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3. Chemical and biological aspects of Amaryllidaceae alkaloids

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Abstract. The Amaryllidaceae alkaloids represent a large (over 300 alkaloids have been isolated) and still expanding group of biogenetically related isoquinoline alkaloids that are found exclusively in plants belonging to this family. In spite of their great variety of pharmacological and/or biological properties, only galanthamine is used therapeutically. First isolated from *Galanthus* species, this alkaloid is a long-acting, selective, reversible and competitive inhibitor of acetylcholinesterase, and is used for the treatment of Alzheimer's disease. Other Amaryllidaceae alkaloids of pharmacological interest will also be described in this chapter.

Introduction

The Amaryllidaceae are richly represented in the tropics and have pronounced centers of diversity in South-Africa and the Andean region.

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Some genera are also found in the Mediterranean area and temperate regions of Asia.

A particular characteristic of Amaryllidaceae is a consistent presence of an exclusive group of alkaloids, which have been isolated from the plants of all the genera of this family. The Amaryllidaceae alkaloids represent a large and still expanding group of isoquinoline alkaloids, the majority of which are not known to occur in any other family of plants. Since the isolation of the first alkaloid, lycorine, from *Narcissus pseudonarcissus* in 1877, substantial progress has been made in examining the Amaryllidaceae plants, although they still remain a relatively untapped phytochemical source [1]. At present, over 300 alkaloids have been isolated from plants of this family [2] and, although their structures vary considerably, these alkaloids are considered to be biogenetically related.

The large number of structurally diverse Amaryllidaceae alkaloids are classified mainly into nine skeleton types, for which the representative alkaloids are: norbelladine, lycorine, homolycorine, crinine, haemanthamine, narciclasine, tazettine, montanine and galanthamine (Fig. 1). With the aim of unifying the numbering system of the different skeleton types, Ghosal's model will be used in this review [3].

As the alkaloids of the Amaryllidaceae family species fall mainly into one of these subgroups, they can serve as a classifying tool for including genera and species in this family. Recently, Unver and Jin have proposed subgroups for some skeleton types, according to the structures of new alkaloids isolated from *Galanthus* species [4,5]. Furthermore, although it is unusual to find other types of alkaloids in this family, if present, they are always accompanied by typical Amaryllidaceae alkaloids. The classical example is the reported presence of the mesembrane (*Sceletium*) alkaloids, generally found in the Aizoaceae family [6,7], in a few species of Amaryllidaceae such as *Hymenocallis arenicola*, *Crinum oliganthum*, *Narcissus pallidulus* and *Narcissus triandrus* [8-10]. In turn, the unexpected isolation of (-)-capnoidine and (+)-bulbocapnine from *Galanthus nivalis* subsp. *cilicicus* is the first report of the occurrence of classical isoquinoline alkaloids in a typical member of the Amaryllidaceae [11].

Plants of the Amaryllidaceae family have been used for thousands of years as herbal remedies. The alkaloids from their extracts have been the object of active chemical investigation for nearly 200 years. Over the past three decades many have been isolated, screened for different biological activities, and synthesized by a number of research groups.

The structural elucidation of the Amaryllidaceae alkaloids and their biological profiles, as well as their synthesis, have been summarized in the last few years [12-14], which, together with the regular publications of the journal

