Research on the Alkaloids of Amaryllidaceae Plants: Genera *Lycoris* and *Hippeastrum*

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Abbreviations and symbols

AChE	Acetylcholinesterase
AD	Alzheimer's Disease
APG	Angiosperm Phylogeny Group
BSTFA	*N,O*-Bis-(trimethylsilyl)trifluoroacetamide
br	Broad
Calcd	Calculated
CD	Circular Dichroism
COSY	Correlation Spectroscopy
d	Doublet
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
dddd	Doublet of doublet of doublet of doublets
DW	Dry Weight
EI	Electron Ionization (also Electron Impact)
EIMS	Electron Impact Mass Spectrometry
GAL	Galanthamine
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HMBC	Heteronuclear Multiple Bond Correlation spectroscopy
HPLC	High Performance Liquid Chromatography
HR-ESI-MS	High Resolution-Electrospray Ionization-Mass Spectrometry
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Correlation spectroscopy
IC_{50}	Half maximal inhibitory concentration
IR	Infrared spectroscopy
J	Coupling constant
L-Phe	L-Phenylalanine
L-Tyr	L-Tyrosine
m	Multiplet
M	Molecular
MHz	Megahertz
MS	Mass Spectrometry
m/z	Mass/charge
NOESY	Nuclear Overhauser Effect Spectroscopy
NTPDase	Nucleoside Triphosphate Diphosphohydrolase

ppm	Parts per million
PTLC	Preparative Thin Layer Chromatography
q	Quartet
R. I.	Retention Index
r-DA	retro-Diels-Alder
NMR	Nuclear Magnetic Resonance spectroscopy
^{1}H NMR	Proton Nuclear Magnetic Resonance spectroscopy
^{13}C NMR	Carbon-13 Nuclear Magnetic Resonance spectroscopy
1D NMR	One-dimensional Nuclear Magnetic Resonance spectroscopy
2D NMR	Two-dimensional Nuclear Magnetic Resonance spectroscopy
TIC	Total Ion Current
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
UV	Ultraviolet
VLC	Vacuum Liquid Chromatography
WHO	World Health Organization
$[\alpha]_{D}$	Optical rotation
δ	Chemical shit
$[\theta]$	Molar ellipticity

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1. INTRODUCTION

1. Introduction

1.1. Natural products

Natural products can generate a variety of goods and services, including medicine, food products and new materials. Additionally, natural areas are increasingly becoming centers of tourism. Active principles of natural origin are sometimes the treatment of choice for certain illnesses, and generate benefits by meeting the pharmaceutical demand.

The history of natural products dates back practically to the emergence of human civilization (Lahlou, 2013). Since then, natural products have played a considerable role in health protection and disease prevention. The ancient civilians of Mesopotamia, Egypt, China, Indian, and Greece provided written evidence for the use of natural sources for curing diverse diseases (Dias et al., 2012). The usefulness of plants for treating diseases was recorded in the Emperor Shennung's classic herbals of China (2700 BC) and Eber's papyrus in Egypt (1550 BC). However, it was not until the 19th century that scientists isolated active components from various medicinal plants. Friedrich Sertuerner isolated morphine from *Papaver somniferum* in 1806, and since then natural products have been extensively screened for their medicinal purposes. A recent review of natural products as sources of new drugs over the 30 years from 1981 to 2010 composed by Newman and Cragg is a very significant contribution to this area of investigation. In their review, they point out that around 35% molecular structures approved as drugs correspond to compounds of natural and semisynthetic derivatives, while 30% are synthetic molecular compounds inspired by natural products or with a pharmacophore developed from a natural compound (Newman and Cragg, 2012). They consider the rapid identification of effective, novel lead structures is a vital necessity, now made posible by the emergence of novel screening systems and the explosion of genetic information (Cragg and Newman, 2013).

According to studies conducted by the World Health Organization (WHO), about 80% of the world's population relies on traditional medicine (WHO, 2002), and

available data suggests that the market for traditional medicine is still substantial (WHO, 2014). The output of Chinese materia medica was estimated to amount to US$ 83.1 billion in 2012, an increase of more than 20% from the previous year. Out-of-pocket spending for natural products in the United States was US$ 14.8 billion in 2008 by WHO's investigation last year (WHO, 2014). Over all, many natural products and synthetically modified natural product derivatives have been successfully developed for clinical use to treat human diseases in almost all therapeutic areas.

1.2. The Amaryllidaceae family

The Amaryllidaceae are a family of herbaceous, mainly perennial and bulbous (rarely rhizomatous) flowering plants included in the monocot order Asparagales. According to the latest update and classification of the Angiosperm Phylogeny Group (APG), the family Amaryllidaceae J.St.-Hil. has three subfamilies: Agapanthoideae, Allioideae and Amaryllidoideae (Chase et al., 2009; APG III, 2009), supported by studies of molecular biology and taxonomy (Meerow et al., 1999; Meerow and Snijman, 2006). These subfamilies were previously regarded as families: Agapanthaceae, Alliaceae and Amaryllidaceae, respectively.

Important misunderstandings as a result of using both classifications and the presence or absence of alkaloids may occur, since the concept used more widely associated with the term "Amaryllidaceae" implies only a subfamily of the current taxon.

The species of the subfamily Amaryllidoideae are preferably distributed in tropical and subtropical regions, but also in temperate zones. Thus, one of its distinguishing features is great adaptability. We can find the species of this subfamily in mountainous areas of the Andes, Mediterranean, temperate Asia, Oceania and southern Africa (Meerow and Snijman, 1998). The species of the subfamily Amaryllidoideae are perennial bulbous herbs, often with showy flowers, which provide ornamental value. The subfamily comprises about 54 genera and 796 species. Taking into consideration the whole Amaryllidaceae family, including the Alliaceae and Agapanthaceae subfamilies, these values reach 73 genera and about 1600 species (Dutilh et al., 2013).

1.3. The Amaryllidaceae alkaloids

Since the first isolation of lycorine from *Lycoris radiata*, almost 450 structurally diverse alkaloids have been isolated from plants of the Amaryllidaceae family. The alkaloids of this family represent a large and still expanding group of isoquinoline alkaloids, which are classified mainly into nine skeleton types. The representative alkaloids are: norbelladine, lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine and galanthamine (Bastida et al., 2006) (Figure 1.1). Their general chemical characteristics can be summarized as follows: a fundamental ring system composed of a C_1-C_6 and an N-C_2-C_6 building block, derived from the amino acids L-phenylalanine and L-tyrosine, respectively. They are moderately weak bases (pKa values between 6 and 9). Commonly, each alkaloid contains one nitrogen atom, which is secondary, tertiary or even quaternary. Typically, the carbon content varies from 16 to 20 atoms, depending on the substituents of the ring system (Bastida and Viladomat, 2002).
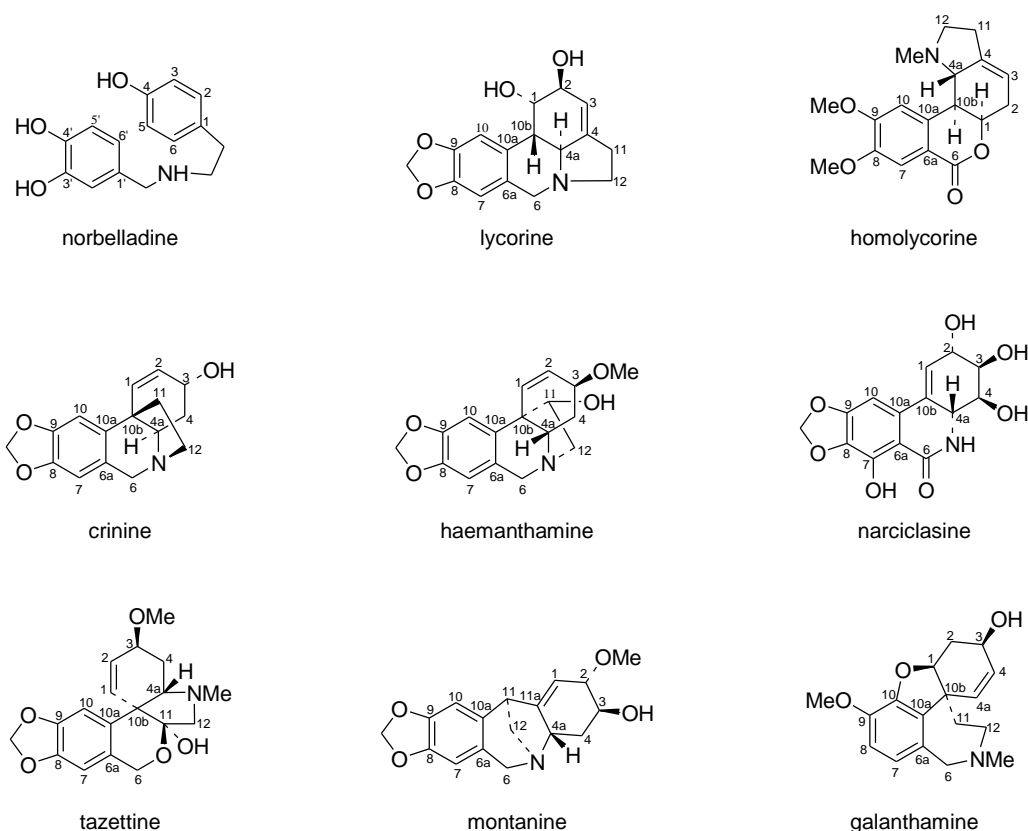
Figure 1.1: Amaryllidaceae alkaloid types.

1.3.1. Biosynthesis and structural types of Amaryllidaceae alkaloids

The biosynthetic pathway of the Amaryllidaceae alkaloids usually follows four stages, starting with the enzyme preparation of precursors from the amino acids L-phenylalanine (L-Phe) and L-tyrosine (L-Tyr). Although L-Phe and L-Tyr are closely related in chemical structure, they are not interchangeable in plants (Bastida et al., 2006).

In the Amaryllidaceae alkaloids, L-Phe serves as a primary precursor of the C_6-C_1 fragment, corresponding to ring A and the benzylic position (C-6), whereas, L-Tyr is the precursor of ring C, the two-carbon side chain (C-11 and C-12) and nitrogen, C_6-C_2-N. The conversion of L-Phe to the C_6-C_1 unit requires the loss of two carbon atoms from the side chain as well as the introduction of at least two oxygenated substituents into the aromatic ring, which occurs through cinnamanic acid or its derivatives, involving the participation of the enzyme phenylalanine ammonia lyase (PAL). The fragmentation of the cinnamic acid involves oxidation of the β-carbon to the ketone or acid level, where the final product is protocatechuic aldehyde. On the other hand, L-Tyr is degraded no further than tyramine before incorporation into the Amaryllidaceae alkaloids (Figure 1.2) (Machocho, 2000; Bastida et al., 2011; Pigni, 2013).

The second stage involves merging the biosynthesis of tyramine and the protocatechuic aldehyde, resulting in norbelladine by forming a Schiff's base. This reaction occupies a pivotal position since it represents the entry of primary metabolites into a secondary metabolic pathway (Figure 1.2) (Bastida et al., 2011).

Norbelladine can undergo oxidative coupling of phenols in Amaryllidaceae plants, once ring A has been suitably protected by methylation, which is considered as the third step (Figure 1.3).

Finally, the last stage includes a series of sequential reactions resulting in the diversification into the other eight skeletons shown in Figure 1.4. *Ortho-para'* phenol oxidative coupling of *O*-methylnorbelladine results in the formation of a lycorine-type
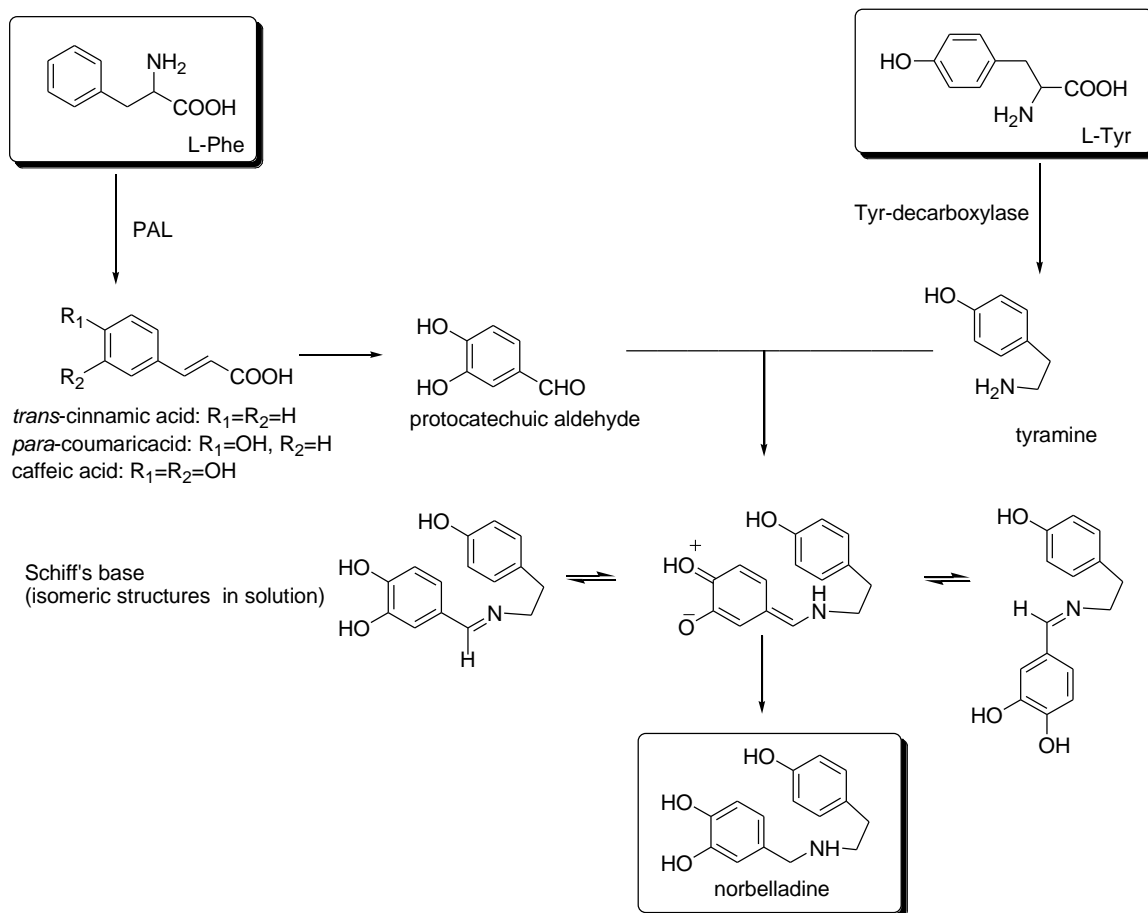
Figure 1.2: Biosynthetic pathway to norbelladine.
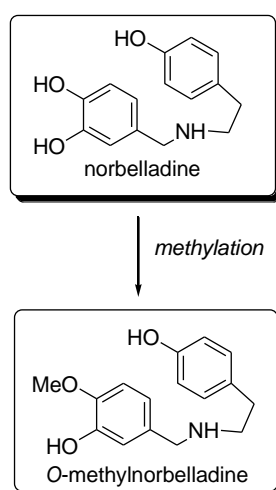
Figure 1.3: Methylation pathway to *O*-methylnorbelladine
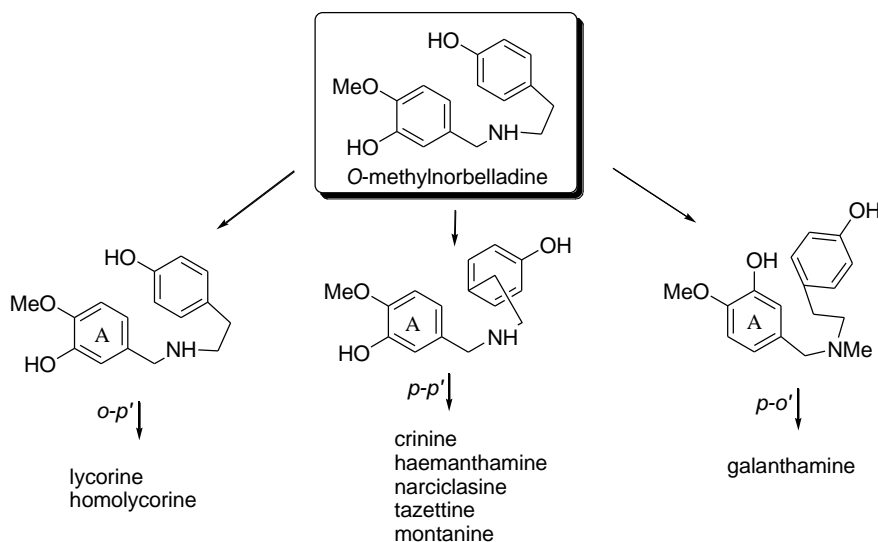
Figure 1.4: Phenol oxidative coupling in Amaryllidaceae alkaloids.

skeleton, from which homolycorine-type compounds proceed as well. The galanthamine-type is the only skeleton which originates from *para-ortho'* phenol oxidative coupling. And *para-para'* phenol oxidative coupling leads to the formation of crinine, haemanthamine, tazettine, narciclasine and montanine structures (Figure 1.4) (Bastida et al., 2006). Subsequent transformations may involve oxidation, reduction, ring opening and rotation, where one alkaloid may be converted into another (Machocho, 2000).

1.3.2. Other structural types of Amaryllidaceae alkaloids

Although the majority of alkaloids found in the Amaryllidoideae family can be cataloged in one of nine skeleton types discussed so far, some structures could be described as representatives of new types that are not common in the family (Figure 1.4).

The new group of alkaloids was led by galanthindol. This compound, initially isolated from *Galanthus plicatus* ssp. *byzantinus*, is described as a representative of an unfused indole ring (Unver et al., 2003; Unver, 2007). Lycosinine A and its derivatives (Yang et al., 2005) have the same origin. In fact, both structures are very similar to ismine (Suau et al., 1990), and are considered the products of catabolism of the alkaloid (Bastida et al., 2006).

Galasine was isolated from *Galathus elwesii*. Its molecule is linked to infinite

one-dimensional chains by intermolecular hydrogen bonds between the hydroxyl group and the *N*-atom of a neighboring molecule (Latvala et al., 1995).

Possibly cherylline, augustamine and graciline derivatives represent the most distinct structures within the classic nine skeleton types. Cherylline is an unusual phenolic alkaloid first isolated from *Crinum powellii* (Brossi et al., 1970). Augustamine was described in *Crinum augustum* (Ali et al., 1983) and some of its derivatives were isolated years later from *Crinum kirkii* (Machocho et al., 2004). Graciline and its derivatives have been isolated from various species of the genus *Galanthus* (Noyan et al., 1998; Unver et al., 2001).
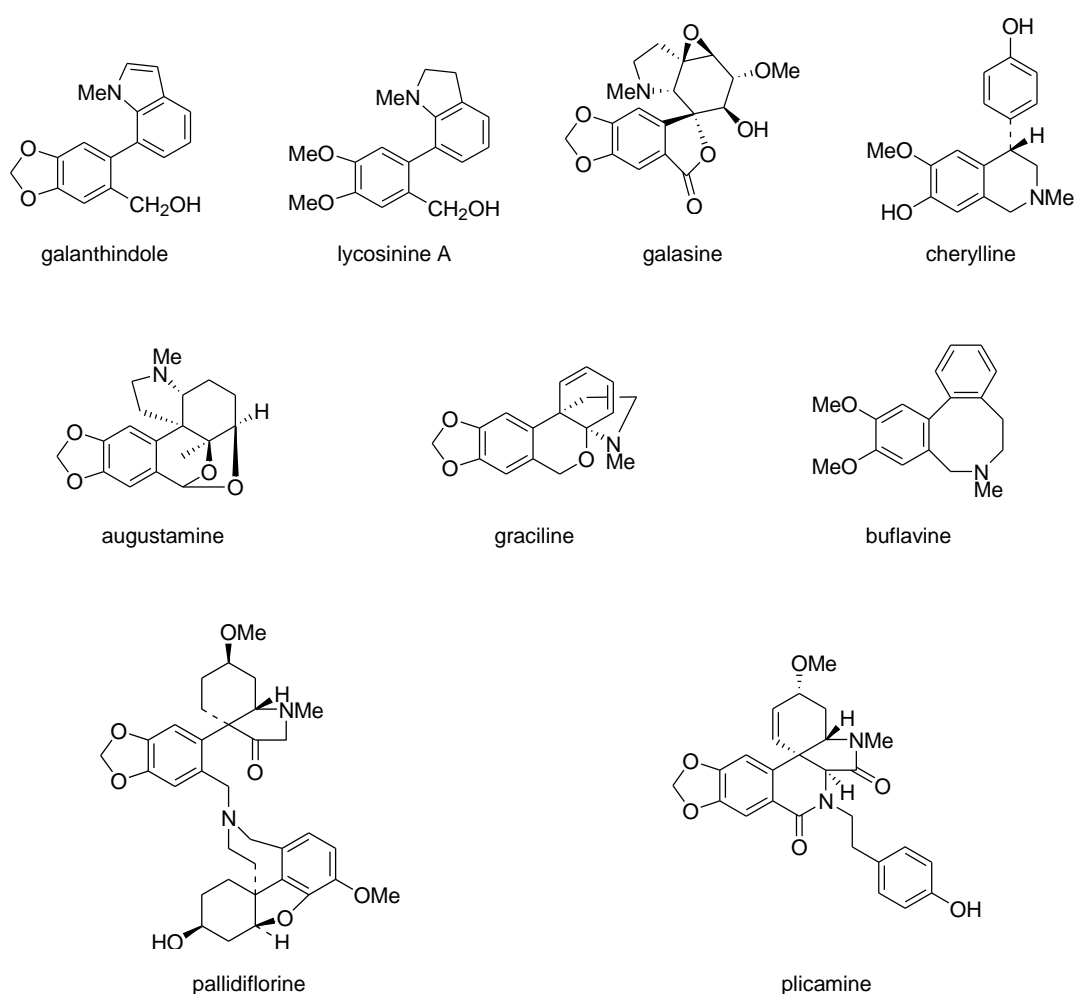
Figure 1.4: Other structural types of Amaryllidaceae alkaloids.

Buflavine and 8-*O*-demethylbulflavine were isolated for the first time from the bulbs of *Boophane flava* (Viladomat et al. 1995). Their structures have been established by spectroscopic methods and are representative of the unusual natural Amaryllidaceae

alkaloids with an eight-membered *N*-heterocyclic ring, which had been previously reported only from *Galanthus nivalis* (Hoshino, 1998).

Pallidiflorine was obtained from *Narcissus pallidiflorus* (Codina et al., 1990), and its formation can be explained by the attack of the nitrogen of *N*-demethyllycoramine on C-6' of tazzettine with opening of the B ring and formation of the keto group (Hoshino, 1998). Plicamine derivatives isolated from the genus *Galanthus* (Unver et al., 1999), *Cyrtanthus* (Brine et al., 2002) and more recently in *Narcissus* (de Andrade et al., 2012a) have been proposed as alkaloids of a new skeleton type within this family (Unver, 2007). However, its similarity to the skeleton of the tazettine should be considered.

1.3.3. Other alkaloids

Mesembrano-type or *Sceletium* alkaloids, which are generally present in the Aizoaceae family, have been isolated in some species of the Amaryllidoideae subfamily. Thus, mesembrenol was obtained from *Crinum oliganthum* (Doepke et al., 1981) and mesembrenone was isolated and characterized from *Narcissus pallidulus* (Bastida et al., 1989). Recently, numerous mesembrano-type alkaloids form *Narcissus triandrus* have been identified and isolated, among which we can highlight 4'-*O*-demethylmesembrenone and 6-epimesembranol (Figure 1.5) (Pigni et al., 2013).

In addition, bulbocapnine and capnoidine, found in typical Fumarioideae, Lauraceae and Papaveraceae plants, have been isolated from *Galanthus nivalis* subsp. *cilicicus* (Figure 1.5) (Kaya et al., 2004).

On the opposite side, in two cases the isolation and characterization of typical Amaryllidaceae alkaloids have been described in other plant families. Thus, the crinamine alkaloid isolated from *Dioscorea dregeana* was the first Amaryllidaceae alkaloid reported in another botanical family, the Dioscoreaceae (Mulholland et al., 2002). Recently, some alkaloids with a homolycorine and narciclasine skeleton were identified in *Hosta plantaginea* (Asparagaceae) (Wang et al., 2007b). These secondary metabolites could constitute a tool of great interest in chemotaxonomic studies of the Amaryllidaceae family.

mesembrenol   mesembrenone   4'-O-demetilmesembrenone   6-epimesembranol

bulbocapnine   capnoidine
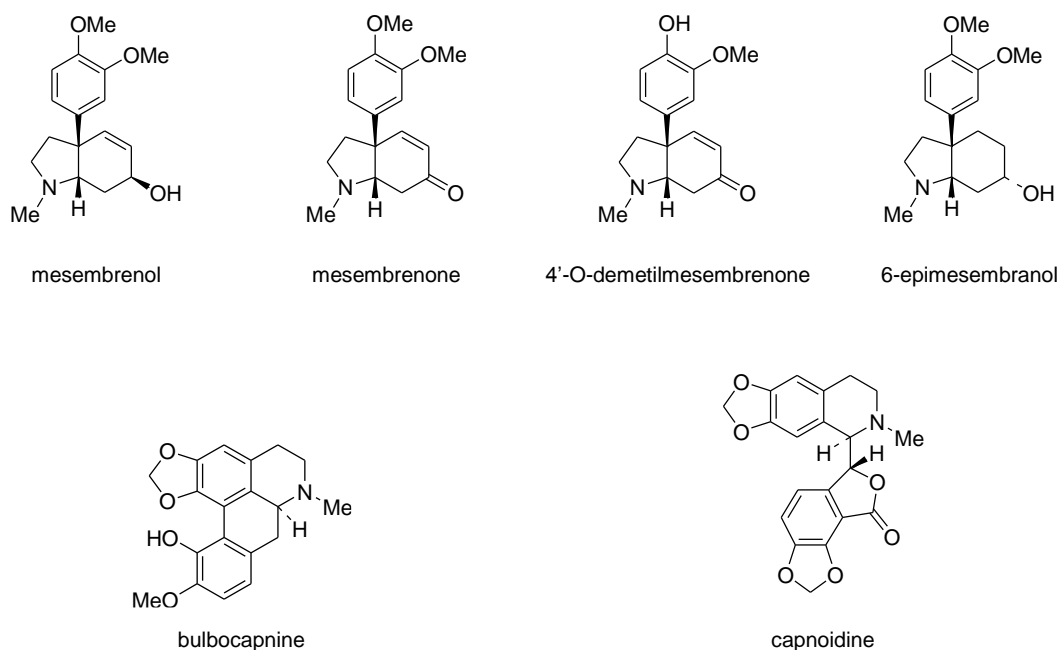
Figure 1.5: Other alkaloids.

1.4. Alkaloids from the genus *Lycoris*

The *Lycoris* genus is distributed in Asia, mainly in China, Japan, Korea, Laos, Myanmar, Pakistan, Thailand, Vietnam and so forth. Until April of 2015, 22 species and one hybrid of this genus were recognized by the *World Checklist of Selected Plant Families*. Notably, there are 15 species (ten endemic) in China.

The plants of *Lycoris* are perennial bulb herbs. The bulbs are subglobose to ovoid, with a brown or black skin. Leaves are ligulate, appearing before or after anthesis, 30-60 cm long and only 0.5-2 cm broad. The scape is erect, simple and solid, 30-70 cm tall, bearing a terminal umbel of four to eight flowers, which can be white, creamy, gold, pink or bright red. Perianths, sometimes with an undulate margin, are funnelform, consisting of six oblanceolate or narrowly elliptic tepals. The long filiform stamens are inserted at the throat of the perianth tube. Most flower from July to September. The ovary is trilocular with few ovules, and the fruit is a three-valved capsule containing several black subglobose seeds (Ji and Meerow, 2000; Yu et al., 2006).

The taxonomy of the genus *Lycoris* is difficult because of its wide distribution area and easy natural hybridization. To distinguish and classify the genus, morphology

(Zhou et al., 2005, 2006), cytology (In et al., 1996), and genetics aspects have been considered (Xie et al., 2007). In genomics research, RAPD (random amplified polymorphism DNA) and ISSR (inter-simple sequence repeats) molecular markers (Roh et al., 2002; Zhang et al., 2002; Deng et al., 2006), ITS (internal transcribed spacers) sequencing (Chen et al., 2009; Quan et al., 2012), isozyme (Lee et al., 2001), *trn*L-F sequencing and phylogenetic clustering (Yuan et al., 2008) have been applied in research into the genetic relationship among *Lycoris* species, as well as the taxonomy of the genus (Jiang et al., 2009b).

Phytochemical studies of the genus *Lycoris* began at the end of the 19th century. The first study was carried out with *L. radiata*, which yielded the alkaloids lycorine (**1**) and sekisanine (tazettine) (**88**) (Morishima, 1899). In the 1930s, Kondo *et al.* initiated a series of in-depth studies on *Lycoris* alkaloids, resulting in the elucidation of the structures of lycorine (**1**), tazettine (**88**), lycoramine (**105**) and homolycorine (**32**) (Kondo and Ikeda, 1940; Kondo and Katura, 1940; Kondo et al., 1932, 1954). Galanthamine (**97**) was isolated from *Lycoris* species in 1957 (Boit and Ehmke, 1957). In 1959, two new phenolic alkaloids, norpluviine (**9**) and 8-*O*-demethylhomolycorine (**40**), were isolated from *L. radiata* (Uyeo and Yanaihara, 1959). From the 1960s to the 1980s, exhaustive research work led to the first isolation of sanguinine (**100**) from *L. sanguinea*, as well as *O*-demethyllycoramine (**107**) from *L. radiata*; at the same time, other species such as *L. squamigera*, *L. guangxiensis* and *L. chinensis* were studied (Hung and Ma, 1964; Takagi and Yamaki, 1974; Kobayashi et al., 1976, 1980; Li et al., 1987; Ma et al., 1987). In the 1990s, Kihara *et al.* isolated a new alkaloid from flowers of *L. incarnata* named incartine (**22**), which has been proposed as a biosynthetic intermediate in the conversion of galanthine (**5**) to narcissidine (**17**) (Kihara et al., 1994). Two new alkaloids, norsanguinine (**102**) and 3'-hydroxybutanoylnorsanguinine (**103**), were isolated and characterized from the bulbs of *L. sanguinea*, in addition to five known Amaryllidaceae alkaloids (Abdallah, 1995).

During the first decade of the current century, the continued investigation of *Lycoris* plants yielded further novel structures. Reported in *L. radiata* bulbs, lycosinine A (**113**) and lycosinine B (**114**) were classified as a new structural group

(galanthindole-type), in addition to the unusual lycorine-type alkaloids, lycoranine A (**23**) and lycoranine B (**24**), all of them completely characterized by spectroscopic methods (Yang et al., 2005; Wang et al., 2009). Moreover, a new montanine-type alkaloid named squamigine (**95**), together with 3-*O*-ethyltazettinol (**90**) and 2*R*-hydroxy-*N*,*O*-dimethylnorbelladine (**116**), were isolated from the bulbs of *L. squamigera* and *L. aurea* (Pi et al., 2009; Takayama et al., 2009).

In the last five years, investigations in the genus *Lycoris* have increased rapidly, and a number of new alkaloids have been isolated, especially in the species *Lycoris radiata*. Studies with bulbs of *L. radiata* resulted in the isolation of 14 new alkaloids, including 1-hydroxyungeremine (**27**), 5,6-dehydrolycorine (**28**) and 5,6-dehydrodihydrolycorine (**29**), classified as lycorine-type; 3-acetyl-6*β*-acetyoxybulbispermine (**66**), 6*β*-acetoxycrinamine (**73**), 6*β*-acetoxybulbispermine (**74**), and 6*β*-acetyl-8-hydroxy-9-methoxycrinamine (**75**), belonging to the haemanthamine-type; four new homolycorine-type alkaloids, 2*α*-methoxy-6-*O*-ethyloduline (**49**), 8-*O*-acetylhomolycorine-*N*-oxide (**52**), 2*α*-hydroxy-8-*O*-demethylhomolycorine-*N*-oxide (**54**), and 8,9-methylenedioxylhomolycorine-*N*-oxide (**56**), together with 5,6-dihydro-*N*-methyl-2-hydroxyphenanthridine (**84**), *O*-demethyllycoramine-*N*-oxide (**111**), and *N*-methoxy-carbonyl-2-demethylnorisocorydione (**124**) (Feng et al., 2011; Hao et al., 2013; Huang et al., 2013; Li et al., 2013; Liu et al., 2015).

Similarly, the new alkaloids 1,2-dihydroxyanhydrolycorin-6-one (**15**), 1,2-dihydroxyanhydrolycorine-*N*-oxide (**31**), *O*-*n*-butyllycorenine (**39**) 2*α*-hydroxy-6-*O*-*n*-butyloduline (**50**), 8-*O*-demethyhomolycorine-*N*-oxide (**55**), 8-demethyldehydrocrebanine (**121**), and 2-demethylisocorydione (**123**) have been isolated from *Lycoris aurea* (Jin et al., 2014; Song et al., 2014). In addition, further work by Wu et al. resulted in the isolation of two new alkaloids, lycosprenine (**14**) and 2*α*-methoxy-6-*O*-methyl- lycorenine (**37**), from the bulb of *Lycoris sprengeri*, which was the first time new alkaloids have been reported in this species (Wu et al., 2014). Three new bulbocapnine-type alkaloids, 2-hydroxyanhydrolycorine-*N*-oxide (**30**), *N*-methoxycar- bonylnandigerine (**119**), and *N*-methoxycarbonyllindcarpine (**120**),

together with 10-*O*-methylhernovine-*N*-oxide (**125**) were extracted from *Lycoris caldwellii* (Cao et al., 2013).

To the best of our knowledge, seventeen species from the genus *Lycoris* have been phytochemically studied to date and 125 alkaloids have been found and classified in different structural types (Table 1.1, Figure 1.6-1.15).

Table 1.1: Alkaloids reported in the genus *Lycoris*.

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L. incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
Lycorine-type																	
lycorine (**1**)	1,2,3,42	1	1,5,6,27,39,42		1,7,8,9,42	11	1,42	1	1,12,42	1,14,42	1,15,17,18,20,21,23,24,27,28,37,42	42	1	40,42	25,29,31	1,27,32,42	1
pseudolycorine (**2**)			27			11				42	21,27,37				29	27,32	
9-*O*-methylpseudolycorine (**3**)										42						42	
11-methoxylycorine (**4**)										42	37					42	
galanthine (**5**)	42		42		42		42		1242		16	42		40,42	29	42	
hippamine (**6**)											37						
caranine (**7**)	42		42		42		42		42	42	18,42	42		42		42	
pluviine (**8**)			39		7		42			42	37	42		40		27,42	
norpluviine (**9**)	42		27								27,28						
assoanine (**10**)										42							
hippadine (**11**)														40			
anhydrolycorine (**12**)	42		42		42		42		42	42	42	42		42		42	
2-hydroxyanhydrolycorin-6-one (**13**)			38														
lycosprenine (**14**)														40			
1,2-dihydroxyanhydrolycorin-6-one (**15**)			38														

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L. incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
narcissidine (**16**)														40			
ungiminorine (**17**)									12		18						
6-oxodihydrolycorine (**18**)											15						
dihydrolycorine (**19**)											15,37						
epizephyranthine (**20**)											37						
amarbellisine (**21**)										14							
incartine (**22**)	42		42		42		42		12,42		34	42		42		42	
lycoranine A (**23**)											19						
lycoranine B (**24**)											19						
11,12-dehydroanhydrolycorine (**25**)	42		42		42		42	42	42	42	42	42		42		42	
ungeremine (**26**)											34						
1-hydroxyungeremine (**27**)											36						
5,6-dehydrolycorine (**28**)											35						
5,6-dehydrodihydrolycorine (**29**)											15						
2-hydroxyanhydrolycorine-*N*-oxide (**30**)				41													
1,2-dihydroxyanhydrolycorine-*N*-oxide (**31**)			38														
Homolycorine-type																	
homolycorine (**32**)	2,3		5,10,27,39		7		42				15,17,21,24,27, 28,33,42	42		40		27	

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L.incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
2α-hydroxyhomolycorine (**33**)											33						
lycorenine (**34**)	2,3		27		7						21,24,27,33			40		27	
deoxylycorenine (**35**)			5								17,23,42			40			
O-methyllycorenine (**36**)			39								33,37						
2α-methoxy-6-*O*-methyllycorenine (**37**)														40			
O-ethyllycorenine (**38**)											33,37						
O-*n*-butyllycorenine (**39**)			39														
8-*O*-demethylhomolycorine (**40**)							42				17,20,23,24,33,42	42					
9-*O*-demethylhomolycorine (**41**)	3										37,33						
9-*O*-demethyl-2α-hydroxyhomolycorine (**42**)											37						
oduline (**43**)			10								33						
2α-hydroxyoduline (**44**)			10								37						
2α-hydroxy-6-*O*-methyloduline (**45**)	3		10,39								37						
2α-methoxy-6-*O*-methyloduline (**46**)			10								15,33						
ungerine (**47**)											18						
hippeastrine (**48**)	2,3		5,10,39		7						15,17,18,23,24,28,37,42					27	

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L.incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
2α-methoxy-6-O-ethyloduline (**49**)											33						
2α-hydroxy-6-O-n-butyloduline (**50**)			39														
homolycorine-N-oxide (**51**)											35						
8-O-acetylhomolycorine-N-oxide (**52**)											15						
9-O-demethylhomolycorine-N-oxide (**53**)											34						
2α-hydroxy-8-O-demethylhomolycorine-N-oxide (**54**)											36						
8-O-demethyhomolycorine-N-oxide (**55**)			38								36						
8,9-methylenedioxyl homolycorine-N-oxide (**56**)											35						
Crinine-type																	
crinine (**57**)			39		7	11				14	28						
macowine (**58**)										13							
buphanamine (**59**)											28						
crinamidine (**60**)											28						
undulatine (**61**)											28						
Haemanthamine-type																	
haemanthamine (**62**)	2,3		5,42		42				42		26,28,42			40,42	31	32	
haemanthidine (**63**)/ epihaemanthidine (**64**) *	3		5		7						17,18,20			40	31	32,42	

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L.incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
3-methyl-6β-acetoxybulbispermine (**65**)											35						
3-acetyl-6β-acetyoxybulbispermine (**66**)											35						
vittatine (**67**)											17,26,28					27	
oxovittatine (**68**)											34						
11-hydroxyvittatine (**69**)										14	18					32	
8-*O*-demethylmaritidine (**70**)											16						
6α-hydroxycrinamine (**71**)										13							
6β-hydroxycrinamine (**72**)										13	16						
6β-acetoxycrinamine (**73**)											15,36						
6β-acetoxybulbispermine (**74**)											35						
6β-acetyl-8-hydroxy-9-methoxycrinamine (**75**)											36						
apohaemanthamine (**76**)											34						
Narciclasine-type																	
narciclasine (**77**)	3				7					14	18,23,37			40	31	32	
lycoricidine (**78**)	3										15,23				31	32	
5,6-dihydrobicolorine (**79**)											15			40			
crinasiadine (**80**)														40			
N-isopentylcrinasiadine (**81**)														40			
arolycoricidine (**82**)															31		

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L. incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
arolycoricidinol (**83**)															31		
5,6-dihydro-*N*-methyl-2-hydroxyphenanthridine (**84**)			38								35						
trisphaeridine (**85**)										42	16,33			40,42			
bicolorine (**86**)											16						
ismine (**87**)											34						
Tazettine-type																	
tazettine (**88**)	42		6,,27,42		42		42			13,42	17,21,22,27,28,42	42		40,42	31	27,32,42	
deoxytazettine (**89**)										42	42						
3-*O*-ethyltazettinol (**90**)			4														
pretazettine (**91**)											24,25						
6-*O*-methylpretazettine (**92**)																32	
Montanine-type																	
montanine (**93**)	42		42		42					42						32,42	
pancracine (**94**)			6							14	15,37						
squamigine (**95**)										14,42	37					27,32	
montabuphine (**96**)											37			40			
Galanthamine-type																	
galanthamine (**97**)	1,2,3, 42	1	1,5,6,27,39, 42		1,7,8,9, 42	11	1,42	1	1,12, 42	1,13, 42	1,15,17,18,20,21, 22,23,27,28,37,42		1	40,42	25,29, 30,31	1,27,32, 42	1

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L.incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
epigalanthamine (**98**)																27	
norgalanthamine (**99**)			5,39			11										32	
sanguinine (**100**)			5,42		42				12	42	16,37				25,29,30	32	
N-allylnorgalanthamine (**101**)						11											
norsanguinine (**102**)															29		
3'-hydroxybutanoylnorsanguinine (**103**)															29		
narwedine (**104**)	42					11				42						42	
lycoramine (**105**)	1,3,42	1	1,6,27,42		1,7,8,42	11	1,42	1	1,12,42	1,13,42	1,17,18,20,21,22,23,24,27,28,37,42		1	40,42	30,31	1,27,32,42	1
3-epilycoramine (**106**)					7												
O-demethyllycoramine (**107**)			5						12	42	16,17,18,24,37					32	
norlycoramine (**108**)	42				42				42	42	18,42			42		42	
galanthamine-*N*-oxide (**109**)											37						
lycoramine-*N*-oxide (**110**)											37						
O-demethyllycoramine-*N*-oxide (**111**)											37						
Miscellaneous																	
galanthindole (**112**)			42							42							
lycosinine A (**113**)			5								19						
lycosinine B (**114**)			5								19						

Compound	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. caldwellii*	*L. chinensis*	*L. guanxiensis*	*L. haywaedii*	*L. houdyshelii*	*L.incarnata*	*L. longituba*	*L. radiata*	*L. radiata var pumila*	*L. rosea*	*L. sprengeri*	*L. sanguinea*	*L. squamigera*	*L. straminea*
cheryline (**115**)					42												
2*R*-hydroxy-*N*,*O*-dimethylnorbelladine (**116**)																32	
hostasinine A (**117**)	3																
7-demethoxyhostasine (**118**)											18						
N-methoxylcarbonylnandigerine (**119**)				41													
N-methoxycarbonyllindcarpine (**120**)				41													
8-demethyldehydrocrebanine (**121**)			38														
isocorydione (**122**)			38														
2-demethylisocorydione (**123**)			38														
N-methoxycarbonyl-2-demethylnorisoco rydione (**124**)											36						
10-*O*-methylhernovine-*N*-oxide (**125**)				41													

* haemanthidine (**63**) and epihaemanthidine (**64**) are found as a mixture of epimers.

