 164(14), 138(20), 137(9), 111(13)
Galanthamine (**13**)*	2395	11.26	1.75	12.93	6.60	287(83)	286(100), 270(13), 244(24), 230(12), 216(33), 174(27), 115(12)
Vittatine (**17**)*	2472	–	0.34	–	–	271(100)	228(25), 199(95), 187(85), 173(28), 128(32), 115(33), 56(22)
3-O-Methyl-epimacowine (**2**)*	2477	–	–	14.68	13.45	287 (100)	272(39), 256(34), 217(71), 203(21), 174(18), 157(18), 128(14)
Narwedine (**14**)*	2483	0.98	–	0.72	tr	285(84)	284(100), 242(18), 216(20), 199(18), 174(31), 128(16), 115(16)
Galanthindol (**23**)*	2487	–	–	–	1.11	281(100)	280(7), 264(13), 263(17), 262(20), 252(15), 204(7), 1(14), 132(8)
Anhydrolycorine (**12**)**	2501	–	1.84	–	5.31	251(43)	250(100), 192(13), 191(11), 165(4), 164(3), 139(2), 124(7)
Nerinine (**5**)*	2509	2.38	5.75	0.36	0.91	347(<1)	222(1), 207(2), 179(1), 164(1), 110(8), 109(100), 108(18), 94(2)
8-O-Demethylmaritidine (**16**)*	2510	–	2.41	–	tr	273(100)	256(22), 230(20), 201(83), 189(42), 174(22), 128(23), 115(24)
7-Methoxy-O-methyllycorenine (**4**)*	2538	1.60	–	–	–	361(<1)	330(8), 221(10), 191(2), 110(8), 109(100), 108(15), 94(2), 83(2)
11-Oxohaemanthamine (**3**)*	2585	1.50	tr	–	–	299(<1)	271(100), 270(37), 240(10), 238(10), 211(23), 181(77), 152(20)
Aulicine (**1**)*	2607	43.65	5.47	–	–	319(100)	304(19), 288(37), 246(18), 233(73), 218(19), 206(26), 163(7)
Haemanthamine (**15**)*	2641	30.3	71.58	–	–	301(14)	272(100), 257(10), 240(16), 181(21), 214(12), 211(14), 128(8)
Tazettine (**19**)/Pretazettine (**20**)[d,*]	2653	tr	tr	–	0.62	331(31)	316(15), 298(23), 247(100), 230(12), 201(15), 181(11), 152(7)
11-Hydroxyvittatine (**18**)*	2728	–	–	–	9.50	287(5)	258(100), 211(15), 186(20), 181(23), 153(13), 128(24), 115(23)
Lycorine (**10**)*	2746	–	9.26	0.89	41.89	287(31)	286(19), 268(24), 250(15), 227(79), 226(100), 211(7), 147(15)
Homolycorine (**7**)*	2767	2.43	–	3.21	–	315(<1)	206(<1), 178(2), 109(100), 150(1), 108(22), 94(3), 82(3)
Albomaculine (**6**)*	2815	7.16	–	66.41	13.39	345(<1)	221(1), 193(1), 165(1), 110(10), 109(100), 108(25), 94(2), 82(3)
Pseudolycorine (**11**)*	2823	–	0.64	–	4.02	289(23)	270(21), 252(12), 228(100), 214(10), 147(17), 111(18), 82(10)
2α-Methoxyhomolycorine (**8**)**	2870	–	–	–	0.64	345(<1)	206(<1), 178(2), 150(1), 139(100), 124(64), 96(5), 94(5), 81(3)
2α,7-Dimethoxyhomolycorine (**9**)*	2962	–	–	0.80	1.88	375(<1)	236(<1), 139(100), 124(54), 221(2), 193(2), 96(3), 94(3), 81(2)

RI: Retention Index.

[a] Alkaloid percentage in the total mixture of alkaloids from *H. aulicum*.

[b] Alkaloid percentage in the total mixture of alkaloids from *H. calyptratum*.

[c] Traces <0.20 of TIC.

[d] Tazettine detection by GC-MS mean identification of both alkaloids tazettine (**19**) and pretazettine (**20**) (de Andrade et al., 2012b).

* Alkaloids identified using an in-home MS database.

** Alkaloids identified using the NIST 05 database; recursive procedure, HR-MS and literature data.

Table 2. ^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of aulicine (1) (400 MHz, $CDCl_3$).