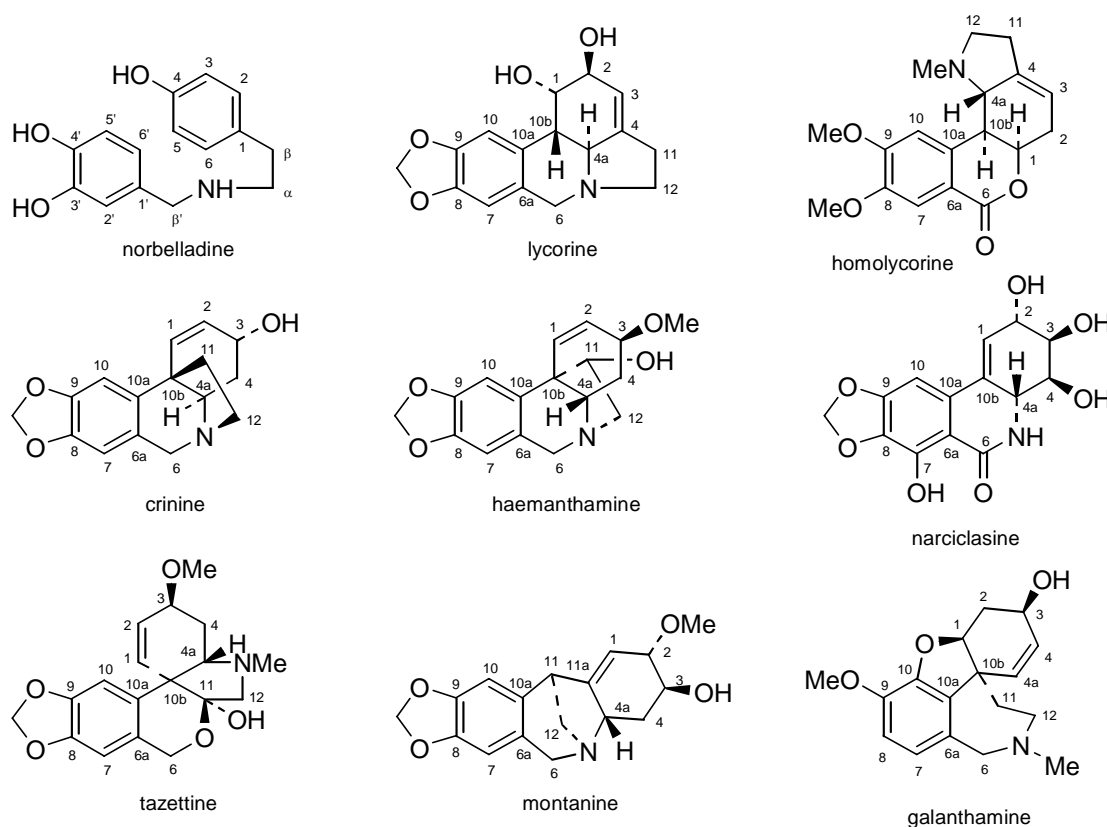


Figure 1. Amaryllidaceae alkaloid types.

Natural Products Reports [5,15-17] over the last decade, represents a valuable source of information.

The present review provides coverage of the biosynthesis, NMR spectroscopy and biological activity of the Amaryllidaceae alkaloids up to the end of 2010.

1. Biosynthetic pathways

Most of the biosynthetic research done on Amaryllidaceae alkaloids was carried out in the sixties and early seventies. Since then, the only noteworthy study has been the biosynthesis of galanthamine and related alkaloids [18]. As in most alkaloid biosyntheses, that of the Amaryllidaceae follows a pattern made up of certain steps.

1.1. Enzymatic preparation of the precursors

Although L-phenylalanine (L-phe) and L-tyrosine (L-tyr) are closely related in chemical structure, they are not interchangeable in plants. In the Amaryllidaceae alkaloids, L-phe serves as a primary precursor of the C₆-C₁

fragment, corresponding to ring A and the benzylic position (C-6), and L-tyr is the precursor of ring C, the two-carbon side chain (C-11 and C-12) and nitrogen, C₆-C₂-N. The conversion of L-phe to the C₆-C₁ 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 is performed via cinnamic acids. The presence of the enzyme phenylalanine ammonia lyase (PAL) has been demonstrated in Amaryllidaceae plants [19] and the elimination of ammonia mediated by this enzyme is known to occur in an antiperiplanar manner to give *trans*-cinnamic acid, with loss of the β -*pro*-S hydrogen [20]. Thus, it may be expected that L-phe would be incorporated into Amaryllidaceae alkaloids with retention of the β -*pro*-R hydrogen. However, feeding experiments in *Narcissus* 'King Alfred' showed that tritium originally present at C- β of L-phe, whatever the configuration, was lost in the formation of several haemanthamine and homolycorine type alkaloids, which led to the conclusion that fragmentation of the cinnamic acids involves oxidation of C- β to ketone or acid level, the final product being protocatechuic aldehyde or its derivatives (Fig. 2). On the other hand, L-tyr is degraded no further than tyramine before incorporation into the Amaryllidaceae alkaloids.