1) Yuan et al., 2010; 2) Boit et al., 1958; 3) Jitsuno et al., 2011; 4) Pi et al., 2009; 5) Yang et al., 2005; 6) Wang et al., 2007a; 7) Ma et al., 1987; 8) Mu et al., 2010; 9) Mu et al., 2009; 10) Liao et al., 2012; 11) Li et al., 1987; 12) Kihara et al., 1994; 13) Liang et al., 2010; 14) Zhao et al., 2011; 15) Feng et al., 2011; 16) Wang et al., 2010; 17) Kihara et al., 1991; 18) Wang et al., 2011; 19) Wang et al., 2009; 20) Yang et al., 2010; 21) Jiang and Liu, 2009; 22) Ao et al., 2008; 23) Numata et al., 1983; 24)Kobayashi et al., 1980; 25) Kobayashi et al., 1976; 26) Uyeo et al., 1966; 27) Hung and Ma, 1964; 28) Takagi et al., 1968; 29) Abdallah, 1995; 30) Kobayashi et al., 1991; 31) Takagi and Yamaki, 1974; 32) Kitajima et al., 2009; 33) Huang et al., 2013; 34) Liu et al., 2013; 35) Hao et al., 2013; 36) Liu et al., 2015; 37) Li et al., 2013; 38) Song et al., 2014; 39) Jin et al., 2014; 40) Wu et al., 2014); 41) Cao et al., 2013; 42) Guo et al., 2014.
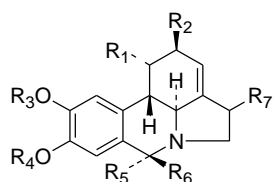
1 R$_1$=OH, R$_2$=OH, R$_3$+R$_4$=CH$_2$, R$_5$=H, R$_6$=H, R$_7$=H   lycorine

2 R$_1$=OH, R$_2$=OH, R$_3$=H, R$_4$=Me, R$_5$=H, R$_6$=H, R$_7$=H   pseudolycorine

3 R$_1$=OH, R$_2$=OH, R$_3$=Me, R$_4$=Me, R$_5$=H, R$_6$=H, R$_7$=H   9-*O*-methylpseudolycorine

4 R$_1$=OH, R$_2$=OH, R$_3$+R$_4$=CH$_2$, R$_5$=H, R$_6$=H, R$_7$=OMe   11-methoxylycorine

5 R$_1$=OH, R$_2$=OMe, R$_3$=Me, R$_4$=Me, R$_5$=H, R$_6$=H, R$_7$=H   galanthine

6 R$_1$=OH, R$_2$=OMe, R$_3$+R$_4$=CH$_2$, R$_5$=H, R$_6$=H, R$_7$=H   hippamine

7 R$_1$=OH, R$_2$=H, R$_3$+R$_4$=CH$_2$, R$_5$=H, R$_6$=H, R$_7$=H   caranine

8 R$_1$=OH, R$_2$=H, R$_3$=Me, R$_4$=Me, R$_5$=H, R$_6$=H, R$_7$=H   pluviine

9 R$_1$=OH, R$_2$=H, R$_3$=Me, R$_4$=H, R$_5$=H, R$_6$=H, R$_7$=H   norpluviine
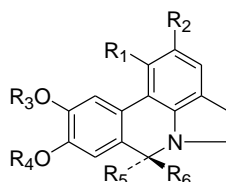
10 R$_1$=H, R$_2$=H, R$_3$=Me, R$_4$=Me, R$_5$=H, R$_6$=H   assoanine

11 R$_1$=H, R$_2$=H, R$_3$+R$_4$=CH$_2$, R$_5$+R$_6$=O   hippadine

12 R$_1$=H, R$_2$=H, R$_3$+R$_4$=CH$_2$, R$_5$=H, R$_6$=H   anhydrolycorine

13 R$_1$=H, R$_2$=OH, R$_3$+R$_4$=CH$_2$, R$_5$+R$_6$=O   2-hydroxyanhydrolycorin-6-one

14 R$_1$=H, R$_2$=OMe, R$_3$=Me, R$_4$=Me, R$_5$+R$_6$=O   lycosprenine

15 R$_1$=OH, R$_2$=OH, R$_3$+R$_4$=CH$_2$, R$_5$+R$_6$=O   1,2-dihydroxyanhydrolycorin-6-one
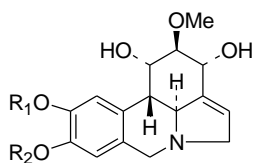
16 R$_1$=Me, R$_2$=Me   narcissidine

17 R$_1$+R$_2$=CH$_2$ ungiminorine
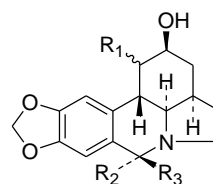
18 R$_1$=*α*-OH, R$_2$+R$_3$=O   6-oxodihydrolycorine

19 R$_1$=*α*-OH, R$_2$=H, R$_3$=H   dihydrolycorine

20 R$_1$=*β*-OH, R$_2$=H, R$_3$=H   epizephyranthine
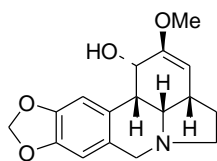
21 amarbellisine
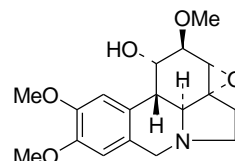
22 incartine

Figure 1.6: Lycorine-type alkaloid structures I.
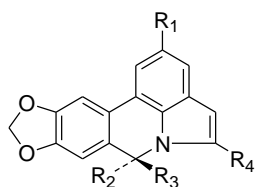
23 R_1=OMe, R_2+R_3=O, R_4=H   lycoranine A

24 R_1=OMe, R_2+R_3=O, R_4=Me   lycoranine B

25 R_1=H, R_2=H, R_3=H, R_4=H   11,12-dehydroanhydrolycorine
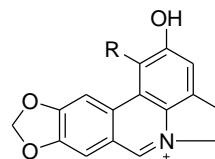
26 R=H   ungeremine

27 R=OH   1-hydroxyungeremine
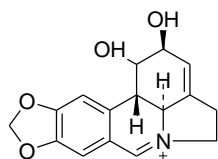
28 5,6-dehydrolycorine
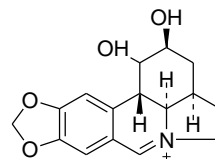
29 5,6-dehydrodihydrolycorine
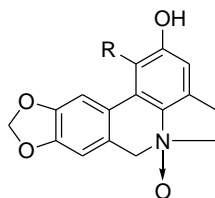
30 R=H   2-hydroxyanhydrolycorine-*N*-oxide

31 R=OH   1,2-dihydroxyanhydrolycorine-*N*-oxide

Figure 1.7: Lycorine-type alkaloid structures II.
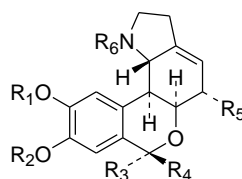
32 R$_1$=Me, R$_2$=Me, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   homolycorine

33 R$_1$=Me, R$_2$=Me, R$_3$+R$_4$=O, R$_5$=OH, R$_6$=Me   2α-hydroxyhomolycorine

34 R$_1$=Me, R$_2$=Me, R$_3$=OH, R$_4$=H, R$_5$=H, R$_6$=Me   lycorenine

35 R$_1$=Me, R$_2$=Me, R$_3$=H, R$_4$=H, R$_5$=H, R$_6$=Me   deoxylycorenine

36 R$_1$=Me, R$_2$=Me, R$_3$=OMe, R$_4$=H, R$_5$=H, R$_6$=Me   *O*-methyllycorenine

37 R$_1$=Me, R$_2$=Me, R$_3$=OMe, R$_4$=H, R$_5$=OMe, R$_6$=Me   2α-methoxy-6-*O*-methyllycorenine

38 R$_1$=Me, R$_2$=Me, R$_3$=OEt, R$_4$=H, R$_5$=H, R$_6$=Me   *O*-ethyllycorenine

39 R$_1$=Me, R$_2$=Me, R$_3$=O-*n*-C$_4$H$_9$, R$_4$=H, R$_5$=H, R$_6$=Me   *O*-*n*-butyllycorenine

40 R$_1$=Me, R$_2$=H, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   8-*O*-demethylhomolycorine

41 R$_1$=H, R$_2$=Me, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   9-*O*-demethylhomolycorine

42 R$_1$=H, R$_2$=Me, R$_3$+R$_4$=O, R$_5$=OH, R$_6$=Me   9-*O*-demethyl-2α-hydroxyhomolycorine

43 R$_1$+R$_2$=CH$_2$, R$_3$=OH, R$_4$=H, R$_5$=H, R$_6$=Me   oduline

44 R$_1$+R$_2$=CH$_2$, R$_3$=OH, R$_4$=H, R$_5$=OH, R$_6$=Me   2α-hydroxyoduline

45 R$_1$+R$_2$=CH$_2$, R$_3$=OMe, R$_4$=H, R$_5$=OH, R$_6$=Me   2α-hydroxy-6-*O*-methyloduline

46 R$_1$+R$_2$=CH$_2$, R$_3$=OMe, R$_4$=H, R$_5$=OMe, R$_6$=Me   2α-methoxy-6-*O*-methyloduline

47 R$_1$+R$_2$=CH$_2$, R$_3$+R$_4$=O, R$_5$=OMe, R$_6$=Me   ungerine

48 R$_1$+R$_2$=CH$_2$, R$_3$+R$_4$=O, R$_5$=OH, R$_6$=Me   hippeastrine

49 R$_1$+R$_2$=CH$_2$, R$_3$=OEt, R$_4$=H, R$_5$=OMe, R$_6$=Me   2α-methoxy-6-*O*-ethyloduline

50 R$_1$+R$_2$=CH$_2$, R$_3$=O-*n*-C$_4$H$_9$, R$_4$=H, R$_5$=OH, R$_6$=Me   2α-hydroxy-6-*O*-*n*-butyloduline
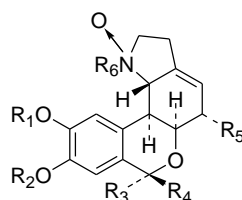
51 R$_1$=Me, R$_2$=Me, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   homolycorine-*N*-oxide

52 R$_1$=Me, R$_2$=Ac, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   8-*O*-acetylhomolycorine-*N*-oxide

53 R$_1$=H, R$_2$=Me, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   9-*O*-demethylhomolycorine-*N*-oxide

54 R$_1$=Me, R$_2$=H, R$_3$+R$_4$=O, R$_5$=OH, R$_6$=Me   2α-hydroxy-8-*O*-demethylhomolycorine-*N*-oxide

55 R$_1$=Me, R$_2$=H, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   8-*O*-demethylhomolycorine-*N*-oxide

56 R$_1$+R$_2$=CH$_2$, R$_3$+R$_4$=O, R$_5$=H, R$_6$=Me   8,9-methylenedioxylhomolycorine-*N*-oxide

Figure 1.8: Homolycorine-type alkaloid structures.

57 R₁+R₂=CH₂   crinine
58 R₁= Me, R₂=H   macowine

59 buphanamine

60 R₁=OH   crinamidine
61 R₁=OMe   undulatine
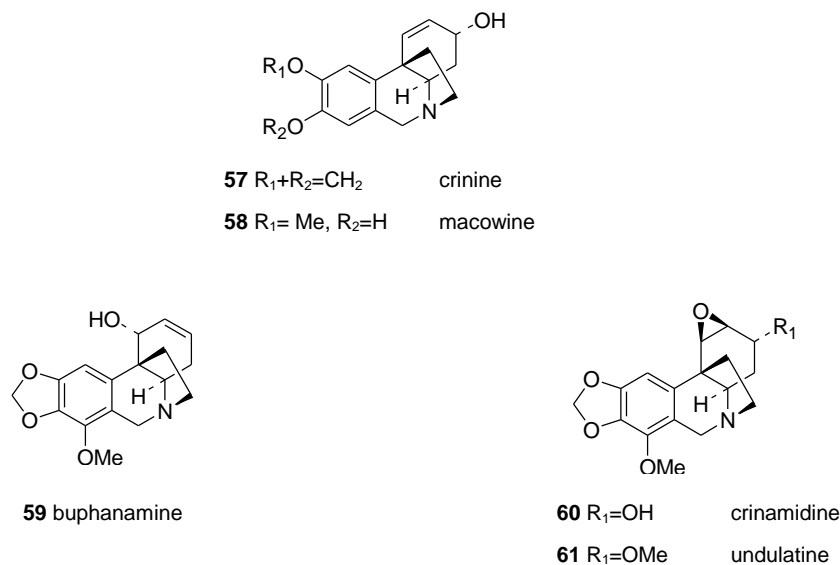
Figure 1.9: Crinine-type alkaloid structures.

62 R₁=OMe, R₂=H, R₃+R₄=CH₂, R₅=H, R₆=H, R₇=OH   haemanthamine

63 R₁=OMe, R₂=H, R₃+R₄=CH₂, R₅=H, R₆=OH, R₇=OH   haemanthidine

64 R₁=OMe, R₂=H, R₃+R₄=CH₂, R₅=OH, R₆=H, R₇=OH   epihaemanthidine

65 R₁=H, R₂=OMe, R₃+R₄=CH₂, R₅=OAc, R₆=H, R₇=OH   3-methyl-6β-acetoxybulbispermine

66 R₁=H, R₂=OAc, R₃+R₄=CH₂, R₅=OAc, R₆=H, R₇=OH   3-acetyl-6β-acetyoxybulbispermine

67 R₁=OH, R₂=H, R₃+R₄=CH₂, R₅=H, R₆=H, R₇=H   vittatine

68 R₁+R₂=O, R₃+R₄=CH₂, R₅=H, R₆=H, R₇=H   oxovittatine

69 R₁=OH, R₂=H, R₃+R₄=CH₂, R₅=H, R₆=H, R₇=OH   11-hydroxyvittatine

70 R₁=OH, R₂=H, R₃=Me, R₄=H, R₅=H, R₆=H, R₇=H   8-*O*-demethylmaritidine

71 R₁=H, R₂=OMe, R₃+R₄=CH₂, R₅=H, R₆=OH, R₇=OH   6α-hydroxycrinamine

72 R₁=H, R₂=OMe, R₃+R₄=CH₂, R₅=OH, R₆=H, R₇=OH   6β-hydroxycrinamine

73 R₁=H, R₂=OMe, R₃+R₄=CH₂, R₅=OAc, R₆=H, R₇=OH   6β-acetoxycrinamine

74 R₁=H, R₂=OH, R₃+R₄=CH₂, R₅=OAc, R₆=H, R₇=OH   6β-acetoxybulbispermine

75 R₁=H, R₂=OMe, R₃=Me, R₄=H, R₅=OAc, R₆=H, R₇=OH   6β-acetyl-8-hydroxy-9-methoxycrinamine

76 apohaemanthamine

Figure 1.10: Haemanthamine-type alkaloid structures.
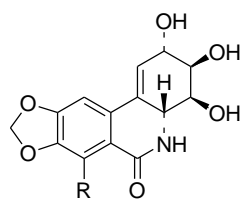
77 R=OH   narciclasine

78 R=H   lycoricidine
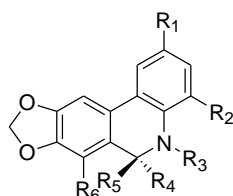
79 R_1=H, R_2=H, R_3=Me, R_4=H, R_5=H, R_6=H   5,6-dihydrobicolorine

80 R_1=H, R_2=H, R_3=H, R_4+ R_5=O, R_6=H   crinasiadine

81 R_1=H, R_2=H, R_3=CH$_2$CH$_2$CH(CH$_3$)$_2$, R_4+R_5=O, R_6=H   *N*-isopentylcrinasiadine

82 R_1=H, R_2=OH, R_3=H, R_4+R_5=O, R_6=H   arolycoricidine

83 R_1=H, R_2=OH, R_3=H, R_4+R_5=O, R_6=OH   arolycoricidinol

84 R_1=OH, R_2=H, R_3=Me, R_4=H, R_5=H, R_6=H   5,6-dihydro-*N*-methyl-2-hydroxyphenanthridine
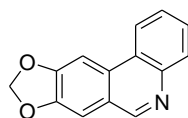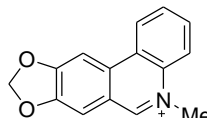
85 trisphaeridine   **86** bicolorine   **87** ismine
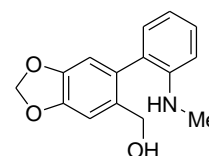
Figure 1.11: Narciclasine-type alkaloid structures.
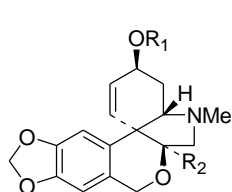
88 R_1=Me, R_2=OH   tazettine   **91** R=H   pretazettine

89 R_1=Me, R_2=H   deoxytazettine   **92** R=Me   6-*O*-methylpretazettine

90 R_1=Et, R_2=OH   3-*O*-ethyltazettinol

Figure 1.12: Tazettine-type alkaloid structures.

93 R=Me   montanine
94 R=H   pancracine

95 squamigine

96 montabuphine
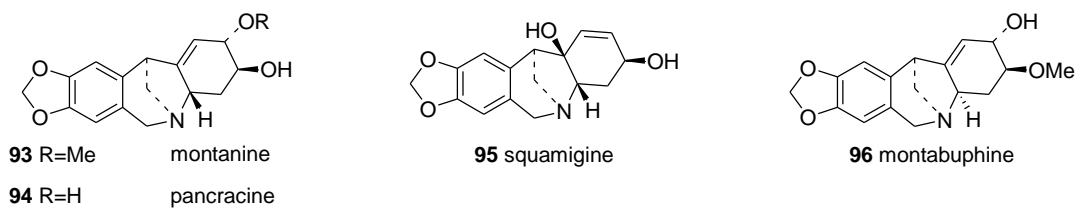
Figure 1.13: Montanine-type alkaloid structures.

97 R$_1$=OH, R$_2$=H, R$_3$=Me, R$_4$=Me   galanthamine
98 R$_1$=H, R$_2$=OH, R$_3$=Me, R$_4$=Me   epigalanthamine
99 R$_1$=OH, R$_2$=H, R$_3$=H, R$_4$=Me   norgalanthamine
100 R$_1$=OH, R$_2$=H, R$_3$=Me, R$_4$=H   sanguinine
101 R$_1$=OH, R$_2$=H, R$_3$=CH$_2$CHCH$_2$, R$_4$=Me   *N*-allylnorgalanthamine
102 R$_1$=OH, R$_2$=H, R$_3$=H, R$_4$=H   norsanguinine
103 R$_1$=OCOCH$_2$CHOHMe, R$_2$=OH, R$_3$=H, R$_4$=H   3'-hydroxybutanoylnorsanguinine
104 R$_1$+R$_2$=O, R$_3$=Me, R$_4$=Me   narwedine

105 R$_1$=OH, R$_2$=H, R$_3$=Me, R$_4$=Me   lycoramine
106 R$_1$=H, R$_2$=OH, R$_3$=Me, R$_4$=Me   3-epilycoramine
107 R$_1$=OH, R$_2$=H, R$_3$=Me, R$_4$=H   *O*-demethyllycoramine
108 R$_1$=OH, R$_2$=H, R$_3$=H, R$_4$=Me   norlycoramine

109 galanthamine-*N*-oxide

110 R=Me   lycoramine-*N*-oxide
111 R=H   *O*-demethyllycoramine-*N*-oxide

Figure 1.14: Galanthamine-type alkaloid structures.
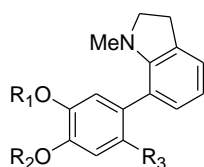
112 R$_1$+R$_2$=CH$_2$, R$_3$=CH$_2$OH   galanthindole

113 R$_1$=Me, R$_2$=Me, R$_3$=CH$_2$OH   lycosinine A

114 R$_1$=Me, R$_2$=Me, R$_3$=CHO   lycosinine B
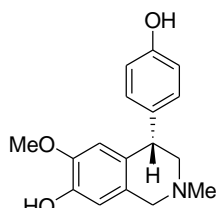
115 cheryline
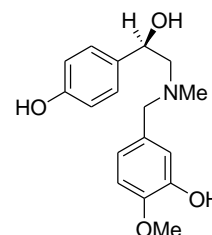
116 2*R*-hydroxy-*N,O*-dimethylnorbelladine
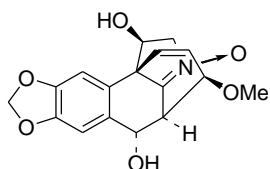
117 hostasinine A
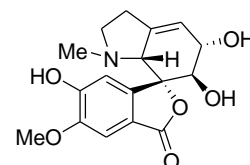
118 7-demethoxyhostasine
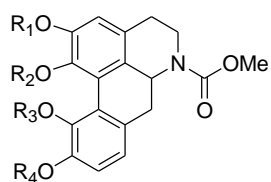
119 R$_1$+R$_2$=CH$_2$, R$_3$=Me, R$_4$=Me   *N*-methoxycarbonylnandigerine

120 R$_1$=H, R$_2$=Me, R$_3$=H, R$_4$=Me   *N*-methoxycarbonyllindcarpine
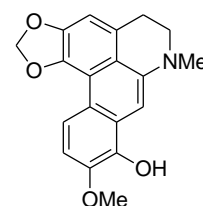
121 8-demethyldehydrocrebanine
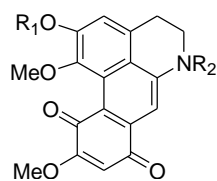
122 R$_1$= Me, R$_2$= Me,   isocorydione

123 R$_1$= H, R$_2$= Me,   2-demethylisocorydione

124 R$_1$= H, R$_2$= COOCH$_3$ *N*-methoxycarbonyl-2-demethylnorisocorydione
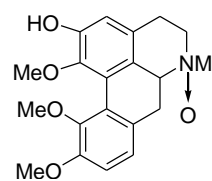
125 10-*O*-methylhernovine-*N*-oxide

Figure 1.15: Miscellaneous

1.5. Alkaloids from the genus *Hippeastrum*

A review on the genus *Hippeastrum* by our research group has been recently published in *Revista Latinoamericana de Qu ímica* (de Andrade et al., 2012).

As a co-author of this publication, I have included it as Appendix I, even though it does not form part of the work presented in this thesis.

1.6. GC-MS (Gas Chromatography-Mass Spectrometry) and NMR (Nuclear Magnetic Resonance)

The analysis of alkaloids from plant extracts, as well as identifying new compounds of Amaryllidaceae species, has been possible through the development of two fundamental techniques that have become part of routine application procedures: gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR). Extensive research conducted during the last 50 years in the field of Amaryllidaceae alkaloids has achieved the characterization of certain patterns for various structural types. This enables a rapid identification of known compounds and the elucidation of detailed structures in the case of new isolated products.

1.6.1. GC-MS

Gas chromatography, which was introduced in the 1950s, is a known technique with the ability to separate components in a mixture, which involves sample volatilization by heating. The equipment includes a column with the stationary phase, an inert carrier gas, and a detector. Only molecules that can be vaporized without decomposition are suitable for this analysis. Moreover, the mass spectrometer is a basic instrument that measures the mass to charge (m/z) of ions in the gas phase, providing information on the abundance of each ion, and offers the possibility of being coupled to a detector (Kitson et al., 1996). Generally, organic compounds have distinctive fragmentation patterns after being ionized, which allows identification by comparison with previously obtained data. The combination of both techniques is a powerful tool

commonly known as GC-MS, which has a relatively low cost, as well as high resolution and efficiency.

During the 60s and 70s, numerous studies on Electron Impact Mass Spectrometry (EIMS) of Amaryllidaceae alkaloids were conducted, allowing characteristic fragmentation patterns to be established for various kinds of skeletons (Bastida et al., 2006).

Extracts of Amaryllidaceae are usually complex mixtures with a large number of compounds. The GC-MS technique, either electron impact (EI) or chemical ionization (CI), has proved to be a very useful method for a rapid separation and detection of components. Amayllidaceae alkaloids can be analyzed without prior derivatization since they retain their particular patterns of fragmentation under the conditions of GC, allowing the identification of previously characterized compounds or providing valuable structural information when it comes to new molecules (Berkov et al., 2005). Notably, small changes in the stereochemistry of these alkaloids often cause significant differences in the mass spectrum of the stereoisomers (Duffield et al., 1965; Berkov et al., 2012b).

Recently, GC-MS validation has been reported as a method of choice for quality control of plant materials used in the production of galantamine (Berkov et al., 2011a), demonstrating the advantages of its use in qualitative analysis and quantity of these plants, even compared with other methodologies (Gotti et al., 2006).

The further development of the new methods, as well as the characterization of other structures, has generated well-documented information of considerable diagnostic value for identifying this group of alkaloids. Therefore, it is worth making some comments on the most representative cases. The examples described below demonstrate the value of GC-MS methodology in identifying Amaryllidaceae alkaloids, although it is not suitable for all structures.

1.6.1.1. Lycorine type

The molecular peak appears with appreciable intensity, and generally suffers the loss of water, as well as C-1 and C-2 and their substituents, by a r-DA fragmentation

(Fig 1.16). Interestingly, the loss of water from the molecular ion depends on the stereochemistry of the hydroxyl group at C-2, and does not occur in acetyl derivatives. Thus, in the mass spectrum of lycorine the relative intensity is low, while in 2-epilycorine it is the base peak (Bastida et al., 2006).
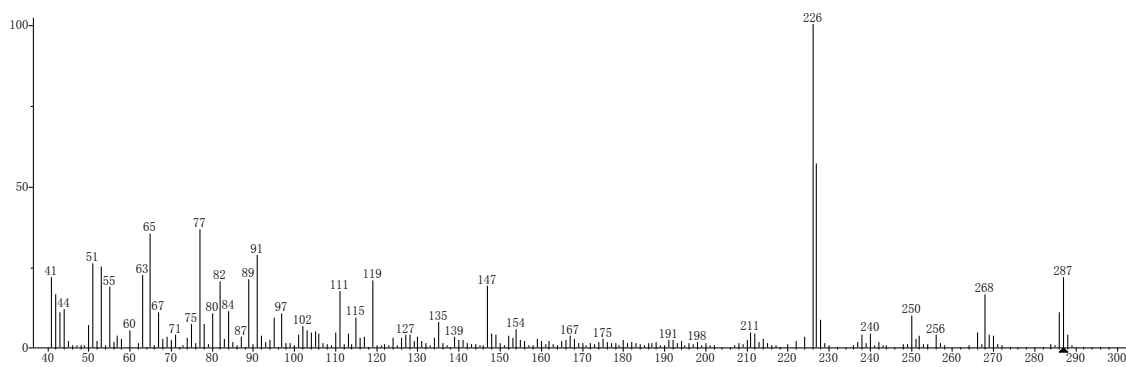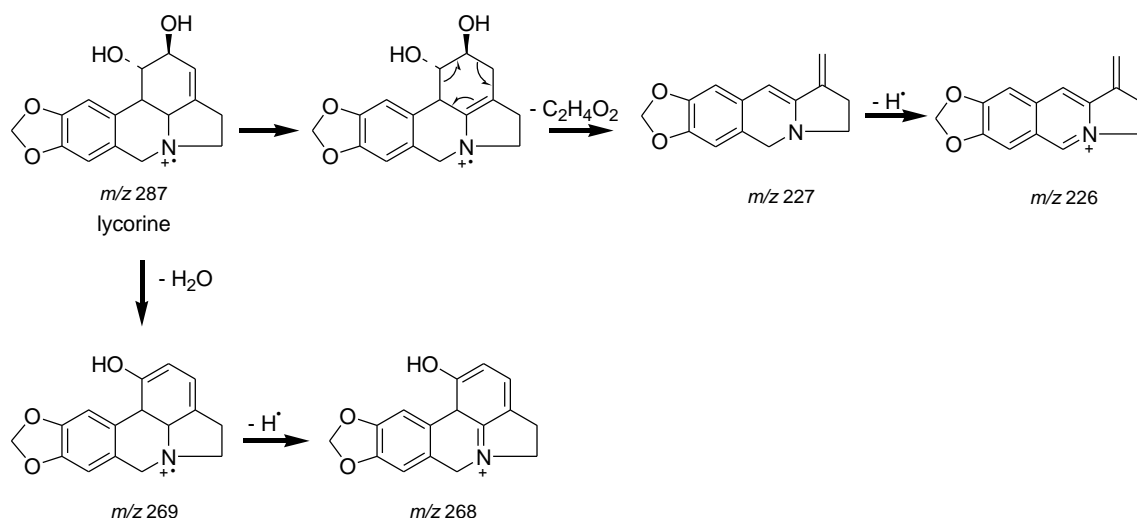
Figure 1.16: Mass fragmentation pattern of lycorine.

1.6.1.2. Homolycorine type

In this type of alkaloid, the cleavage of the labile bonds in ring C by a r-DA reaction is predominant, generating two fragments: the most characteristic one represents the pyrrolidine ring (together with the substituents at position 2), and the other (a less-abundant fragment) encompasses the aromatic lactone or hemilactone moiety. Therefore, in the case of homolycorine, the base peak is observed at *m/z* 109, while hippeastrine (with a hydroxyl group at C-2) presents at *m/z* 125. A significant aspect is the low abundance of the molecular ion (Figure 1.17) (Bastida et al., 2006).
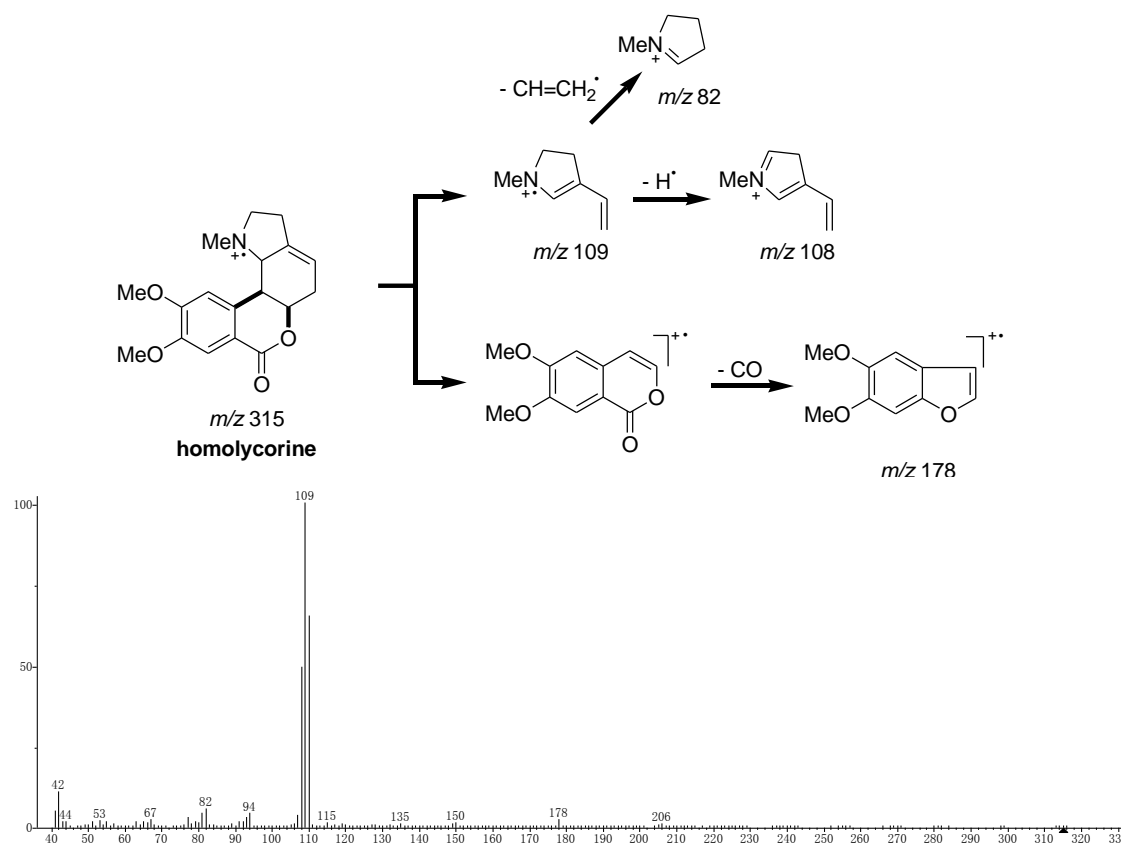
Figure 1.17: Mass fragmentation pattern of homolycorine.

1.6.1.3. Crinine and Haemanthamine types

Various fragmentations of the two types of alkaloids have been studied in detail. In most cases, the molecular ion is the base peak. The aromatic ring plays a significant role in the stabilization of the ions, which is retained in all fragments of high mass, while the nitrogen atom is often lost. The fragmentation mechanisms are initiated by the rupture of the bond β to the nitrogen atom, which implies the opening of the C-11/C-12 bridge. Several described characteristic patterns have taken into account the presence of substituents at various positions, saturation of the C ring and the influence of stereochemistry. There are three patterns of fragmentation: loss of CH_3OH, C_2H_6N and CHO (Figure 1.18) (Bastida and Viladomat, 2002).
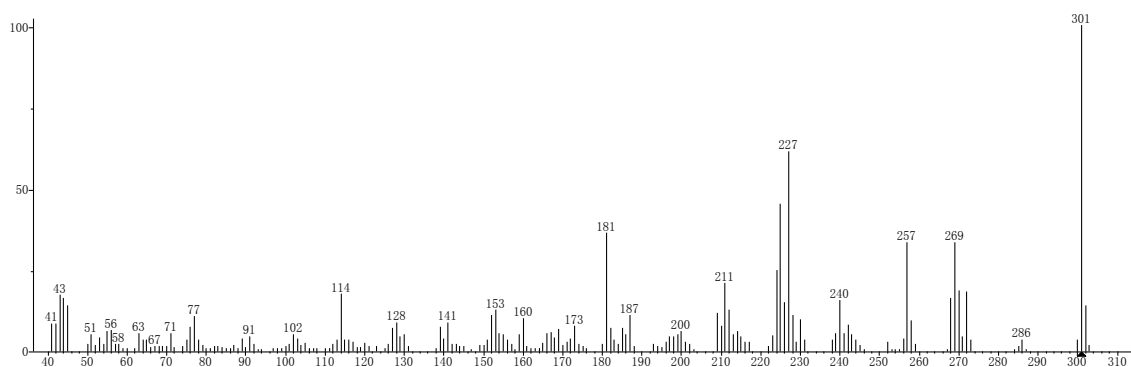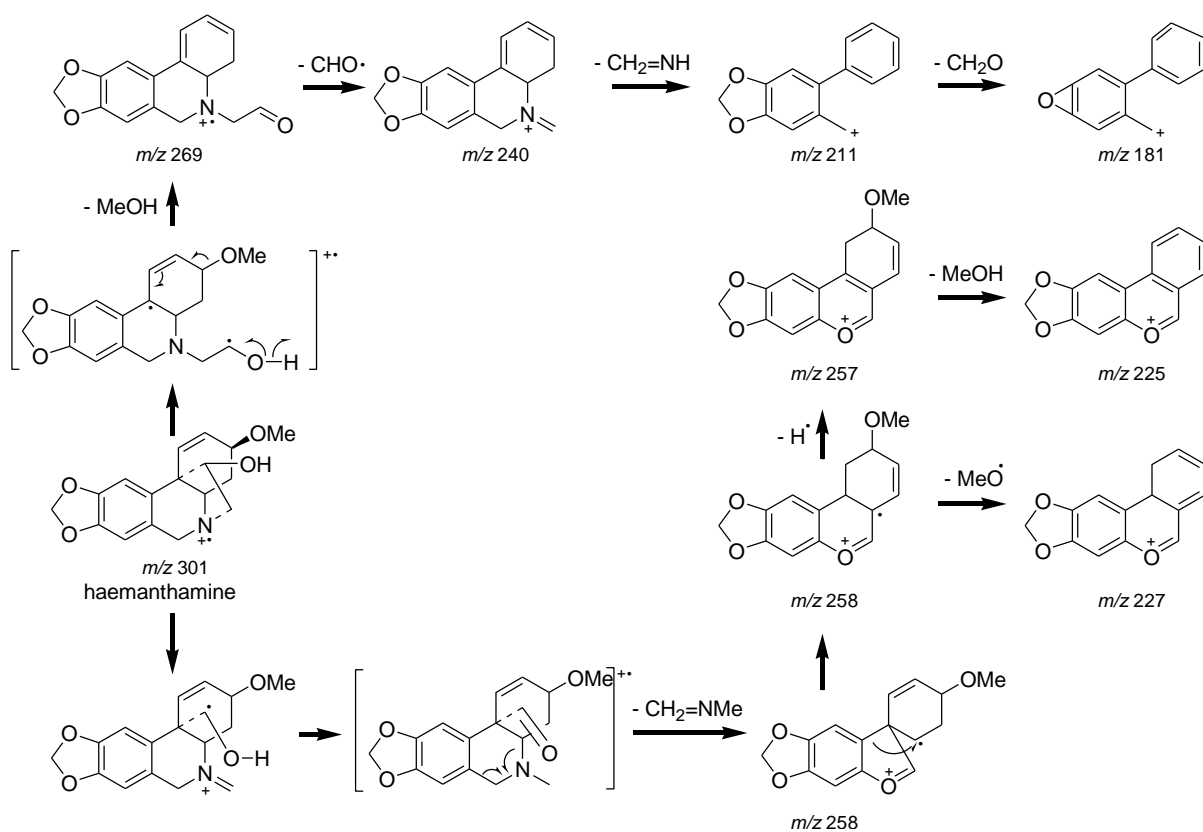
Figure 1.18: Mass fragmentation patterns of haemanthamine.

1.6.1.4. Tazettine type

The tazettine skeleton is a good example to illustrate how small changes in stereochemistry can be reflected in the fragmentation patterns. Criwelline and Tazettine only differ in the methoxy group setting at C-3, but this is enough to produce significant variations in their mass spectra. Consequently, in the MS of tazettine, with a *β*-configuration of the methoxy group at C-3, the dominant ion occurs at *m/z* [M⁺ -84], following a C-ring fragmentation by an r-DA process. In contrast, the mass spectrum of its epimer criwelline contains a peak of low abundance at *m/z* [M⁺-84]. Ions occur in

both stereoisomers owing to the successive loss of a methyl radical and water from the molecular ion (Figure 1.19) (Bastida et al., 2006).
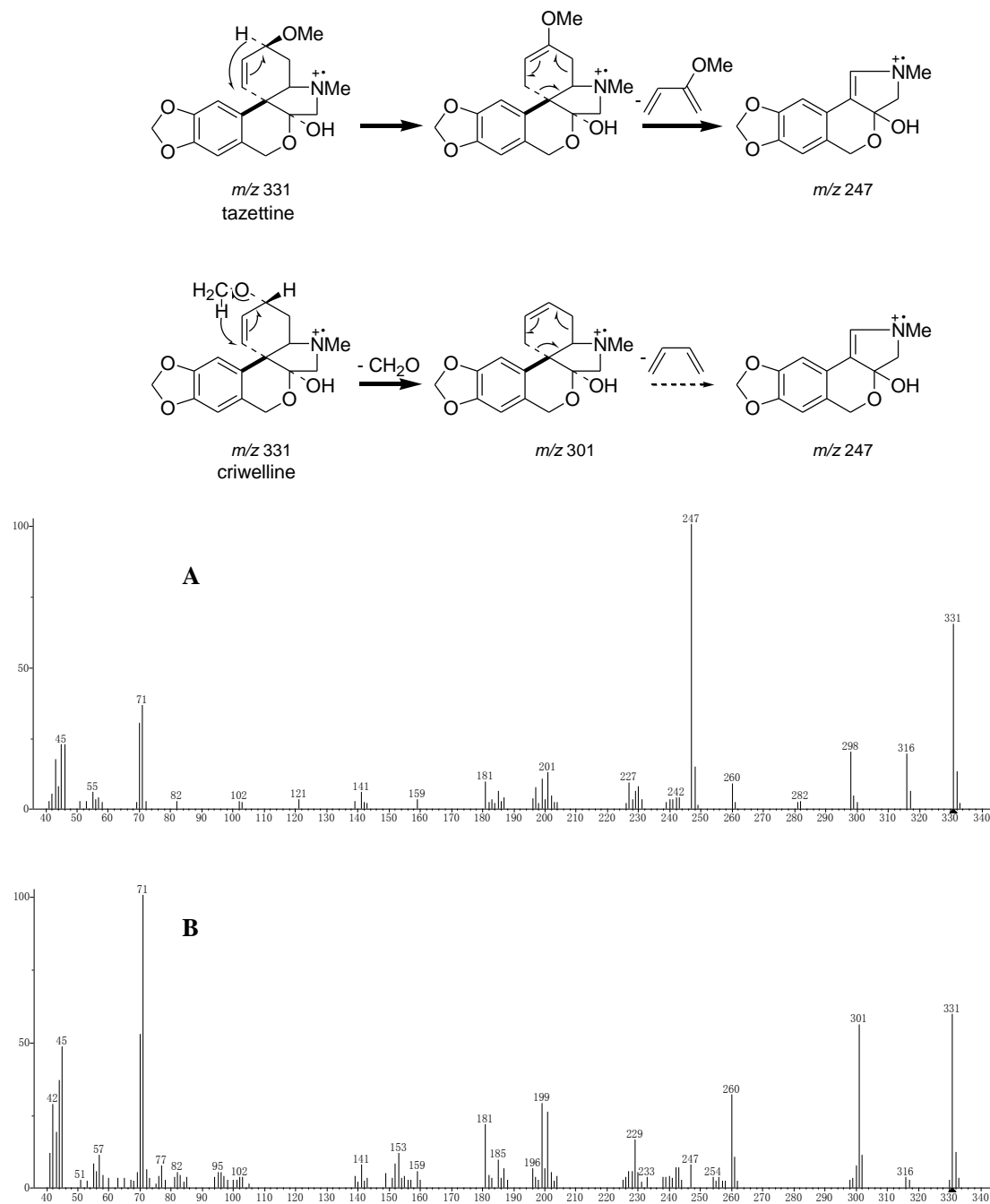
Figure 1.19: Mass fragmentation patterns of tazettine (A) and criwelline (B).

1.6.1.5. Montanine type

The mass spectral fragmentation patterns observed for alkaloids containing the 5,11-methanomorphanthridine nucleus depend on the substituents at C-2 and C-3. Their nature, as well as their particular configuration, have a considerable effect. Therefore, all the alkaloids that possess a methoxyl group give rise to an $[M-31]^+$ ion. The

configuration of the C-2 substituent has a considerable effect on the extent to which the r-DA fragmentation ion is observed. There is a definite enhancement of this fragmentation when C-2 has an *α*-configuration (Figure 1.20) (Bastida and Viladomat, 2002).
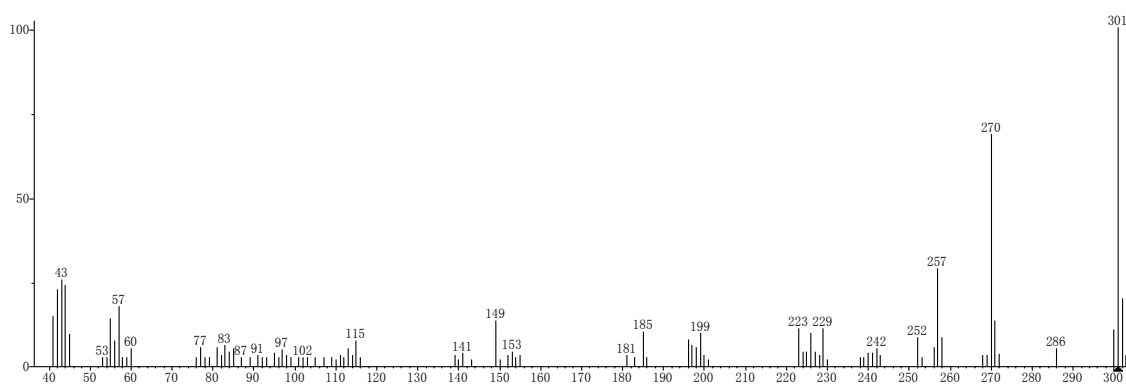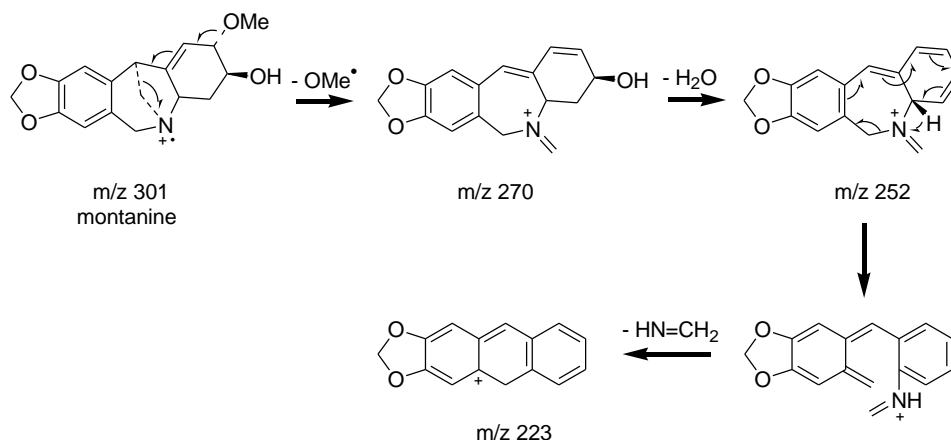
Figure 1.20: Mass fragmentation patterns of montanine.