Position	δ_H (J in Hz)	COSY	NOESY	HSQC	HMBC
1α (ax)	1.77 *td* (14.0, 4.4)	H-1β, H-2α, H-2β	H-1β, H-2α	26.8 *t*	C-2, C-10b, C-11
1β (eq)	3.10–3.20 *m*	H-1α, H-2α, H-2β	H-1α, H-2β, H-11*exo*		C-10b
2α (eq)	2.04 *m*	H-1α, H-1β, H-2β, H-3	H-1α, H-2β, H-3, 3-OMe	27.7 *t*	
2β (ax)	1.44 *tdd* (13.5, 11.5, 4.0)	H-1α, H-1β, H-2α, H-3	H-1β, H-2α, H-4β, H-11*exo*		C-3
3 (ax)	3.10–3.20 *m*	H-2α, H-2β, H-4α, H-4β	H-2α, H-4α, H-4a	77.6 *d*	3-OMe
4α (eq)	2.13 *br d* (12.4)	H-3, H-4β, H-4a	H-3, H-4β, H-4a, 3-OMe	33.8 *t*	C-10b
4β (ax)	1.21 *q* (12.4)	H-3, H-4α, H-4a	H-2β, H-4α, H-11*exo*, H-12*exo*		C-2, C-3, C-4a
4a	2.93 *dd* (12.4, 5.2)	H-4α, H-4β	H-3, H-4α, H-6α	67.9 *d*	C-4, C-6, C-10a, C-11, C-12
6α	4.38 *d* (16.8)	H-6β, H-7	H-4a, H-6β, H-7	62.7 *t*	C-6a, C-7, C-10a, C-12
6β	3.71 *d* (16.8)	H-6α, H-7	H-6α, H-7, H-12*endo*		C-4a, C-6a, C-7, C-10a, C-12
6a				130.1 *s*	
7	6.10 *s*	H-6α, H-6β, 8-OMe	H-6α, H-6β, 8-OMe	101.0 *d*	C-6, C-8, C-9, C-10a
8				150.2 *s*	
9				133.9 *s*	
10				146.8 *s*	
10a				126.0 *s*	
10b				43.2 *s*	
11*endo*	1.90 *ddd* (12.0, 8.8, 3.2)	H-11*exo*, H-12*endo*, H-12*exo*	H-11*exo*, H-12*endo*	36.5 *t*	C-4a, C-10b
11*exo*	2.23 *ddd* (12.4, 10.4, 6.4)	H-11*endo*, H-12*endo*, H-12*exo*	H-1β, H-2β, H-4β, H-11*endo*, H-12*exo*		C-1, C-10a, C-10b, C-12
12*endo*	2.78 *ddd* (12.8, 8.8, 6.4)	H-11*endo*, H-11*exo*, H-12*exo*	H-6β, H-11*endo*, H-12*exo*	52.2 *t*	C-4a, C-6, C-11
12*exo*	3.36 *ddd* (12.8, 10.0, 3.2)	H-11*endo*, H-11*exo*, H-12*endo*	H-4β, H-11*exo*, H-12*endo*		C-6
3-OMe	3.38 *s* (3H)		H-2α, H-4α	55.6 *q*	C-3
8-OMe	3.80 *s* (3H)	H-7	H-7	55.7 *q*	C-8
9-OMe	3.87 *s* (3H)			61.0 *q*	C-9

435 **Table 3** ^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of 3-O-methyl-epimacowine (2) (500
436 MHz, $CDCl_3$).