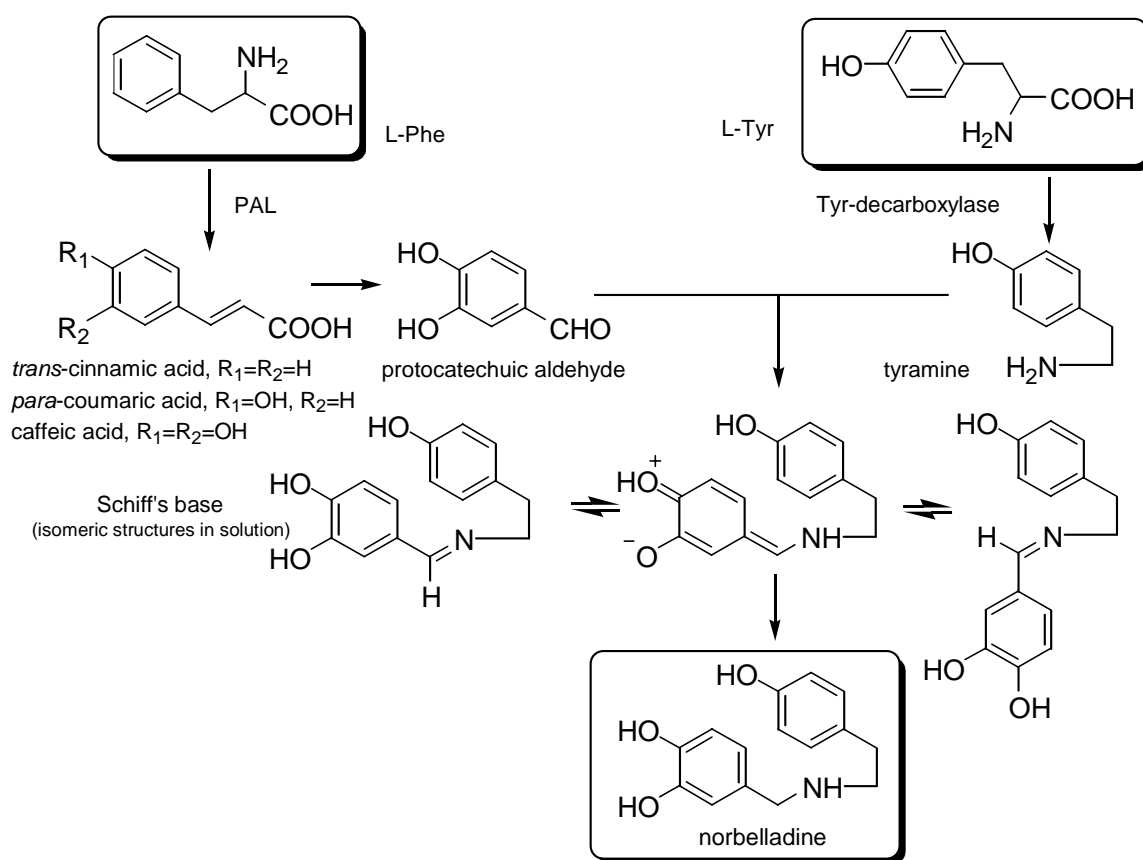


Figure 2. Biosynthetic pathway to norbelladine.

1.2. Primary cyclization mechanisms

Tyramine and protocatechuic aldehyde or its derivatives are logical components for the biosynthesis of the precursor norbelladine. This pivotal reaction represents the entry of primary metabolites into a secondary metabolic pathway. The junction of the amine and the aldehyde results in a Schiff's base, two of which have been isolated up to now from several *Crinum* species: craugsodine [21] and isocraugsodine [22]. The existence of Schiff's bases in nature as well as their easy conversion into the different ring-systems of the Amaryllidaceae alkaloids suggest that the initial hypothesis about this biosynthetic pathway was correct.