1.6.1.6. Galanthamine type

This series of structures are probably the most studied among the Amaryllidaceae alkaloids. In this type of structure, the intense molecular ion as well as the $[M^+-1]$ peak, the breaking of ring C (losing a C_4H_6O fragment), and the elimination of elements of ring B (including the nitrogen atom) are characteristic (Figure 1.21). This behavior is similar for the dihydro derivatives. Recently, GC-MS methodology has been used for detailed analysis of the various galanthamine-type skeletons, and has been established as a routine technique for the study of plant extracts that contain these alkaloids (Berkov et al., 2012b).
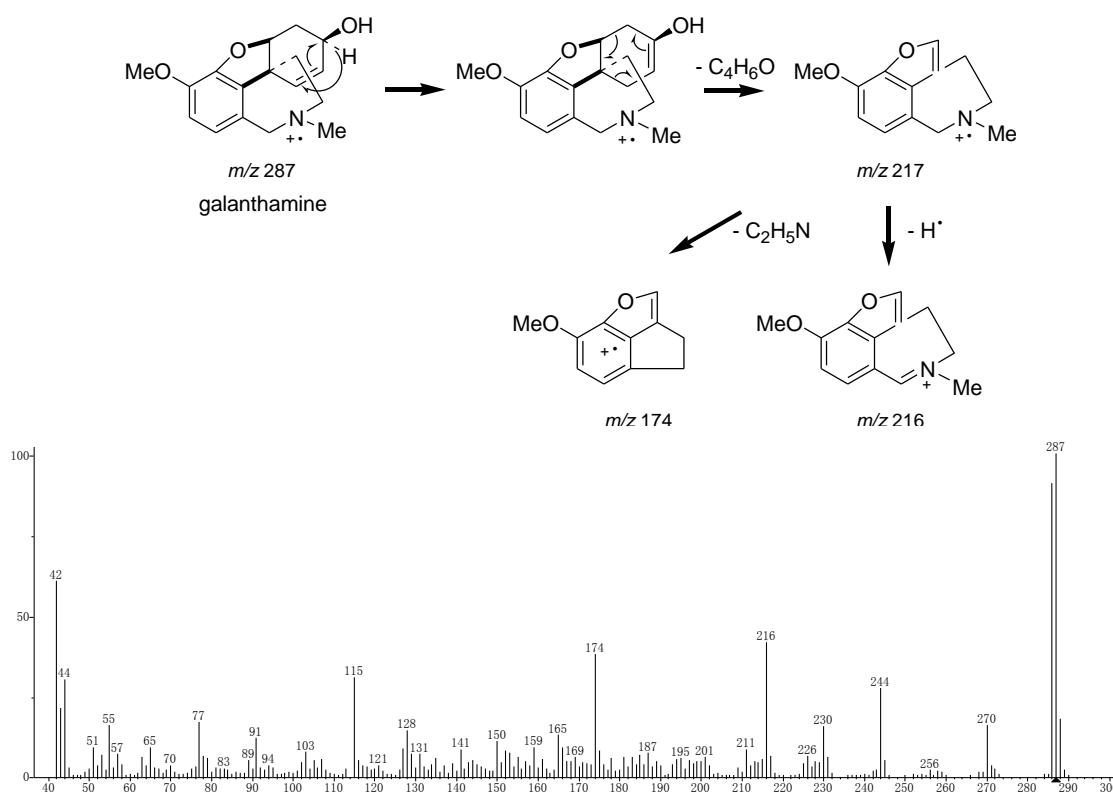
Figure 1.21: Mass fragmentation patterns of galanthamine.

1.6.2. Proton nuclear magnetic resonance

Nuclear magnetic resonance (NMR) spectroscopy is a research technique that exploits the magnetic properties of certain atomic nuclei. It determines the physical and chemical properties of atoms or the molecules in which they are contained. It relies on the phenomenon of nuclear magnetic resonance and can provide detailed information about the structure, dynamics, reaction state, and chemical environment of molecules. It is also a kind of absorption spectrum, like infrared (IR) or ultraviolet (UV). The NMR technique is mainly applied in the identification of pure organic compounds, but in recent years it has also been extended to the analysis of mixtures in the field of metabolomics, which is being used in many plant extract studies, including analysis of the species and varieties of Amaryllidaceae (Kim et al., 2010; Lubbe et al., 2010).

^{1}H NMR spectroscopy provides the most extensive and fundamental information on the different structural types of Amaryllidaceae alkaloids, while its combination with ^{13}C NMR spectroscopy and two-dimensional NMR techniques (2D-NMR) has facilitated structural assignments and the settling of their stereochemistry.

The most significant features of the spectra of ^{1}H NMR alkaloids of Amaryllidaceae have been outlined, providing keys for their identification (Bastida et al., 2011). In general, the aromatic region (6.5-8.5ppm) defines the skeleton type, whereas the observation of substitution of the aromatic ring corresponds to one or more methoxyl signals around 3.6-4.0 ppm, or the presence of the typical signal of methylenedioxy about 6.0 ppm. In many structures, the benzylic C-6 position is saturated, as in lycorine, galanthamine and haemanthamine. The presence of an AB system is characteristic of these protons. Interestingly, the chemical shift is influenced by the orientation of the free electron pair of the nitrogen atom. In addition to these common features, it is worth mentioning some peculiarities for each type of alkaloid (Bastida et al., 2006).

1.6.2.1. Lycorine type

Key features of the ^{1}H NMR spectrum of lycorine and its derivatives are the two singlets of the para-oriented aromatic protons, with a single olefinic proton and the two doublets as an AB system corresponding to the benzylic position 6. The deshielding observed in the *β*-proton positions 6 and 12, in relation to their counterparts, is due to the effect of the *cis*-lone pair of the nitrogen atom. Generally, alkaloids isolated from the genus *Narcissus, Lycoris* and *Hippeastrum* show a *trans* B/C ring junction, with a constant coupling between protons 4a-10b of about 11 Hz. Only kirkine form *Narcissus* and amarbellisine from *Lycoris* have a *cis* B/C ring junction, with a smaller coupling constant (8 Hz) (Bastida et al., 2011; Zhao et al., 2011).

1.6.2.2. Homolycorine type

These compounds include a characteristic group that can be a lactone, a hemiacetal or a cyclic ether. Generally, in the ^{1}H NMR spectrum, there are two singlets for the *para*-oriented aromatic protons. In lactone alkaloids, the deshielding of H-7 is caused by the *peri*-carbonyl group. The hemiacetal alkaloids always show the substituent at C-6 in *α*-disposition.

Most of these alkaloids belong to a single enantiomeric series containing a *cis* B/C ring junction, which is congruent with the small size of the coupling constants between

protons H-1 and H-10b. In the *Narcissus* genus no exception to this rule has been observed. In addition, the high value of the constant between *α*-orientation H-4a and H-10b (*J* ~ 10 Hz) is only consistent with a *trans*-diaxial relationship. The exception is hippapiline from the genus *Hippeastrum* with a *β*-orientation of H-1 and H-10b.

Typically, ring C has a vinyl proton. If position 2 is replaced by a hydroxyl, methoxy or acetyl group, it always displays an *α*-disposition. The *N*-methyl group is often found in the range of 2.0-2.2 ppm, but in the case of alkaloids with the saturated C ring, some empirical correlations have been described for the stereochemistry of the connections between rings B/C and C/D, where more deshielded signals are reported (Jeffs and Mueller, 1988). The H-12*α* is more deshielded than H-12*β* as a consequence of the *cis*-lone pair of the nitrogen atom (Bastida and Viladomat, 2002).

1.6.2.3. Crinine and Haemanthamine types

The absolute configuration of these alkaloids is determined by the circular dichroism (CD) spectrum. The alkaloids of the genus *Narcissus* are exclusively of the haemanthamine-type, whereas in some genera such as *Brunsvigia*, *Boophane* etc., the crinine-type alkaloids are predominant. The alkaloids from the genus *Lycoris* and *Hippeastrum* include both haemanthamine and crinine types. It is also reported that the alkaloids isolated from the *Narcissus* genus do not show additional substitutions in the aromatic ring apart from those of C-8 and C-9. On the contrary, in the genera where crinine-type alkaloids predominate, the presence of compounds with a methoxy substituent at C-7 is quite common.

Using CDCl$_3$ as the solvent, the magnitude of the coupling constants between each olefinic proton (H-1 and H-2) and H-3 gives information about the configuration of the C-3 substituent. Thus, in those alkaloids in which the two-carbon bridge (C-11 and C-12) is *cis* to the substituent at C-3, H-1 shows an allylic coupling with H-3 ($J_{1,3}$ ~1-2 Hz) and H-2 shows a smaller coupling with H-3 ($J_{2,3}$ ~ 0-1.5 Hz), as occurs in crinamine. On the contrary, in the corresponding C-3 epimeric series, e.g. haemanthamine, a larger coupling between H-2 and H-3 ($J_{2,3}$ ~5 Hz) is shown, the coupling between H-1 and H-3 being undetectable. This rule is also applicable to the crinine-type alkaloids.

In the haemanthamine series, there is frequently an additional W coupling of H-2 with the equatorial H-4, while the axial proton H-4 shows a large coupling with H-4a ($J_{4\alpha, 4a}$ ~13 Hz) due to their *trans*-diaxial disposition. The same is applicable in the crinine series.

The pair of alkaloids with a hydroxy substituent at C-6, like papyramine/ 6-epipapyramine, haemanthidine/ 6-epihaemanthidine etc, appear as a mixture of epimers not separable even by HPLC (Bastida et al., 2011).

1.6.2.4. Tazettine type

The presence of an *N*-methyl group (2.4-2.5 ppm) distinguishes this type of alkaloid from crinine and haemanthamine types, from which they proceed biosynthetically. What is more, the spectrum of ^{1}H NMR always shows the signal corresponding to the methylenedioxy group (Bastida et al., 2011).

1.6.2.5. Narciclasine type

In the alkaloids of this type, the only aromatic proton appears as a singlet, the chemical shift exceeding 7 ppm. Additionally, the compounds do not exhibit the classic double bond in C-1/C-10b, and show a *trans* fusion ring B/C, confirmed by its coupling constant J_{4a-10b} (Bastida et al., 2006).

1.6.2.6. Montanine type

The absolute configuration of montanine-type alkaloids must be determined by CD. Their ^{1}H NMR spectrum is very similar to that of lycorine-type alkaloids, but their structures can be distinguished by analysis of the COSY spectrum. The most shielded signals in montanine-type alkaloids are attributed to protons H-4 and show correlation with H-3 and H-4a, while the highly shielded lycorine-type alkaloid signals correspond to two protons at positions 11 and 12 (Bastida and Viladomat, 2002).

1.6.2.7. Galanthamine type

Among the Amaryllidaceae alkaloids, only the galanthamine-type show an *ortho*-coupling constant (~ 8 Hz) between both aromatic protons of ring A. The assignment of the substituent stereochemistry at C-3 is made in relation with the coupling constants of the olefinic protons H-4 and H-4a. When the coupling constant

$J_{3, 4}$ is about 5Hz, the substituent is pseudoaxial, while if it is ~ 0 Hz this indicates that the substituent at C-3 is pseudoequatorial.

This type of alkaloids usually show the presence of an *N*-methyl group, although *N*-formyl has also occasionally been reported. The presence of the furan ring causes a deshielding effect on H-1 (Bastida et al., 2006).

1.6.3. Carbon^{13} nuclear magnetic resonance

^{13}C NMR spectroscopy has been widely used for determining the carbon skeleton of Amaryllideceae alkaloids. Overall, the ^{13}C NMR spectra of Amarillydeceae alkaloids can be divided in two regions. The low-field region (> 90 ppm) contains signals of the carbonyl group, the olefinic and aromatic carbons, as well as that of the methylenedioxy group. The other signals corresponding to the saturated carbon resonances are found in the high-field region, the *N*-methyl being the only characteristic group, easily recognizable by a quartet signal between 40 and 46 ppm. The effect of a substituent (OH, OMe, OAc) on the carbon resonances is of considerable importance in localizing the position of the functional groups (Bastida et al., 2006).

1.6.4. Two-dimensional nuclear magnetic resonance spectroscopy

Finally, as already mentioned, two-dimensional experiments are important for proper allocation of ^{1}H NMR and ^{13}C NMR signals, especially in the case of unknown structures. 2D NMR techniques are used more broadly, and include the following:

✓ ^{1}H-^{1}H COSY (Correlated Spectroscopy), in which the correlations observed correspond to direct couplings between protons. It is quite useful in assigning the geminal and vicinal links.

✓ ^{1}H-^{1}H NOESY (Nuclear Overhauser Effect Spectroscopy) provides information on the spatial proximity of protons, and therefore has great value in defining the stereochemistry.

✓ ^{1}H-^{13}C HSQC (Heteronuclear Single Quantum Correlation) shows correlations between ^{1}H-^{13}C directly linked, allowing adequate allocation of all carbons, except the quaternary.

✓ ^{1}H-^{13}C HMBC (Heteronuclear Multiple Bond Correlation) is extremely useful in determining correlations between long-range ^{1}H-^{13}C, and allows identification of quaternary carbons by observing their correlation with protons located three links away.

1.7. Biological activities

The Amaryllidaceae alkaloids have shown a broad range of pharmacological and biological activities, including acetylcholinesterase (AChE) inhibition, and antitumoral, antibacterial, antifungal, antiviral and antimalarial activities (Bastida et al., 2006; Jin, 2009). Until now, only galanthamine is being marketed as a hydrobromide salt (Razadyne®, Reminyl®) for the palliative treatment of mild to moderate Alzheimer's disease (AD), but the significant activities of other alkaloids in the family demonstrated in recent years could favour their therapeutic use in the near future (Bastida et al., 2011).

1.7.1. Lycorine type

Lycorine, which is one of the most frequently occurring alkaloids in Amaryllidaceae plants, has been found in all *Lycoris* species. Generally speaking, the compound has been reported as a potent inhibitor of ascorbic acid biosynthesis, cell growth and division, and organogenesis in higher plants, algae and yeasts, inhibiting the cell cycle during the interphase (Bastida et al., 2006). In addition, lycorine is a novel contributor to the control of the length of mammalian cell life (Onishi et al., 2012).

The lycorine series compounds have recently emerged as novel inhibitors of AChE, in some instances with higher levels of activity than galanthamine, making them attractive targets for natural product and synthetically-driven structure-activity relationship studies. Thus, in the past decade lycorine has emerged as a promising lead in therapeutic approaches towards AD and may advance into the clinical stage in the future (Nair and van Staden, 2012). Additionally, lycorine induces cell-cycle arrest in the G0/G1 phase in K562 cells via histone deacetylase (HDAC) inhibition, which exhibits significant antitumor activity (Li et al., 2012b). Other studies of lycorine have

reported potential antinociceptive, anti-inflammatory, hepatoprotective and hypotensive activities (McNulty et al., 2010; Schmeda-Hirschmann et al., 2000). Lycorine isolated from *H. santacarina* has remarkable inhibitory activity of the enzymes NTPDase and ecto-5'-nucleotidase from *Trichomonas vaginalis*, which contributes to an increased susceptibility of this parasite to the host immune response. Lycorine has also demonstrated anti-*T.vaginalis* activity, involving a mechanism of cell death induction associated with paraptosis rather than the apoptosis observed in tumor cells (Giordani et al., 2010, 2011, 2012). In particular, the alkaloid shows biological activity against *Entamoeba histolytica* (Machocho et al., 1998), while structures of lycorine exhibited both antimalarial and cytotoxic activity in tests with 2 strains of cultured *Plasmodium falciparum* and cytotoxicity in BL6 mouse melanoma cells (Campbell et al., 1998, 2000; Ramires et al., 2001).

Similarly, galanthine shows AChE inhibitory, cytotoxic and antiproliferative activities (Jensen et al., 2011; Dalecka et al., 2013). 5,6-Dehydrolycorine, in turn, exhibits significant cytotoxic activities against HL-60 (IC_{50} values<10μM), and antimalarial activities against the two strains of *P. falciparum* (Hao et al., 2013). Pseudolycorine shows good activity in *in vitro* assays against *Trypanosoma brucei rhodesiense*, *T. cruzi* and *Plasmodium falciparum* (Osorio et al., 2010). It also exhibits *in vitro* activity against three medically important RNA viruses, Japanese encephalitis (JE), yellow fever (YF) and dengue type-4 viruses (flaviviruses) (Gabrielsen et al., 1992b). Furthermore, the alkaloid has anticancer activity as well as remarkable antileukemic activity (Furusawa et al., 1971, 1973; Suzuki et al., 1974; Pan et al., 1979; Kong et al., 1982). Dihydrolycorine, and pseudolycorine halts HeLa cell growth at 10^{-1} mM or lower concentrations (Jimenez et al., 1976). What is more, dihydrolycorine has protective effects on myocardial ischemia reperfusion injury rats, focal cerebral ischemia-reperfusion injury rats, and hypoxia/reoxygenation injury rats (Jiang and Gong, 2005; Jiang et al., 2006, 2007, 2009a, 2010; Zhang et al., 2008). Additionally, it exhibits hypotensive, protective effects in ischemia and reperfusion brain damage (Chen et al., 1993; Zhang et al., 2007). Ungiminorine displays a mild inhibitory effect on AChE (Ingkaninan et al., 2000). The lycorine-type alkaloid, amarbellisine, has a pronounced

antiproliferative effect, shows antibacterial activity against the Gram-positive *Staphylococcus aureus*, exhibits activity against the Gram-negative *Escherichia coli,* and also shows antifungal activity against *Candida albicans* (Evidente et al., 2004, 2009).

1.7.2. Homolycorine type

Generally, the homolycorine-type alkaloids, such as homolycorine, 8-*O*-demethylhomolycorine, dubiusine, hippeastrine, lycorenine, and *O*-methyllycorenine, present cytotoxic effects on c-fibroblastic LMTK cells, Molt 4 lymphoma, HepG2 human hepatoma, LNCaP human prostate cancer, HT, HCT-116 and HeLa cell lines (Bastida et al., 2006; Jokhadze et al., 2011). Dubiusine, lycorenine and 8-*O*-demethylhomolycorine also show DNA binding activity comparable to that of vinblastine. Additionally, dubiusine, homolycorine, 8-*O*-demethylhomolycorine, and lycorenine have a hypotensive effect on the arterial pressure of normotensive rats (Bastida et al., 2006). Homolycorine, 2α-hydroxyoduline, oduline, hippeastrine, 2α-hydroxy-6-*O*-methyloduline, and 2α-methoxy-6-*O*-methyloduline from *Lycoris aurea* have been evaluated for anticancer efficacy both *in vivo* and *in vitro* using murine sarcoma S180 cells, indicating that their anticancer effects, at least in part, are the result of inducing cancer cell apoptosis (Liao et al., 2012).

Lycorenine demonstrates a vasodepressor action ascribed to the maintenance of its adrenergic blocking action, and produces bradycardia by modifying vagal activity. Another feature of lycorenine is its analgesic activity. Homolycorine possesses high antiretroviral activity, accompanied by low therapeutic indices; it is also an inductor of delayed hypersensitivity in animals. Hippeastrine, in turns, exhibits antiviral activity against Herpes simplex type 1 and highly pathogenic avian influenza virus H5N1, antifungal activity against *C. albicans* and also weak insect antifeedant activity (Bastida et al., 2006; He et al., 2013). In particular, the compounds ungerine and hippeastrine have an effect in the inhibition and treatment of muscular atrophy (Adams et al. 2012). 2α-methoxy-6-*O*-ethyloduline and 2α-methoxy-6-*O*-methyloduline showed weak antiviral activities against the flu virus A (Huang et al., 2013).

1.7.3. Crinine type

As well as AChE inhibitory activity (Elgorashi et al., 2004; Abou-Donia et al., 2012), crinine shows antiproliferative effects against human tumor cell lines (Berkov et al., 2011b). In particular, it displays affinity to the serotonin reuptake transport protein (Elgorashi et al., 2006), and interaction with P-glycoprotein mediated calcein-AM efflux effect (Eriksson et al., 2012).

The crinine-type alkaloid buphanamine has affinity to the serotonin transporter and shows important anti-proliferative effects, being well-tolerated even at a high concentration (Evidente et al., 2009; Neergaard et al., 2009). Similarly, undulatine exhibits promising AChE and prolyl oligopeptidase inhibitory activities (Cahlikova et al., 2013).

1.7.4. Hemanthamine type

Hemanthamine and hemanthidine display pronounced cell growth inhibitory activities against a variety of tumor cells, such as Rauscher viral leukemia, Molt 4 lymphoma, BL6 mouse melanoma, HepG2 human hepatoma, HeLa, LNCaP human prostate cancer, HT, Glioma Cells, glioblastoma, melanoma, non-small-cell lung and metastatic cancers, C-6 (rat glioma cells), CHO-K1 (Chinese hamster ovary cells) and non-tumoral fibroblastic LMTK cells, and p53-negative human leukemic Jurkat cells (Bastida et al., 2006; van Goietsenoven et al., 2010a; Luchetti et al., 2012; Havelek et al., 2013; Katoch et al., 2013).

Additionally, hemanthamine exhibits antiviral activity against Herpes simplex type 1 and highly pathogenic avian influenza virus H5N1 (He et al., 2013). Furthermore, the alkaloid has an antiprotozoal effect, showing good activity in *in vitro* assays against *T. brucei rhodesiense*, *T. cruzi* and *P. falciparum* (Osorio et al., 2010). In particular, the antimalarial activity against strains of chloroquine-sensitive *P. falciparum* observed in hemanthamine and hemanthidine can be attributed to the methylenedioxybenzene part of the molecule and the tertiary nitrogen without methyl (Bastida et al., 2006).

Moreover, hemanthamine has hypotensive, antioxidant and anticonvulsant effects (Oloyede et al., 2010). Like lycorine, hemanthidine has stronger analgesic and anti-inflammatory activity than aspirin (Bastida et al., 2006).

Among the other alkaloids of this type, vittatine has been found to potentiate the analgesic effect of morphine, and exhibits antibacterial activity against the Gram-positive *S. aureus* and the Gram-negative *E. coli* (Evidente et al., 2004; Bastida et al., 2006). 11-Hydroxyvittatine and 8-*O*-demethylmaritidine present antimicrobial activity (Abou-Donia et al., 2008), and the latter also exhibits significant AChE inhibition activity (Kulhankova et al., 2013). The compound 6-hydroxycrinamine, in turn, which has two epimers, 6α-hydroxycrinamine and 6β-hydroxycrinamine, possesses AChE inhibitory activity and is toxic to the neuroblastoma cells (Adekanmi et al., 2012). Additionally, 6β-hydroxycrinamine shows cytotoxicity against HL-60, A-549, and MCF-7 cells (Feng et al., 2011).

1.7.5. Tazettine type

Tazettine exhibits AChE inhibitory activity, and also shows promising human plasma butyrylcholinesterase inhibitory activity (Cahlikova et al., 2011; Sarikaya et al., 2013). Tazettine is also mildly active against certain tumor cell lines with cytotoxicity when tested on fibroblastic LMTK, HCT-116 and HeLa cell lines. It also displays weak hypotensive and antimalarial activities and interacts with DNA (Bastida et al., 2006; Jokhadze et al., 2011). One feature of tazettine is its affinity to the serotonin-reuptake transport protein (Elgorashi et al., 2006). Finally, tazettine and pretazettine demonstrate notable *in vitro* activity against *T. cruzi* (de Andrade et al., 2012a). It should be emphasized that tazettine is an isolation artefact of chemically labile pretazettine (de Andrade et al. 2012a), the latter being far more interesting due to its anticancer and antiviral activities (Bastida et al. 2006).

Several studies in the literature report excellent antiproliferative effects of pretazettine on human MDR1-gene-transfected L5178 mouse lymphoma cells (Zupko et al., 2009). In addition, pretazettine displays cytotoxicity against fibroblastic LMTK cell lines and inhibits HeLa cell growth, being therapeutically effective against advanced

Rauscher leukemia, Ehrlich ascites carcinoma, spontaneous AKR lymphocytic leukemia, Lewis lung carcinoma, and Molt4 lymphoid cells. Notably, pretazettine has also been shown to be active against Rauscher leukemia virus, Herpes simplex type 1 virus and selected RNA-containing flavoviruses (Japanese encephalitis, yellow fever, and dengue) and bunyaviruses (Punta Toro and Rift Valley fever) in organ culture (Bastida et al., 2006)

1.7.6. Narciclasine type

Narciclasine displays marked proapoptotic and cytotoxic activities. It involves the impairment of actin cytoskeleton organization by targeting GTP-ases, including RhoA and the elongation factor eEF1A, making it a promising GTP-ase targeting agent against brain cancers (van Goietsenoven et al., 2013). Narciclasine was also found to possess potent inhibitory activity against Human Cytochrome P450 (McNulty et al., 2011). Moreover, narciclasine induces marked apoptosis-mediated cytotoxic effects in two kinds of human cancer cells, human MCF-7 breast and PC-3 prostate carcinoma cells (Dumont et al., 2007). Narciclasine also impairs eEF1A-related actin bundling activity, EEF1A being a potential target to combat melanomas (van Goietsenoven et al., 2010b). Finally, narciclasine activates Rho and stress fibers in glioblastoma cells (Lefranc et al., 2009).

Narciclasine, an antimitotic alkaloid, affects cell division at the metaphase stage and inhibits protein synthesis in eukaryotic ribosomes. In particular, it also retards DNA synthesis and inhibits calprotectin-induced cytotoxicity at a concentration more than 10-fold lower than lycorine. The important effects of narciclasine seem to arise from the functional groups and conformational freedom of its C-ring, with the 7-hydroxyl group believed to play a considerable role in its biological activity (Bastida et al., 2006).

Additionally, narciclasine displays a prophylactic effect on the adjuvant arthritis model in rats. The alkaloid is active against *Corynebacterium fascians*, inhibits the pathogenic yeast *Cryptococcus neoformans*, and modifies the growth of the pathogenic bacterium *Neisseria gonorrhoeae*. Antiviral activity has been observed against RNA-containing flaviviruses and bunyaviruses (Bastida et al., 2006).

In plants, narciclasine is a potent inhibitor, showing a broad range of effects, including the ability to inhibit seed germination and seedling growth of some plants in a dose-dependent manner, interacting with hormones in some physiological responses (Bastida et al., 2006).

Some alkaloids of this type, such as lycoricidine, possess anti-HCV activity (Chen et al., 2013). Lycoricidine also exhibits *in vitro* activity against the important RNA viruses, JE, YF and dengue-4, flaviviruses, vunyaviruses, Punta Toro and Rift Valley fever virus (Gabrielsen et al., 1992a, 1992b).

Arolycoricidine presents inhibitory activity against African trypanosomiasis *T. brucei rhodesiense* and also has significant antimalarial activity against drug-resistant *P. falciparum* K1 (Kaya et al., 2011). Trisphaeridine possesses antiretroviral activities, accompanied by low therapeutic indices (Bastida et al., 2006).

1.7.7. Montanine type

Like other Amaryllidaceae alkaloids, montanine has psychopharmacol activities including anxiolytic, antidepressive, anticonvulsive, antiproliferative, antiinflammatory, antioxidant, antimicrobial, and inhibition of the AChE activity effects (da Silva et al., 2006, 2008; Castilhos et al., 2007; Pagliosa et al., 2010). Montanine also exhibits an effect on binding to the serotonin transporter protein *in vitro*, and presents low binding affinity to P-glycoprotein (Stafford et al., 2013).

In turn, pancracine, which shows antibacterial activity against *S. aureus* and *Pseudomonas aeroginosa*, also shows a weak activity against *T. brucei rhodesiense*, *T. cruzi*, and *P. falciparum* (Bastida et al., 2006).

1.7.8. Galanthamine type

Galanthamine is a long-acting, selective, reversible and competitive inhibitor of AChE and an allosteric modulator of the neuronal nicotinic receptor for acetylcholine. AChE is responsible for the degradation of acetylcholine at the neuromuscular junction, in peripheral and central cholinergic synapses. Galanthamine has the ability to cross the blood-brain barrier and to act within the central nervous system (Berkov et al., 2012a).

Galanthamine, therefore, is the most studied Amaryllidaceae alkaloid in terms of biological activity, clinical response, tolerance and safety, being marketed as a hydrobromide salt under the name of Razadine®, formerly Reminyl® (de Andrade et al., 2012b).

Galanthamine has other noteworthy pharmacological actions, including an ability to amplify the nerve-muscle transfer, affecting membrane ionic processes. Besides this, galanthamine acts as a mild analeptic, shows an analgesic power as strong as morphine, compensates for the effects of opiates on respiration, relieves jet lag, fatigue syndrome, male impotence, and alcohol dependence, and when applied in eye drops, reduces the intraocular pressure. It also acts as a hypotensive and has a weak antimalarial activity (Bastida et al., 2006).

Recently, more effects of galanthamine have been found. The alkaloid presents a protective effect on myocardial ischemia-reperfusion injury in rats (Li et al., 2012a). Galanthamine plus estradiol treatment enhances cognitive performance in aged ovariectomized rats (Gibbs et al., 2011). Moreover, the compound also has an inhibitory effect on tumor necrosis factor alpha (TNF-α) release in rats with lipopolysaccharide-induced peritonitis, and the vagus nerve plays a role in the process of the action of galanthamine (Liu et al., 2010).

After the therapeutic success of galanthamine, the search for new AChE inhibitors has intensified. Consequently, epigalanthamine, with a hydroxyl group at the α-position, and narwedine, with a keto group at C-3, are also reported as active AChE inhibitors, but about 130 times less powerful than galanthamine. The loss of the methyl group at the N atom, as in *N*-demethylgalanthamine, decreases the activity 10-fold. Hydrogenation of the C_4-C_{4a} double bond, as in lycoramine, results in a complete loss of AChE inhibitory activity (de Andrade et al., 2012b).

On the other hand, sanguinine, which has a hydroxyl group at C-9 instead of a methoxyl group, is an even more powerful inhibitor of AChE than galanthamine (Torras-Claveria et al., 2013). Quite recently, *N*-allylnorgalanthamine, was isolated from the bulbs of *Lycoris guangxiensis* and inhibits AChE considerably more than the approved drug galanthamine (Li et al., 1987; Berkov et al., 2008).

1.7.9. Other types

Hostasinine A from *Lycoris albiflora* exhibits potent cytotoxic activities against not only HL-60 cells but also HSC-2 cells (Jitsuno et al., 2011).

2. OBJECTIVES

2. Objectives

Overall, the main objective of this thesis is the chemical and biological study of the Chinese genus *Lycoris*. To our knowledge, there are only two reports in the literature about the use of GC-MS for the identification of alkaloids in *Lycoris* species (*L. radiata* and *L. aurea*) (Sun et al., 2012; Wang et al., 2007a). Consequently, the aim is to provide detailed alkaloid profiles of a range of *Lycoris* species for the first time. In addition, the other general aim is to study two species from the South American genus *Hippeastrum*, namely *Hippeatsrum papilio* and *H. calyptratum*.

Specific Objectives:

➤ To provide alkaloid profiles of species belonging to the genus *Lycoris*. The application of GC-MS to evaluate and quantify the alkaloids in *L. albiflora*, *L. aurea*, *L. chinensis*, *L. haywardii, L. incarnata*, *L. longituba*, *L. radiata*, *L. sprengeri*, *L. squamigera* and *L. radiata* var. *pumila*, all from China.

➤ To evaluate the alkaloid content of the species *Hippeastrum papilio* and *H. calyptratum* through GC-MS. To characterize new compounds, and provide complete spectral data for unknown compounds, using different spectroscopic techniques including NMR, IR, UV, ORD, CD and HRMS.

➤ To identify species with potential pharmaceutical interest due to a high content of compounds showing remarkable bioactivity.

➤ To carry out activity tests by *in vitro* assays on new alkaloids against the parasitic protozoa *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*, as well as to evaluate cytotoxicity by use of L-6 myoblast cells.

➤ To contribute to the taxonomical revision of the species under study, based on the presence of certain types of alkaloids as chemical markers.

3. RESULTS

3. Results

3.1. Article 1

Analysis of Bioactive Amaryllidaceae Alkaloid Profiles in *Lycoris* Species by GC-MS

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Analysis of Bioactive Amaryllidaceae Alkaloid Profiles in *Lycoris* Species by GC-MS

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The genus *Lycoris*, a group of Amaryllidaceae plants distributed in temperate regions of Eastern Asia, is already known for containing representative alkaloids typical of this botanical family with a wide range of biological activities (for example, lycorine and galanthamine). In the present work, the alkaloid profiles of nine species, *L. albiflora, L. aurea, L. chinensis, L. haywardii, L. incarnata, L. longituba, L. radiata, L. sprengeri,* and *L. squamigera*, and one variety (*L. radiata* var. *pumila*) have been evaluated by GC-MS. Structures belonging to the lycorine-, homolycorine-, haemanthamine-, narciclasine-, tazettine-, montanine- and galanthamine-series were identified and quantified, with galanthamine- and lycorine-type alkaloids predominating and usually showing a high relative abundance in comparison with other alkaloids of the extracts. Interestingly, *L. longituba* revealed itself to be a potential commercial source of bioactive alkaloids. In general terms, our results are consistent with the alkaloid profiles reported in the literature for previously studied species.

Keywords: *Lycoris*, Amaryllidaceae Alkaloids, GC-MS, Lycorine, Galanthamine.

The Amaryllidaceae family is comprised of about 1100 perennial bulbous species, classified into 85 genera, and distributed throughout the tropics and warm temperate regions of the world. It is one of the 20 most important alkaloid-containing plant families [1]. *Lycoris*, a genus belonging to the Amaryllidaceae, includes 22 species and one hybrid, and is found in temperate woodlands of Eastern Asia. In particular, fifteen of these species grow in China, of which 10 are endemic [2].

The Amaryllidaceae alkaloids represent a large and still expanding group of isoquinoline alkaloids, usually classified into nine skeleton types whose representative compounds are: norbelladine, lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine and galanthamine. Biogenetically, these structures are the result of an intramolecular oxidative coupling of the key intermediate *O*-methylnorbelladine, derived from the amino acids L-phenylalanine and L-tyrosine. *Ortho-para´* phenol oxidative coupling of *O*-methylnorbelladine results in the formation of a lycorine-type skeleton, from which homolycorine-type alkaloids are derived. The galanthamine-type skeleton is the only one originating from *para-ortho´* phenol oxidative coupling; while *para-para´* coupling leads to the formation of crinine, haemanthamine, tazettine, narciclasine and montanine structures (Figure 1) [3].

To date, almost 500 structurally diverse alkaloids have been isolated from plants of this family, showing a broad range of biological effects, including acetylcholinesterase (AChE)-inhibitory, antitumor, antibacterial, antifungal, antiviral and antimalarial activities [4]. Lycorine is one of the most frequently occurring alkaloids in Amaryllidaceae species and has been found in all *Lycoris* species. It has been reported as a potent inhibitor of ascorbic acid synthesis, cell growth and division, and organogenesis in higher plants, algae and yeasts, inhibiting the cell cycle during the interphase [3]. In addition, recent studies have reported potential antinociceptive, anti-inflammatory, hepatoprotective and hypotensive activities [5,6]. In terms of bioactivity, the most prominent alkaloid of the group is galanthamine, a long-acting, selective, reversible and competitive inhibitor of AChE, the enzyme responsible for the degradation of acetylcholine at the neuromuscular junction in peripheral and central cholinergic synapses. AChE inhibition is the current strategy of choice for the treatment of mild and moderate stages of Alzheimer´s disease. Besides its good inhibitory activity, a dual mechanism of action as an allosteric modulator of the neuronal nicotinic receptor for acetylcholine has been proposed for galanthamine, which also has the ability to cross the blood-brain barrier and act within the central nervous system [1]. As a result, its use was approved by the FDA in 2001, and galanthamine is currently marketed as a hydrobromide salt under the name of Razadine® (formerly Reminyl®)[7]. Finally, among other notable structures previously reported in the *Lycoris* genus are tazettine and montanine. The former has also shown AChE-inhibitory activity [8, 9], but is considered to be an isolation artifact of the chemically labile pretazettine [10]. Montanine, a widespread alkaloid in the genus, has psychopharmacological activities including anxiolytic, antidepressive and anticonvulsive, as well as antiproliferative, antiinflammatory, antioxidant, antimicrobial, and AChE-inhibitory effects [11-14].

The extracts obtained from Amaryllidaceae plants usually show complex alkaloid profiles. Traditionally, isolation and identification of alkaloids has been achieved through a combination of chromatography, and IR, UV, NMR and CD techniques, which can be time-consuming and laborious. Phytochemical study of the genus *Lycoris* began at the end of the 19th century. The first study was carried out with *L. radiata*, which yielded the alkaloids lycorine and sekisanine (tazettine) [15]. In the 1930s, Kondo *et al.* initiated a

series of in-depth studies on *Lycoris* alkaloids, resulting in the elucidation of the structures of lycorine, tazettine, lycoramine and homolycorine [16-19]. Galanthamine was first found in *Lycoris* species in 1957 [20]. In 1959 two new phenolic alkaloids (norpluviine and 8-*O*-demethylhomolycorine) were isolated from *L. radiata* [21]. From the 1960s to the 1980s, exhaustive research work led to the first isolation of sanguinine from *L. sanguinea*, as well as *O*-demethyllycoramine from *L. radiata*; at the same time, other species such as *L. squamigera*, *L. guangxiensis* and *L. chinensis* were studied [22-27]. In the 1990s, Kihara *et al.* isolated a new alkaloid from flowers of *L. incarnata* named incartine, which has been proposed as a biosynthetic intermediate in the conversion of galanthine to narcissidine [28]. Two new alkaloids, norsanguinine and norbutsanguinine, were isolated and characterized from the bulbs of *L. sanguinea*, in addition to five known Amaryllidaceae alkaloids [29]. During the first decade of the current century, the continued investigation of *Lycoris* plants yielded further novel structures. Reported in *L. radiata* bulbs, lycosinine A and lycosinine B were classified as a new structural group (galanthindole-type), in addition to the unusual lycorine-type alkaloids, lycoranine A and lycoranine B, all of them completely characterized by spectroscopic methods [30, 31]. Moreover, a new montanine-type alkaloid named squamigine, together with 2*R*-hydroxy-*N*,*O*-dimethylnorbelladine and 3-*O*-ethyltazettinol, were isolated from the bulbs of *L. squamigera* and *L. aurea* [32, 33]. Recent studies with bulbs of *L. radiata* resulted in the isolation of 5,6-dehydrodihydrolycorine, 6*β*-acetoxycrinamine, 8-*O*-acetylhomolycorine-*N*-oxide, 5,6-dehydrolycorine, 3*α*,6*β*-diacetylbulbispermine, 3*α*-hydroxy-6*β*-acetylbulbispermine, 8,9-methylene-dioxylhomolycorine-*N*-oxide, 5,6-dihydro-5-methyl-2-hydroxyphenanthridine, and 2*α*-methoxy-6-*O*-ethyloduline [34-36].

To summarize, fifteen species of *Lycoris*, *L. albiflora*, *L. anhuiensis*, *L. aurea*, *L. chinensis*, *L. guangxiensis*, *L. haywardii*, *L. houdyshelii*, *L. incarnata*, *L. longituba*, *L. radiata*, *L. rosea*, *L. sanguinea*, *L. sprengeri*, *L. squamigera* and *L. straminea* have been phytochemically studied to date, and around one hundred alkaloids of mainly 8 structural types have been found and classified, with lycorine- and galanthamine-type alkaloids predominating in all the species (Table 1).

The aim of the present work was to analyze the alkaloid content of 9 *Lycoris* species collected in China, using a simple and rapid methodology that combines the advantages of gas chromatography-mass spectrometry (GC-MS) for alkaloid profiling and direct quantification from dry plant material. GC-MS is a proven useful, rapid and specific method with good sensitivity for the investigation and identification of complex alkaloid mixtures of different groups from various plants, requiring a very low quantity of plant material and no derivatization step [37, 38]. To our knowledge, there are only two reports in the literature about the use of this method for the identification of alkaloids in *Lycoris* species (*L. radiata* and *L. aurea*) [39, 40]. In the current work, the alkaloid profiles of *L. albiflora*, *L. aurea*, *L. chinensis*, *L. haywardii, L. incarnata*, *L. longituba*, *L. radiata*, *L. sprengeri*, *L. squamigera* and the variety *L. radiata* var. *pumila*, all from China, were evaluated and quantified by GC-MS. The aim was to contribute to a better knowledge of the variability and quantification of *Lycoris* alkaloids.
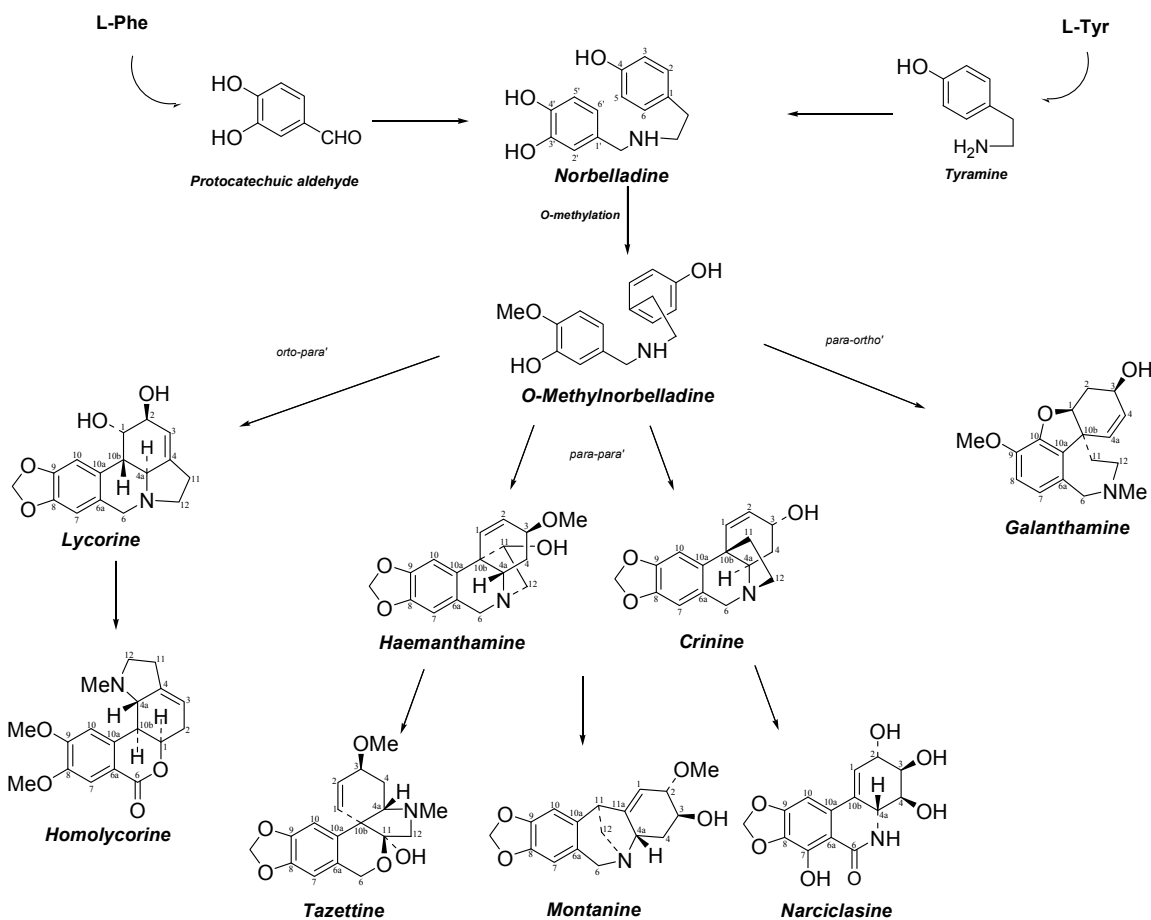

Figure 1: Biosynthetic pathway of *Lycoris* alkaloids with representative compounds.