Position	δ_H (*J* in Hz)	COSY	NOESY	HSQC	HMBC
1	6.48 *dd* (10.0, 2.0)	H-2	H-2, H-10	129.1 *d*	C-3, C-4a, C-10a, C-11
2	5.84 *dt* (10.0, 1.5)	H-1	H-1, H-3, 3-OMe	129.2 *d*	C-4, C-10b
3	4.00 *ddt* (10.5, 5.5, 2.0)	H-4α, H-4*β*	H-2, H-4α, H-4a, 3-OMe	76.3 *d*	C-1, 3-OMe
4α	2.29 *m*	H-3, H-4*β*, H-4a	H-3, H-4a, H-4*β*	30.8 *t*	C-2, C-3, C-4a, C-10b
4*β*	1.58 *ddd* (13.5, 12.0, 10.5)	H-3, H-4α, H-4a	H-4α, H-11*exo*, H-12*exo*		C-3, C-4a, C-10b
4a	3.28 *dd* (13.5, 4.0)	H-4α, H-4*β*	H-3, H-4α, H-6α	66.8 *d*	C-12
6α	4.45 *d* (16.5)	H-6*β*	H-4a, H-6*β*, H-7	61.5 *t*	C-6a, C-7, C-10a, C-12
6*β*	3.82 *d* (17.0)	H-6α	H-6α, H-7, H-12*endo*		C-4a, C-6a, C-7, C-10a, C-12
6a				125.0 *s*	
7	6.59 *s*		H-6α, H-6*β*	113.0 *d*	C-6, C-9, C-10a
8				144.3 *s*	
9				145.3 *s*	
10	6.78 s		H-1, 9-OMe	104.9 *d*	C-6a, C-8, C-10a, C-10b
10a				136.7 *s*	
10b				44.7 *s*	
11*endo*	2.20 *ddd* (12.0, 9.0, 4.5)	H-11*exo*, H-12*endo*, H-12*exo*	H-11*exo*, H-12*endo*	44.8 *t*	C-4a, C-10a, C-10b, C-12
11*exo*	2.12 *ddd* (12.0, 10.5, 6.0)	H-11*endo*, H-12*endo*, H-12*exo*	H-4*β*, H-11*endo*, H-12*exo*		C-1, C-10a, C-10b, C-12
12*endo*	2.95 *ddd* (13.0, 9.0, 6.0)	H-11*endo*, H-11*exo*, H-12*exo*	H-6*β*, H-11*endo*, H-12*exo*	53.2 *t*	C-4a, C-6, C-10b
12*exo*	3.50 *ddd* (13.0, 10.5, 4.5)	H-11*endo*, H-11*exo*, H-12*endo*	H-4*β*, H-12*endo*, H-11*exo*		C-6
3-OMe	3.42 *s* (3H)		H-2, H-3	56.2 *q*	C-3
9-OMe	3.89 *s* (3H)		H-10	56.2 *q*	C-9

437

438

439

440

441

442 **Table 4** ^{1}H NMR, COSY, and HSQC data of 11-oxohaemanthamine (3) (500 MHz, $CDCl_3$).

Position	δ_H (*J* in Hz)	COSY	HSQC
1	6.54 *d* (10.0)	H-2	126.8 *d*
2	6.21 *ddd* (10.0, 5.5, 1.5)	H-1, H-3	129.5 *d*
3	3.84 *ddd* (5.5, 3.5, 2.0)	H-2, H-4α; H-4β	71.8 *d*
4α	1.47 *td* (14.0, 4.0)	H-3; H-4β, H-4a	29.8 *t*
4β	2.25 *br d* (14.0)	H-3; H-4α, H-4a	
4a	3.55 *m*	H-4α; H-4β	61.5 *d*
6α	3.83 *d* (17.0)	H-6β, H-7	60.6 *t*
6β	4.58 *d* (17.0)	H-6α, H-7	
7	6.52 *s*	H-6α, H-6β	106.9 *d*
10	6.83 *s*		104.2 *d*
12*endo*	3.27 *dd* (18.5, 1.5)	H-12*exo*	59.3 *t*
12*exo*	3.56 *d* (18.5)	H-12*endo*	
3-OMe	3.37 *s* (3H)		56.8 *q*
OCH_2O	5.92 2*d* (1.5)		101.3 *t*

443

444

445

446

447 **Table 5** ^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of 7-methoxy-O-methyllycorenine (4)
448 (500 MHz, CD_3OD).

Position	δ_H (*J* in Hz)	COSY	NOESY	HSQC	HMBC
1	4,40 *br d* (6.5)	H-2α, H-2β, H-3, H-10b	H-2α, H-2β, H-10b, 6-OMe	67.0 *d*	C-3, C-4a, C-6, C-10a
2α	2,67 *ddt* (19.0, 6.5, 3.0)	H-1, H-2β, H-3, H-4a	H-1, H-2β, H-3	32.5 *t*	
2β	2,29 *dt* (19.5, 3.0)	H-1, H-2α, H-3, H-4a	H-1, H-2α, H-3		
3	5,55 *br s*	H-1, H-2α, H-2β, H-4a, H-11α/β	H-2α, H-2β, H-11α/β	118.1 *d*	
4				140.2 *s*	
4a	2,92 *br d* (10.0)	H-2α, H-2β, H-3, H-10b	NMe	69.2 *d*	
6β	5,52 *s*		6-OMe	97.8 *d*	C-1, C-7, C-6a, C-10a, 6-OMe
6a				121.7 *s*	
7				153.2 *s*	
8				142.9 *s*	
9				154.7 *s*	
10	6.85 *s*	9-OMe	H-10b, 9-OMe, NMe	110.0 *d*	C-6a, C-8, C-9, C-10a, C-10b, C-7
10a				134.1 *s*	
10b	2.47 *dd* (10.0, 2.0)	H-1, H-4a	H-1, H-10, H-12α	44.1 *d*	C-4a, C-6a, C-10, C-10a
11α/β	2.49–2.58 *m*	H-3, H-12α, H-12β	H-3, H-12α	28.6 *t*	
12α	3.22 *ddd* (10.5, 7.5, 3.0)	H-11α/β, H-12β	H-10b, H-11α/β, H-12β, NMe	57.7 *t*	
12β	2.42 *m*	H-11α/β, H-12α	H-12α, NMe		
6-OMe	3.51 *s* (3H)		H-1, H-6β	55.6 *q*	C-6
7-OMe	3.89 *s* (3H)			61.5 *q*	C-7
8-OMe	3.82 *s* (3H)			61.2 *q*	C-8
9-OMe	3.87 *s* (3H)	H-10	H-10	56.6 *q*	C-9
NMe	2.11 *s* (3H)		H-4a, H-10, H-12α, H-12β	44.0 *q*	C-4a, C-12