1.3. Enzymatic preparation of intermediates

In 1957, Barton and Cohen [23] proposed that norbelladine or related compounds could undergo oxidative coupling in Amaryllidaceae plants, once ring A had been suitably protected by methylation, resulting in the different skeletons of the Amaryllidaceae alkaloids (Fig. 3). The key intermediate in most of cases is *O*-methylnorbelladine.

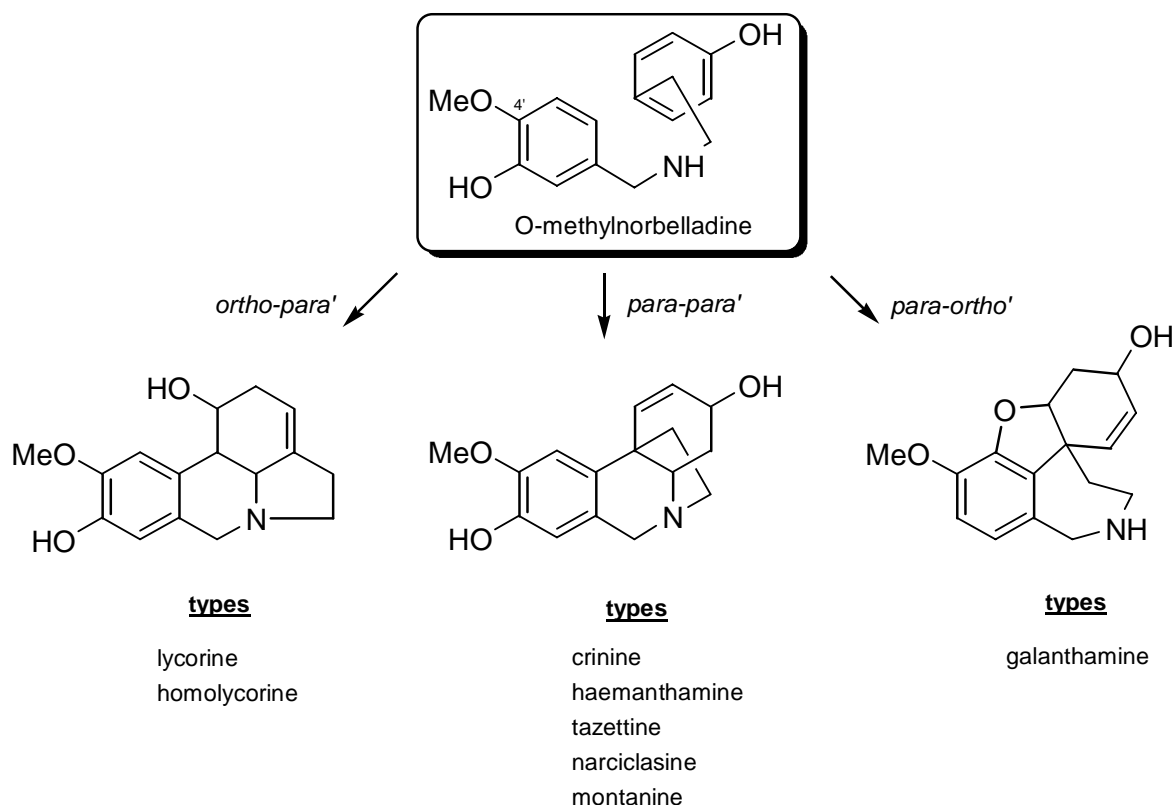


Figure 3. Phenol oxidative coupling in Amaryllidaceae.

1.4. Secondary cyclization, diversification and restructuring

Secondary cyclization is produced by an oxidative coupling of *O*-methylnorbelladine.

1.4.1. Lycorine and homolycorine types

The alkaloids of this group are derivatives of the pyrrolo[*de*]phenanthridine (lycorine type) and the 2-benzopirano-[3,4-*g*]indole (homolycorine type) skeletons, and both types originate from an *ortho-para'* phenol oxidative coupling (Fig. 4).