Table 1: Different types of alkaloids in *Lycoris* species.

Structural Type	*L. albiflora*	*L. anhuiensis*	*L. aurea*	*L. chinensis*	*L. guangxiensis*	*L. haywardii*	*L. houdyshelii*	*L. incarnata*	*L. longituba*	*L. radiata*	*L. radiata* var. *pumila*	*L. rosea*	*L. sanguinea*	*L. sprengeri*	*L. squamigera*	*L. straminea*
Lycorine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Homolycorine	+		+	+					+						+	
Crinine			+	+						+	+					
Haemanthamine	+		+	+	+				+	+				+		+
Narciclasine	+			+	+				+	+				+		+
Tazettine			+						+	+				+		+
Montanine			+						+	+					+	
Galanthamine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Other	+			+						+					+	

GC-MS analysis of the bulbs of different species of *Lycoris* resulted in the identification of 31 alkaloids, the majority of them belonging to the lycorine, homolycorine, haemanthamine, narciclasine, tazettine, montanine and galanthamine types, together with one unusual alkaloid known as cherylline. In general, the results coincided with previously reported alkaloids found in the genus, including lycorine and galanthamine. The alkaloid profile of the extracts of all the studied species was dominated by alkaloids arising from *ortho-para´* (lycorine-type) and *para-ortho´* (galanthamine-type) oxidative coupling of *O*-methylnorbelladine (Table 2). The number of alkaloids detected varied among extracts, from 10 in *L. incarnata* and *L. radiata* var. *pumila* to 20 in *L. longituba*.

In all the species, lycorine- and galanthamine-type alkaloids were predominant. *L. longituba* and *L. sprengeri* showed the highest content of lycorine-type compounds, with values above 2.5 mg GAL/g DW. The maximum level of galanthamine-type alkaloids was detected in *L. longituba* bulbs (4.75 mg GAL/g DW), while

Table 2: Alkaloid profiles of studied species. Values are expressed as μg GAL/g DW.

Alkaloids	M+	Base Peak	RI	*L. albiflora*	*L. aurea*	*L. chinensis*	*L. haywardii*	*L. incarnata*	*L. longituba*	*L. radiata*	*L. radiata* var. *pumila*	*L. sprengeri*	*L. squamigera*
Lycorine-type				863.2	1445.9	1113.1	1280.1	1291.7	2572.4	2037.9	976.4	2514.3	1912.2
Lycorine	287	226	2766.1	370.7	502.8	360.8	349.7	337.6	656.9	923.8	298.7	687.4	257.1
Pseudolycorine	289	228	2837.2	-	-	-	-	-	224.8	-	-	-	-
Galanthine	317	242	2703.4	trace	222.0	trace	228.0	239.8	-	-	-	337.4	467.5
Pluviine	287	242	2571.7	-	-	-	trace	-	237.3	-	223.1	-	trace
Norpluviine	273	228	2602.2	trace	-	-	-	-	-	-	-	trace	-
Caranine	271	226	2537.4	245.4	248.5	264.3	236.3	237.7	260.1	299.3	trace	267.1	trace
Methylpseudolycorine	303	242	2792.3	-	-	-	-	-	490.8	-	-	-	233.1
Incartine	333	332	2457.8	trace	trace	trace	trace	224.4	-	-	-	446.9	265.1
2-Dehydroxylycorine	271	250	2551.3	-	trace	-	-	-	222.2	226.2	-	224.3	-
*Galanthine derivative**	315	240	2782.1	-	trace	trace	trace	-	-	-	-	trace	240.5
Anhydrolycorine	251	250	2516.2	trace	222.0	228.0	221.3	trace	226.7	283.8	222.7	227.2	216.1
11,12-Dehydroanhydrolycorine	249	248	2622.1	247.1	250.7	259.9	244.7	252.2	253.7	304.7	231.9	324.0	232.9
Assoanine	267	266	2581.4	-	-	-	-	-	trace	-	-	-	-
Homolycorine-type				-	-	-	550.5	-	-	1408.8	534.2	-	-
Homolycorine	315	109	2765.0	-	-	-	238.0	-	-	256.8	245.7	-	-
8-*O*-Demethylhomolycorine	301	109	2822.0	-	-	-	312.5	-	-	659.3	288.4	-	-
Hippeastrine	315	125	2903.4	-	-	-	-	-	-	266.0	-	-	-
O-Methyllycorenine	331	109	2487.9	-	-	-	-	-	-	226.7	-	-	-
Haemanthamine-type				-	251.5	224.3	-	trace	-	trace	-	286.2	trace
Haemanthamine	301	272	2644.3	-	251.5	224.3	-	trace	-	trace	-	286.2	-
Haemanthidine	317	317	2731.0	-	-	-	-	-	-	-	-	-	trace
Narciclasine-type				-	-	-	-	-	trace	-	-	trace	-
Trisphaeridine	233	233	1866.0	-	-	-	-	-	trace	-	-	trace	-
Tazettine-type				221.1	238.3	368.5	275.7	-	863.3	692.1	226.4	331.9	300.8
Tazettine	331	247	2655.0	221.1	238.3	368.5	275.7	-	863.3	692.1	226.4	331.9	300.8
Deoxytazettine	315	231	2546.6	-	-	-	-	-	trace	trace	-	-	-
Montanine-type				260.3	314.9	353.8	-	-	831.3	-	-	-	327.0
Montanine	301	301	2637.3	260.3	314.9	353.8	-	-	607.2	-	-	-	327.0
Pancratinine C	287	176	2600.2	-	-	-	-	-	224.1	-	-	-	-
Galanthamine-type				721.6	1455.6	1786.1	895.5	722.4	4754.3	2386.5	411.7	2425.2	1804.0
Galanthamine	287	286	2403.7	trace	674.1	618.0	227.1	227.4	836.0	425.0	trace	289.9	501.1
Sanguinine	273	273	2427.6	-	550.9	380.0	-	-	241.5	-	-	-	-
Lycoramine	289	288	2430.3	458.5	230.6	538.0	668.4	264.3	1808.6	1694.7	411.7	1870.0	1046.4
O-Demethyllycoramine	274	275	2459.7	-	-	-	-	-	344.9	-	-	-	-
Norlycoramine	274	275	2471.7	263.1	-	249.4	-	230.7	1523.3	266.8	-	265.4	256.5
Narwedine	285	284	2485.3	trace	-	-	-	-	trace	-	-	-	trace
Other-type				-	-	-	-	-	-	-	-	-	-
Cherylline	285	242	2574.0	-	-	trace	-	-	-	-	-	-	-
Total				2066.2	3706.2	3845.8	3001.8	2014.1	9021.3	6525.3	2148.6	5557.6	4343.9

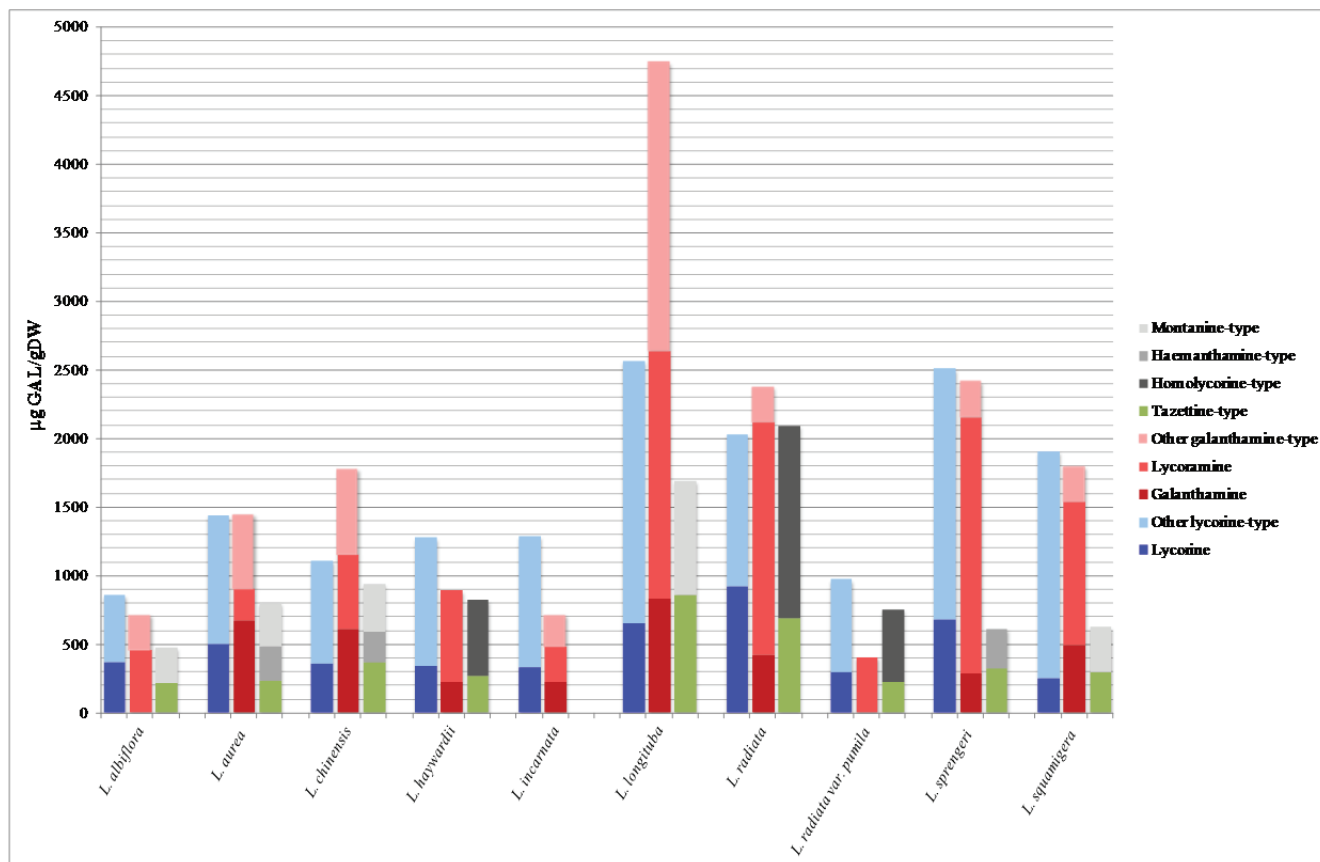*Although not reported in the literature, the MS of this compound indicates a structure derived from galanthine.

Figure 2: Alkaloid and alkaloid-type predominance in extracts of the genus *Lycoris*.

the lowest level was found in the bulbs of *L. radiata* var. *pumila* (0.4 mg GAL/g DW). Tazettine-type alkaloids were present in all the species, with the exception of *L. incarnata*, while the montanine type was not detected in five of the ten extracts analyzed. Homolycorine-type alkaloids were only found in *L. radiata*, *L. radiata* var. *pumila* and *L. haywardii*. Narciclasine-type alkaloids were present as mere traces in *L. longituba* and *L. sprengeri*. Although in low quantities (values less than 0.29 mg GAL/g DW), the haemanthamine type was detected in five species, *L. aurea*, *L. chinensis*, *L. radiata*, *L. sprengeri* and *L. squamigera* (Table 2; Figure 2).

In a more detailed examination of the alkaloid profiles, *L. albiflora* showed the presence of lycorine-, tazettine-, montanine- and galanthamine-type alkaloids, while those with homolycorine-, haemanthamine- and narciclasine-type skeletons were not detected. The alkaloid profile of *L. aurea* and *L. squamigera* was similar, with lycorine, haemanthamine, tazettine, montanine, and galanthamine types found in both species, but not narciclasine-type nor homolycorine-type alkaloids. In *L. chinensis* galanthamine-type (1786.1 μg GAL/g DW) and lycorine-type (1113.1 μg GAL/g DW) alkaloids predominated, with a relatively low amount of tazettine (368.5 μg GAL/g DW), only traces of the haemanthamine type, and no homolycorine-type alkaloids detected. Lycorine-, homolycorine-, tazettine- and galanthamine-type alkaloids were found in *L. haywardii*. The only species in which tazettine was not detected was *L. incarnata*, which showed mainly lycorine- and galanthamine-type, as well as traces of haemanthamine-type alkaloids. It should be noted that the total alkaloid content of *L. incarnata* was the lowest of all the species studied (2014.1 μg GAL/g DW). On the other hand, the species with the highest total content of alkaloids was *L. longituba*, which showed the maximum values of

galanthamine, lycorine, montanine and tazettine types. In *L. radiata* and its variety, *L. radiata* var. *pumila*, the most important feature was the noticeable abundance of homolycorine-type compounds. While both showed similar alkaloid profiles, containing lycorine, homolycorine, tazettine and galanthamine types, the quantity of each type was far higher in *L. radiata* than the variety. As shown in Table 2, lycorine, haemanthamine, narciclasine, tazettine and galanthamine types occurred in bulbs of *L. sprengeri*, but homolycorine and montanine skeletal types were not detected.

Regarding specific alkaloids, the extracts contained mainly lycorine, tazettine, galanthamine and lycoramine. Lycorine was the predominant alkaloid of its series (about 30%) in all species. The highest lycorine-containing species was *L. radiata*, which yielded almost 1 mg GAL/g DW. *L. longituba* and *L. sprengeri* also contained a large amount lycorine (656.9 μg GAL/g DW and 687.4 μg GAL/g DW, respectively). Almost the only tazettine-type alkaloid found was tazzetine, with the highest abundance in *L. longituba* (863.3 μg GAL/g DW). The other predominant alkaloids detected were galanthamine and lycoramine, both of them classified in the same group due to structural similarity, the only difference being an extra double bond at position 4-4a in galanthamine. The quantity of galanthamine was quite considerable in *L. aurea*, *L. chinensis*, *L. radiata* and *L. squamigera*, but particularly high in *L. longituba* (836.0 μg GAL/g DW). *L. aurea* and *L. chinensis* contained more galanthamine than lycoramine, which was the reverse in the other species. *L. longituba*, *L. radiata*, *L. sprengeri* and *L. squamigera* showed the highest amounts of lycoramine.

Figure 2 shows the alkaloid profile of each extract as a bar graph. The results are grouped into 3 main bars: two of them comprise lycorine- and galanthamine-type alkaloids, colored blue and red,

respectively, to highlight their predominance, whereas the third one represents all the remaining skeleton types (including tazettine-, homolycorine-, haemanthamine- and montanine-). The lycorine, galanthamine and lycoramine alkaloids are represented by different shades of red or blue, and tazettine is in green, given that these are the most frequently found structures.

The analysis of bioactive Amaryllidaceae alkaloid profiles in 9 species and one variety of *Lycoris* by GC-MS revealed some similarities and differences. This kind of analysis could be useful in guiding the search for compounds with pharmacological activity. Among the species analyzed in the present work, *L. longituba* could be considered as a potential commercial source of bioactive alkaloids (such as galanthamine and lycorine) due to its high content in comparison with the other species. In particular, the galanthamine content is nearly two times the amount calculated for *L. radiata,* which is currently the main source for the production of this drug in China. Finally, the GC-MS technology here applied has demonstrated to be sufficiently sensitive for the detection of Amaryllidaceae alkaloids, allowing the application of quantitative methodologies to obtain valuable information in relatively short times.

Experimental

Plant material: Bulbs of the genus *Lycoris* were collected from Nanjing Botanical Garden (Mem. Sun Yat-Sen) in China (2011-2014) and identified by Professor Gan Yao (Institute of Botany, Jiangsu Province and Chinese Academy of Sciences). Voucher specimens of *L. albiflora* (0653963), *L. aurea* (0653958), *L. chinensis* (0653960), *L. haywardii* (0653964)*, L. incarnata* (0653959), *L. longituba* (0653969), *L. radiata* (0653965), *L. sprengeri* (0653967), *L. squamigera* (0653962) and *L. radiata* var. *pumila* (0653966) have been deposited in the Herbarium of the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences.

Chemicals: Methanol, $CHCl_3$ and NH_4OH (25%) were purchased from SDS (Val de Reuil, France), and H_2SO_4 (96%) from Carlo-Erba (Rodano, Italy). Codeine (purity $\geq$ 99%), used as an internal standard, was purchased from Sigma Aldrich (St. Louis, MO, USA).

Alkaloid extraction: Fifty mg of dried and powdered bulbs of each sample was macerated in 1 mL of methanol at pH 8 for 2 h, together with codeine (0.05 mg) as the internal standard. During this time, the samples were submitted to 15 min in an ultrasonic bath every 30 min. The extract was acidified with 500 μL H_2SO_4 (2%) and the neutral compounds were removed with $CHCl_3$ (2 x 500 μL). The polar fraction was then basified with 200 μL NH_4OH (25%), and the alkaloids extracted with $CHCl_3$ (3 x 500 μL).

GC-MS analysis of alkaloid extracts: Dried alkaloid extracts were re-dissolved in 100 μL of $CHCl_3$ and directly injected into the GC-MS apparatus (Hewlett Packard 6890 coupled with MSD5975; Hewlett Packard, Palo Alto, CA, USA) operating in EI mode at 70eV. An HP-5 MS column (30 m x 0.25 mm i.d., film thickness 0.25 μm) was used. The temperature gradient performed was the following: 100-180°C at 15°C/min, 180-300°C at 5°C/min, 10 min hold at 300°C, and 2 min at 100°C. The injector temperature was 250°C and the flow-rate of carrier gas (helium) was 1 mL/min. A split ratio of 1:5 was applied.

Galanthamine quantification: A calibration curve was constructed for 10 dilutions of galanthamine (5, 10, 25, 50, 100, 200, 400, 500, 700, and 900 μg/mL). The same amount of codeine (0.05 mg) was added to each solution as an internal standard. The peak areas were manually obtained considering selected ions for each compound (usually the base peak of their MS, *i.e. m/z* at 286 for galanthamine, at 299 for codeine). The ratio between the values obtained for galanthamine and codeine in each solution was plotted against the corresponding concentration of galanthamine to obtain the calibration curve and its equation (y = 57.324x + 10.73; R^2 = 0.9981).

GC-MS identification of alkaloids and determination of alkaloid profile: The alkaloids were identified by comparing their GC-MS spectra and Kovats Retention Index (RI) with those of authentic Amaryllidaceae alkaloids previously isolated and identified by spectrometric methods (NMR, UV, CD, MS), by the NIST 05 Database or by literature data. RI values of compounds were measured with a standard *n*-hydrocarbon mixture (C9-C36) using AMDIS 2.64 software.

Alkaloids were quantified considering the area of peaks in each chromatogram. All data were standardized to the area of the internal standard (codeine) and the equation obtained for the calibration curve of galanthamine was used to calculate the quantities expressed as μg GAL, which was finally related to the amount of dried plant material (g DW). It is important to remember that as the peak area does not only depend on the corresponding alkaloid concentration but also on the intensity of the mass spectral fragmentation, this was not an absolute quantification. However, the methodology is considered suitable for comparing the amount of specific alkaloids between samples [38].

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3.2. Article 2

New alkaloids from Hippeastrum papilio (Ravenna) Van Scheepen

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New alkaloids from *Hippeastrum papilio* (Ravenna) Van Scheepen

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Abstract

A new phytochemical study of the indigenous Brazilian species *Hippeastrum papilio* is reported herein. Three novel Amaryllidaceae alkaloids were isolated, including hippapiline (**1**), papiline (**2**) and 3-*O*-(3'-hydroxybutanoyl)haemanthamine (**3**). Their structures were determined by physical and spectroscopic methods. In addition, the known alkaloids haemanthamine (**4**), galanthamine (**5**), narwedine (**6**), 11β-hydroxygalanthamine (**7**), apogalanthamine (**8**) and 9-*O*-demethyllycosinine B (**9**) were identified. The unusual *cis*-B/C ring fusion for the new homolycorine representative hippapiline was ratified by NMR and CD spectroscopy.

Keywords

Introduction

The plant family Amaryllidaceae comprises around 1600 species in 73 genera which are distributed through the tropics and warm, temperate regions of the globe [1,2]. These perennial, bulbous geophytes belong to one of the twenty most significant alkaloid-producing plant families [1,2]. A striking feature of the Amaryllidaceae is the presence of an exclusive group of isoquinoline alkaloids, which are responsible for a wide-range of biological activities [3].

Structurally, these alkaloids have been grouped into nine skeletal-types formed via specific oxidative phenolic couplings from the common amino acid-derived biogenetic precursor norbelladine [3]. The homolycorine-type skeleton is characterized by a *cis*-B/C ring fusion in which H-1 and H-10b are both *α*-oriented. The correct stereochemical characterization of Amaryllidaceae alkaloids thus allows for a better understanding of the biosynthetic pathway diagnostic for a particular skeleton.

Over the past two decades gas chromatography-mass spectrometry (GC-MS) has been applied successfully in the analysis of Amaryllidaceae alkaloids [4]. A preliminary study of *H. papilio* via GC-MS indicated the presence of several unknown structures with MS fragmentation patterns diagnostic of Amaryllidaceae alkaloids [5]. A larger collection of *H. papilio* bulbs was here subjected to a comprehensive phytochemical investigation leading to the identification of hippapiline (**1**), papiline (**2**) and 3-*O*-(3'-hydroxybutanoyl)haemanthamine (**3**) as the novel constituents, in addition to six other well-known Amaryllidaceae alkaloids. The *β*-orientation for both H-1 and H-10b

in compound **1**, uncovered by rigorous spectroscopic analysis, is indicative of an unsual

cis-B/C ring fusion previously not seen for homolycorine-type alkaloids.

Results and Discussion

GC-MS analysis (*Table 1*) revealed that galanthamine (**5**) was the main constituent in the

n-hexane extract (86.3 %), also featuring as one of the major components in the EtOAc

extract (39.0 %) together with haemanthamine (**4**) (26.9 %). These results are in

agreement with a previous study of *H. papilio* in which these same alkaloids were

isolated and identified by NMR and CD spectroscopic techniques [5]. However,

apogalanthamine (**8**) and 9-*O*-demethyllycosinine B (**9**) are now reported in this species

for the first time (*Fig. 1*), whilst narwedine (**6**) as in the previous instance [5] was here

also detectable only in minimal quantity.

Compound **1** had the HRESIMS [M+H]$^+$ signal at *m/z* 318.1706 (calc. for $C_{18}H_{24}NO_4$:

318.1700) and a base peak at *m/z* 109 $[C_7H_{11}N]^+$ in its GC-MS spectrum, arsing from a

retro-Diels-Alder reaction, which is characteristic for a hexahydroindole ring in the

homolycorine series lacking substitution at C-2 [4]. Although the basic structure of a

homolycorine-type alkaloid for compound **1** was readily established by NMR evidence,

the unusual chemical shift and splitting pattern were ratified via comparisons with the

data for 8-*O*-demethyl-6-*O*-methyllycorenine found in the literature [6]. The ^{1}H NMR

data of **1** was atypical in the following three ways: (i) two *para*-oriented aromatic

protons attributed to H-7 and H-10, the latter of which was assignable to the highly

deshielded resonance singlet at δ 8.41 (confirmed by NOESY correlation with the *N*-methyl group); (ii) an uncommon coupling constant (J = 4.5 Hz) observed between H-1 and H-10b and the absence of the distinctive *trans*-diaxial coupling ($J \sim$ 10 Hz) between H-10b and H-4a; (iii) a NOESY correlation between H-4a, H-10b and H-1. This data was thus pivotal in assigning both H-1 and H-10b to the β-face. CD analysis showed that there were positive, negative and positive Cotton effects at 225, 250 and 290 nm, respectively, which are antipodal to those observed for typically *cis*-B/C ring-fused homolycorine-type alkaloids [7]. Taken together, the CD and NMR data (*Table 2*) are in agreement with this novel stereochemical arrangement involving *cis*-B/C ring fusion in **1**, for which the name hippapiline is proposed.

The HRESIMS of **2** suggested a molecular formula $C_{19}H_{24}NO_5$ for $[M+H]^+$ with the parent ion at *m/z* 346.1643 (calcd. 346.1649). The EIMS showed a signal ion at *m/z* 286 $[M-59]^+$ diagnostic for the loss of an acetoxy group. Characteristic NMR signals included: (i) a singlet proton resonance at δ 9.91, indicative of an aldehyde functionality which appears in nonfused dihydroindole lycosinine derivatives [8,9], with the correspondent signal at δ 191.7 (*d*) in the ^{13}C NMR; (ii) two *para*-orientated aromatic protons at δ 7.32 and 7.29, the more deshielded of which was assigned to H-7 due to its NOESY correlation with H-6; (iii) the acetoxy substituent was assigned to C-1 due to the strong deshielding of H-1 (δ 5.45), confirmed by HMBC; (iv) the magnitude of the coupling constant ($J_{4a,10b} = J_{1,10b}$ = 4.5 Hz) together with the observed NOESY correlations were congruent with the *syn*-disposition for H-1, H-10b and H-4a. The IR spectrum of **1** displayed strong absorbances at 1738 and 1674 cm^{-1} for two C=O groups

ascribed to the acetoxy and conjugated aldehyde moieties, respectively. All spectroscopic data together were in agreement with a new compound bearing a nonfused hexahydroindole nucleus for which the name papiline has been proposed. The complete NMR data for compound **2** are listed in *Table 3*.

The new alkaloid **3** exhibited a parent $[M+H]^+$ ion at *m/z* 374.1603 in its HRESIMS spectrum, suggesting the molecular formula $C_{20}H_{24}NO_6$ (calcd. 374.1598). The EIMS fragmentation showed a loss of 87 mass units (*m/z* 287, $[M-87]^+$) characteristic of a 3'-hydroxybutanoyl substituent of unknown absolute configuration [10], which was confirmed by corresponding resonances in the ^1H and ^{13}C NMR spectra (*Table 4*). The remaining NMR data were very similar to those of haemanthamine [3]. Furthermore, a loss of 29 mass units from the molecular ion observed by GC-MS (*m/z* 344, $[M-29]^+$) is typical of haemanthamine derivatives bearing a hydroxyl group at C-11 [4]. The magnitude of the coupling constant between H-2 and H-3 ($J_{2,3}$ = 5.1 Hz) suggested a *trans* relationship between the substituent at C-3 and the 5,10b-ethano bridge [3]. The CD spectrum confirmed **3** as a haemanthamine-type derivative, showing positive and negative Cotton effects at 275 nm and 249 nm, respectively. A full set of NMR data for 3-*O*-(3'-hydroxybutanoyl)haemanthamine (**3**) is shown in *Table 4*.

In summary, a new phytochemical investigation of *H. papilio* led to the identification of nine alkaloids, among which hippapiline, papiline and 3-*O*-(3'-hydroxybutanoyl)hae-manthamine are reported for the first time. The unusual *β*-orientation for both H-1 and H-10b in the homolycorine-type skeleton of hippapiline was confirmed by NMR and CD

spectroscopy. This finding provides new insight on the biosynthesis of homolycorine compounds in particular and Amaryllidaceae alkaloids in general.

Acknowledgments

Experimental part

General

NMR spectra were recorded on a Varian VNMRS 500 MHz (Palo Alto, CA, USA), using $CDCl_3$ as solvent and TMS as the internal standard. Chemical shifts are reported in δ units (ppm) and coupling constants (J) in Hz. EIMS were obtained on an Agilent 6890 + MSD 5975 GC-MS spectrometer (Agilent Technologies, Santa Clara, CA, USA) operating in EI mode at 70 eV. An HP-5 MS column (30 m × 0.25 mm × 0.25 μm) was used. The temperature program was as follows: 100-180 ℃ at 15 ℃ min^{-1}, 1 min hold at 180 ℃, 180-300 ℃ at 5 ℃ min^{-1} and 1 min hold at 300 ℃. The injector temperature was 280 ℃. The flow rate of carrier gas (Helium) was 0.8 ml min^{-1}. The 1:20, 1:10 and 1:5

split ratios were applied, depending on sample concentration. GC-MS results were analyzed using AMDIS 2.64 software (NIST). HRESIMS data were obtained on an LC/MSD-TOF spectrometer (Agilent Technologies, Santa Clara, CA, USA) through direct injection of pure compounds dissolved in H_2O/MeCN (1:1). Optical rotations were measured in $CHCl_3$ at 22 ℃ using a Perkin-Elmer 241 polarimeter (Waltham, MA, USA). A Jasco-J-810 Spectrophotometer (Easton, MD, USA) was used to obtain CD spectra, all recorded in MeOH. UV spectra were obtained on a Dinko UV2310 instrument (Barcelona, Spain). Silica gel (Kieselgel − mesh 0.15/0.30, Val-de-Reuil, France) was used for all vacuum liquid chromatography (VLC) procedures. For thin layer chromatography (TLC), silica gel F_{254} was used as the stationary phase with plate dimensions 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).

Plant material

Hippeastrum papilio was collected during the flowering period (May, 2012) in the south of Brazil (Caxias do Sul - RS). A voucher specimen (ICN-149428) was authenticated by Dr. Julie Dutilh (University of Campinas) and deposited in the Institute of Botany, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre.

Identification of alkaloids by GC-MS

The alkaloids were identified by comparing their GC-MS spectra and Kovats Retention Index (RI) with those of authentic Amaryllidaceae alkaloids previously isolated and

identified by spectrometric methods (NMR, UV, CD, MS) [5,8,11], the NIST 05 Database or literature data. RI values of compounds were measured with a standard *n*-hydrocarbon mixture (C9-C36) using AMDIS 2.64 software. The proportion of each individual compound in the alkaloid fractions analyzed by GC-MS (*Table 1*) is expressed as a percentage of the total alkaloids (TIC – total ion current). The area of the GC-MS peaks depends on the concentration of the corresponding compound and the intensity of their mass spectral fragmentation. Although data given in *Table 1* do not express a real quantification, they can be used for a relative comparison of the amount of the respective alkaloids present in *Hippeastrum papilio*.

Extraction and isolation of alkaloids

Dried bulbs (220 g) of *H. papilio* were crushed and extracted with methanol at room temperature as following: twice using 900 ml for 48 h and twice with 400 ml for 72 h. The extract was evaporated under reduced pressure to yield 55 g. This crude extract was acidified to pH 2 with H_2SO_4 (2% v/v), and extracted with Et_2O (170 ml $\times$ 6) to remove neutral material. The aqueous solution was basified with 25% ammonia up to pH 10, and extracted with *n*-hexane (170 ml $\times$ 13) to give extract A (1.3 g). Another extraction using EtOAc (170 ml $\times$ 20) gave extract B (1.5 g). Extract A yielded galanthamine (**5**, 130.71 mg) and haemanthamine (**4**, 49.5 mg) by SPTLC (CH_2Cl_2/Acetone 10:7; in NH_3 atmosphere). Extract B was subjected to vacuum liquid chromatography (VLC) using a silica gel (7 g) column with a diameter of 2.5 cm and a height of 4.5 cm, eluting with *n*-hexane gradually enriched with EtOAc (0→100%), and then with EtOAc gradually

enriched with MeOH (0→20%). Fractions of 40 ml were collected (340 in total) monitored by TLC (Dragendorff′s reagent, UV 254 nm) and combined according to their profiles. From fractions 11-20, hippapiline (**1**, 22.2 mg) was directly obtained as a crystal. Fractions 90-160 were subjected to SPTLC (*n*-hexane/EtOAc 1:2; and a second time *n*-hexane/EtOAc/MeOH 5:10:7; in NH_3 atmosphere) to give papiline (**2,** 26.7 mg). 9-*O*-demethylcosinine B (**9,** 7.4 mg) was isolated from fractions 61-85 by SPTLC (*n*-hexane/EtOAc 4:1; in NH_3 atmosphere), while 11*β*-hydroxygalanthamine (**7**, 1.2 mg) was obtained via SPTLC (*n*-hexane/EtOAc/MeOH, 10:3:3; in NH_3 atmosphere) from fractions 206-210. A third VLC column (1.5 × 4.5 cm) was performed with fractions 161-205 yielding 176 fractions (10 ml each), of which fractions 69-92 were further purified by SPTLC (*n*-hexane/EtOAc/MeOH, 2:4:1; in NH_3 atmosphere) to furnish 3-*O*-(3'-hydroxybutanoyl)haemanthamine (**3**, 12.5 mg). In addition, a small amount of narwedine (**6**) and apogalanthamine (**8**), which were identified by comparing their GC-EI-MS spectra and Kovats retention indices (RI) with our own library database. All known alkaloids isolated were identified by comparing their physical and spectroscopic data with those of alkaloids previously isolated and characterized by our group [5,8,11].

Hippapiline (**1**): white crystals; $[\alpha]^{24}_D$ +33 (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 316 (2.90), 282 (3.30), 228 (3.64) nm; CD (MeOH, 20 ℃): $\Delta\epsilon_{227}$ +1330, $\Delta\epsilon_{247.5}$ −455, $\Delta\epsilon_{281.5}$ +89, $\Delta\epsilon_{305.5}$ −176; IR ($CHCl_3$) ν_{max}: 3380, 2924, 1733, 1509, 1449, 1275, 1130, 1058, 1043, 960, 913, 880, 802, 758 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR

(CDCl$_3$, 125 MHz) are shown in *Table 2*; EIMS data are detailed in *Table 1*; HREIMS of [M+H]$^+$ *m/z* 318.1706 (calcd. for C$_{18}$H$_{24}$NO$_4$, 318.1700).

Papiline (**2**): amorphous solid; $[\alpha]^{24}_D$ −29 (*c* 0.26, CHCl$_3$); UV (MeOH) λ_{max} (log ε) 318 (3.27), 280 (3.48), 236 (3.76) nm; IR (CHCl$_3$) ν_{max}: 2925, 2854, 1738, 1674, 1595, 1557, 1514, 1455, 1378, 1260, 1148, 1099, 1020, 800 cm^{-1}; for ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz) see *Table 3*; EIMS data are shown in *Table 1*; HREIMS of [M+H]$^+$ *m/z* 346.1643 (calcd. for C$_{19}$H$_{24}$NO$_5$, 346.1649).

3-*O*-(3'-Hydroxybutanoyl)haemanthamine (**3**): amorphous solid; $[\alpha]^{24}_D$ −13 (*c* 0.49, CHCl$_3$); UV (MeOH): λ_{max} (log ε) 292 (3.46), 238 (3.36) nm; CD (MeOH, 20 °C): $\Delta\epsilon_{249}$ −2375, $\Delta\epsilon_{275}$ +1569; IR (CHCl$_3$) ν_{max}: 3377, 2925, 1727, 1504, 1484, 1376, 1295, 1239, 1173, 1064, 1037, 988, 936, 853, 758 cm^{-1}; ^{1}H NMR (CDCl$_3$, 500 MHz) and ^{13}C NMR (CDCl$_3$, 125 MHz) are shown in *Table 4*; EIMS data are detailed in *Table 1*; HREIMS of [M+H]$^+$ *m/z* 374.1603 (calcd. for C$_{20}$H$_{24}$NO$_6$, 374.1598).

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Legends for Figure

Fig. **1**: Alkaloids identified in *Hippeastrum papilio*.

1

2

3

4

5 R₁=OH, R₂=R₃=H
6 R₁+R₂=O, R₃=H
7 R₁=OH, R₂=H, R₃=OH

8

9
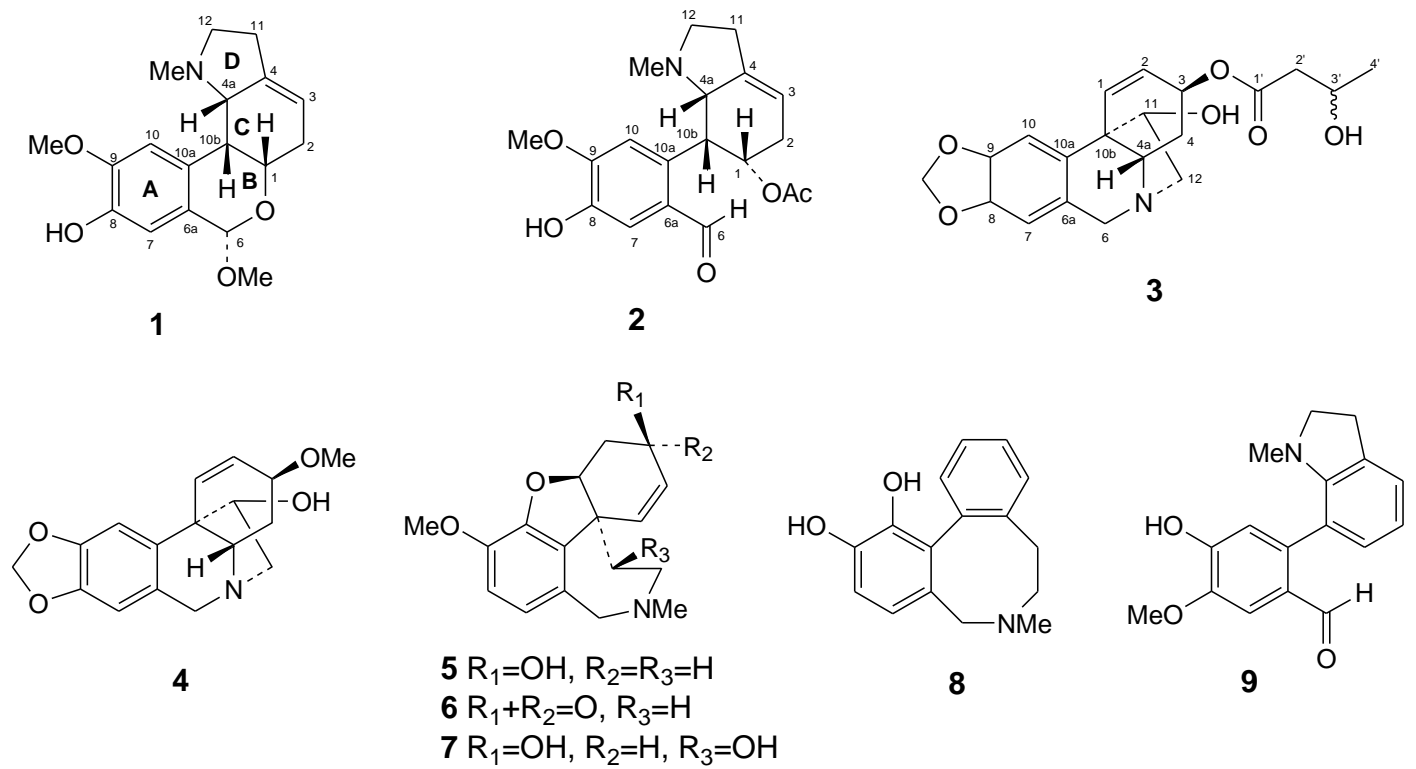
Table **1**: *GC-MS analysis of the alkaloid content of Hippeastrum papilio.*

Alkaloid	RI	%A [a])	%B [b])	M⁺	MS
Apogalanthamine (**8**)	2253	---	3.12	269(88)	268(32), 254(26), 226(61), 211(54), 194(32), 193(50), 166(29), 165(100), 152(30)
Hippapiline (**1**)	2301	---	1.68	317(-)	110(8), 109(100), 108(18), 107(2), 94(24), 81(2), 77(2), 42(2)
Galanthamine (**5**)	2335	86.26	39.01	287(82)	288(14), 286(100), 270(13), 244(26), 230(13), 216(36), 174(30), 115(13)
Narwedine (**6**)	2402	1.31	0.52	285(85)	286(15), 284(100), 242(22), 216(23), 214(10), 199(26), 185(13), 181(12), 178(15), 174(43), 161(11), 153(13), 141(10), 128(22), 115(22), 77(13), 42(22)
9-*O*-Demethyllycosinine B (**9**)	2499	0.24	5.72	283(100)	284(19), 256(11), 255(70), 254(72), 240(30), 239(13), 223(11), 222(33), 210(11), 194(17), 167(10), 44(16)
11*β*-Hydroxygalanthamine (**7**)	2510	traces	7.02	303(21)	302(12), 231(21), 230(100), 213(28), 181(13), 174(13), 115(13), 44(13)
Haemanthamine (**4**)	2556	1.92	26.87	301(12)	273(18), 272(100), 242(15), 240(16), 214(13), 212(14), 211(15), 181(26), 153(10), 128(12), 115(11)
Papiline (**2**)	2565	1.08	10.98	345(-)	286(3), 177(3), 165(1), 122(1), 110(6), 109(100), 108(14), 96(1), 82(3), 81(2), 44(1), 43(3), 42(2)
3-*O*-(3'-Hydroxybutanoyl)haemanthamine (**3**)	3030	---	1.00	373(5)	345(21), 344(95), 270(24), 269(37), 268(25), 240(55), 226(20), 225(30), 224(25), 212(53), 211(27), 210(16), 182(20), 181(100), 153(33), 128(16), 115(17), 45(21)

[a]**) % A:** Alkaloid percentage in the total mixture of alkaloids extracted with *n*-hexane. [b]**) % B:** Alkaloid percentage in the total mixture of alkaloids extracted with EtOAc. All values are expressed as a relative percentage of TIC

Table **2**: *NMR data (500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR, CDCl$_3$) of compound **1**. [δ] in ppm and [J] in Hz.*

Position	H [δ]	COSY	NOESY	C [δ]	HMBC
1	4.29 *ddd* (9.8, 6.8, 4.5)	H-2α, H-2β, H-10b	H-2a, H-2β, H-4a, H-10b	70.5 *d*	
2α	2.41-2.47 *m*	H-1, H-2β, H-3, H-4a, H-11	H-1, H-2β, H-3, 6-OMe	28.9 *t*	
2β	2.25-2.29 *m*	H-1, H-2α, H-3, H-4a, H-11	H-1, H-2α, H-3		
3	5.07 *br s*	H-2α, H-2β, H-4a, H-11	H-2α, H-2β, H-11	115.2 *d*	
4				138.4s	
4a	2.94 *br s*	H-2α, H-2β, H-3, H-10b; H-11	H-1, H-10b, H-12β, NMe	70.6 *d*	
6β	5.44 *s*	H-7	H-7, H-10b, 6-OMe	97.1 *d*	C-1, C-10a, 6-OMe
6a				127.8 *s*	
7	6.88 *s*	H-6β	H-6β	112.4 *d*	C-6, C-8, C-9, C-10a
8				143.7 *s*	
9				145.9 *s*	
10	8.41 *s*	H-10b	9-OMe, NMe	110.6 *d*	C-6a, C-8
10a				126.7 *s*	
10b	3.32 *t* (4.5)	H-1, H-4a, H-10	H-1, H-4a, H-6β, NMe	35.4 *d*	C-1, C-4, C-4a, C-6a
11 (2H)	2.28-2.36 *m*	H-2α, H-2β, H-3, H-4a, H-12α, H-12β	H-3, H-12α, H-12β	28.6 *t*	
12α	3.21 *ddd* (8.7, 6.7, 2.0)	H-11, H-12β	H-11, H-12β, NMe	56.1 *t*	C-4,C-4a
12β	2.18 *dt* (9.8, 8.6)	H-11, H-12α	H-4a, H-11, H-12α, NMe		
6-OMe	3.57 *s*		H-2α, H-6β	55.3 *q*	C-6
9-OMe	3.80 *s*		H-10	55.7 *q*	C-9
NMe	2.44 *s*		H-4a, H-10, H-10b, H-12α, H-12β	40.0 *q*	C-4a, C-12

Table **3**: *NMR data (500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR, CDCl$_3$) of compound **2**. [δ] in ppm and [J] in Hz.*

Position	H [δ]	COSY	NOESY	C [δ]	HMBC
1	5.45 *ddd* (10.0, 5.0, 5.0)	H-2α, H-2β, H-10b	H-2β, H-4a, H-10b	70.9 *d*	C-10a, Me<u>C</u>O
2α	2.00-2.06 *m*	H-1, H-2β, H-3, H-4a, H-11	H-2β, H-3, H-10	27.9 *t*	Me<u>C</u>O
2β	2.44 *dddd* (16.5, 5.5, 4.5, 2.5)	H-1, H-2α, H-3, H-4a, H-11	H-1, H-2α, H-3		
3	5.54 *m*	H-2α, H-2β, H-4a, H-11	H-2α, H-2β, H-11	116.1 *d*	
4				131.1 *s*	
4a	3.10 *s*	H-2α, H-2β, H-3, H-10b, H-11	H-1, H-10b, H-12β, NMe	69.5 *d*	
6	9.91 *s*		H-7, H-10b	191.7.*d*	
6a				127.2 *s*	
7	7.32 *s*		H-6	116.2 *d*	C-8, C-9, C-10a
8				144.6 *s*	
9				150.1 *s*	
10	7.29 *s*	9-OMe	H-2α, H-11, 9-OMe	112.5 *d*	C-6a, C-8, C-9, C-10b
10a				129.0 *s*	
10b	4.74 *t* (4.5)	H-1, H-4a	H-1, H-4a, H-6, NMe	35.3 *d*	C-1, C-4a, C-10
11 (2H)	2.55 *m*	H-2α, H-2β, H-3, H-4a, H-12α, H-12β	H-3, H-10, H-12α, H-12β	28.0 *t*	
12α	3.26 *br s*	H-11, H-12β	H-11, H-12β, NMe	56.3 *t*	
12β	2.33 *q* (9.0)	H-11, H-12α	H-4a, H-11, H-12α, NMe		NMe
9-OMe	3.84 *s*	H-10	H-10	55.8 *q*	C-9
<u>Me</u>CO	1.90 *s*			21.2 *q*	Me<u>C</u>O
Me<u>C</u>O				170.6 *s*	
NMe	2.20 *s*		H-4a, H-10b, H-12α, H-12β	40.9 *q*	C-4a, C-12

Table **4**: *NMR data (500 MHz for ^{1}H- and 125 MHz for ^{13}C-NMR, CDCl$_3$) of compound **3**. [δ] in ppm and [J] in Hz.*

Position	H [δ]	COSY	NOESY	C [δ]	HMBC
1	6.52 *d* (10.1)	H-2	H-2, H-10	129.3 *d*	C-3, C-4a, C-10a, C-10b
2	6.31 *ddd* (10.1, 5.1, 0.7)	H-1, H-3	H-1, H-3	130.2 *d*	C-3, C-4, C-10b
3	5.44 *td* (4.8, 1.7)	H-2, H-4α, H-4β	H-2, H-4α, H-4β	67.2 *d*	C-1, C-2, C-4a, C-1'
4α	2.37 *td* (14.0, 4.6)	H-3, H-4β, H-4a	H-3, H-4β, H-4a H-12*exo*	29.5 *t*	C-4a
4β	1.91 *dd* (14.0, 4.6)	H-3, H-4α, H-4a	H-3, H-4α, H-4a		C-2, C-3, C-4a, C-10b
4a	3.36 *dd* (13.5, 4.5)	H-4α, H-4β	H-4α, H-4β, H-6β	63.0 *d*	C-6, C-12, C-11
6α	3.72 *d* (17.0)	H-6β, H-7	H-6β, H-12*endo*, H-7	61.5 *t*	C-4a, C-6a, C-7, C-10a
6β	4.35 *d* (17.0)	H-6α, H-7	H-4a, H-6α, H-7		C-6a, C-7, C-8, C-10a, C-11, C-12
6a				126.9 *s*	
7	6.51 *s*	H-6α, H-6β	H-6α, H-6β	107.1 *d*	C-6, C-9, C-10a
8				146.6 *s*	
9				146.8 *s*	
10	6.84 *s*		H-1	103.4 *d*	C-6a, C-8, C-10b
10a				134.8 *s*	
10b				50.2 *s*	
11	4.03 *ddd* (6.5, 3.5, 1.5)	H-12*exo*, H-12*endo*	H-12*endo*	80.3 *d*	C-4a
12*exo*	3.27 *dd* (14.0, 3.0)	H-11, H-12*endo*	H-4α, H-12 *endo*	63.6 *t*	C-4a, C-6, C-11
12*endo*	3.41 *dd* (14.0, 6.5)	H-11, H-12*exo*	H-6α, H-11, H-12 *exo*		C-6, C-4a, C-10b, C-11
OCH$_2$O	5.91 *d* (7.5)			101.1 *t*	C-8, C-9
1'				172.4 *s*	C-2'
2'a	2.45 *dd* (16.5, 3.0)	H-2'b, H-3'	H-2'b	43.0 *t*	C-1', C-3', C-4'
2'b	2.36 *dd* (16.5, 9.0)	H-2'a, H-3'	H-2'a		C-1', C-3', C-4'
3'	4.17 *ddq* (9.5, 6.3, 3.3)	H-2'a, H-2'b, H-4'	H-4'	64.4 *d*	
4'	1.19 *d* (6.3)	H-3'	H-3'	22.6 *q*	C-2', C-3'

3.3. Article 3

Crinine-type alkaloids from *Hippeastrum aulicum* and *H. calyptratum*

Jean Paulo de Andrade, **<u>Ying Guo</u>** , Mercè Font-Bardia, Teresa Calvet, Jullie Dutilh, Francesc Viladomat, Carles Codina, Jerald J. Nair, Jose A. Silveira Zuanazzi, Jaume Bastida
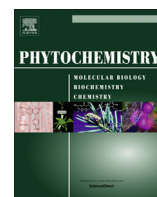
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CrossMark

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,*]

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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*.