449

450

451

452 **Table 6** ^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of nerinine (5) (400 MHz, $CDCl^{3}$).

453

Position	δ_H (*J* in Hz)	COSY	NOESY	HSQC	HMBC
1	4.52 *ddd* (5.6, 2.0, 1.0)	H-2α, H-2β, H-10b	H-2α, H-2β, H-10b	66.3 *d*	C-3, C-4a, C-6
2α	2.65 *ddt* (19.2, 6.0, 2.8)	H-1, H-2β, H-3	H-1, H-2β, H-3	31.9 *t*	
2β	2.34 *dt* (19.2, 2.5)	H-1, H-2α, H-3	H-1, H-2α, H-3		
3	5.47 *br m*	H-2α, H-2β, H-4a, H-11α/β	H-2α, H-2β, H-11α/β	115.8 *d*	
4				141.1 *s*	
4a	2.73 *d* (9.6)	H-3, H-10b	H-6β, H-12β, NMe	67.5 *d*	
6β	6.14 *s*		H-4a, 7-OMe	89.8 *d*	C-1, C-7, C-10a
6a				121.2 *s*	
7				151.3 *s*	
8				141.2 *s*	
9				153.1 *s*	
10	6.77 *s*		H-10b, 9-OMe, NMe	109.1 *d*	C-6a, C-8, C-9, C-10b
10a				133.6 *s*	
10b	2.41 *dd* (9.6, 1.5)	H-1, H-4a	H-1, H-10	44.5 *d*	C-4a, C-6a, C-10, C-10a
11α/β	2.44–2.51 *br m*	H-3, H-12α, H-12β	H-3, H-12α, H-12β	28.4 *t*	
12α	3.14 *m*	H-11α/β, H-12β	H-11α/β, H-12β, NMe	57.1 *t*	C-4, C-4a
12β	2.24 *q* (9.2)	H-11α/β, H-12α	H-4a, H-11α/β, H-12α, NMe		NMe
7-OMe	3.99 *s* (3H)		H-6β	61.4 *q*	C-7
8-OMe	3.87 *s* (3H)			61.0 *q*	C-8
9-OMe	3.86 *s* (3H)		H-10, NMe	56.3 *q*	C-9
NMe	2.06 *s* (3H)		H-4a, H-10, H-12α, H-12β, 9-OMe	44.6 *q*	C-4a, C-12

454

455

456

457

458

459 **Table 7** ^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of albomaculine (6) (400 MHz, $CDCl_3$).

460

Position	δ_H (J in Hz)	COSY	NOESY	HSQC	HMBC
1	4.68 *br m*	H-2α/β, H-3, H-10b	H-2α/β, H-10b	76.3 *d*	C-3, C-4a, C-10a
2α/β	2.55–2.60 *br m*	H-1, H-3, H-11α/β	H-1, H-3	31.0 *t*	C-1, C-3, C-10b
3	5.48 *br m*	H-1, H-2α/β, H-4a, H-11 α/β	H-2α/β, H-11α/β	115.6 *d*	
4				140.6 *s*	
4a	2.72 *d* (10.0)	H-3, H-10b	NMe	66.0 *d*	
6				162.4 *s*	
6a				111.6 *s*	
7				156.3 *s*	
8				142.7 *s*	
9				157.2 *s*	
10	6.78 *s*		H-10b, 9-OMe, NMe	107.4 *d*	C-6a, C-8, C-10b
10a				140.8 *s*	
10b	2.63 *d* (10.0)	H-1, H-4a	H-1, H-10	45.5 *d*	
11α/β	2.45–2.53 *br m*	H-2α/β, H-3, H-12α, H-12β	H-3, H-12α, H-12β	28.1 *t*	C-4
12α	3.13 *ddd* (9.6, 7.2, 3.6)	H-11α/β, H-12β	H-11α/β, H-12β, NMe	56.6 *t*	
12β	2.23 *q* (9.6)	H-11α/β, H-12α	H-11α/β, H-12α		C-11, NMe
7-OMe	3.99 *s* (3H)			62.1 *t*	C-7
8-OMe	3.89 *s* (3H)			61.3 *t*	C-8
9-OMe	3.91 *s* (3H)		H-10, NMe	56.5 *t*	C-9, C-10
NMe	2.05 *s* (3H)		H-4a, H-10, H-12α, 9-OMe	43.7 *t*	