The biological conversion of cinnamic acid via hydroxylated cinnamic acids into the C₆-C₁ unit of norpluviine has been used in a study of hydroxylation mechanisms in higher plants [24]. When [3-³H, β-¹⁴C] cinnamic acid was fed to *Narcissus* 'Texas' a tritium retention in norpluviine of 28% was observed, which is very close to the predicted value resulting from *para*-hydroxylation with hydrogen migration and retention.

In the conversion of *O*-methylnorbelladine into lycorine, the labelling position [3-³H] on the aromatic ring of L-tyr afterwards appears at C-2 of norpluviine, which is formed as an intermediate, the configuration of the tritium apparently being β [25]. This tritium is retained in subsequently formed

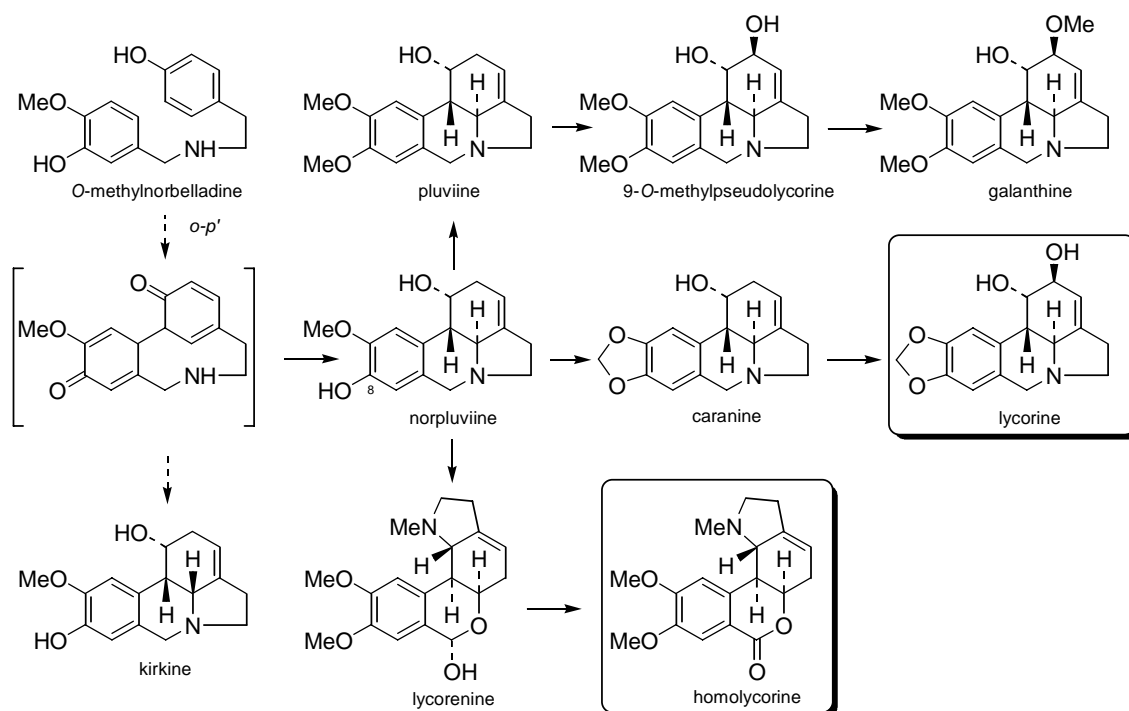


Figure 4. Alkaloids proceeding from an *ortho-para'* coupling.

lycorine, which means that hydroxylation at C-2 proceeds with an inversion of configuration [26] by a mechanism involving an epoxide, with ring opening followed by allylic rearrangement of the resulting alcohol (Fig. 5). Supporting evidence comes from the incorporation of $[2\beta\text{-}^3\text{H}]$ caranine into lycorine in *Zephyranthes candida* [27]. However, an hydroxylation of caranine in *Clivia miniata* occurring with retention of configuration was also observed [28]. Further, $[2\alpha\text{-}^3\text{H}; 11\text{-}^{14}\text{C}]$ caranine was incorporated into lycorine with high retention of tritium at C-2, indicating that no 2-oxo-compound can be implicated as an intermediate.