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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 11β-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

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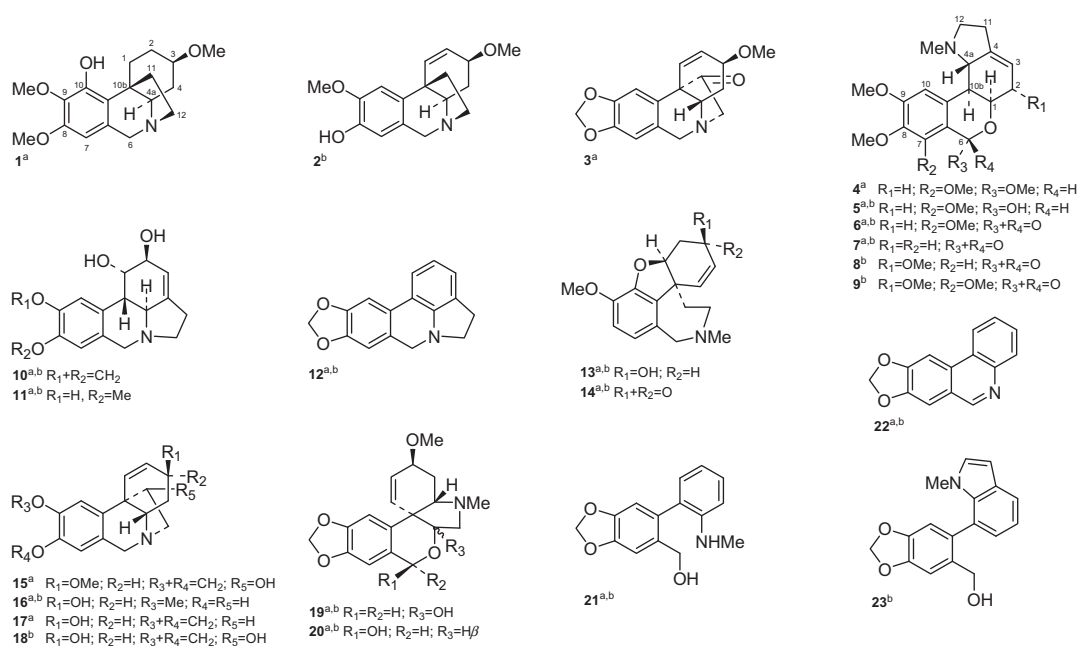*E-mail address:* jaumebastida@ub.edu (J. Bastida).

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

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-dihydroethan-ophenanthridines 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 ^{1}H-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

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(12), 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-11exo		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-11exo		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-11exo, H-12exo		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-12endo		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	
11endo	1.90 ddd (12.0, 8.8, 3.2)	H-11exo, H-12endo, H-12exo	H-11exo, H-12endo	36.5 t	C-4a, C-10b
11exo	2.23 ddd (12.4, 10.4, 6.4)	H-11endo, H-12endo, H-12exo	H-1β, H-2β, H-4β, H-11endo, H-12exo		C-1, C-10a, C-10b, C-12
12endo	2.78 ddd (12.8, 8.8, 6.4)	H-11endo, H-11exo, H-12exo	H-6β, H-11endo, H-12exo	52.2 t	C-4a, C-6, C-11
12exo	3.36 ddd (12.8, 10.0, 3.2)	H-11endo, H-11exo, H-12endo	H-4β, H-11exo, H-12endo		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

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 crinine-type 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 ($J_{3,4β} = 10.5$ 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 ($J_{3,4α} \sim 4.0$ and $J_{3,4β} = 2.0$ 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 (Pabuççuoğlu 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

A

B

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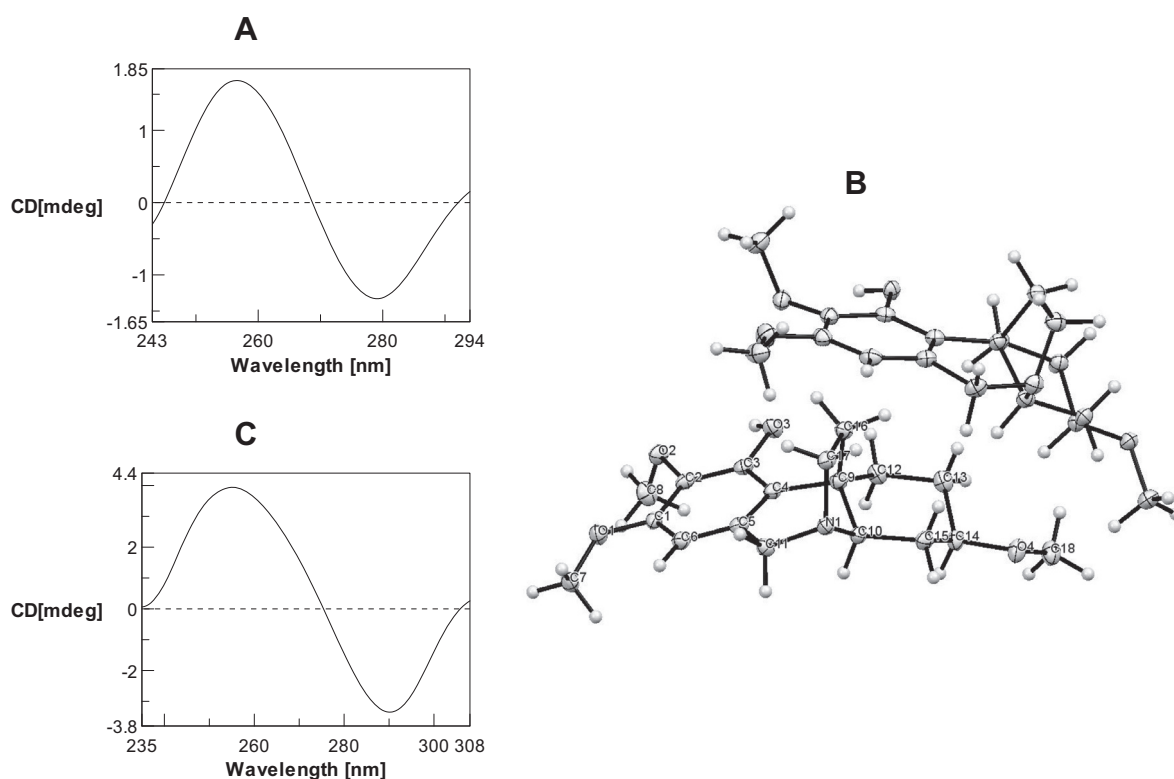
Fig. 2. CD spectrum (A) and ORTEP projection (B) of alkaloid **1**. CD spectrum (C) of alkaloid **2**.

Table 3
^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of 3-*O*-methyl-epimacowine (**2**) (500 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-4β	H-2, H-4α, H-4a, 3-OMe	76.3 *d*	C-1, 3-OMe
4α	2.29 *m*	H-3, H-4β, H-4a	H-3, H-4a, H-4β	30.8 *t*	C-2, C-3, C-4a, C-10b
4β	1.58 *ddd* (13.5, 12.0, 10.5)	H-3, H-4α, H-4a	H-4α, H-11exo, H-12exo		C-3, C-4a, C-10b
4a	3.28 *dd* (13.5, 4.0)	H-4α, H-4β	H-3, H-4α, H-6α	66.8 *d*	C-12
6α	4.45 *d* (16.5)	H-6β	H-4a, H-6β, H-7	61.5 *t*	C-6a, C-7, C-10a, C-12
6β	3.82 *d* (17.0)	H-6α	H-6α, H-7, H-12endo		C-4a, C-6a, C-7, C-10a, C-12
6a				125.0 *s*	
7	6.59 *s*		H-6α, H-6β	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*	
11endo	2.20 *ddd* (12.0, 9.0, 4.5)	H-11exo, H-12endo, H-12exo	H-11exo, H-12endo	44.8 *t*	C-4a, C-10a, C-10b, C-12
11exo	2.12 *ddd* (12.0, 10.5, 6.0)	H-11endo, H-12endo, H-12exo	H-4β, H-11endo, H-12exo		C-1, C-10a, C-10b, C-12
12endo	2.95 *ddd* (13.0, 9.0, 6.0)	H-11endo, H-11exo, H-12exo	H-6β, H-11endo, H-12exo	53.2 *t*	C-4a, C-6, C-10b
12exo	3.50 *ddd* (13.0, 10.5, 4.5)	H-11endo, H-11exo, H-12endo	H-4β, H-12endo, H-11exo		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

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

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
12endo	3.27 dd (18.5, 1.5)	H-12exo	59.3 t
12exo	3.56 d (18.5)	H-12endo	
3-OMe	3.37 s (3H)		56.8 q
OCH$_2$O	5.92 2d (1.5)		101.3 t

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, CHCl$_3$); CD $[\Theta]_\lambda^{20}$: $[\Theta]_{255+}$ 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}; ^{1}H-NMR (CDCl$_3$, 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 $[\Theta]_\lambda^{20}$: $[\Theta]_{254}$ +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 $[\Theta]_\lambda^{20}$: $[\Theta]_{255}$ −3429, $[\Theta]_{320}$ +3298; UV (MeOH) λ_{max}(log ε) 250 (2.94), 295 (2.92), 313 (2.82) nm; IR (CHCl$_3$) ν_{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}(log ε) 222 (4.26), 266 (3.86), 298 (3.34) nm; IR (CHCl$_3$) ν_{max} 2929, 2849, 2783, 1725, 1592, 1334, 1254, 1111, 1022 cm^{-1}; ^{1}H-NMR (CDCl$_3$, 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

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[1] CCDC 963600 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk)

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 β-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 α-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 CDCl$_3$ (CD$_3$OD for **4** and **10**) as the solvent and TMS as the internal standard. Chemical shifts are reported in δ 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 × 0.25 mm × 0.25 μm, 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 H$_2$O-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 F$_{254}$ was used as the stationary phase with a plate dimension of 20 cm × 20 cm × 0.20 mm for analytical TLC and 20 cm × 20 cm × 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 Et$_2$O (4 × 250 ml) and EtOAc

Table 5
^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of 7-methoxy-*O*-methyllycorenine (**4**) (500 MHz, CD$_3$OD).

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

Table 6
^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of nerinine (**5**) (400 MHz, CDCl$_3$).

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

(4 × 250 ml) to remove neutral material. The aqueous solution was basified with ammonia (25%) up to pH 10 and extracted with *n*-hexane (8 × 250 ml) to give extract IA (0.86 g). Another extraction using EtOAc (8 × 250 ml) produced extract IIA (2.0 g), wherein lycorine (**10**) precipitated spontaneously. A final extraction using EtOAc–MeOH (3:1, 3 × 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 × 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 *λ* 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–Me$_2$CO–*n*-hexane–MeOH – 6:2:1:1, in NH$_3$ 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–Me$_2$CO–MeOH–*n*-

BuOH – 4:3:3:2:1, in NH$_3$ atmosphere). The supernatant was loaded onto a VLC column (1.5 × 4 cm) of silica gel (3 g), using *n*-hexane (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–Me$_2$CO–EtOAc–MeOH – 15:10:5:2, in NH$_3$ atmosphere). Fractions 222–250 were combined and subjected to SPTLC (*n*-hexane–EtOAc–Me$_2$CO–MeOH–*n*-BuOH – 4:3:3:2:1, in NH$_3$ 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 × 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–Me$_2$CO–MeOH–*n*-BuOH – 4:3:3:2:1, in NH$_3$ atmosphere) of fractions 134–190 gave **1** (152 mg), **15**

Table 7
^{1}H NMR, COSY, NOESY, HSQC, and HMBC data of albomaculine (**6**) (400 MHz, CDCl$_3$).

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	

(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 Et$_2$O (4 × 250 ml) and EtOAc (4 × 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 × 250 ml) to give extract IC (100 mg). Extraction with EtOAc (8 × 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 × 4 cm column, 10 g of silica gel) using the same solvent system as that for *H. aulicum*. Alkaloid **10** (115 mg) precipitated directly from fractions 93–140. Fractions 71–170 were combined (250 mg) and subjected to VLC (1.5 × 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 (10 ml 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 cm column, starting with *n*-hexane (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 (10 ml each). Albomaculine (**6**, 19.3 mg) was isolated from fractions 69–88 by SPTLC (Me$_2$CO-CH$_2$Cl$_2$ – 3:10, in NH$_3$ atmosphere) together with 2α,7-dimethoxyhomolycorine (**9**, 3.2 mg). Likewise, 3-*O*-methyl-epimacowine (**2**, 18.3 mg) and alkaloid **13** (7.7 mg) were isolated from fractions 89–148 using SPTLC (EtOAc-Me$_2$CO-CH$_2$Cl$_2$-MeOH – 3:1:1:0.5, in NH$_3$ atmosphere).

4.4. Identification of alkaloids by GC–MS

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

4.5. Crystals of aulicine (**1**)

Compound **1** was dissolved in a MeOH-CHCl$_3$ (1:1) mixture under a pentane atmosphere. After 14 days standing at ~5 °C, small crystals of **1** formed and were selected for X-ray crystallography.

4.6. X-ray analysis

A prismatic crystal (0.1 × 0.1 × 0.2 mm) was selected and mounted on a Bruker D8 Venture four-circle diffractometer (Karlsruhe, Germany). Intensities were collected with a multilayer monochromator and a Cu high brilliance microfocus sealed tube using the φ and ω scan-technique. A total of 24158 reflections were measured in the range of 2.93 ⩽ θ ⩽ 74.32, with 6377 of the reflections non-equivalent by symmetry (Rint(on I) = 0.031). Overall, 6028 reflections were assumed to be as observed by applying the condition $I > 2\sigma(I)$. Lorentz-polarisation and absorption corrections were performed.

The structure was solved by direct methods, using the SHELXS computer program (and refined by a full-matrix least-squares method with the SHELX97 computer program (Sheldrick, 2008)) and 24158 reflections, (very negative intensities were not assumed). The function minimised was Σ w ||Fo|^2 − |Fc|^2|^2, where $w = [\sigma^2(I) + (0.0343P)^2 + 0.8335P]^{-1}$, and $P = (|Fo|^2 + 2 |Fc|^2)/3$, *f*, *f′* and *f′* were taken from the International Tables of X-ray Crystallography (1974). All H atoms were computed and refined using a riding model, with an isotropic temperature factor equal to 1.2 times the equivalent temperature factor of the atoms that

are linked. The final R(on F) factor was 0.0298, wR(on $|F|^2$) = 0.074 and goodness of fit = 1.042 for all observed reflections. The number of refined parameters was 423. Max. shift/esd = 0.00, Mean shift/esd = 0.00. Max. and min. peaks in the final difference synthesis were 0.215 and $-0.164\ e\text{Å}^{-3}$, respectively.

Acknowledgements

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4. DISCUSSION

4. Discussion

The thesis is mainly focused on the study of alkaloids from the genera *Lycoris* and *Hippeastrum*. I have divided this chapter into three separate sections: first, analysis of bioactive Amaryllidaceae alkaloid profiles of *Lycoris* species by GC-MS; second, isolation and characterization of new alkaloids of the species *Hippeastrum papilio*; and finally, research on new alkaloids from *Hippeastrum calyptratum*.

4.1. Alkaloid profiles of the genus *Lycoris*

As previously described, the genus *Lycoris* from the Amaryllidaceae family includes 22 species and one hybrid, which are principally found in temperate woodlands of Eastern Asia. Notably, fifteen of these species grow in China, of which 10 are endemic. GC-MS is a rapid and sensitive tool for investigation and identification of complex alkaloid mixtures of different groups from numerous plants, requiring a low quantity of plant samples and no derivatization step. It therefore constitutes an appropriate technical support for analyzing the numerous alkaloids of different *Lycoris* species.

In the present work, nine species, *Lycoris albiflora*, *L. aurea*, *L. chinensis*, *L. haywardii, L. incarnata, L. longituba, L. radiata, L. sprengeri*, and *L. squamigera*, and one variety (*L. radiata* var. *pumila*) were evaluated by GC-MS.

GC-MS analysis of the bulbs of different species of *Lycoris* resulted in the identification of 31 alkaloids belonging to the lycorine, homolycorine, haemanthamine, narciclasine, tazettine, montanine and galanthamine types, together with one unusual alkaloid known as cherylline. In general, the results coincided with previously reported alkaloids found in the genus. The alkaloid profile of the extracts of all the studied species was dominated by alkaloids arising from *ortho-para'* (lycorine-type) and *para-ortho'* (galanthamine-type) oxidative coupling of *O*-methylnorbelladine. The number of alkaloids detected varied among extracts, from 10 in *L. incarnata* and *L. radiata* var. *pumila* to 20 in *L. longituba*.

In all the species, lycorine- and galanthamine-type alkaloids were predominant. *L. longituba* and *L. sprengeri* showed the highest content of lycorine-type compounds, with values above 2.5 mg GAL/g DW. The maximum level of galanthamine-type alkaloids was detected in *L. longituba* bulbs (4.75 mg GAL/g DW), while the lowest level was found in the bulbs of *L. radiata* var. *pumila* (0.4 mg GAL/g DW). Tazettine-type alkaloids were present in all the species, with the exception of *L. incarnata*, while the montanine type was not detected in five of the ten extracts analyzed. Homolycorine-type alkaloids were only found in *L. radiata*, *L. radiata* var. *pumila* and *L. haywardii*. Narciclasine-type alkaloids were present as traces in *L. longituba* and *L. sprengeri*. Although in low quantities (values less than 0.29 mg GAL/g DW), the haemanthamine type alkaloids were detected in five species, *L. aurea*, *L. chinensis*, *L. radiata*, *L. sprengeri* and *L. squamigera* (Table 4.1; Figure 4.1).

Figure 4.1 shows the alkaloid profile of each extract as a bar graph. The results are grouped into 3 main bars: two of them comprise lycorine- and galanthamine-type alkaloids, colored blue and red, respectively, to highlight their predominance, whereas
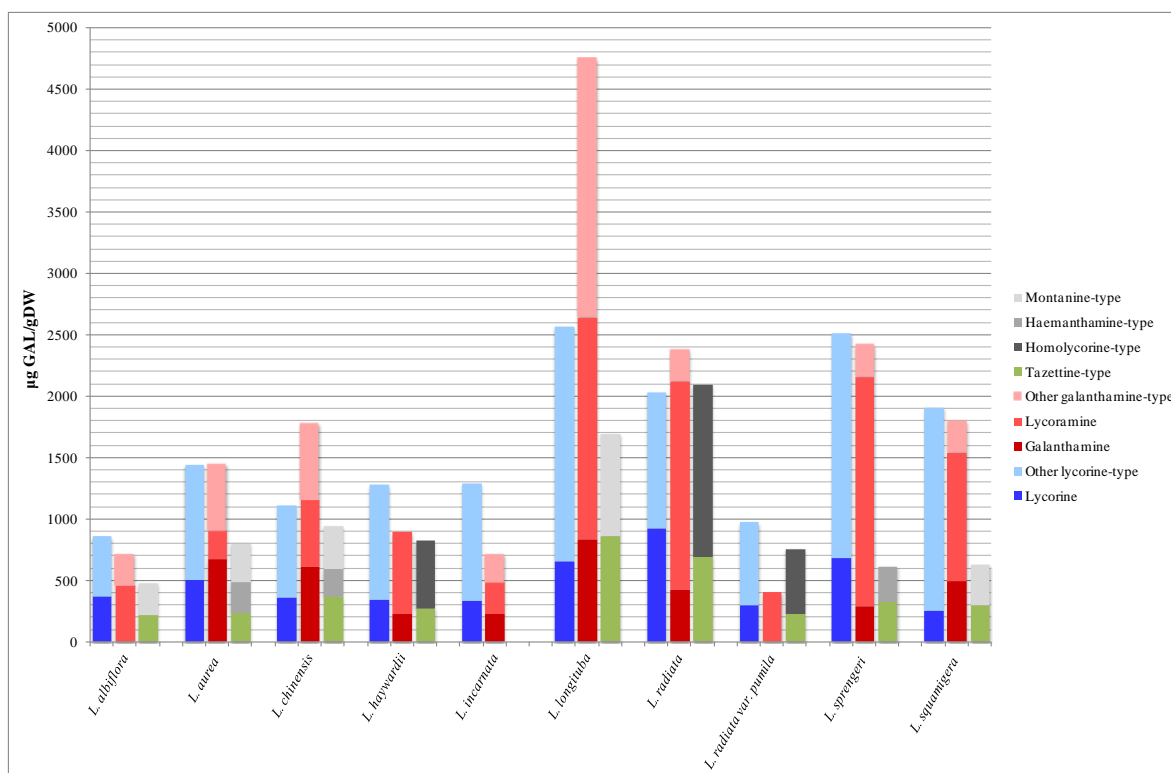
Figure 4.1: Alkaloid and alkaloid-type predominance in extracts of the genus *Lycoris*.

Table 4.1: Alkaloid profiles of studied species. Values are expressed as μg GAL/g DW.

Alkaloids	M$^+$	Base Peak	RI	L. albiflora	L. aurea	L. chinensis	L. haywardii	L. incarnata	L. longituba	L. radiata	L. radiata var. pumila	L. sprengeri	L. squamigera
Lycorine-type				**863.2**	**1445.9**	**1113.1**	**1280.1**	**1291.7**	**2572.4**	**2037.9**	**976.4**	**2514.3**	**1912.2**
lycorine	287	226	2766.1	370.7	502.8	360.8	349.7	337.6	656.9	923.8	298.7	687.4	257.1
pseudolycorine	289	228	2837.2	-	-	-	-	-	224.8	-	-	-	-
galanthine	317	242	2703.4	trace	222.0	trace	228.0	239.8	-	-	-	337.4	467.5
pluviine	287	242	2571.7	-	-	-	trace	-	237.3	-	223.1	-	trace
norpluviine	273	228	2602.2	trace	-	-	-	-	-	-	-	trace	-
caranine	271	226	2537.4	245.4	248.5	264.3	236.3	237.7	260.1	299.3	trace	267.1	trace
methylpseudolycorine	303	242	2792.3	-	-	-	-	-	490.8	-	-	-	233.1
incartine	333	332	2457.8	trace	trace	trace	trace	224.4	-	-	-	446.9	265.1
2-dehydroxylycorine	271	250	2551.3	-	trace	-	-	-	222.2	226.2	-	224.3	-
galanthine derivative*	315	240	2782.1	-	trace	trace	trace	-	-	-	-	trace	240.5
anhydrolycorine	251	250	2516.2	trace	222.0	228.0	221.3	trace	226.7	283.8	222.7	227.2	216.1
11,12-dehydroanhydrolycorine	249	248	2622.1	247.1	250.7	259.9	244.7	252.2	253.7	304.7	231.9	324.0	232.9
assoanine	267	266	2581.4	-	-	-	-	-	trace	-	-	-	-
Homolycorine-type				-	-	-	**550.5**	-	-	**1408.8**	**534.2**	-	-
homolycorine	315	109	2765.0	-	-	-	238.0	-	-	256.8	245.7	-	-
8-*O*-demethylhomolycorine	301	109	2822.0	-	-	-	312.5	-	-	659.3	288.4	-	-
hippeastrine	315	125	2903.4	-	-	-	-	-	-	266	-	-	-
O-methyllycorenine	331	109	2487.9	-	-	-	-	-	-	226.7	-	-	-

Alkaloids	M⁺	Base Peak	RI	*L. albiflora*	*L. aurea*	*L. chinensis*	*L. haywardii*	*L. incarnata*	*L. longituba*	*L. radiata*	*L. radiata var. pumila*	*L. sprengeri*	*L. squamigera*
Haemanthamine-type				-	**251.5**	**224.3**	**-**	**trace**	**-**	**trace**	**-**	**286.2**	**trace**
haemanthamine	301	272	2644.3	-	251.5	224.3	-	trace	-	trace	-	286.2	-
haemanthidine	317	317	2731.0	-	-	-	-	-	-	-	-	-	trace
Narciclasine-type				**-**	**-**	**-**	**-**	**-**	**trace**	**-**	**-**	**trace**	**-**
trisphaeridine	233	233	1866.0	-	-	-	-	-	trace	-	-	trace	-
Tazettine-type				**221.1**	**238.3**	**368.5**	**275.7**	**-**	**863.3**	**692.1**	**226.4**	**331.9**	**300.8**
tazettine	331	247	2655.0	221.1	238.3	368.5	275.7	-	863.3	692.1	226.4	331.9	300.8
deoxytazettine	315	231	2546.6	-	-	-	-	-	trace	trace	-	-	-
Montanine-type				**260.3**	**314.9**	**353.8**	**-**	**-**	**831.3**	**-**	**-**	**-**	**327.0**
montanine	301	301	2637.3	260.3	314.9	353.8	-	-	607.2	-	-	-	327.0
pancratinine C	287	176	2600.2	-	-	-	-	-	224.1	-	-	-	-
Galanthamine-type				**721.6**	**1455.6**	**1786.1**	**895.5**	**722.4**	**4754.3**	**2386.5**	**411.7**	**2425.2**	**1804**
galanthamine	287	286	2403.7	trace	674.1	618.0	227.1	227.4	836.0	425.0	trace	289.9	501.1
sanguinine	273	273	2427.6	-	550.9	380.0	-	-	241.5	-	-	-	-
lycoramine	289	288	2430.3	458.5	230.6	538.0	668.4	264.3	1808.6	1694.7	411.7	1870	1046.4
O-demethyllycoramine	274	275	2459.7	-	-	-	-	-	344.9	-	-	-	-
norlycoramine	274	275	2471.7	263.1	-	249.4	-	230.7	1523.3	266.8	-	265.4	256.5
narwedine	285	284	2485.3	trace	-	-	-	-	trace	-	-	-	trace
Other-type				**-**	**-**	**trace**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
cherylline	285	242	2574.0	-	-	trace	-	-	-	-	-	-	-
Total				2066.2	3706.2	3845.8	3001.8	2014.1	9021.3	6525.3	2148.6	5557.6	4343.9

*Although not reported in the literature, the MS of this compound indicates a structure derived from galanthine

the third one represents all the remaining skeleton types (including tazettine-, homolycorine-, haemanthamine- and montanine-). The lycorine, galanthamine and lycoramine alkaloids are represented by different shades of red or blue, and tazettine is in green, given that these are the most frequently found structures.

4.2. Alkaloids from *Hippeastrum papilio*

Hippeastrum papilio was collected during the flowering period (November, 2012) in the South of Brazil (Caxias do Sul - RS). The dried bulbs (220 g) were crushed and extracted with methanol. After purification, three novel Amaryllidaceae alkaloids from *Hippeastrum papilio* were isolated: hippapiline, papiline and 3-*O*-(3'-hydroxybutanoyl)haemanthamine. Additionally, the known alkaloids haemanthamine, galanthamine, narwedine, 11β-hydroxygalanthamine, apogalanthamine and 9-*O*-demethyllycosinine B were identified (Figure 4.2).

hippapiline

papiline

3-O-(3'-hydroxybutanoyl)haemanthamine

haemanthamine

R₁=OH, R₂=R₃=H   galanthamine

R₁+R₂=O, R₃=H   narwedine

R₁=OH, R₂=H, R₃=OH   11β-Hydroxygalanthamine

apogalanthamine

9-*O*-demethyllycosinine B
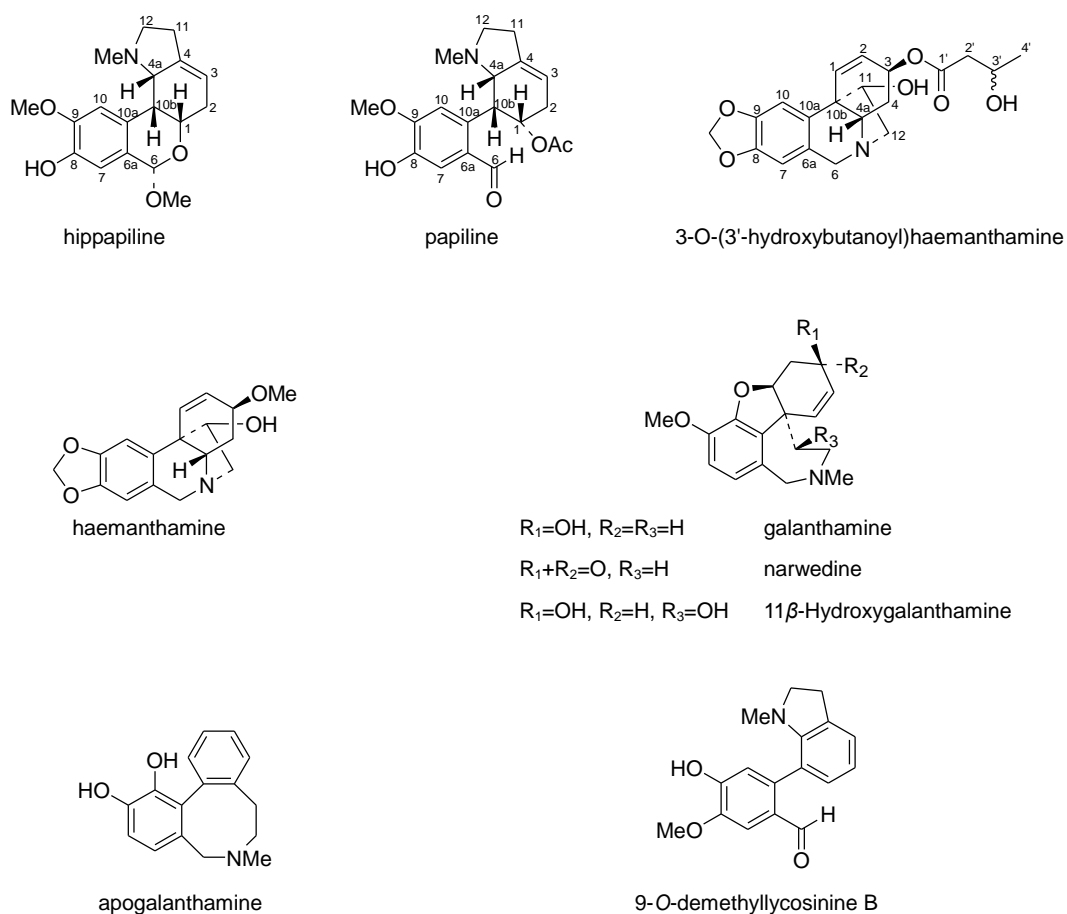
Figure 4.2: Alkaloids from *Hippeastrum papilio*.

4.2.1. GC-MS analysis of *Hippeastrum papilio*

The results of GC-MS analysis revealed that galanthamine was the main constituent in the *n*-hexane extract (86.3 %), also appearing as one of the major components in the EtOAc extract (39.0 %), together with haemanthamine (26.9 %). Apogalanthamine and 9-*O*-demethyllycosinine B are reported in this species for the first time, while narwedine was detected only in small quantities (Table 4.2).

Table 4.2: GC-MS analysis of the alkaloid content of *Hippeastrum papilio.*

Alkaloid	RI	%A*	%B*	M$^+$	MS
apogalanthamine	2253	-	3.12	269(88)	268(32), 254(26), 226(61), 211(54), 194(32), 193(50), 166(29), 165(100), 152(30)
hippapiline	2301	-	1.68	317(-)	110(8), 109(100), 108(18), 107(2), 94(24), 81(2), 77(2), 42(2)
galanthamine	2335	86.26	39.01	287(82)	288(14), 286(100), 270(13), 244(26), 230(13), 216(36), 174(30), 115(13)
narwedine	2402	1.31	0.52	285(85)	286(15), 284(100), 242(22), 216(23), 214(10), 199(26), 185(13), 181(12), 178(15), 174(43), 161(11), 153(13), 141(10), 128(22), 115(22), 77(13), 42(22)
9-*O*-demethyllycosinine B	2499	0.24	5.72	283(100)	284(19), 256(11), 255(70), 254(72), 240(30), 239(13), 223(11), 222(33), 210(11), 194(17), 167(10), 44(16)
11*β*-hydroxygalanthamine	2510	traces	7.02	303(21)	302(12), 231(21), 230(100), 213(28), 181(13), 174(13), 115(13), 44(13)
haemanthamine	2556	1.92	26.87	301(12)	273(18), 272(100), 242(15), 240(16), 214(13), 212(14), 211(15), 181(26), 153(10), 128(12), 115(11)
papiline	2565	1.08	10.98	345(-)	286(3), 177(3), 165(1), 122(1), 110(6), 109(100), 108(14), 96(1), 82(3), 81(2), 44(1), 43(3), 42(2)
3-*O*-(3'-hydroxybutanoyl)haemanthamine	3030	-	1	373(5)	345(21), 344(95), 270(24), 269(37), 268(25), 240(55), 226(20), 225(30), 224(25), 212(53), 211(27), 210(16), 182(20), 181(100), 153(33), 128(16), 115(17), 45(21)

% A: Alkaloid percentage in the total mixture of alkaloids extracted with *n*-hexane. **% B:** Alkaloid percentage in the total mixture of alkaloids extracted with EtOAc. All values are expressed as a relative percentage of TIC.

As metioned before, Galanthamine, an AChE inhibitor marketed as a hydrobromide salt (Razadyne®, Reminyl®) for the treatment of Alzheimer's disease

(AD), is obtained from Amaryllidaceae plants, especially those belonging to the genera *Leucojum*, *Narcissus*, *Lycoris* and *Ungernia*. The growing demand for galanthamine has prompted searches for new sources of this compound, as well as other bioactive alkaloids for the treatment of AD (de Andrade et al., 2011). *H. papilio* could be considered as a potential commercial source of bioactive alkaloids due to its high content of galanthamine (86.26% of *n*-hexane alkaloids extraction and 39.0% in the total mixture of alkaloids extracted with EtOAc).

4.2.2. Hippapiline

The alkaloid hippapiline showed HRESIMS $[M+H]^+$ at *m/z* 318.1706 (calc. for $C_{18}H_{24}NO_4$ − 318.1700) and a base peak at *m/z* 109 by GC-MS analysis, which is characteristic of the hexahydroindol ring in the homolycorine series, without a substitution at C-2 (Berkov et al., 2008). Although the basic structure of a homolycorine-type compound was established by NMR data, an unusual shifting and splitting pattern in comparison with the compound 8-*O*-demethyl-6-*O*-methyllycorenine (Wang et al., 2007b) was noticed, which proved to be crucial for the correct characterization of hippapiline.

It was also noteworthy that the ^{1}H NMR data of hippapiline showed some atypical characteristics: two *para*-oriented aromatic protons attributed to H-7 and H-10, the latter assigned to the highly deshielded singlet at *δ* 8.41 (confirmed by NOESY correlation with the *N*-methyl group); an uncommon coupling constant (*J* = 4.5 Hz) observed between H-1 and H-10b and the absence of the distinctive *trans*-diaxial coupling constant between H-10b and H-4a; a NOESY correlation between H-4a and H-1. All these data were essential for the correct assignment of hippapiline, consistent with a *β*-orientation of H-1 and H-10b.

Furthermore, CD analysis was carried out to confirm the consequent *cis*-4 B/C ring fusion in hippapiline, wherein the positive, negative, and positive Cotton effects, observed at ca. 225, 250, and 290 nm, respectively, were completely opposite to those observed for the representative *cis*-3 B/C fusion of other homolycorine-type compounds (Wagner et al., 1996). Consequently, the CD analysis and NMR data were in agreement

with a *cis*-4 B/C ring fusion.

4.2.3. Papiline

The HRESIMS of papiline suggested a molecular formula $C_{19}H_{24}NO_5$ for $[M+H]^+$ with a parent ion at *m/z* 346.1643 (calcd. 346.1649). The EIMS showed a molecular ion $[M]^+$ at *m/z* 345 and a loss typical of an acetoxy group (*m/z* 286, $[M-59]^+$).

Characteristic NMR signals included: (i) a singlet at δ 9.91, indicative of an aldehyde function, as occurs in nonfused dihydroindol lycosinine derivatives (Sebben, 2005; Yang et al., 2005), and a signal at δ 191.7 in ^{13}C NMR confirmed the aldehyde carbonyl group; (ii) two *para*-oriented aromatic protons at δ 7.32 and 7.29, the more deshielded of which was assigned to H-7, due to its NOESY correlation with H-6; (iii) the acetoxy substituent was assigned at C-1, due to the strong deshielding effect observed for H-1 (δ 5.45), confirmed by HMBC; (iv) the magnitude of the coupling constant ($J_{4a,10b} = J_{1,10b} = 4.5$ Hz), together with the observed NOESY correlations confirmed the same orientation for H-1, H-10b and H-4a. Moreover, its IR spectrum displayed strong absorbance at 1738 and 1674 cm^{-1}, indicating two C=O groups ascribed as acetoxy and aldehyde groups, respectively. All data were in agreement with a new compound bearing a nonfused hexahydroindol nucleus.

4.2.4. 3-*O*-(3'-hydroxybutanoyl)haemanthamine

The new alkaloid 3-*O*-(3'-hydroxybutanoyl)haemanthamine exhibited a parent $[M+H]^+$ ion at *m/z* 374.1603 in its HRESIMS spectrum, suggesting the molecular formula $C_{20}H_{24}NO_6$ (calcd. 374.1598). The ^1H NMR data were very similar to those of haemanthamine, even though the presence of a 3'-hydroxybutanoyl substituent was also observed (carbonyl group at δ 172.4). Furthermore, a loss of 29 units from the molecular ion observed by GC-MS (*m/z* 344, $[M-29]^+$) is typical of haemanthamine derivatives bearing a hydroxyl group at C-11 (Kreh et al., 1995). The assignment of H-3 at lower fields (δ 5.44), 1.62 ppm more deshielded than its homologue in haemanthamine, supported another 3-*O*-(3'-hydroxybutanoyl) alkaloid. The magnitude of CD spectra established the α-orientation of the 5,10b-ethano bridge.

4.3. Alkaloids from *Hippeastrum calyptratum*

A crinine-type alkaloid, 3-*O*-methyl-epimacowine was identified in the indigenous Brazilian species *Hippeastrum calyptratum*. Furthermore, the ongoing search for alkaloids in the Amaryllidaceae species using GC-MS resulted in the identification of 18 alkaloids (Table 4.3, Figure 4.3).

Table 4.3: GC-MS analysis of the alkaloid content of *Hippeastrum calyptratum*.