461

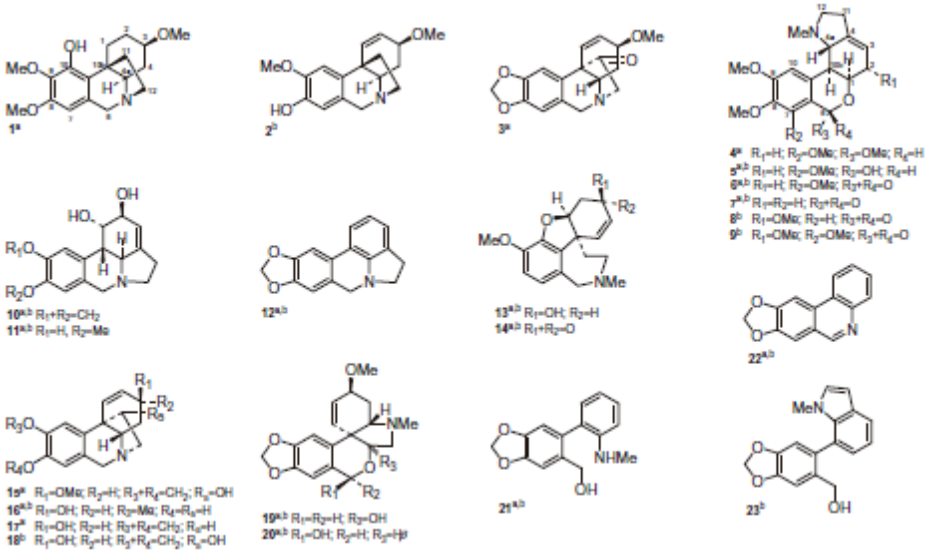
462

463

464 **Figure 1**

465

466



467

468

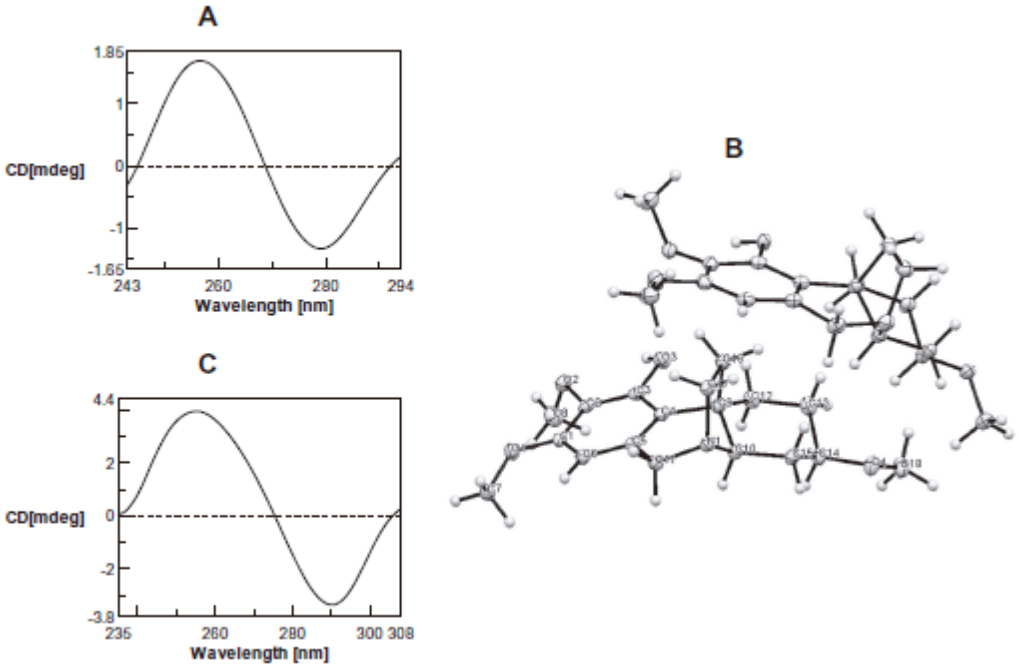
469

470

471 **Figure 2**

472

473



474