Alkaloid	RI	%A*	%B*	M⁺	MS
ismine	2280	-	0.45	257(35)	238(100), 211(6), 196(8), 168(6), 154(3), 106(4), 77(3)
trisphaeridine	2282	-	tr	223(100)	222(38), 167(8), 165(9), 164(14), 138(20), 137(9), 111(13)
galanthamine	2395	12.93	6.6	287(83)	286(100), 270(13), 244(24), 230(12), 216(33), 174(27), 115(12)
3-*O*-methyl-epimacowine	2477	14.68	13.45	287 (100)	272(39), 256(34), 217(71), 203(21), 174(18), 157(18), 128(14)
narwedine	2483	0.72	tr	285(84)	284(100), 242(18), 216(20), 199(18), 174(31), 128(16), 115(16)
galanthindol	2487	-	1.11	281(100)	280(7), 264(13), 263(17), 262(20), 252(15), 204(7), 191(14),
anhydrolycorine	2501	-	5.31	251(43)	250(100), 192(13), 191(11), 165(4), 164(3), 139(2), 124(7)
nerinine	2509	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	2510	-	tr	273(100)	256(22), 230(20), 201(83), 189(42), 174(22), 128(23), 115(24)
tazettine /pretazettine	2653	-	0.62	331(31)	316(15), 298(23), 247(100), 230(12), 201(15), 181(11), 152(7)
11-hydroxyvittatine	2728	-	9.5	287(5)	258(100), 211(15), 186(20), 181(23), 153(13), 128(24), 115(23)
lycorine	2746	0.89	41.89	287(31)	286(19), 268(24), 250(15), 227(79), 226(100), 211(7), 147(15)
homolycorine	2767	3.21	-	315(<1)	206(<1), 178(2), 109(100), 150(1), 108(22), 94(3), 82(3)
albomaculine	2815	66.41	13.39	345(<1)	221(1), 193(1), 165(1), 110(10), 109(100), 108(25), 94(2), 82(3)
pseudolycorine	2823	-	4.02	289(23)	270(21), 252(12), 228(100), 214(10), 147(17), 111(18), 82(10)
2α-methoxyhomolycorine	2870	-	0.64	345(<1)	206(<1), 178(2), 150(1), 139(100), 124(64), 96(5), 94(5), 81(3)
2α,7-dimethoxyhomolycorine	2962	0.8	1.88	375(<1)	236(<1), 139(100), 124(54), 221(2), 193(2), 96(3), 94(3), 81(2)

% A: Alkaloid percentage in the total mixture of alkaloids extracted with *n*-hexane. **% B:** Alkaloid percentage in the total mixture of alkaloids extracted with EtOAc. All values are expressed as a relative percentage of TIC.

4.3.1. 3-*O*-methyl-epimacowine

The new crinine alkaloid 3-*O*-methyl-epimacowine from *H. calyptratum* exhibited a parent $[M+H]^+$ ion at *m/z* 288.1595 in its HRESIMS spectrum, suggesting the molecular formula $C_{17}H_{22}NO_3$ (calcd. 288.1594). The NMR data were similar to those of macowine (Nair et al., 2000), 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 3-*O*-methylepi-

macowine the large coupling constant ($J_{3,4\beta}$ = 10.5 Hz) suggested a pseudoequatorial disposition for the 3-methoxyl substituent and therefore a *cis* relationship with 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.

3-*O*-methyl-epimacowine

R₁=H; R₂=OMe; R₃=OH; R₄=H   nerinine

R₁=H; R₂=OMe; R₃+R₄=O   albomaculine

R₁=R₂=H; R₃+R₄=O   homolycorine

R₁=OMe; R₂=H; R₃+R₄=O   2α-methoxyhomolycorine

R₁=OMe; R₂=OMe; R₃+R₄=O   2α,7-dimethoxyhomolycorine

R₁=OH; R₂=H   galanthamine

R₁+R₂=O   narwedine

R₁=OH; R₂=H; R₃=Me;R₄=R₅=H   8-*O*-demethylmaritidine

R₁=OH; R₂=H; R₃+R₄=CH₂; R₅=OH   11-hydroxyvittatine

R₁+R₂=CH₂   lycorine

R₁=H, R₂=Me   pseudolycorine

R₁=R₂=H; R₃=OH   tazettine

R₁=OH; R₂=H; R₃=Hβ   pretazettine

ismine

galanthindol

anhydrolycorine

trisphaeridine
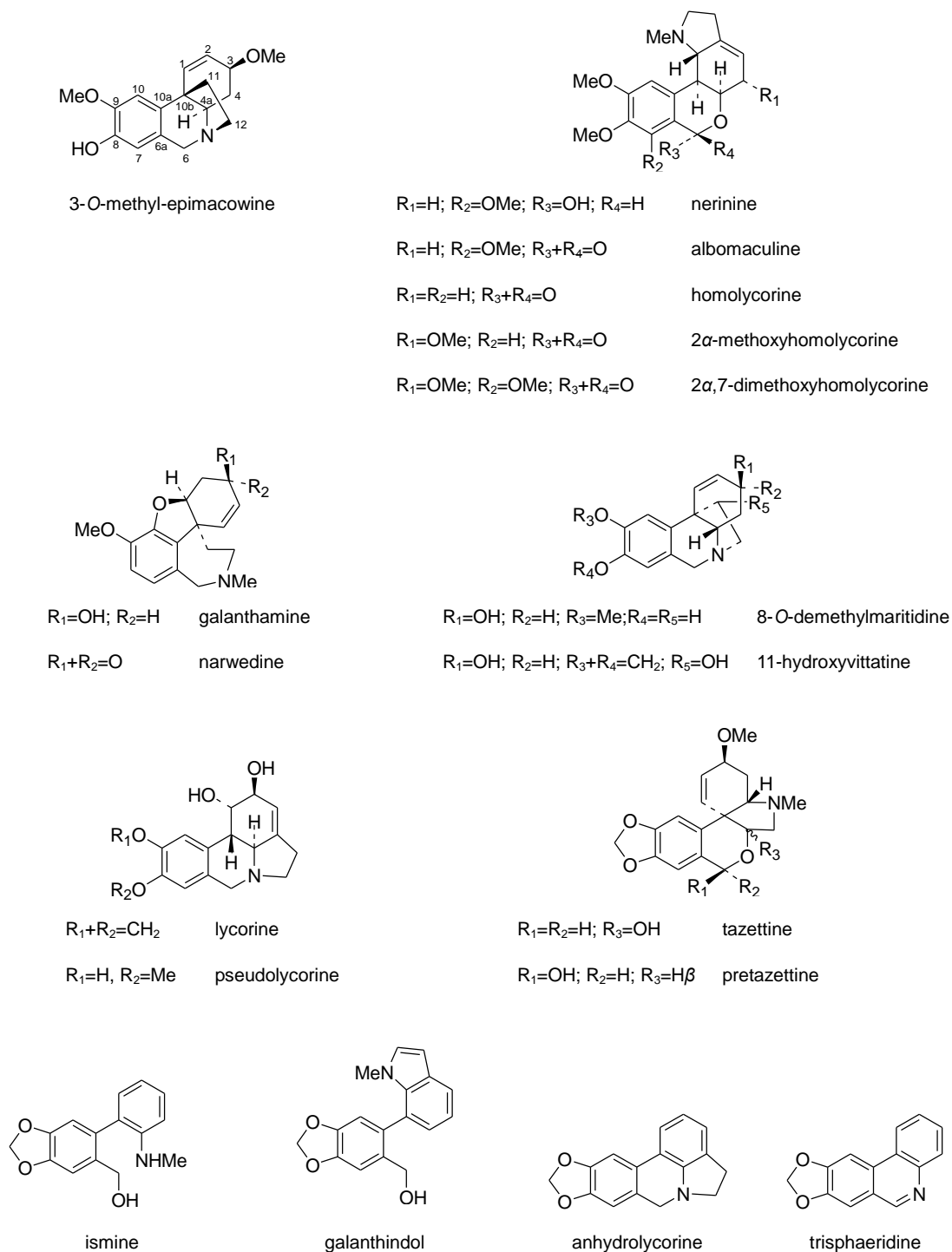
Figure 4.3: Alkaloids from *Hippeastrum calyptratum*.

4.4. Parasitic protozoa *in vitro* assays

Parasites are organisms that live in or on other organisms (their hosts) and benefit by obtaining nutrients at their hosts' expense. They continue to present a threat for the wellbeing of man and his domesticated animals in all parts of the world. There are over 45000 named species of protozoa and about 10000 are parasitic in invertebrates and in almost every species of vertebrate (Machocho, 2000).

The assays on parasitic protozoa were carried out at the Swiss Tropical Insitute (STI), Basel. Table 4.4 lists the parasites, the parasite strain and stage, and the standard used for each parasite. The values are expressed in inhibitory doses (IC_{50}) in μg/ml. Cytotoxicity was evaluated by use of L-6 myoblast cells, and expressed in inhibitory doses (IC_{50}) in μg/ml as well (Table 4.4).

Table 4.4: Parasitic protozoa *in vitro* assays.

Parasite	Strain(s)	Stage	Reference drug
Trypanosoma brucei rhodesiense	STIB 900	trypomastigotes	melarsoprol
Trypanosoma cruzi	Tulahuen C4	amastigotes	benznidazole
Leishmania donovani	MHOM-ET-67/L82	amastigotes	miltefosine
Plasmodium falciparum	NF54	IEF	chloroquine
Cytotoxicity	L-6		podophyllotoxin

Values expressed in μg/ml.

4.4.1. *Trypanosoma brucei rhodesiense*

Trypanosoma brucei rhodesiense is a protozoan subspecies causing Rhodesian trypanosomiasis; it is transmitted by tsetse flies, especially *Glossina morsitans* in humans; various game animals can act as reservoir hosts.

Table 4.5: *In vitro* assays against *T.b.rhodesiense*.

Compound	*T.b.rhodesiense* (IC_{50})
melarsoprol	0.0035
hippapiline	17
papiline	0.523
3-*O*-(3'-hydroxybutanoyl)haemanthamine	2.13

Values expressed in μg/ml.

Four alkaloids were tested against *Trypanosoma brucei rhodesiense* (strain STIB 900, stage trypomastigotes). The alkaloid papiline showed favorable activity with an IC_{50} of 0.523 μg/ml. Melarsoprol, which was used as standard, had an IC_{50} of 0.0035μg/ml. The other alkaloids, 3-*O*-(3'-hydroxybutanoyl)haemanthamine, and hippapiline, showed very low activities with an IC_{50} of 2.13 and 17 μg/ml, respectively (Table 4.5).

4.4.2. *Trypanosoma cruzi*

Trypanosoma cruzi is a species of parasitic euglenoid protozoan, which infects millions of people in South and Central America and is effective in about 100-150 species of wild and domesticated mammals (Machocho, 2000).

The new alkaloids from *H. papilio* were tested against *T. cruzi* (strain Tulahuen C4, stage amastigotes). The alkaloid 3-*O*-(3'-hydroxybutanoyl)haemanthamine showed mild activity with an IC_{50} of 2.965 μg/ml. Hippapiline and papiline showed no activity. The standard benznidazole had an IC_{50} of 0.6595μg/ml (Table 4.6).

Table 4.6: *In vitro* assays against *T. cruzi*.

Compound	*T.cruzi* (IC_{50})
benznidazole	0.6595
hippapiline	61.85
papiline	47.7
3-*O*-(3'-hydroxybutanoyl)haemanthamine	2.965

Values expressed in μg/ml.

4.4.3. *Leishmania donovani*

Leishmania donovani is one of the leishmania parasites that is prevalent throughout tropical and temperate regions including Africa (mostly in Sudan), China, India, Nepal, southern Europe, Russia and South America. It is responsible for thousands of deaths every year and has spread to 88 countries, with 350 million people at constant risk of infection and 0.5 million new cases a year (Machocho, 2000; Desjeux, 2004).

Papiline and 3-*O*-(3'-hydroxybutanoyl)haemanthamine showed low activity against *L. donovani* (stain MHOM-ET-67/L82, stage amastigotes) with an IC_{50} of 2.83, 4.62 μg/ml, respectively. Miltefosine, the standard, had an IC_{50} of 0.085 μg/ml (Table

4.7).

Table 4.7: *In vitro* assays against *L.donovani*.

Compound	*L.donovani* (IC$_{50}$)
miltefosine	0.085
hippapiline	19.85
papiline	2.83
3-*O*-(3'-hydroxybutanoyl)haemanthamine	4.62

Values expressed in μg/ml.

4.4.4. *Plasmodium falciparum*

Plasmodium falciparum is one of the parasites causing malaria, which is a serious disease in the world, with over 500 million people at risk in tropical and subtropical regions, especially in Africa (Machocho, 2000).

Table 4.8: *In vitro* assays against *P. falciparum*.

Compound	*P. falciparum* (IC$_{50}$)
chloroquine	0.002
hippapiline	30.8
papiline	17.3
3-*O*-(3'-hydroxybutanoyl)haemanthamine	0.987

Values expressed in μg/ml.

The alkaloids were tested against *P. falciparum* (strain NF54, stage IEF). The alkaloid 3-*O*-(3'-hydroxybutanoyl)haemanthamine showed low activity with an IC$_{50}$ of 0.987 μg/ml. Hippapiline and papiline showed no activity. The standard is chloroquine, which had an IC$_{50}$ of 0.002 μg/ml (Table 4.8).

4.4.5. Cytotoxicity

Cytotoxicity is the quality of being toxic to cells. The cytotoxicity of the alkaloids was evaluated on myoblasts (L-6 cells). 3-*O*-(3'-hydroxybutanoyl)haemanthamine showed very low activity against L-6 cells with an IC$_{50}$ of 1.731 μg/ml compared with the standard podophyllotoxin, which had an IC$_{50}$ of 0.005μg/ml (Table 4.9).

Table 4.9: *In vitro* assays of cytotoxicity against myoblasts (L-6 cells).

Compound	Cytotoxicity L-6 (IC_{50})
podophyllotoxin	0.005
hippapiline	55.4
papiline	44.5
3-*O*-(3'-hydroxybutanoyl)haemanthamine	1.731

Values expressed in μg/ml.

5. CONCLUSIONS

5. Conclusions

➤ Information about the variability and quantity of *Lycoris* alkaloids has been provided. Specifically, the alkaloid profiles of nine species of the genus *Lycoris*, *L. albiflora*, *L. aurea*, *L. chinensis*, *L. haywardii*, *L. incarnata*, *L. longituba*, *L. radiata*, *L. sprengeri*, and *L. squamigera*, and one variety (*L. radiata* var. *pumila*), have been evaluated by GC-MS. Structures belonging to the lycorine-, homolycorine-, haemanthamine-, narciclasine-, tazettine-, montanine- and galanthamine-series were identified and quantified. Galanthamine- and lycorine-type alkaloids predominating and usually showing a high relative abundance in comparison with other alkaloids of the extracts.

➤ *L. longituba* could be considered as a potential commercial source of bioactive alkaloids (such as galanthamine and lycorine) due to its high content in comparison with the other species.

➤ The GC-MS technology has been confirmed as sufficiently sensitive for the detection and identification of Amaryllidaceae alkaloids, allowing the application of quantitative methodologies to obtain valuable information in relatively short periods of time.

➤ The phytochemical investigation of the Brazilian species *Hippeastrum papilio* led to the identification of nine alkaloids, among which hippapiline, papiline, and 3-*O*-(3'-hydroxybutanoyl)haemanthamine are reported here for the first time. An unusual *cis*-B/C ring fusion in the homolycorine alkaloid hippapiline was confirmed by NMR and CD spectroscopy.

➤ A new crinine-type alkaloid, 3-*O*-methyl-epimacowine, was indentified in the species *Hippeastrum calyptratum*. In addition, the absolute stereochemistry of the 5,10b-ethano bridge in the crinine variants was determined by circular dichroism and X-ray crystallographic analysis, affording the first direct evidence for the presence of crinine-type alkaloids in the genus *Hippeastrum*.

➢ Parasitic protozoa *in vitro* assays were performed on the new alkaloids from *H. papilio*. The alkaloid papiline showed favorable activity against *Trypanosoma brucei rhodesiense* but low activity against *Leishmania donovani*. Additionally, 3-*O*-(3'-hydroxybutanoyl)haemanthamine showed mild activity against *T. cruzi,* low activity against *L. donovani* and *Plasmodium falciparum* and also showed low cytotoxicity against myoblasts (L-6 cells).

Overall, the research has once again demonstrated the enormous potential of the Amaryllidaceae (subfamily Amaryllidoideae) plants as sources of bioactive products and novel alkaloids, and confirmed the importance of continuing research on species not yet explored.

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7. APPENDICES

7. Appendices

7.1. Appendix I :

Alkaloids from the *Hippeastrum genus*: chemistry and biological activity

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ALKALOIDS FROM THE *HIPPEASTRUM* GENUS: CHEMISTRY AND BIOLOGICAL ACTIVITY

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ABSTRACT

In recent years alkaloids from the genus *Hippeastrum* have been shown to exhibit a broad spectrum of biological activities, including antiparasitic, antiproliferative, apoptosis-induced, psychopharmacological, acetylcholinesterase-inhibitory, among others. This work presents a brief chemical and biological review of the alkaloids found in the genus *Hippeastrum*.

Keywords: Amaryllidaceae, "hippeastroid" clade, *Hippeastrum*, montanine, candimine, 11β-hydroxygalanthamine.

RESUMEN

En los últimos años, los alcaloides aislados del género *Hippeastrum* han mostrado un amplio espectro de actividades incluyendo, entre otras, la antiparasitaria, antiproliferativas, inductoras de apoptosis, psicofarmacológicas y como inhibidores de la acetilcolinesterasa. En este trabajo se presenta una breve revisión química y biológica de los alcaloides del género *Hippeastrum*.

Palabras clave: Amaryllidaceae, clado "hippeastroid", *Hippeastrum*, montanina, candimina, 11β-hidroxigalantamina.

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1. INTRODUCTION

Hippeastrum is a well-known ornamental Amaryllidaceae genus from South America, particularly Brazil. The Amaryllidaceae family is one of the 20 most important alkaloid-containing plant families, comprising about 1100 perennial bulbous species classified in 85 genera. A particular characteristic of Amaryllidaceae plants is the consistent presence of a large, exclusive and still expanding group of isoquinoline alkaloids, the majority of which are not known to occur in any other plant family (Bastida *et al.*, 2006).

Their highly particular skeleton arrangements and broad spectrum of biological activities have prompted numerous chemical and pharmacological studies of this group of alkaloids. As an example, the well-known Amaryllidaceae alkaloid galanthamine (**50**) is a long-acting, selective, reversible and competitive inhibitor of the acetylcholinesterase enzyme (Thomsen *et al.*, 1998) as well as acting as an allosterically potentiating ligand in nicotinic acetylcholine receptors (Maelicke *et al.*, 2001). Due to these attributes, galanthamine (**50**) is one of the most important drugs used for the clinical management of Alzheimer's disease (AD) and is also useful in poliomyelitis and other neurological diseases. It is marketed as a hydrobromide salt under the name of Razadyne® (formerly Reminyl®). As a result of these and other activities demonstrated by the other skeleton-types (da Silva *et al.*, 2006; McNulty *et al.*, 2007; Giordani *et al.*, 2010a, 2011a), plants from the Amaryllidaceae family are currently seen as an important source of new and bioactive molecules.

The Amaryllidaceae are found mainly in the Southern Hemisphere, especially in South Africa and South America, which are considered to be the primary and secondary centers of diversification, respectively, of this family (Ito *et al.*, 1999). Recent nrDNA ITS sequence studies have divided the American Amaryllidaceae species in Andean tetraploid and extra-Andean "hippeastroid" clades. In addition, a probable Brazilian origin of the *Hippeastrum* genus has been accepted, based on its nrDNA ITS sequences (Meerow *et al.*, 2000). The *Hippeastrum* genus comprises approximately 70 species (Judd *et al.*, 1999), 34 being found in Brazil with 22 endemics (Dutilh, 2010). Although few of them have been studied to date, compounds with remarkable biological activity have been isolated in *Hippeastrum* species. Presented here is a brief overview of the phytochemical and biological studies of the *Hippeastrum* genus up to May, 2012.

2. GEOGRAPHICAL DISTRIBUTION, TAXONOMICAL ASPECTS

The *Hippeastrum* genus is distributed from Mexico and the West Indies to Argentina, the majority in eastern Brazil, the Peruvian Andes and Bolivia. It basically consists of large herbs of annual leaves, mostly hysteranthous, sessile, rarely persistent, and subpetiolate. Generally, the leaves are more than 2 cm wide. The scape is hollow with 2 free bracts. The flowers (2-13) are usually large and mostly purple or red. They are funnelform, zygomorphic, declinate, usually with a short tube and paraperigonal fibriae or with a callose ridge present at the throat. The stamens are fasciculate and declinate-ascendent. The stigma is trifid or shortly 3-lobed. The seeds are dry, flattened, obliquely winged or irregularly discoid, hardly ever turgid and globose or subglobose, with a brown or black phytomelanous testa (Dahlgren *et al.*, 1985; Meerow and Snijman, 1998).

A diploidism of 2n=22 is characteristic of the *Hippeastrum* genus, which is inarguably monophyletic with the exception of a single species, *Hippeastrum blumenavium*. This was first described as *Griffinia blumenavia* Koch and Bouche ex Carr and further studies are required to clarify its correct position (Meerow *et al.*, 2000).

The beauty of their flowers has led to numerous *Hippeastrum* species being grown as ornamentals after hybridization (Meerow and Snijman, 1998), although in horticultural circles the use of the name "*Amaryllis*" for this genus persists (Meerow *et al*, 1997).

3. BIOSYNTHESIS AND STRUCTURAL TYPES OF AMARYLLIDACEAE ALKALOIDS

As mentioned above, the consistent presence of an exclusive group of isoquinoline alkaloids is the outstanding feature of the Amaryllidaceae plant species. Amaryllidaceae alkaloids are formed biogenetically by intramolecular oxidative coupling of the key intermediate *O*-methylnorbelladine, derived from the amino acids L-phenylalanine and L-tyrosine (Bastida *et al.*, 2006). Most of them can be classified into nine skeleton-types (Figure 1), namely lycorine, crinine, haemanthamine, narciclasine, galanthamine, tazettine, homolycorine, montanine and norbelladine (Bastida *et al.*, 2006). *Ortho-para'* phenol oxidative coupling of the precursor *O*-methylnorbelladine results in the formation of a lycorine-type skeleton, from which homolycorine-type compounds proceed. *Para-para'* phenol oxidative coupling leads to the formation of crinine, haemanthamine, tazettine, narciclasine and montanine structures. The galanthamine-type skeleton is the only one that originates from *para-ortho'* phenol oxidative coupling (Bastida *et al.*, 2006). In the present review, the numbering system according to Ghosal *et al.* (1985) has been adopted for the structures (Figure 1).
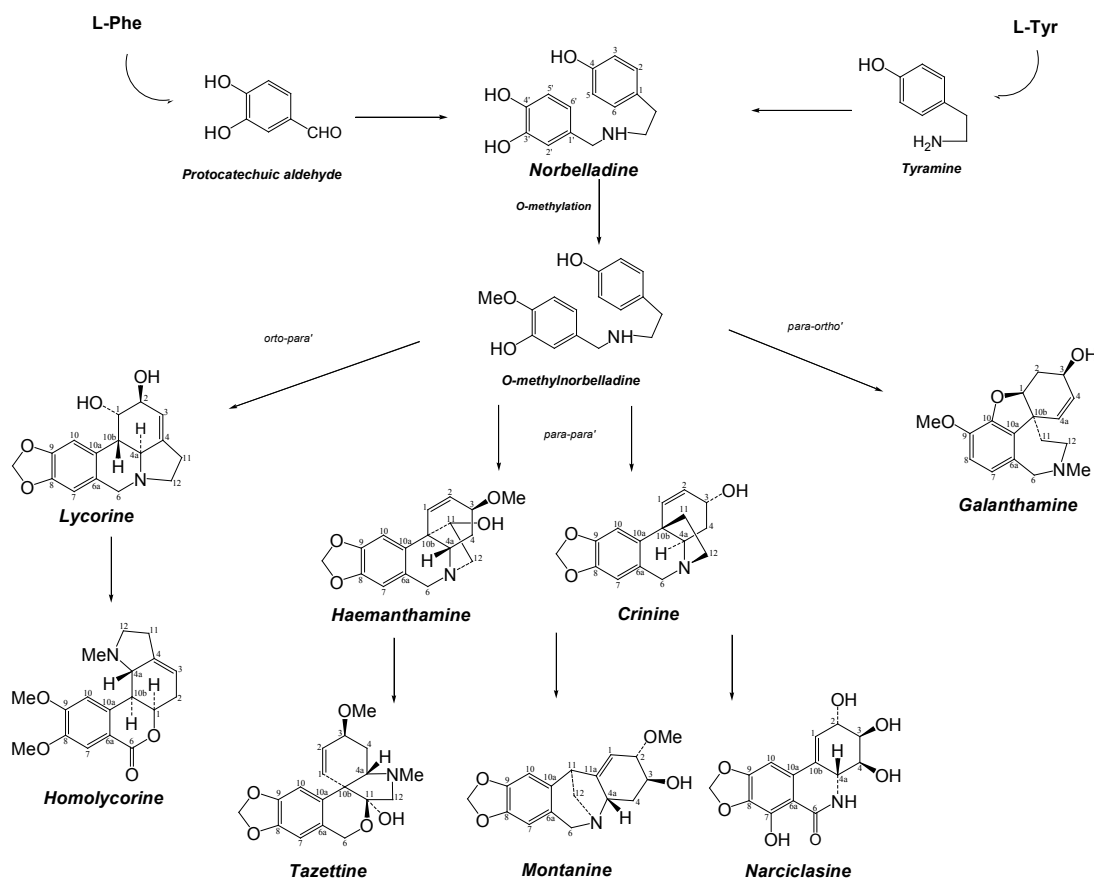

Figure 1. Biosynthetic pathway of the main skeleton-type found in the genus *Hippeastrum.*
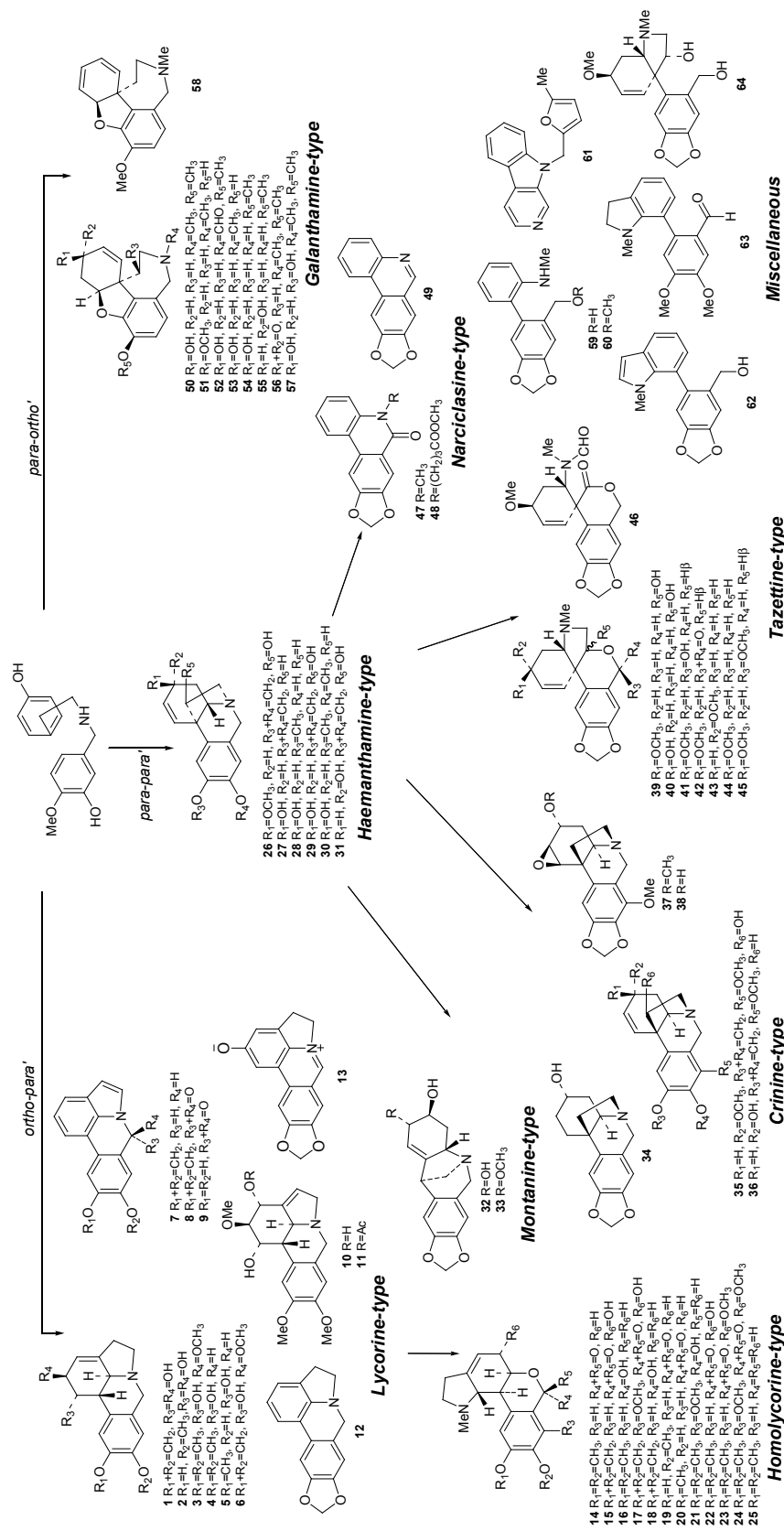
Some new structural subgroups have been proposed recently (Ünver, 2007). Graciline and plicamine-type alkaloids have been found in species of *Galanthus*, *Cyrthanthus* and *Narcissus* (Ünver *et al.*, 1999; Brine *et al.*, 2002; de Andrade *et al.*, 2012). The biogenetic pathway of gracilines possibly originates from the 6-hydroxy derivatives of haemanthamine-type alkaloids (Noyan *et al.*, 1998), while plicamine-type alkaloids most probably proceed from the tazettine-type skeleton, considering their structural similarities. Augustamine-type alkaloids represent a very rare structure found in *Crinum* species (Ali *et al.*, 1983; Machocho *et al.*, 2004). Galanthindole (**62**) is another example of an unusual compound isolated from the *Galanthus* genus and also found in *Hippeastrum* genus. It has been classified as a new skeleton-type (Ünver *et al.*, 2003), although the possibility that it is an artifact from the homolycorine series should be considered. Another uncommon alkaloid found in *Hippeastrum* was a simple carboline, isolated from *Hippeastrum vittatum* (Youssef, 2001).

A few alkaloids commonly found in other plant families have also been described in Amaryllidaceae plants, for example, the mesembrane-type alkaloids, which were isolated in *Narcissus* species (Bastida *et al.*, 2006) despite being typical of the genus *Sceletium* (Aizoaceae). Phtalideisoquinoline-, benzyltetrahydroisoquinoline- and aporphine-type alkaloids were found in *Galanthus trojanus* (Kaya *et al.*, 2004, 2011), being most commonly associated with Papaveraceae and Fumariaceae. Tyramine-type protoalkaloids, which are biosynthesized in Poaceae, Cactaceae, some algae and fungi, have also been found in *Galanthus* and *Leucojum* species (Berkov *et al.*, 2008, 2011a, 2011b). However, it should be borne in mind that these unusual alkaloids have always been isolated together with typical Amaryllidaceae alkaloids. To date, nearly 500 alkaloids have been isolated from amaryllidaceous plants (Zhong, 2005).

4. DISTRIBUTION OF ALKALOIDS IN THE GENUS *HIPPEASTRUM*

Phytochemical studies of the genus *Hippeastrum*, as well as of other genera of the Amaryllidaceae family, started in the early 1950s. The alkaloids reported in the genus *Hippeastrum* are summarized in Table 1 and their respective structures are shown in Figure 2. The first phytochemical study was described with varieties of *H. vittatum*, which yielded the alkaloids tazettine (**39**) and lycorine (**1**) (Boit, 1954). Two years later, a new phytochemical study of the same species yielded the alkaloids haemanthamine (**26**), homolycorine (**14**), hippeastrine (**15**), and vittatine (**27**), as well as tazettine (**39**) and lycorine (**1**) (Boit, 1956). In 1957, a study of *H. bifidum* only yielded lycorine (**1**) (Boit and Döpke, 1957). One year later, galanthamine (**50**) was found for the first time in a *Hippeastrum* species, specifically in *H. rutilum*, although it was isolated as a minor compound (Boit *et al.*, 1958). The work carried out in the 1950s and 60s was notable for the isolation of montanine (**33**) in *H. aulicum* along with some crinine-type representatives (Boit and Döpke, 1959). The main research on the genus in these two decades can be found by searching for the authors Boit, HG and Döpke, W.

There was little phytochemical research on *Hippeastrum* species between the 1970s and 1990s. An interesting study was carried out with *H. vittatum* grown in Egypt in different years, which allowed the elucidation of the alkaloids pancracine (**32**) (formerly hippagine) and hippadine (**8**) (El Mohgazi *et al.*, 1975; Ali *et al.*, 1981, 1984). A phytochemical study of *H. añañuca* from Chile yielded a new alkaloid but with undefined stereochemistry (Pacheco *et al.*, 1978). Quirion *et al.*, (1991) isolated the new compound 3-*O*-acetylnarcissidine (**11**) from *H. puniceum*, and Döpke *et al.*, (1995a) isolated a new phenantridone alkaloid named phamine (**48**) from *H. equestre*. Several known alkaloids were also isolated

Figure 2. Structure of the alkaloids reported in the genus *Hippeastrum*.

Table 1. Alkaloids reported in the genus *Hippeastrum*.

	H. vittatum	H. bifidum	H. rutilum	H. rachyandrum	H. aulicum	H. hybrids*	H. candidum	H. johnsonii	H. añañuca	H. bicolor	H. equestre	H. puniceum	H. solandriflorum	H. glaucescens	H. morelianum	H. psittacinum	H. striatum	H. santacatarina	H. papilio	H. breviflorum
Lycorine-type																				
Lycorine (**1**)	1,2,12,29,30	3	4,5	5	6	7,8,21	8	10	14	16	11,17,18,22,23		24	28		29	29	29,33		
Pseudolycorine (**2**)					6			10			11									
Galanthine (**3**)					6	7,8					17,18									
Pluviine (**4**)						7														
Norpluviine (**5**)					6															
Hippamine (**6**)						8														
11,12-Dehydroanhydrolycorine (**7**)																29		29		29
Hippadine (**8**)	12,15																			
Hippacine (**9**)	9,12,13																			
Narcissidine (**10**)				5	6	7,8														
3-O-Acetylnarcissidine (**11**)							8					20								
Anhydrolycorine (**12**)	29																			
Ungeremine (**13**)													24					29		
Homolycorine-type																				
Homolycorine (**14**)	2		4,5						14											
Hippeastrine (**15**)	2,12		4,5			7,8,21					17,18,22,23			29		29,31				
Lycorenine (**16**)				5																
Candimine (**17**)															29,32					
Oduline (**18**)						9														
9-O-Demethylhomolycorine (**19**)											25									
8-O-Demethylhomolycorine (**20**)																29				29
Nerinine (**21**)															29					
2-Hydroxyhomolycorine (**22**)															29					
2-Methoxyhomolycorine (**23**)															29					
2α,7-Dimethoxyhomolycorine (**24**)															29,32					
Deoxylycorenine (**25**)																				29
Haemanthamine-type																				
Haemanthamine (**26**)	2		4,5			7,8,21				16	18				32			29	29,34	
Vittatine (**27**)	2,12,13,29,30					21						20	24					29	29,34	
8-O-Demethylmaritidine (**28**)	29																			
11-Hydroxyvittatine (**29**)						21					25	20							29,34	
Maritidine (**30**)									14											
Hamayne (**31**)													24		32			29		
Montanine-type																				
Pancracine (former hippagine) (**32**)	12,19,29					21														
Montanine (**33**)	29,30				6	21														

	H. vittatum	*H. bifidum*	*H. rutilum*	*H. brachyandrum*	*H. aulicum*	*H. hybrids**	*H. candidum*	*H. johnsonii*	*H. añañuca*	*H. bicolor*	*H. equestre*	*H. puniceum*	*H. solandriflorum*	*H. glaucescens*	*H. morelianum*	*H. psittacinum*	*H. striatum*	*H. santacatarina*	*H. papilio*	*H. breviflorum*
Crinine-type																				
Crinidine (34)						8														
Ambelline (35)				5	6	7														
Powelline (36)						7,8														
Undulatine (37)				5																
Crinamidine (38)				5		7														
Tazettine-type																				
Tazettine (39)	1,2, 12					8,21		10			11,17,18 22,23,26			28,29	29,32	29		29	29	29
3-O-demethyltazettine (40)											26			28	32	31				
Pretazettine (41)											25			29						
3-*Epi*-macronine (42)											25				29,32	29		29		29
3-*Epi*-deoxytazettine (43)														29	29	29				29
Deoxytazettine (44)														29	29	29		29		29
6-Methoxypretazettine (45)															29	29				29
Tazettamide (46)																29				
Narciclusine-type																				
N-Methylcrinasiadine (47)											22									
Phamine (48)											22,23									
Trisphaeridine (49)											22			29	29,32	29	29	29	29	29
Galanthamine-type																				
Galanthamine (50)	29		4,5			7,8					18			29	29	29	29	29	29,34	
Chlidanthine (51)					6															
N-Formylnorgalanthamine (52)														29						
Sanguinine (53)														29					29	
N-Demethylgalanthamine (54)														29						
3-*Epi*-norgalanthamine (55)														29						
Narwedine (56)														29					29,34	
11β-hydroxygalanthamine (57)																			29,34	
Anhydrogalanthamine (58)														29						
Miscellaneous																				
Ismine (59)	27										25		24		29	29				29
O-Methylismine (60)	27																			
Vittacarboline (61)	27																			
Galanthindole (62)															29	29				29
Lycosinine B (63)																				29
Egonine (64)											26									

1) Boit, 1954; 2) Boit, 1956; 3) Boit and Döpke, 1957; 4) Boit et al., 1958; 5) Boit and Döpke, 1959; 6) Boit and Döpke, 1960a; 7) Boit and Döpke, 1960b; 8) Döpke, 1962; 9) Döpke and Bienert, 1966; 10) Rao et al., 1971; 11) Rao and Vimaladevi, 1972; 12) El Mohgazi et al., 1975; 13) Ali et al., 1981; 14) Pacheco et al., 1978; 15) Ali et al., 1981; 16) Sepúlveda et al., 1981; 17) Alam and Murav'eva, 1982; 18) Murav'eva and Alam, 1982; 19) Ali et al., 1984; 20) Quirion et al., 1991; 21) Müsge et al., 1994; 22) Döpke et al., 1995a; 23) Döpke et al, 1995b; 24) Bastida et al., 1996; 25) Pham et al., 1997; 26) Pham et al., 1999; 27) Youssef, 2001; 28) Hofmann et al., 2003; 29) de Andrade et al., Personal communication, 12 June 2012; 30) da Silva et al., 2008; 31) Pagliosa et al., 2010; 32) Giordani et al., 2011b; 33) Giordani et al., 2011a; 34) de Andrade et al., 2011. * mixture of hybrids from *Hippeastrum*.

from *H. equestre* and submitted to circular dichroism studies (Wagner *et al.*, 1996). A few years later, *H. equestre* yielded another new alkaloid, egonine (**64**) (Pham *et al.*, 1999). This structure has been related as a typical *Sceletium* mesembrine-type alkaloid (Aizoaceae), although its similarity with tazettine-type skeleton should be considered.

Phytochemical studies of *H. vittatum* flowers in 2001 yielded a representative alkaloid of the carboline group named vittacarboline (**61**), as well as the new alkaloid *O*-methylismine (**60**) (Youssef, 2001). A rapid phytochemical study of *H. glaucescens* provided lycorine (**1**), pretazettine (**41**) and tazettine (**39**), but not all alkaloid fractions were studied (Hoffman *et al.*, 2003). In the last decade, most *Hippeastrum* studies have been focused on the biological activity of alkaloids isolated from the genus, although the new alkaloids 2*α*,7-dimethoxyhomolycorine (**24**) and 11*β*-hydroxygalanthamine (**57**) found in *H. morelianum* and *H. papilio*, respectively, should be mentioned (Giordani *et al.*, 2011b, de Andrade *et al.*, 2011).

In the last decade, the GC-MS technique has proved to be very effective for rapid separation and identification of complex mixtures of Amaryllidaceae alkaloids obtained from low mass samples (Kreh *et al.*, 1995, Berkov *et al.*, 2008, 2011a). The high resolution ability of the capillary column and numerous EI-MS spectra available in the literature allow the identification and quantification of known Amaryllidaceae alkaloids, avoiding time-consuming and laborious isolation procedures. This technique has been much applied with the genera *Pancratium*, *Galanthus*, *Leucojum* and *Narcissus* (Kreh *et al.*, 1995; Torras-Claveria *et al.*, 2010; Berkov *et al.*, 2011b; de Andrade *et al.*, 2012). The only study applying GC-MS in the genus *Hippeastrum,* carried out with species from South Brazil, identified more compounds than had been isolated previously and two species were found to

produce significant levels of galanthamine (**50**) (de Andrade *et al.*, Personal communication, 12 June 2012).

To the best of our knowledge, 19 species, including hybrids, from the genus *Hippeastrum* have been phytochemically studied to date. Sixty-four different alkaloids with defined structures have been isolated, while fourteen remain undefined (Boit and Döpke, 1959, 1960a, 1960b; Pacheco *et al.*, 1978; de Andrade *et al.*, Personal communication, 12 June 2012). Table 1 and Figure 2 summarize the alkaloids found in the genus *Hippeastrum.*

5. BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF THE ALKALOIDS FOUND IN *HIPPEASTRUM*

Like most Amaryllidaceae alkaloids, the compounds found in the genus *Hippeastrum* have been little evaluated for their biological activity. However, some of them have demonstrated a broad spectrum of interesting properties.

5.1. *Ortho-para'* phenolic coupling
5.1.1 Lycorine-type
Lycorine (**1**) is probably the most frequent occurring alkaloid in Amaryllidaceae plants and has been found in almost all *Hippeastrum* species. This compound possesses a vast array of biological properties, being reported as a potent inhibitor of ascorbic acid synthesis, cell growth and division, and organogenesis in higher plants, algae and yeasts, inhibiting the cell cycle during the interphase (Bastida *et al.*, 2006). Additionally, lycorine (**1**) exhibits antiviral, anti-inflammatory, antifungal and antiprotozoan activities (Çitoğlu *et al.*, 1998; McNulty *et al.*, 2009; Giordani *et al.*, 2010b, 2011a). Lycorine (**1**) has also been shown to have insect antifeedant activity (Evidente *et al.*, 1986), as does 3-*O*-acetylnarcissidine (**11**), isolated from *H. puniceum*, which is particularly active against the polyphagous

insect *Spodora littoralis* but not against the olphage *Leptinotarsa decemlineata* (Santana *et al.*, 2008).

As a potential chemotherapeutic drug, lycorine (**1**) has been studied as an antiproliferative agent against a number of cancer cell lines (Likhitwitayawuid *et al.*, 1993; McNulty *et al.*, 2009). The *in vitro* mode of action in a model HL-60 leukemia cell line is associated with suppressing tumor cell growth and reducing cell survival via cell cycle arrest and induction of apoptosis. Furthermore, lycorine (**1**) was able to decrease tumor cell growth and increase survival rates with no observable adverse effects in treated animals, thus being a good candidate for a therapeutic agent against leukaemia (Liu *et al.*, 2004, 2007; Liu *et al.*, 2009).

Lycorine (**1**) isolated from *H. santacatarina* showed remarkable inhibitory activity of the enzymes NTPDase and ecto-5'-nucleo- tidase from *Trichomonas vaginalis,* which contributes to an increased susceptibility of this parasite to the host immune response (Giordani *et al.*, 2010b). Lycorine (**1**) was also demonstrated to have anti-*T. vaginalis* activity, involving a mechanism of cell death induction associated with paraptosis rather than the apoptosis observed in tumor cells. This mechanism also differs from the one associated with other pro-apoptotic compounds tested against *T. vaginalis* such as staurosporine, doxorubicin, etoposide and methyl jasmonate. The authors have called for additional molecular studies for a better characterization of the different cell death mechanisms (Giordani *et al.*, 2011a). Lycorine (**1**) has also been tested *in vitro* against human immunodeficiency virus type 1 (HIV-1), results of antiviral showed low inhibition of the replication of HIV-1(NL4-3) with an $EC_{50} > 0.5$ µg/ml with infected lymphoid MT-4 human cells (Reyes-Chilpa *et al.*, 2011).

Compared to other lycorine-type alkaloids, anhydrolycorine (**12**) showed a greater ability to inhibit ascorbic acid syn-thesis (Evidente *et al.*, 1986). Analgesic, hypotensive and antiparasitic activities have been reported for galanthine (**3**). Ungeremine (**13**) has shown acetylcholinesterase inhibitory activity (Bastida *et al.*, 2006). In summary, the lycorine skeleton-type is a promising target for further biological assessments.

5.1.2 *Homolycorine-type*
Homolycorine (**14**), 8-*O*-demethylhomolycorine (**20**) and hippeastrine (**15**) are well-known cytotoxic alkaloids. Homolycorine (**14**) has also shown high antiretroviral activity, while hippeastrine (**15**) is active against *Herpes simplex* type 1. Homolycorine (**14**) and 8-*O*-demethylhomolycorine (**20**) have a hypotensive effect on normotensive rats. In addition, hippeastrine (**15**) shows antifungal activity against *Candida albicans* and also possesses a weak insect antifeedant activity (Bastida *et al.*, 2006). Candimine (**17**), first found in *H. candidum* (Döpke, 1962), has been tested against *Trichomonas vaginalis* and found to inhibit the *T. vaginalis* enzymes NTPDase and ecto-5'-nucleotidase to a greater extent than lycorine (**1**) (Giordani *et al.*, 2010b). Candimine (**17**) was also active against *T. vaginalis*, apparently inducing cell death by paraptosis, as in the case of lycorine (**1**) (Giordani *et al.*, 2010a). Homolycorine (**14**) and 8-*O*-demethylhomolycorine (**20**) were tested against the parasitic protozoa *Trypanosoma cruzi*, *Trypanosoma brucei rhodesiense*, *Leishmania donovani* and *Plasmodium falciparum* but showed no significant activity (de Andrade *et al.*, 2012). However, the bioactivity of most homolycorine-type alkaloids is largely unknown.

5.2. *Para-para'* phenolic coupling
5.2.1. *Haemanthamine-type*
Haemanthamine (**26**), as well as crinamine, has proven to be a potent inducer of apoptosis in tumor cells at micromolar concentrations (McNulty *et al.*, 2007). This

compound also possesses antimalarial activity against strains of chloroquine-sensitive *Plasmodium falciparum*, hypotensive effects and antiretroviral activity (Bastida *et al.*, 2006; Kaya *et al.*, 2011). Vittatine (**27**), isolated from *H. vittatum*, and maritidine (**30**), have shown cytotoxic activity against HT29 colon adenocarcinoma, lung carcinoma and RXF393 renal cell carcinoma (Bastida *et al.*, 2006; da Silva *et al.*, 2008). Vittatine (**27**) also showed antibacterial activity against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*, as well as 11-hydroxyvittatine (**29**) (Kornienko and Evidente, 2008).

5.2.2. *Crinine-type*
The alkaloids crinine, 6-hydroxybuphanidrine and 6-ethoxybuphanidrine showed antiproliferative effects against human tumor cell lines, crinine being the most active (Berkov *et al.*, 2011c). A comparative study of skeleton-types concluded that the crinine-type alkaloid buphanamine was the most promising, since it showed important anti-proliferative effects and was well tolerated even at high concentration (Evidente *et al.*, 2009). Further evaluations are needed to gain more insight into the biological activity of the crinine-type skeleton.

5.2.3. *Tazettine-type*
The alkaloids 3-*epi*-macronine (**42**) and tazettine (**39**) showed moderate cytotoxic activity. Tazettine (**39**) is an isolation artefact of chemically labile pretazettine (**41**) (de Andrade *et al.*, 2012), the latter being far more interesting due to its antiviral and anticancer activities (Bastida *et al.*, 2006). Pretazettine (**41**) shows cytotoxicity against fibroblastic LMTK cell lines and inhibits HeLa cell growth, being therapeutically effective against advanced Rauscher leukemia, Ehrlich ascites carcinoma, spontaneous AKR lymphocytic leukemia, and Lewis lung carcinoma (Bastida *et al.*, 2006). Pretazettine (**41**) isolated from *H. psittacinum* was tested for its ability to inhibit the

AChE enzyme but showed no significant result (Pagliosa *et al.*, 2010).

5.2.4. *Montanine-type*
This group has very few representatives. The alkaloids montanine (**33**) and pancracine (**32**) have been isolated in different periods from *Hippeastrum* species growing in Europe and South America, such as *H. vittatum*. In recent work montanine (**33**) showed anxiolytic-, antidepressant- and anticonvulsant-like effects in mice (da Silva *et al.*, 2006). Montanine (**33**) and vittatine (**27**) were also submitted to an antiproliferative study, the former showing the highest level of cytotoxicity (da Silva *et al.*, 2008). Furthermore, montanine (**33**) significantly inhibited AChE activity at concentrations of 1 milimolar, and 500 and 100 micromolar using the Ellman method (Pagliosa *et al.*, 2010). Pancracine (**32**) showed antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as weak activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi* and *Plasmodium falciparum* (Bastida *et al.*, 2006). The montanine-type skeleton represents one of the most interesting alkaloids for biological evaluations due to its remarkable and broad spectrum of activities.

5.2.5. *Narciclasine-type*
Trisphaeridine (**49**) has a high retroviral activity but a low therapeutic index (Bastida *et al.*, 2006). Narciclasine and pancratistatin are the most studied alkaloids of this group but they have never been found in the *Hippeastrum* genus. Both compounds show strong antimitotic and antitumoral activities (Bastida *et al.*, 2006). No biological evaluation of the alkaloids *N*-methylcrinasiadine (**47**) and phamine (**48**) has been carried out to date.

5.3. *Para-ortho'* phenolic coupling
5.3.1. *Galanthamine-type*
Galanthamine (**50**) is a long-acting, selective, reversible and competitive inhibitor of

acetylcholinesterase (AChE) and an allosteric modulator of the neuronal nicotinic receptor for acetylcholine. Its action increases acetylcholine levels, thus facilitating cholinergic synapses and helping in the management of patients suffering certain stages of AD (Maelicke *et al.*, 2001; Bastida *et al.*, 2006; Heinrich and Teoh, 2004). Galanthamine (**50**), therefore, is the most studied Amaryllidaceae alkaloid in terms of biological activity, clinical response, tolerance and safety, being marketed as a hydrobromide salt under the name of Razadine®, formerly Reminyl®. Galanthamine (**50**) has superior pharmacological profiles and higher tolerance than the original AChE inhibitors physostigmine or tacrine (Grutzendler and Morris, 2001).

After the therapeutic success of galanthamine (**50**), the search for new AChE inhibitors has intensified. *Epi*-galanthamine, with a hydroxyl group at the *α*-position, and narwedine (**56**), with a keto group at C3, are also active AChE inhibitors, but about 130-times less powerful than galanthamine (**50**) (Thomsen *et al.*, 1998). The loss of the methyl group at the *N* atom, as in *N*-demethylgalanthamine (**54**), decreases the activity 10-fold. The alkaloids habranthine and its new epimer 11*β*-hydroxygalathamine (**57**), isolated from *H. papilio*, which shows a hydroxyl-substituent at C11, were both also *ca.* 10-times less active than galanthamine (**50**) (López *et al.*, 2002; de Andrade *et al.*, 2011). Hydrogenation of the C4-C4a double bond, as in lycoramine, results in a complete loss of AChE inhibitory activity (López *et al.*, 2002).

On the other hand, sanguinine (**53**), which has a hydroxyl group at C9 instead of a methoxyl group, is *ca.* 10 times more active than galanthamine (**50**). Recently, *N*-alkylated galanthamine derivatives were isolated from *Leucojum* species and were also *ca.* 10 times more active than galanthamine (**50**). It has been suggested that these naturally occurring AChE inhibitors can act as ecological pesticides, since the AChE-inhibitory activity of synthetic pesticides, such as phospho-organic derivatives, is non-reversible (Houghton *et al.*, 2006).

Galanthamine (**50**) has also been tested *in vitro* against human immunodeficiency virus type 1 (HIV-1), results of antiviral assays indicated that galanthamine (**50**), as well as its structural isomer chlidanthine (**51**) and galanthamine *N*-oxide, did not showed inhibition of the replication of HIV-1(NL4-3) with infected lymphoid MT-4 human cells, but they were also not toxic to non infected cells showing EC_{50} and $CC_{50} >$ 20 μg/ml), respectively (Reyes-Chilpa *et al.*, 2011). The galanthamine-type skeleton is currently the most studied group in terms of biological activity.

5.4. Miscellaneous

Ismine (**59**) shows a significant hypotensive effect on rats and cytotoxicity against Molt 4 lymphoid and LMTK fibroblastic cell lines (Bastida *et al.*, 2006). Recently, extracts from *H. breviflorum* showing different ratios between lycosinine B (**63**) and lycorine (**1**) by HPLC demonstrated significant anti-*Trichomonas vaginalis* activity (Vieira *et al.*, 2011). To date, the alkaloids vittacarboline (**61**), galanthindole (**62**) and *O*-methylismine (**60**) have not been biologically evaluated.

6. CONCLUSION

Over the last 50 years, the bulbous genus *Hippeastrum* has yielded 64 different alkaloids, together with others whose structures remain undefined. Further studies on the isolation of these compounds are called for, especially after recent biological studies showing their significant antiparasitic, psychopharmacological and AChE-inhibitory activities. Notably, some *Hippeastrum* species are able to produce a high level of galanthamine (**50**), comparable with species

of other genera currently being used for the commercial production of this alkaloid. The lack of biological activity shown by most of the alkaloids found in the *Hippeastrum* genus may be due to the small amounts isolated. Consequently, their synthesis or *in silico* studies will facilitate further bioactivity assessment.

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7.2. Appendix Ⅱ : Supplementary data for new alkaloids

7.2.1. Hippapiline
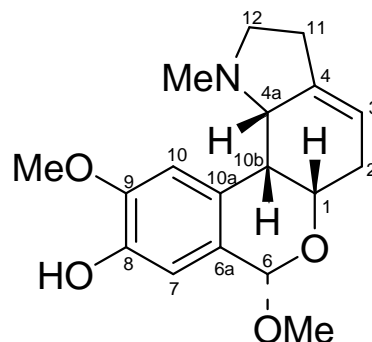
Hippapiline (**1**): $[\alpha]^{24}_D$ +33 (c 0.15, CHCl$_3$); CD $[\theta]^{20}_\lambda$: $[\theta]_{227}$ +1330, $[\theta]_{247.5}$ −455, $[\theta]_{281.5}$ +89, $[\theta]_{305.5}$ −176; UV (MeOH) λ_{max} (log ε) 316 (2.90), 282 (3.30), 228 (3.64), 205 (4.27) nm; IR (CHCl$_3$) ν_{max}: 3380, 2924, 1733, 1509, 1449, 1275, 1130, 1058, 1043, 960, 913, 880, 802, 758 cm^{-1}; HREIMS of [M+H]$^+$ m/z 318.1706 (calcd. for C$_{18}$H$_{24}$NO$_4$, 318.1700).

Figure S1: Physical and spectroscopic data of compound **1**.
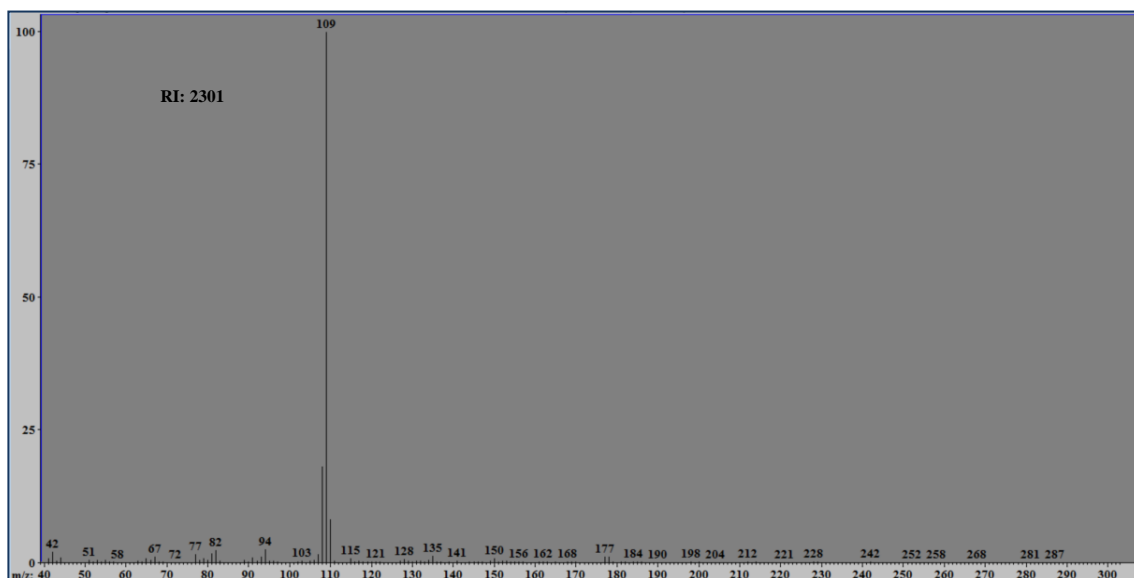
Figure S2: GC-MS spectrum of compound **1**.
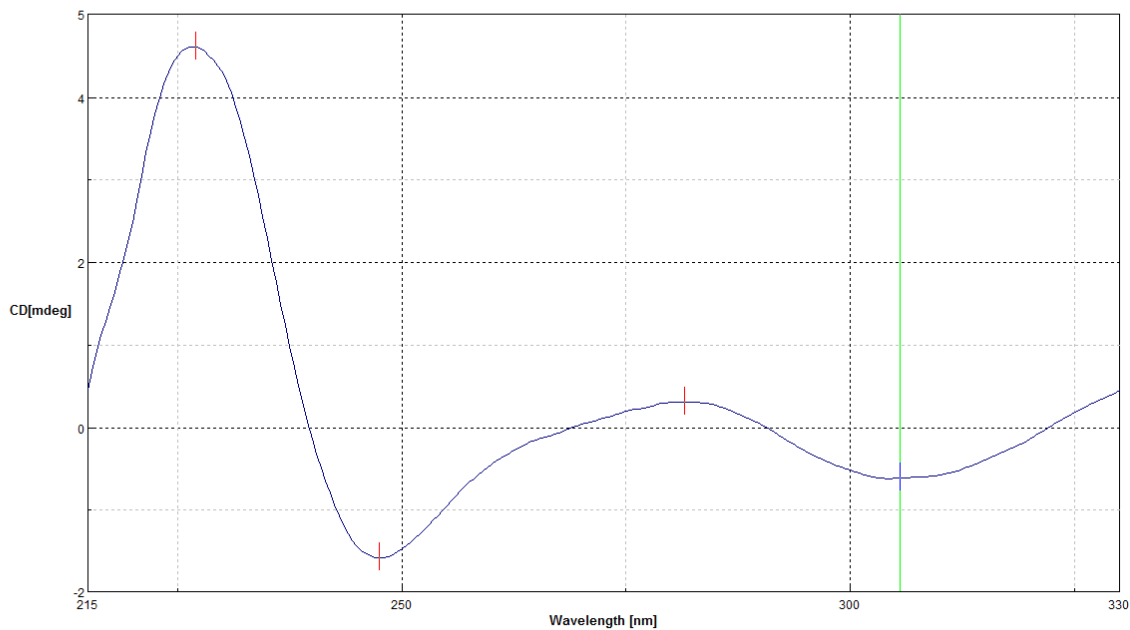
Figure S3: CD spectrum of compound **1**.

Table S1: ^{1}H NMR, COSY, NOESY, HMBC (500MHz, CDCl$_3$) and ^{13}C NMR(125MHz, CDCl$_3$) data of compound **1**.

| Position	H δ (*J* in Hz)	COSY	NOESY	C δ	HMBC
1	4.29 *ddd* (9.8, 6.8, 4.5)	H-2α, H-2β, H-10b	H-2a, H-2β, H-4a, H-10b	70.5 *d*	
2α	2.41-2.47 *m*	H-1, H-2β, H-3, H-4a, H-11	H-1, H-2β, H-3, 6-OMe	28.9 *t*	
2β	2.25-2.29 *m*	H-1, H-2α, H-3, H-4a, H-11	H-1, H-2α, H-3		
3	5.07 *br s*	H-2α, H-2β, H-4a, H-11	H-2α, H-2β, H-11	115.2 *d*	
4				138.4 *s*	
4a	2.94 *br s*	H-2α, H-2β, H-3, H-10b; H-11	H-1, H-10b, H-12β, NMe	70.6 *d*	
6β	5.44 *s*	H-7	H-7, H-10b, 6-OMe	97.1 *d*	C-1, C-10a, 6-OMe
6a				127.8 *s*	
7	6.88 *s*	H-6β	H-6β	112.4 *d*	C-6, C-8, C-9, C-10a
8				143.7 *s*	
9				145.9 *s*	
10	8.41 *s*	H-10b	9-OMe, NMe	110.6 *d*	C-6a, C-8
10a				126.7 *s*	
10b	3.32 *t* (4.5)	H-1, H-4a, H-10	H-1, H-4a, H-6β, NMe	35.4 *d*	C-1, C-4, C-4a, C-6a
11 (2H)	2.28-2.36 *m*	H-2α, H-2β, H-3, H-4a, H-12α, H-12β	H-3, H-12α,,H-12β	28.6 *t*	
12α	3.21 *ddd* (8.7, 6.7, 2.0)	H-11, H-12β	H-11, H-12β, NMe	56.1 *t*	C-4, C-4a
12β	2.18 *dt* (9.8, 8.6)	H-11, H-12α	H-4a, H-11, H-12α, NMe		
6-OMe	3.57 *s*		H-2α, H-6β	55.3 *q*	C-6
9-OMe	3.80 *s*		H-10	55.7 *q*	C-9
NMe	2.44 *s*		H-4a, H-10, H-10b, H-12α, H-12β	40.0 *q*	C-4a, C-12

Figure S4: ^{1}H NMR spectrum of compound **1**.
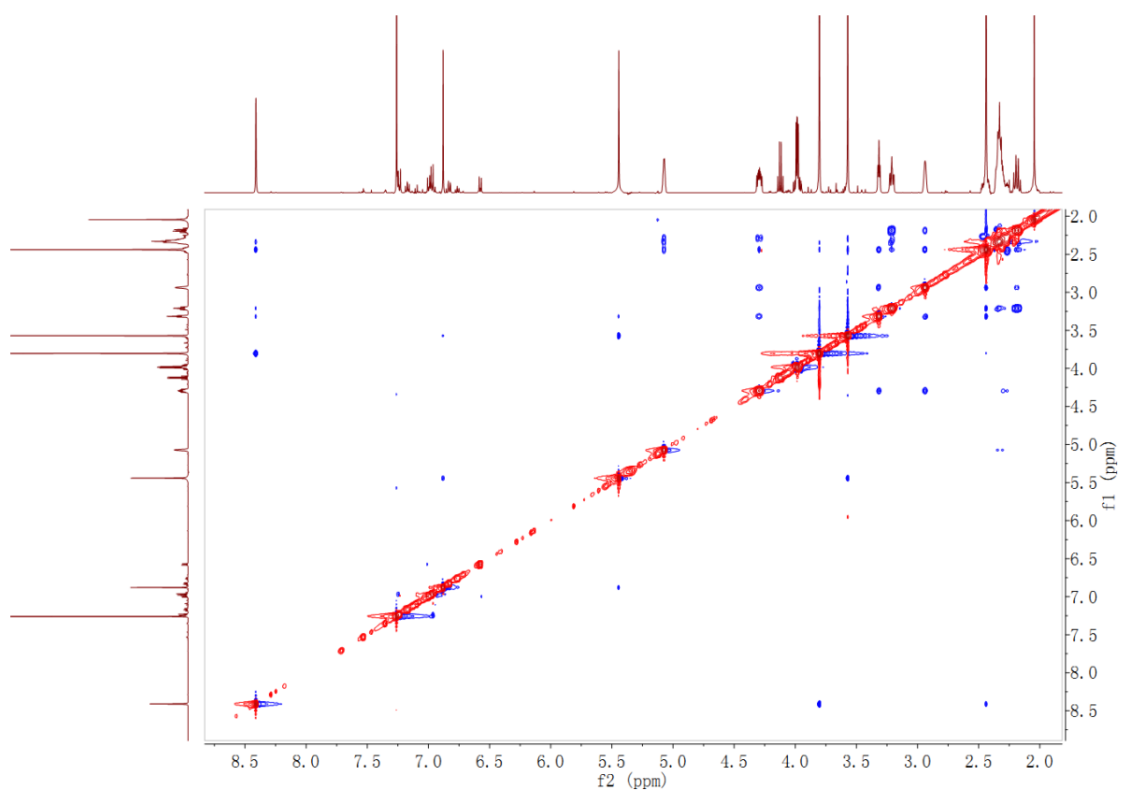
Figure S5: NOESY spectrum of compound **1**.
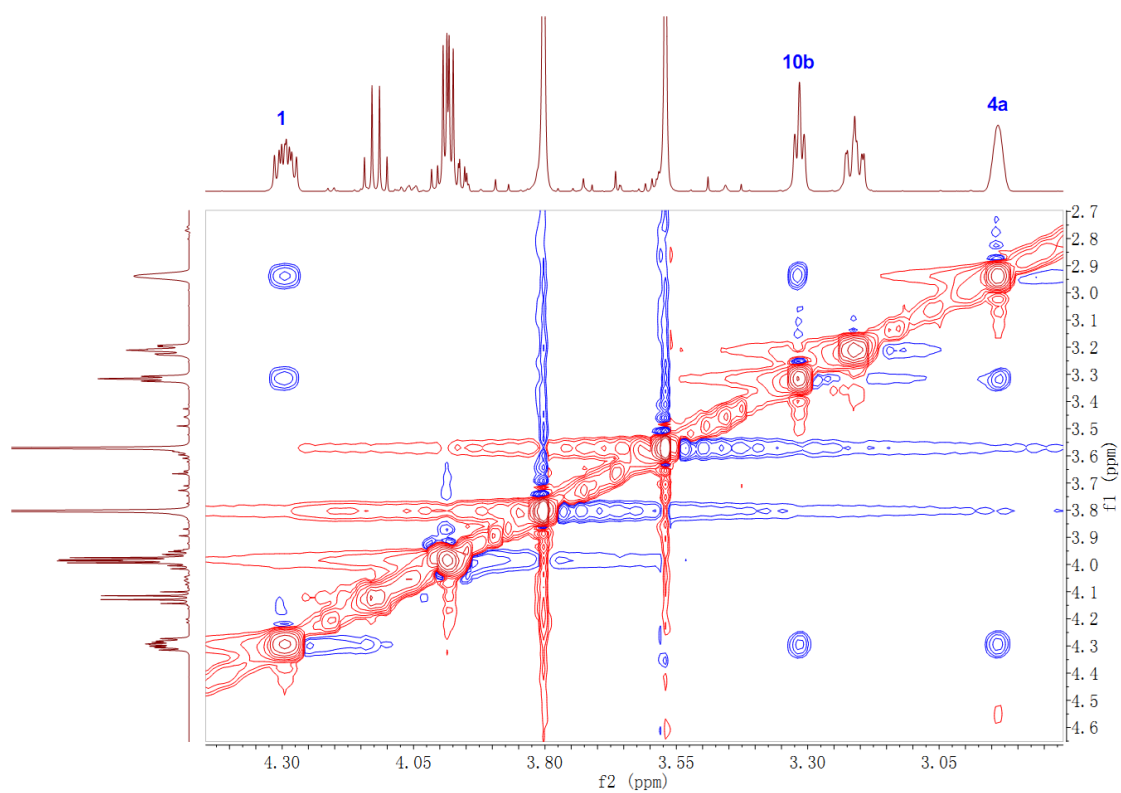
Figure S6: NOESY enlargement of compound **1**.
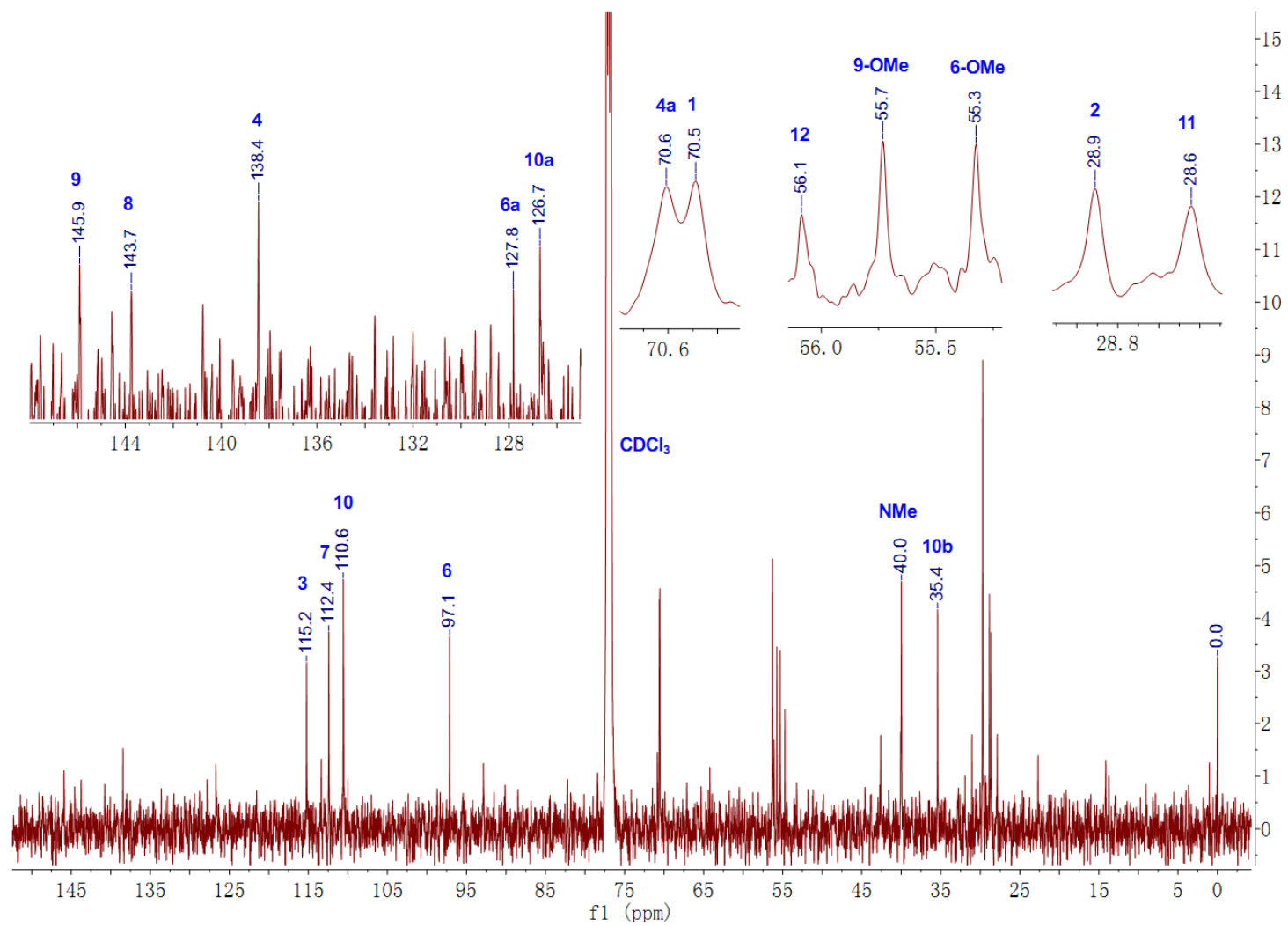
Figure S7: ^{13}C NMR spectrum of compound **1**.

7.2.2. Papiline
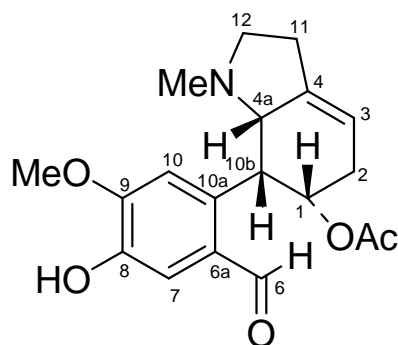
Papiline (**2**): $[\alpha]^{24}_{D}$ −29 (*c* 0.26, CHCl$_3$); CD $[\theta]^{20}_{\lambda}$: $[\theta]_{237}$ 171, $[\theta]_{253}$ -72, $[\theta]_{260}$ 81, $[\theta]_{268}$ -67, $[\theta]_{278}$ 94, $[\theta]_{289}$ -131; UV (MeOH) λ_{max} (log ε) 318 (3.27), 280 (3.48), 236 (3.76), 205 (4.20) nm; IR (CHCl$_3$) ν_{max}: 2925, 2854, 1738, 1674, 1595, 1557, 1514, 1455, 1378, 1260, 1148, 1099, 1020, 800 cm^{-1}; HREIMS of [M+H]$^{+}$ *m/z* 346.1643 (calcd. for C$_{19}$H$_{24}$NO$_5$, 346.1649).

Figure S8: Physical and spectroscopic data of compound **2**.
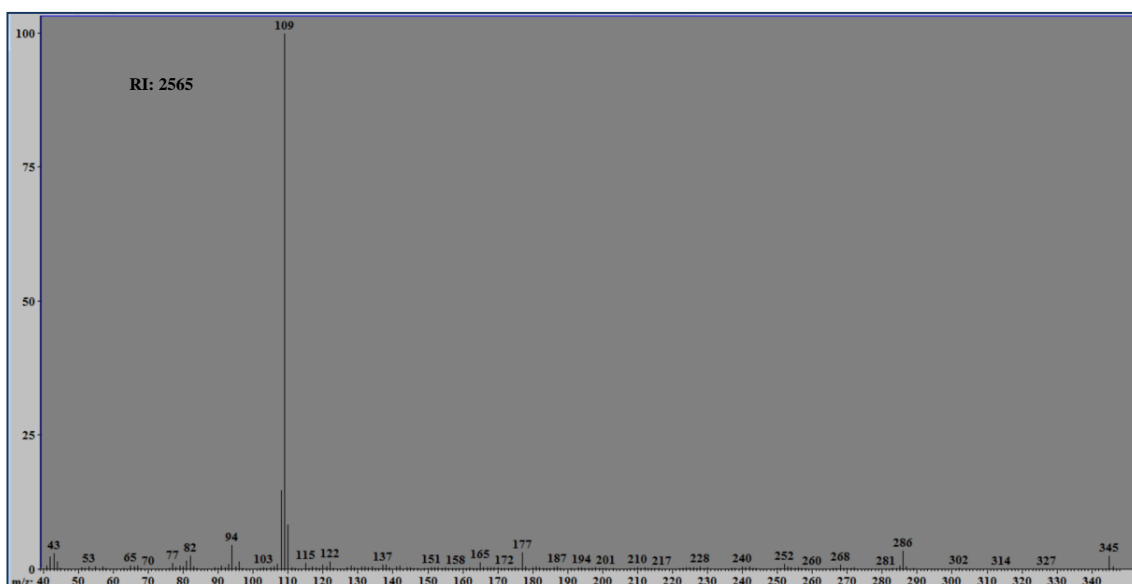
Figure S9: GC-MS spectrum of compound **2**.
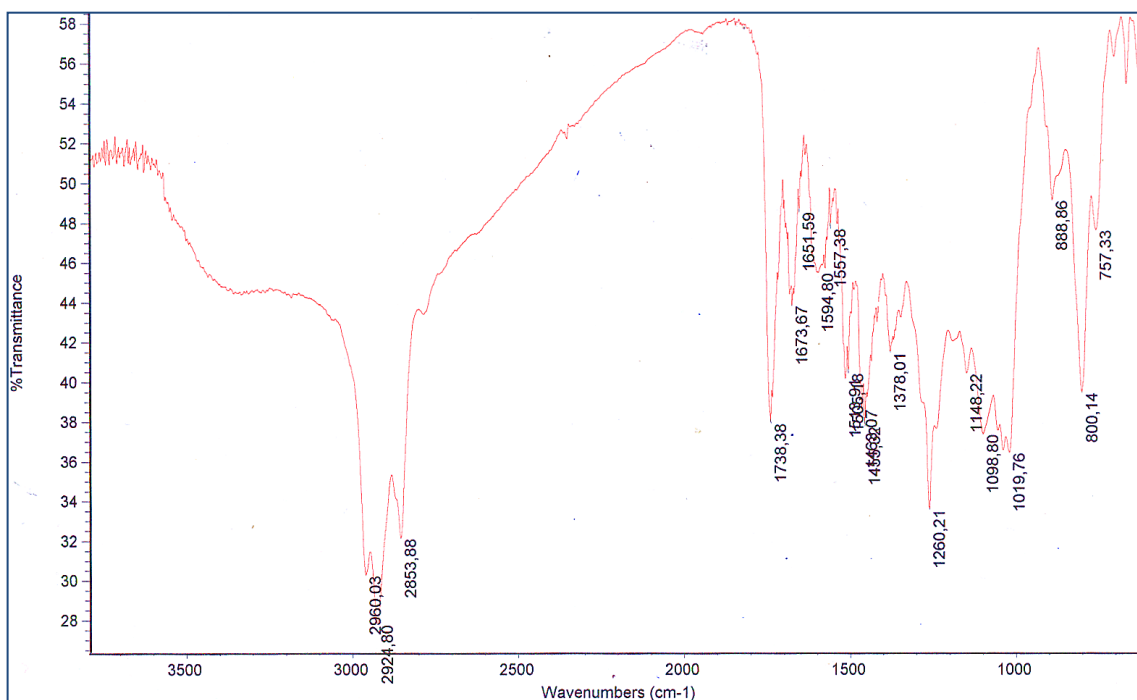
Figure S10: IR spectrum of compound **2**.

Table S2: ^{1}H NMR, COSY, NOESY, HMBC (500MHz, CDCl$_3$) and ^{13}C NMR(125MHz, CDCl$_3$) data of compound **2**.

Position	H δ (*J* in Hz)	COSY	NOESY	C δ	HMBC
1	5.45 *ddd* (10.0, 5.0, 5.0)	H-2α, H-2β, H-10b	H-2β, H-4a, H-10b	70.9 *d*	C-10a, Me<u>C</u>O
2α	2.00-2.06 *m*	H-1, H-2β, H-3, H-4a, H-11	H-2β, H-3, H-10	27.9 *t*	Me<u>C</u>O
2β	2.44 *dddd* (16.5, 5.5, 4.5, 2.5)	H-1, H-2α, H-3, H-4a, H-11	H-1, H-2α, H-3		
3	5.54 *m*	H-2α, H-2β, H-4a, H-11	H-2α, H-2β, H-11	116.1 *d*	
4				131.1 *s*	
4a	3.10 *s*	H-2α, H-2β, H-3, H-10b, H-11	H-1, H-10b, H-12β, NMe	69.5 *d*	
6	9.91 *s*		H-7, H-10b	191.7 *d*	
6a				127.2 *s*	
7	7.32 *s*		H-6	116.2 *d*	C-8, C-9, C-10a
8				144.6 *s*	
9				150.1 *s*	
10	7.29 *s*	9-OMe	H-2α, H-11, 9-OMe	112.5 *d*	C-6a, C-8, C-9, C-10b
10a				129.0 *s*	
10b	4.74 *t* (4.5)	H-1, H-4a	H-1, H-4a, H-6, NMe	35.3 *d*	C-1, C-4a, C-10
11 (2H)	2.55 *m*	H-2α, H-2β, H-3, H-4a, H-12α, H-12β	H-3, H-10, H-12α,,H-12β	28.0 *t*	
12α	3.26 *br s*	H-11, H-12β	H-11, H-12β, NMe	56.3 *t*	
12β	2.33 *q* (9.0)	H-11, H-12α	H-4a, H-11, H-12α, NMe		NMe
9-OMe	3.84 *s*	H-10	H-10	55.8 *q*	C-9
<u>Me</u>CO	1.90 *s*			21.2 *q*	Me<u>C</u>O
Me<u>C</u>O				170.6 *s*	
NMe	2.20 *s*		H-4a, H-10b, H-12α, H-12β	40.9 *q*	C-4a, C-12

Figure S11: ^{1}H NMR data of compound **2**.
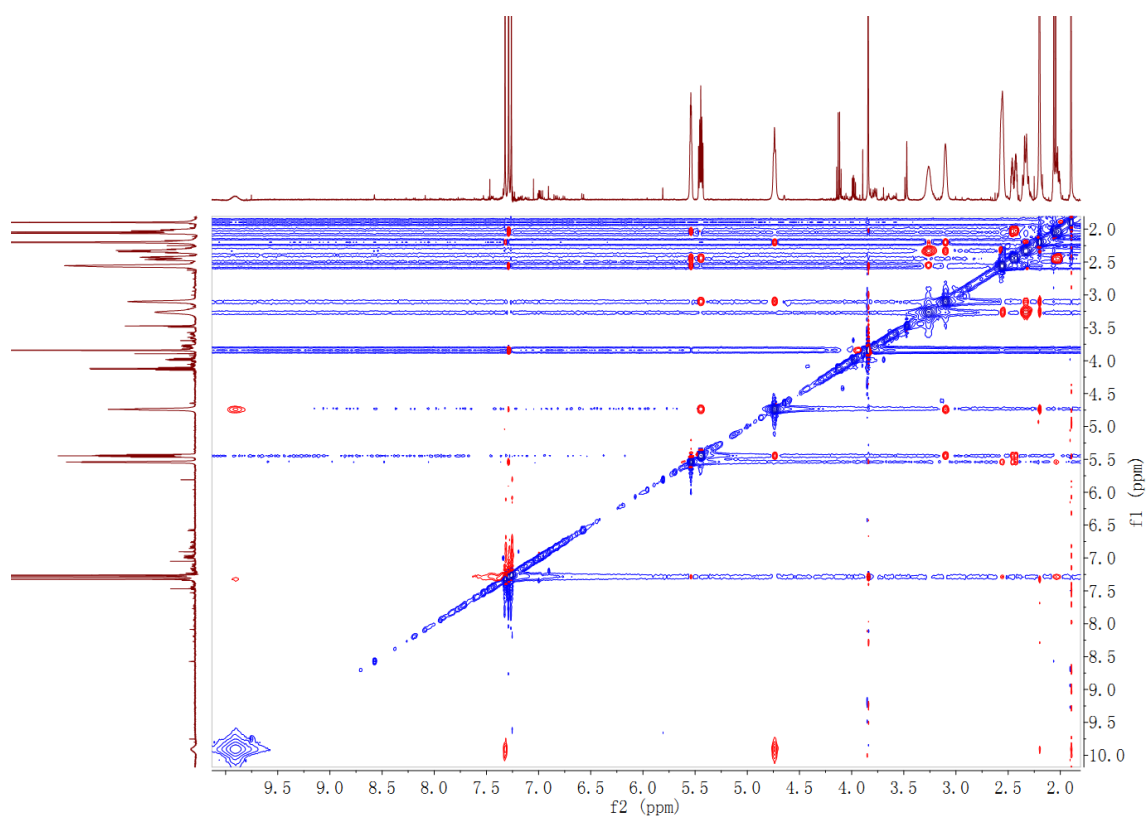
Figure S12: NOESY spectrum of compound **2**.
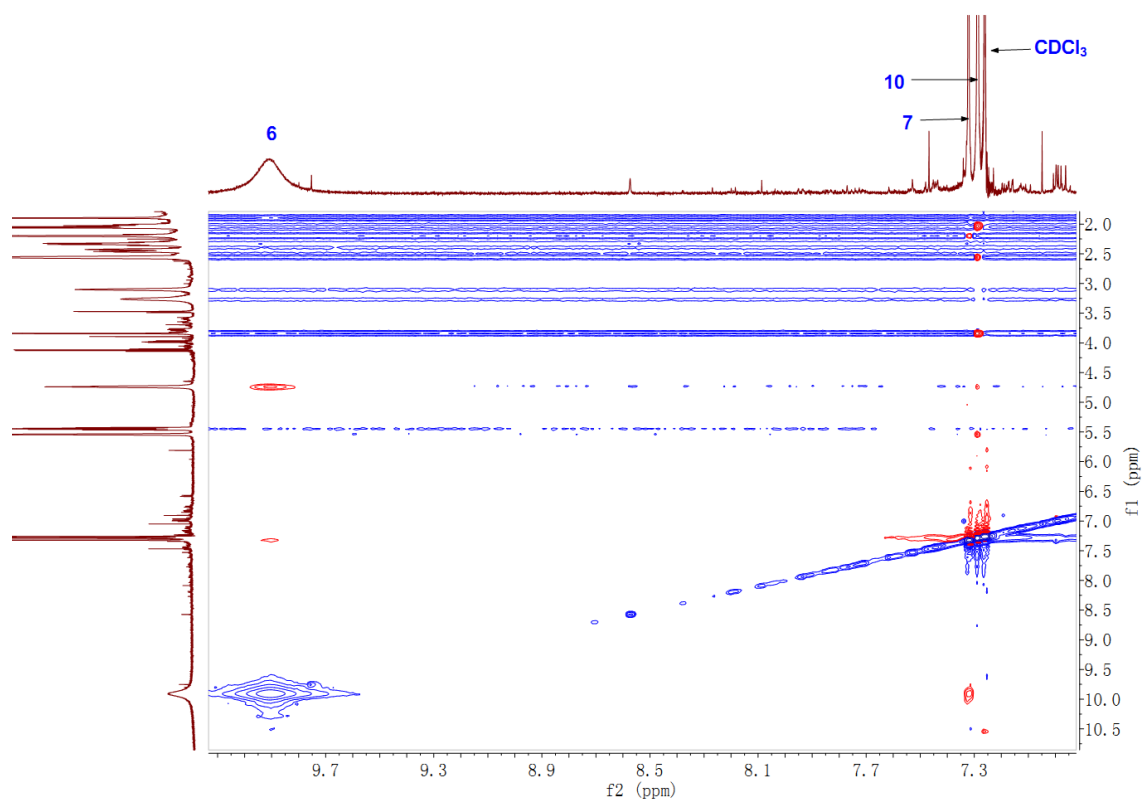
Figure S13: NOESY enlargement of compound **2**.
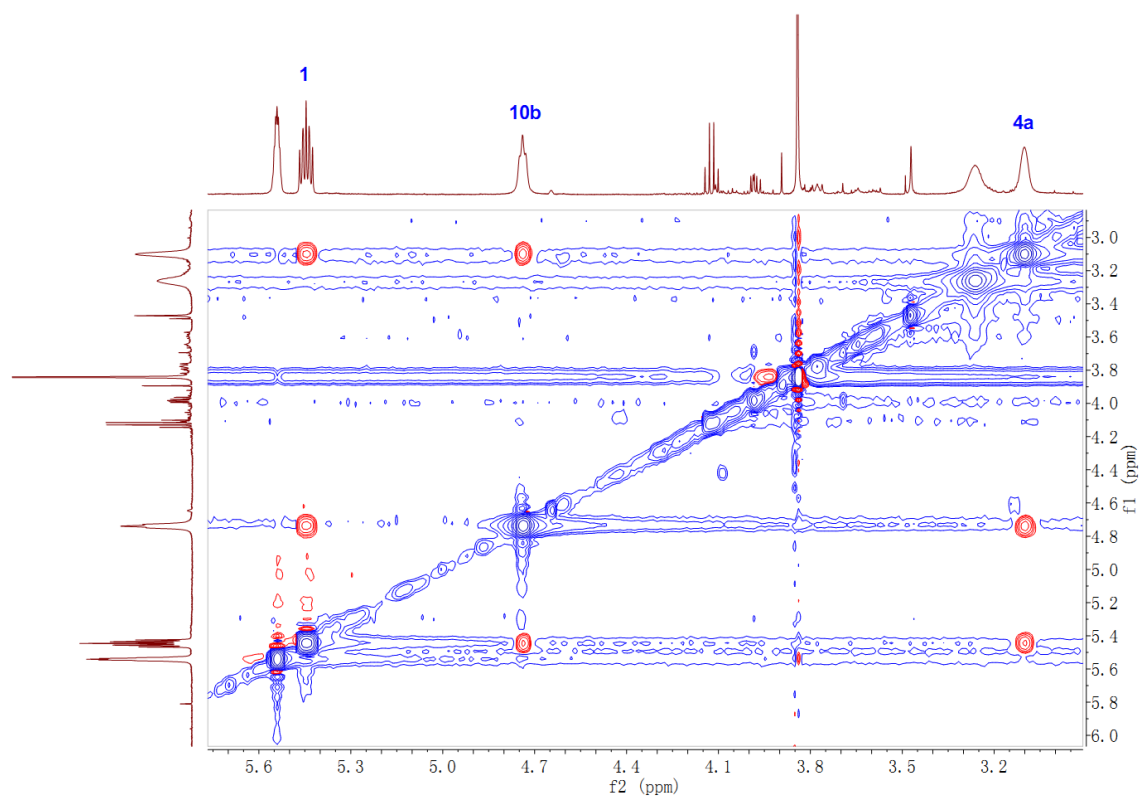
Figure S14: NOESY enlargement of compound **2**.
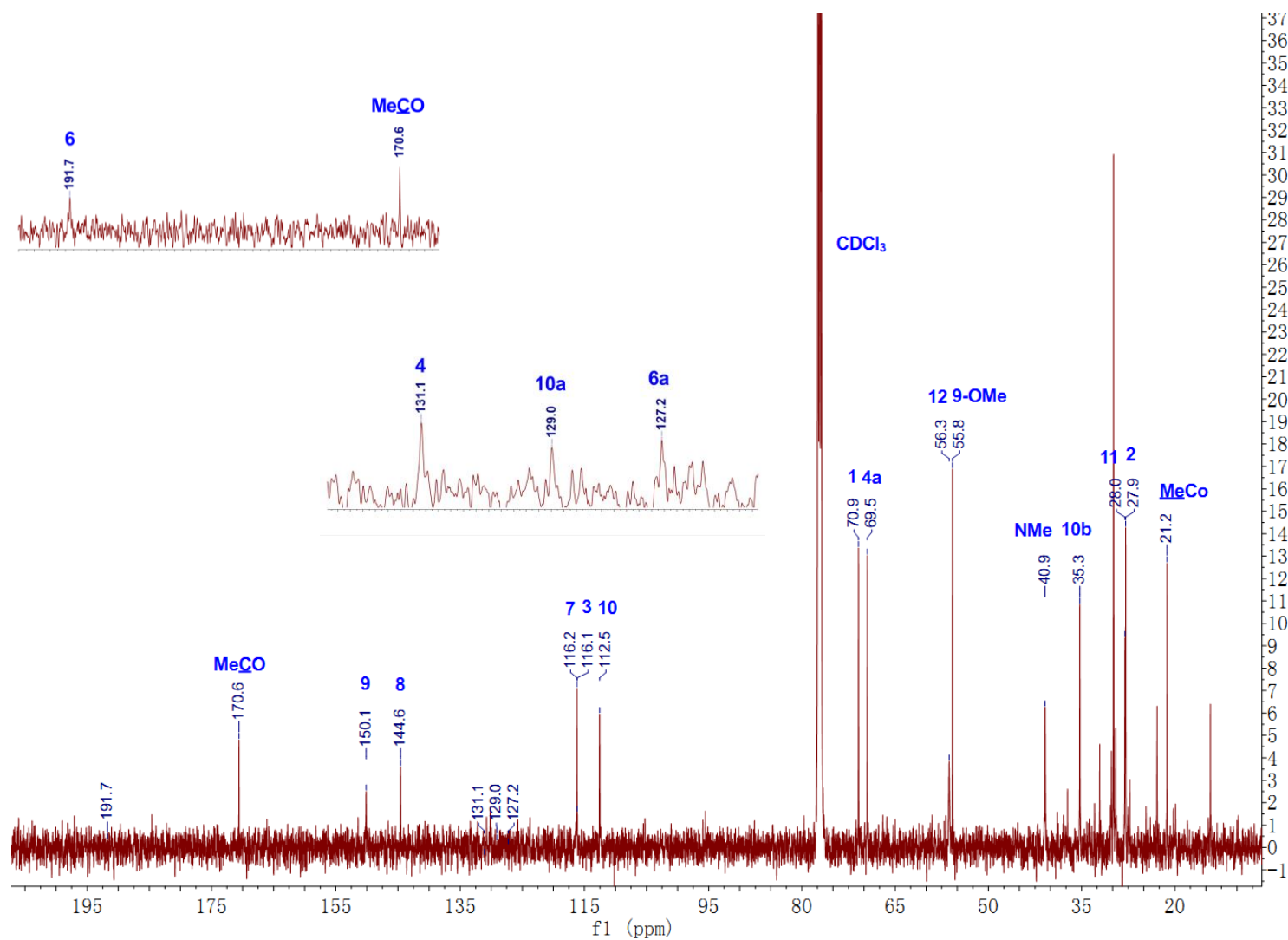
Figure S15: ^{13}C NMR spectrums of compound **2**.

7.2.3. 3-*O*-(3'-Hydroxybutanoyl)haemanthamine
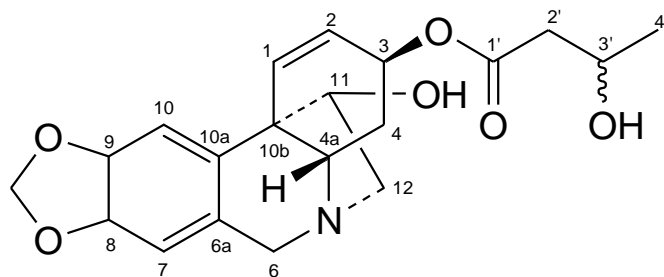
3-*O*-(3'-Hydroxybutanoyl)haemanthamine (**3**): amorphous solid; $[\alpha]^{24}_D$ −13 (*c* 0.49, CHCl$_3$); CD $[\theta]^{20}_\lambda$: $[\theta]_{249}$ −2375, $[\theta]_{275}$ +1569; UV (MeOH): λ_{max} (log ε) 292 (3.46), 238 (3.36), 206 (4.40) nm; IR (CHCl$_3$) ν_{max}: 3377, 2925, 1727, 1504, 1484, 1376, 1295, 1239, 1173, 1064, 1037, 988, 936, 853, 758 cm^{-1}; HREIMS of [M+H]$^+$ *m/z* 374.1603 (calcd. for C$_{20}$H$_{24}$NO$_6$, 374.1598).

Figure S16: Physical and spectroscopic data of compound **3**.
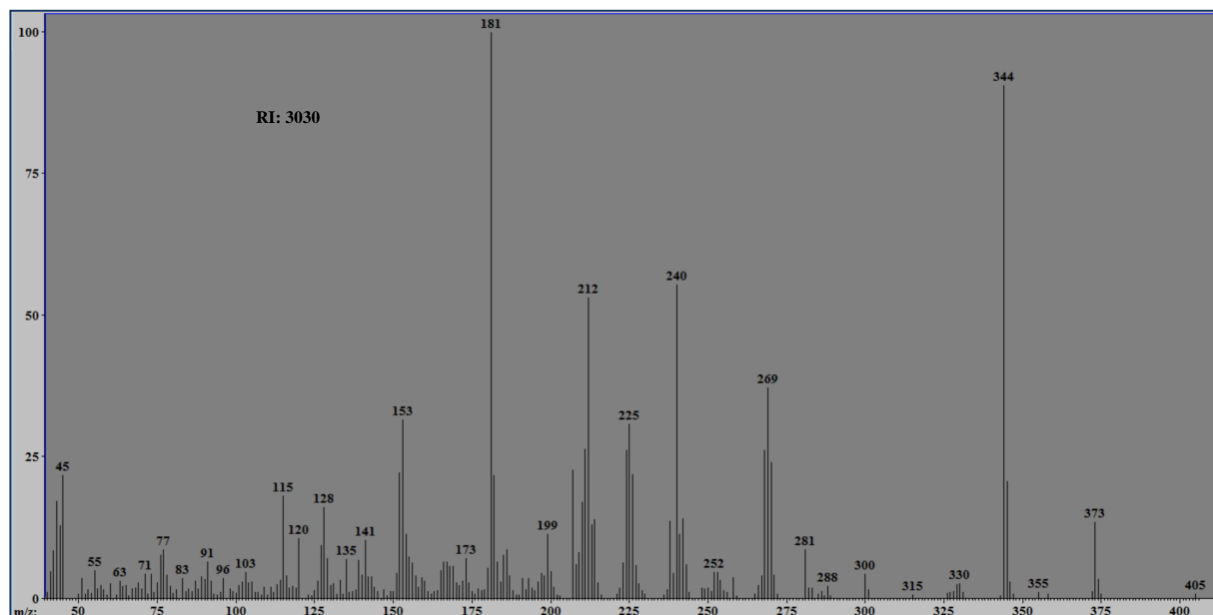
Figure S17: GC-MS spectrum of compound **3**.
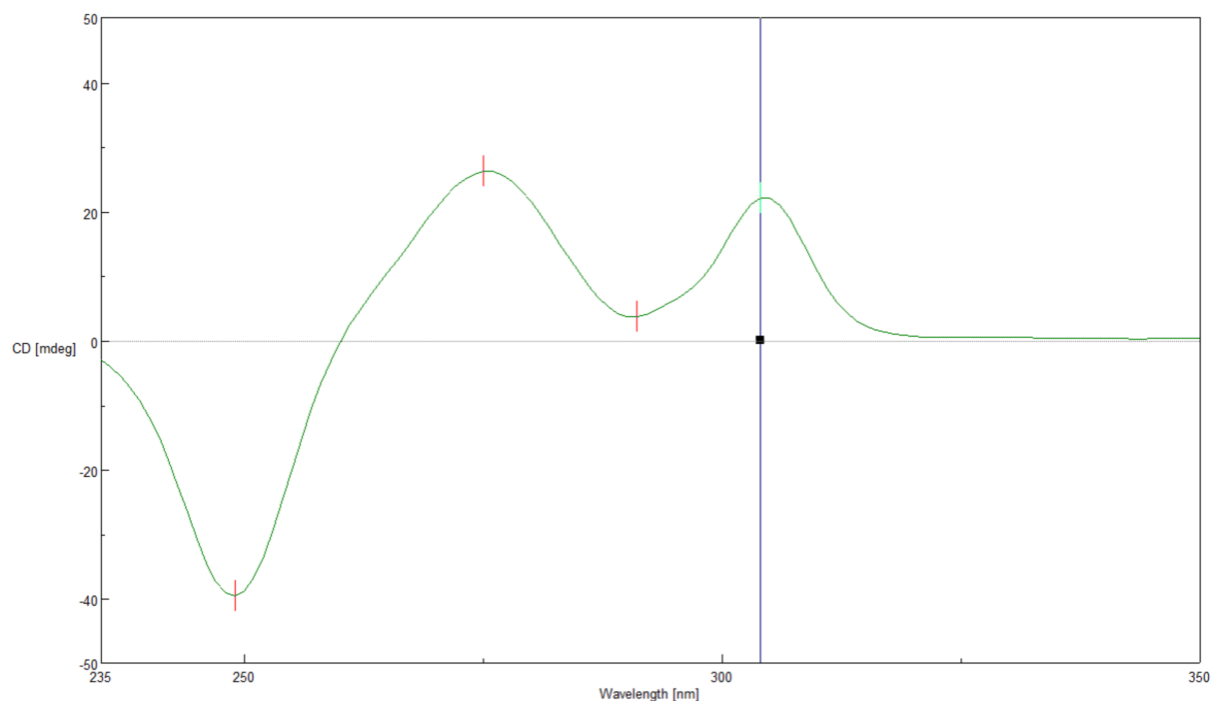
Figure S18: CD spectrum of compound **3**.

Table S3: ^{1}H NMR, COSY, NOESY, HMBC (500MHz, CDCl$_3$) and ^{13}C NMR(125MHz, CDCl$_3$) data of compound **3**.

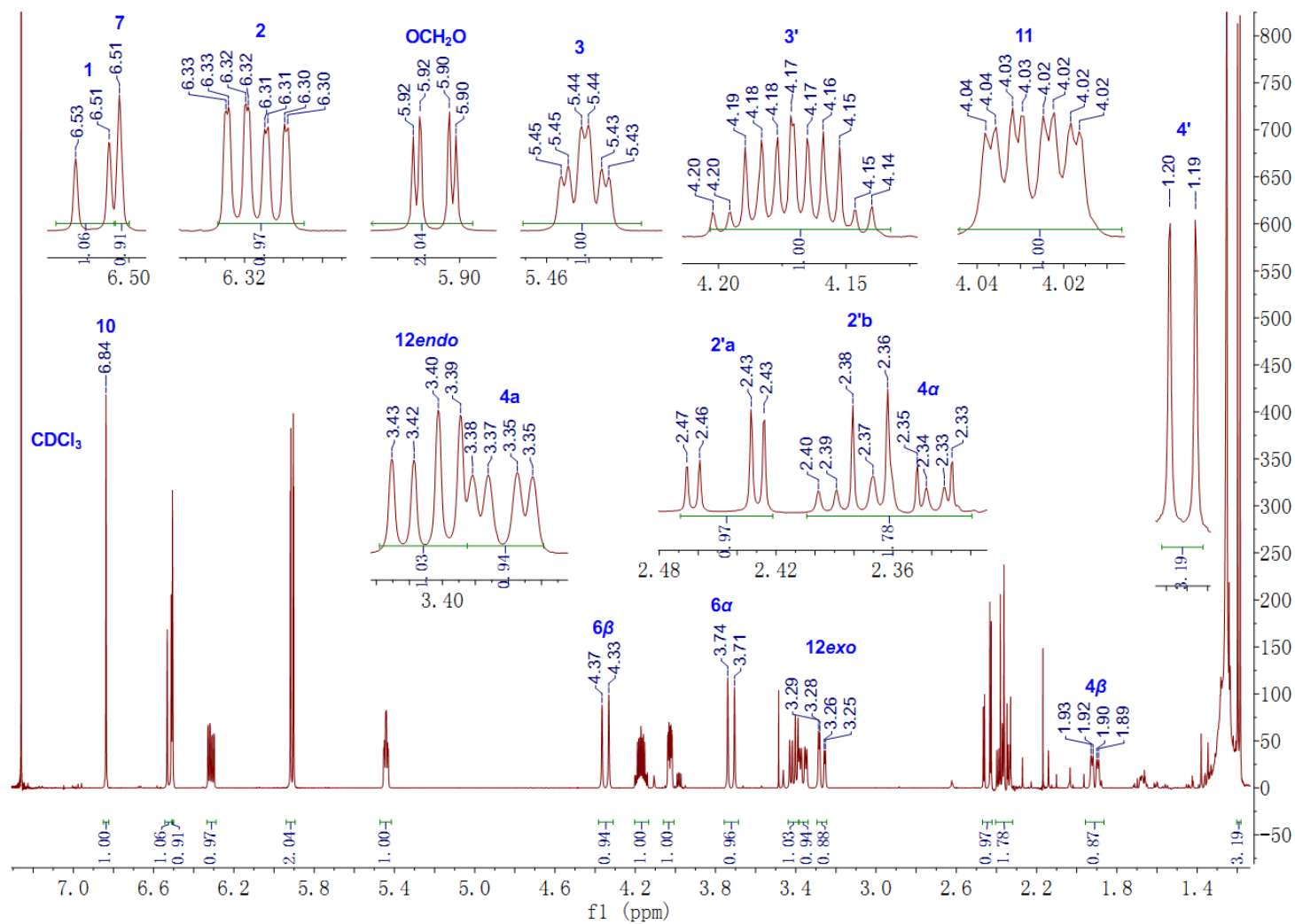| Position	H δ (*J* in Hz)	COSY	NOESY	Cδ	HMBC
1	6.52 *d* (10.1)	H-2	H-2, H-10	129.3 *d*	C-3, C-4a, C-10a, C-10b
2	6.31 *ddd* (10.1, 5.1, 0.7)	H-1, H-3	H-1, H-3	130.2 *d*	C-3, C-4, C-10b
3	5.44 *td* (4.8, 1.7)	H-2, H-4α, H-4β	H-2, H-4α, H-4β	67.2 *d*	C-1, C-2, C-4a, C-1'
4α	2.37 *td* (14.0, 4.6)	H-3, H-4β, H-4a	H-3, H-4β, H-4a H-12*exo*	29.5 *t*	C-4a
4β	1.91 *dd* (14.0, 4.6)	H-3, H-4α, H-4a	H-3, H-4α, H-4a		C-2, C-3, C-4a, C-10b
4a	3.36 *dd* (13.5, 4.5)	H-4α, H-4β	H-4α, H-4β, H-6β	63.0 *d*	C-6, C-12, C-11
6α	3.72 *d* (17.0)	H-6β, H-7	H-6β, H-12*endo*, H-7	61.5 *t*	C-4a, C-6a, C-7, C-10a
6β	4.35 *d* (17.0)	H-6α, H-7	H-4a, H-6α, H-7		C-6a, C-7, C-8, C-10a, C-11, C-12
6a				126.9 *s*	
7	6.51 *s*	H-6α, H-6β	H-6α, H-6β	107.1 *d*	C-6, C-9, C-10a
8				146.6 *s*	
9				146.8 *s*	
10	6.84 *s*		H-1	103.4 *d*	C-6a, C-8, C-10b
10a				134.8 *s*	
10b				50.2 *s*	
11	4.03 *ddd* (6.5, 3.5, 1.5)	H-12*exo*, H-12*endo*	H-12*endo*	80.3 *d*	C-4a
12*exo*	3.27 *dd* (14.0, 3.0)	H-11, H-12*endo*	H-4α, H-12 *endo*	63.6 *t*	C-4a, C-6, C-11
12*endo*	3.41 *dd* (14.0, 6.5)	H-11, H-12*exo*	H-6α, H-11, H-12 *exo*		C-6, C-4a, C-10b, C-11
OCH$_2$O	5.91 *d* (7.5)			101.1 *t*	C-8, C-9
1'				172.4 *s*	C-2'
2'a	2.45 *dd* (16.5, 3.0)	H-2'b, H-3'	H-2'b	43.0 *t*	C-1', C-3', C-4'
2'b	2.36 *dd* (16.5, 9.0)	H-2'a, H-3'	H-2'a		C-1', C-3', C-4'
3'	4.17 *ddq* (9.5, 6.3, 3.3)	H-2'a, H-2'b, H-4'	H-4'	64.4 *d*	
4'	1.19 *d* (6.3)	H-3'	H-3'	22.6 *q*	C-2', C-3'

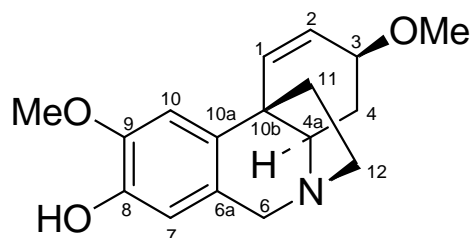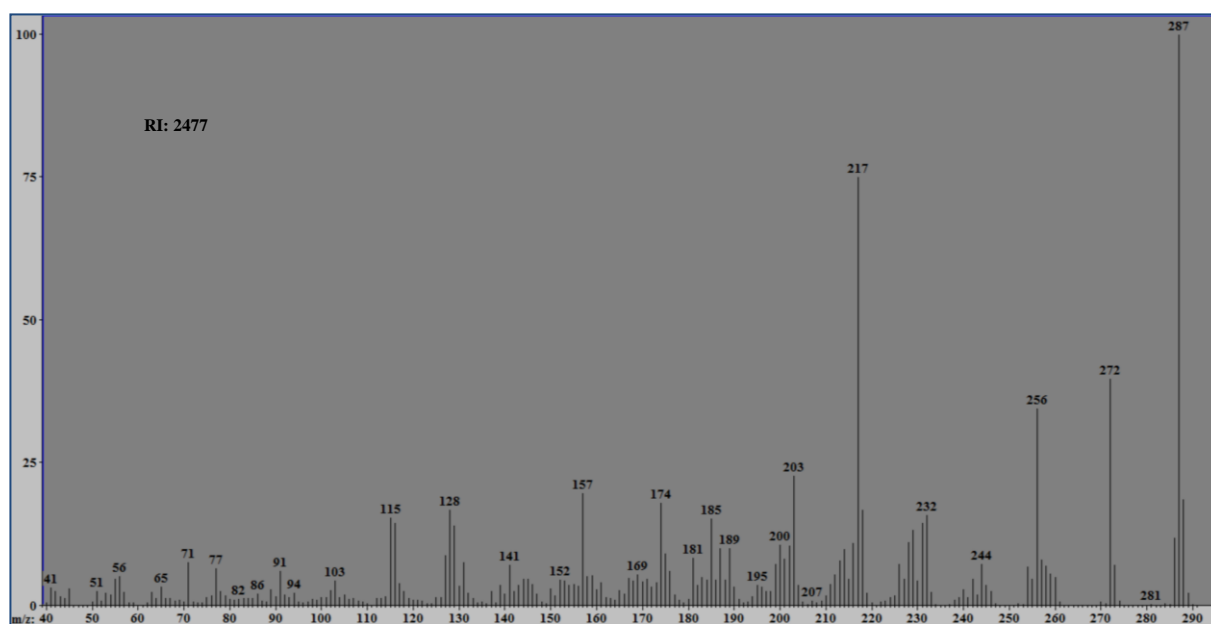**Figure S19:** ^{1}H NMR spectrum of compound **3**.
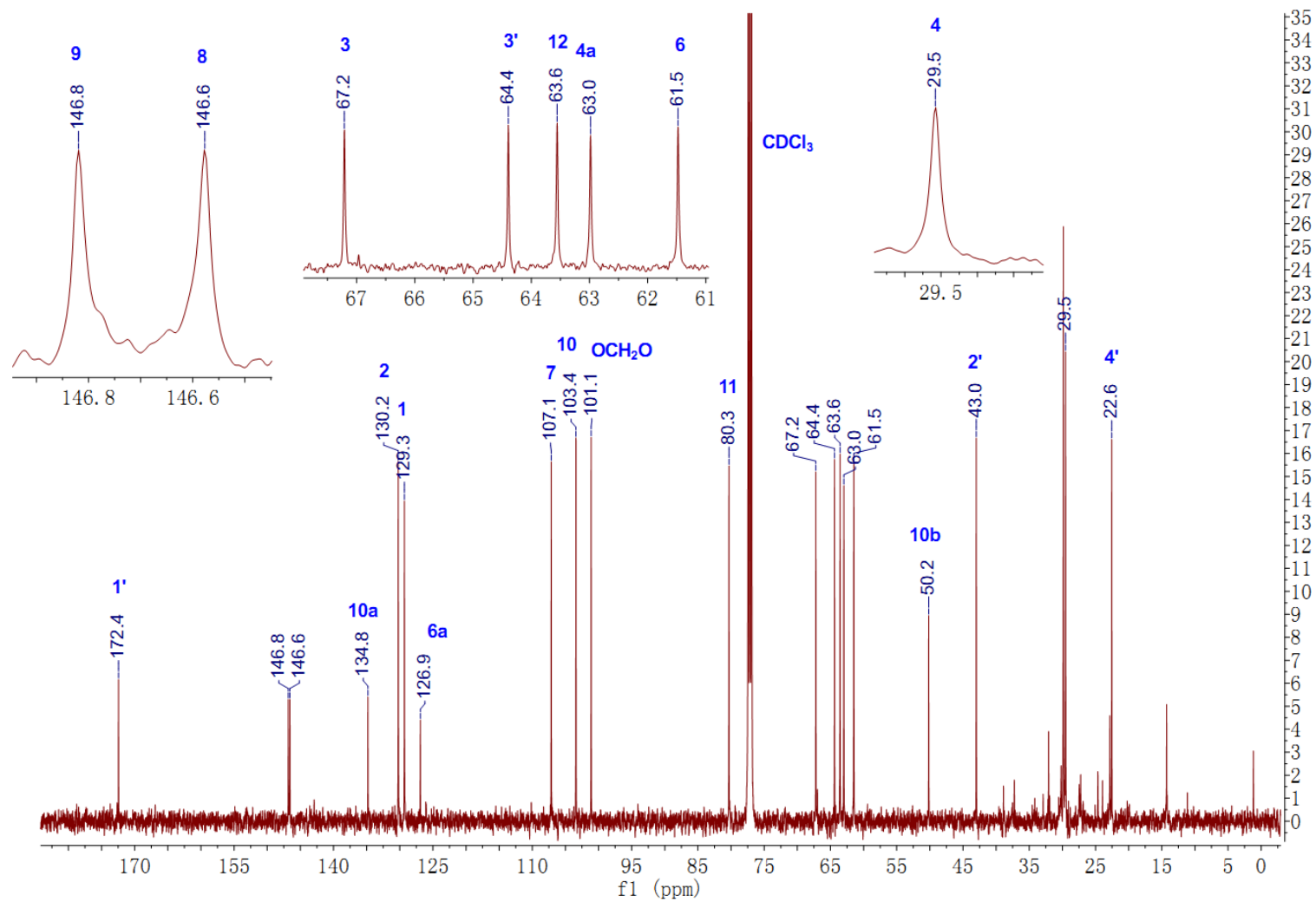
Figure S20: ^{13}C NMR spectrum of compound **3**.
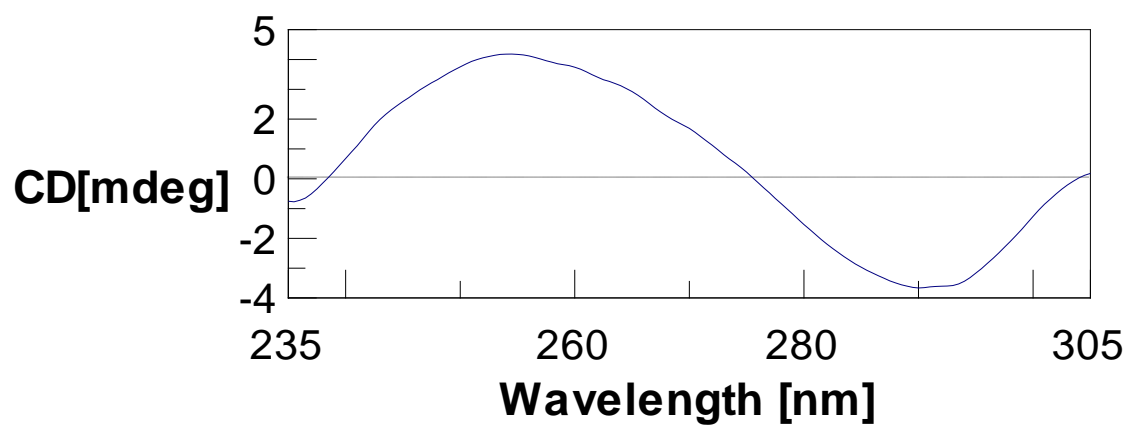
7.2.4. 3-*O*-methyl-epimacowine

3-*O*-Methyl-.epimacowine (**4**): $[\alpha]^{22}_D$ -47 (*c* 0.42, CHCl$_3$); CD $[\Theta]^{20}_\lambda$: $[\Theta]_{254}$ +2528, $[\Theta]_{290}$ +2214; UV (MeOH) λ_{max}(log ε) 230 (3.31), 288 (3.23) nm; IR (CHCl$_3$) ν_{max} 2925, 2854, 1507, 1461, 1312, 1277, 1218, 1097, 753 cm^{-1}; HRESIMS of [M + H]$^+$ *m/z* 288.1595 (calcd for C$_{17}$H$_{22}$NO$_3$, 288.1594).

Figure S21: Physical and spectroscopic data of compound **4**.

Figure S22: GC-MS spectrum of compound **4**.

Figure S23: CD spectrum of compound **4**.

Table S4: ^{1}H NMR, COSY, NOESY, HMBC (500MHz, CDCl$_3$) and ^{13}C NMR (125MHz, CDCl$_3$) data of compound **4**.

| POSITION	δ$_H$ (*J* in Hz)	COSY	NOESY	HSQC	HMBC
1	6.48 *dd* (10.5, 2.0)	H-2	H-2, H-10	128.9 *d*	C-3, C-4a, C-10a
2	5.84 *dt* (10.0, 1.5)	H-1	H-1, H-3, 3-OMe	129.0 *d*	C-4, C-10b
3	4.00 *ddt* (10.5, 5.5, 2.0)	H-4α, H-4β	H-2, H-4α, H-4a, 3-OMe	76.2 *d*	C-1, C-3OMe
4α	2.29 *br dt* (12.0, 4.0)	H-3, H-4β, H-4a	H-3, H-4a, H-4β, 3-OMe	30.7 *t*	C-2, C-3, C-4a, C-10b
4β	1.58 *ddd* (13.4, 12.0, 10.5)	H-3, H-4α, H-4a	H-4α, H-11*exo*, H-12*exo*, 3-OMe		C-2, C-3, C-4a, C-10b
4a	3.28 *dd* (13.0, 4.0)	H-4α, H-4β	H-3, H-4α, H-6α	66.7 *d*	C-3, C-4, C-6, C-10a, C-11, C-12
6α	4.45 *d* (17.0)	H-6β	H-4a, H-6β, H-7	61.3 *t*	C-6a, C-7, C-10a, C-12
6β	3.82 *d* (16.5)	H-6α	H-6α, H-7, H-12*endo*		C-4a, C-6a, C-7, C-10a, C-12
6a				124.9 *s*	
7	6.59 *s*		H-6α, H-6β	112.9 *d*	C-6, C-9, C-10a
8				144.2 *s*	
9				145.1 *s*	
10	6.78 s		H-1, 9-OMe	104.8 *d*	C-6a, C-8, C-10a, C-10b
10a				136.6 *s*	
10b				44.5 *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*	44.7 *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-4β, 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-6β, H-11*endo*, H-12*exo*	53.0 *t*	C-4a, C-6, C-10b
12*exo*	3.50 *ddd* (12.5, 10.5, 4.5)	H-11*endo*, H-11*exo*, H-12*endo*	H-4β, H-12*endo*, H-11*exo*		C-6, C-10b
3-OMe	3.42 *s* (3H)		H-2, H-3, H-4α, H-4β	56.0 *q*	C-3
9-OMe	3.89 *s* (3H)		H-10	56.1 *q*	C-9
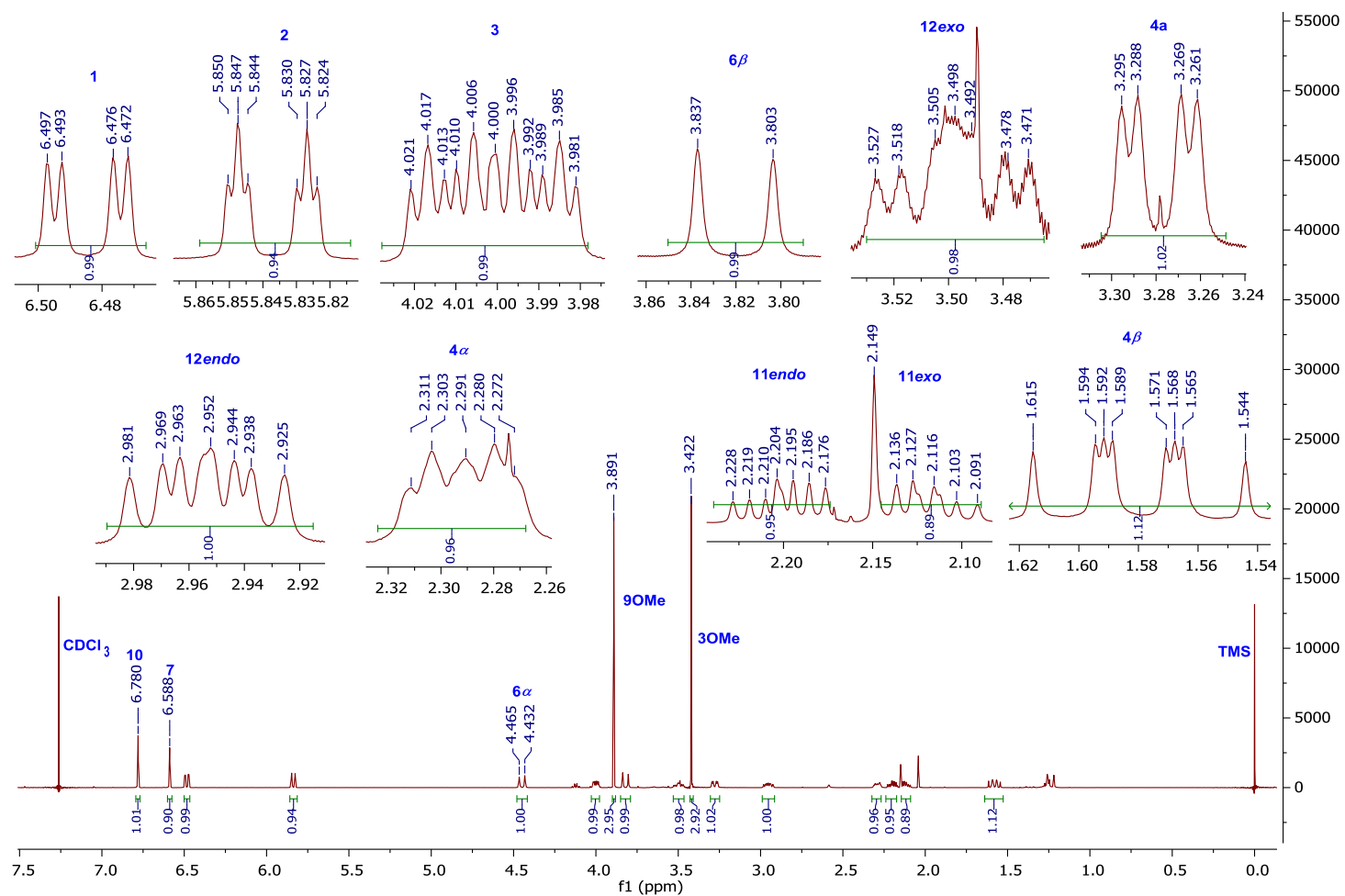
Figure S24: ^{1}H NMR spectrum of compound **4**.
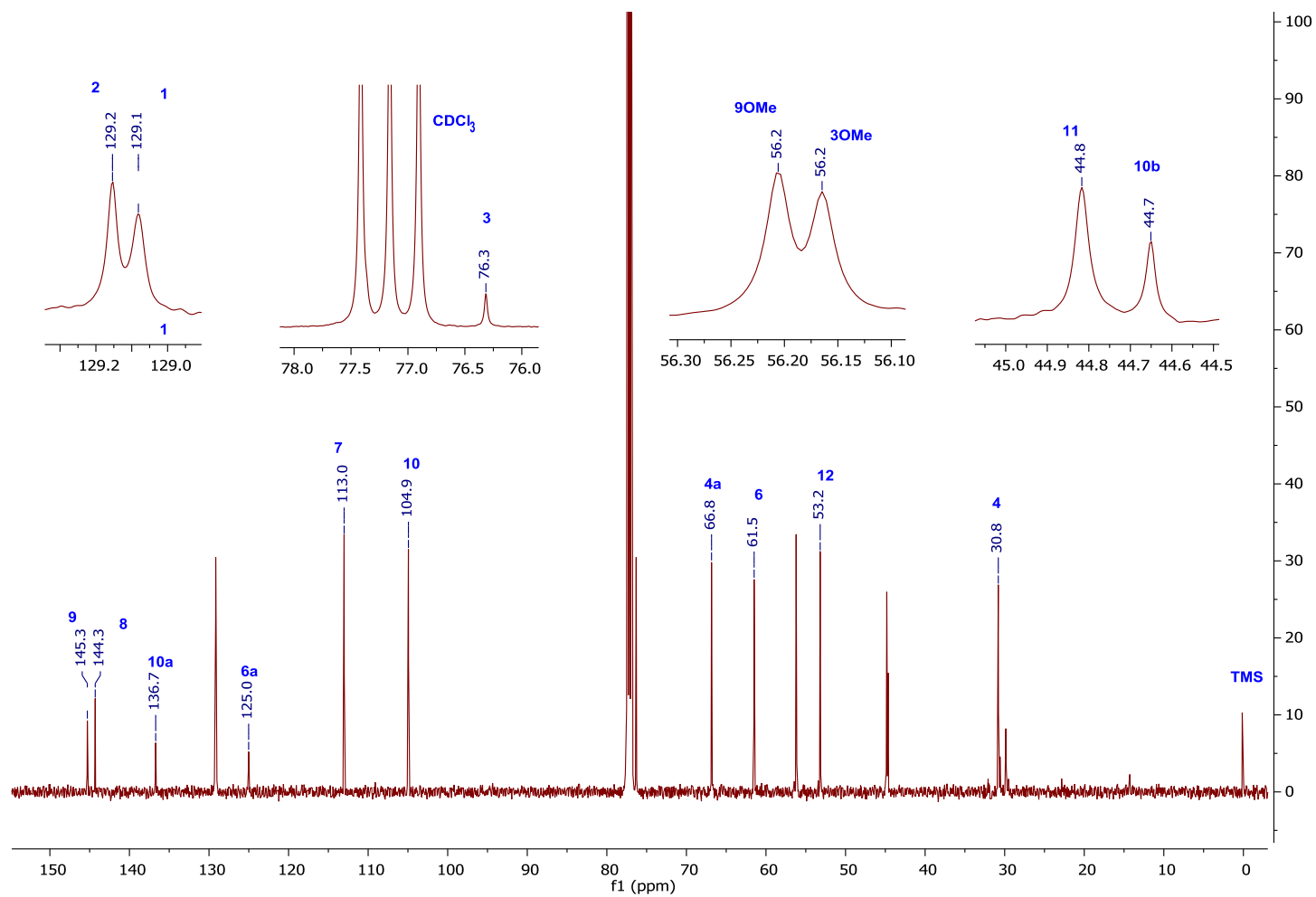
Figure S25: ^{13}C NMR spectrum of compound **4**.

7.3. Appendix Ⅲ: Photos of flowers of Amaryllidaceae

7.3.1. Genus *Lycoris*

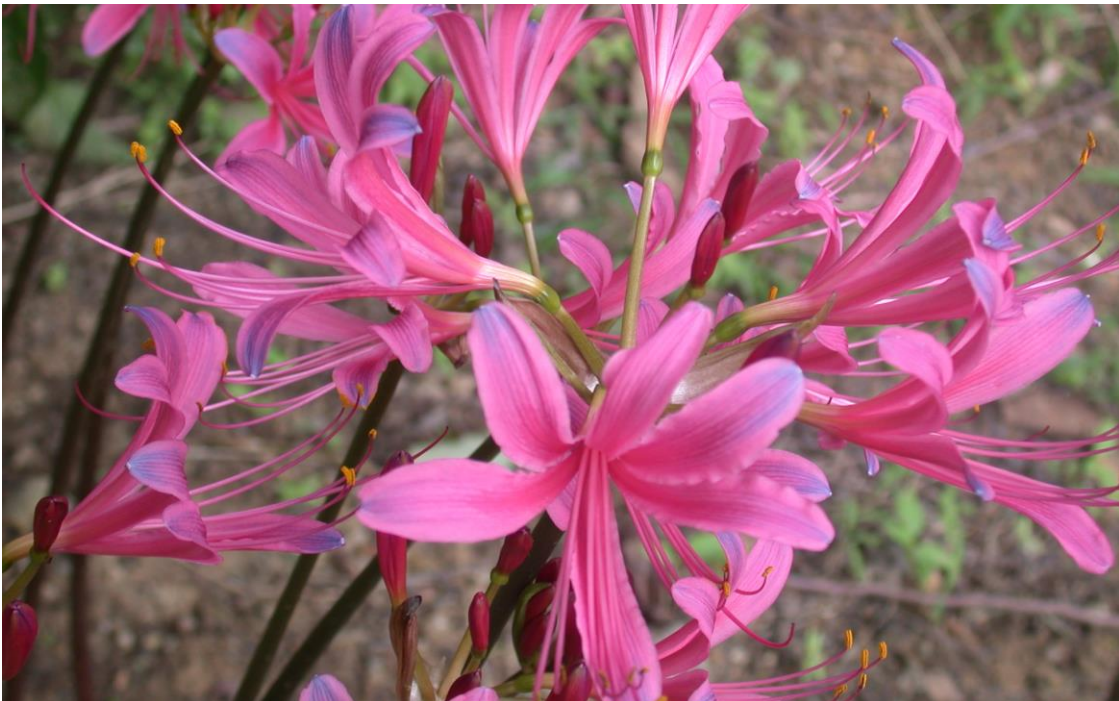#### 7.3.1.1. *Lycoris albiflora*

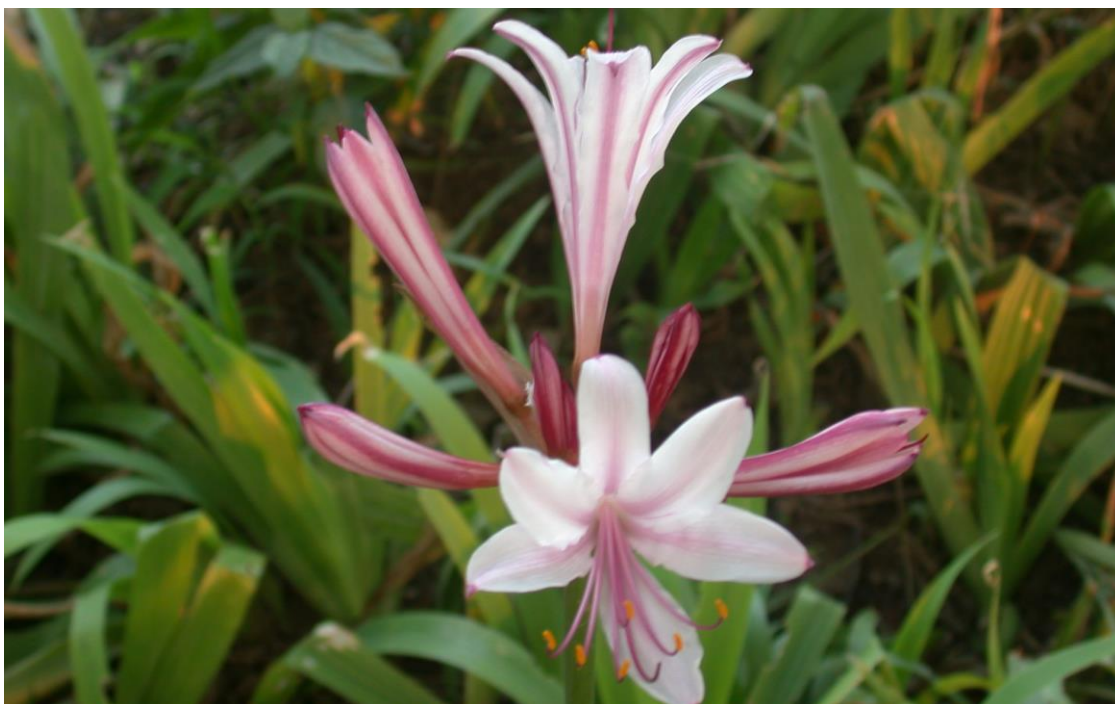#### 7.3.1.2. *Lycoris aurea*

7.3.1.3. *Lycoris chinensis*

7.3.1.4. *Lycoris haywardii*

7.3.1.5. *Lycoris incarnata*

7.3.1.6. *Lycoris longituba*

7.3.1.7. *Lycoris radiata*

7.3.1.8. *Lycoris radiata* var. *pumila*

7.3.1.9. *Lycoris sprengeri*

7.3.1.10. *Lycoris squamigera*

7.3.2. Genus *Hippeastrum*

7.3.2.1. *Hippeastrum papilio*

7.3.2.2. *Hippeastrum calyptratum*