The conversion of the *O*-methoxyphenol to the methylenedioxy group may occur late in the biosynthetic pathway. Tritiated norpluviine is converted to tritiated lycorine by *Narcissus* 'Deanna Durbin', which not only demonstrates the previously mentioned conversion but also indicates that the C-2 hydroxyl group of lycorine is derived by allylic oxidation of either norpluviine or caranine [29].

Regarding the conversion of $[2\beta\text{-}^3\text{H}, 8\text{-OMe-}^{14}\text{C}]$ pluviine into galanthine, in *Narcissus* 'King Alfred', the retention of 79% of the tritium label confirms that hydroxylation of C-2 may occur with inversion of configuration [30].

It was considered [31] that another analogous epoxide could give narcissidine in the way shown by loss of the *pro-S* hydrogen from C-11, galanthine being a suitable substrate for epoxidation. Labelled $[\alpha\text{-}^{14}\text{C}, \beta\text{-}^3\text{H}]$ -*O*-methylnorbelladine, when fed to *Narcissus* 'Sempre Avanti' afforded galanthine (98% of tritium retention) and narcissidine (46% tritium retention). Loss of hydrogen from C-11 of galanthine was therefore stereospecific. In the nineties, Kihara et al. [32] isolated a new alkaloid, incartine, from flowers of *Lycoris incarnata*, which could be considered as the biosynthetic intermediate of this pathway (Fig. 6).

The biological conversion of protocatechuic aldehyde into lycorenine, which proceeds via *O*-methylnorbelladine and norpluviine, first involves a reduction of the aldehyde carbonyl, and afterwards, in the generation of lycorenine, oxidation of this same carbon atom. The absolute stereochemistry

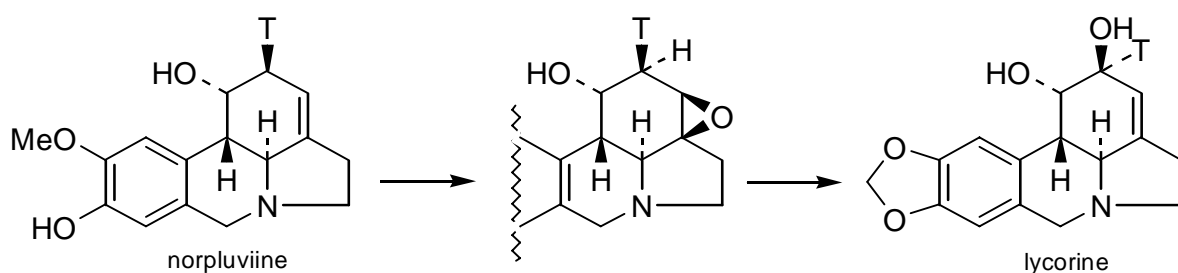


Figure 5. Biosynthesis of lycorine with inversion of the configuration.

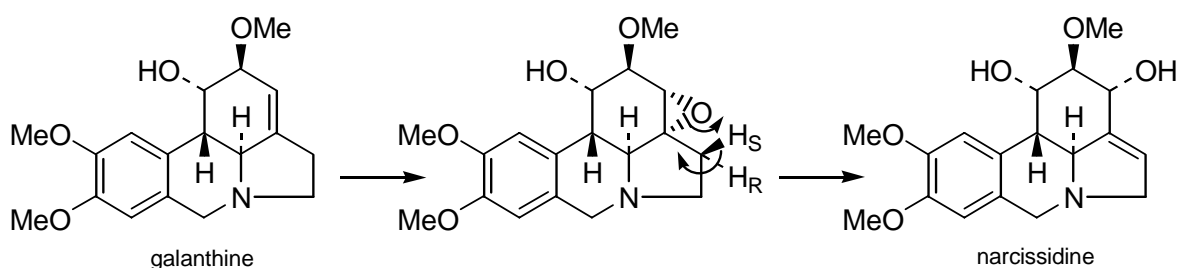


Figure 6. Conversion of galanthine to narcissidine *via* epoxide.

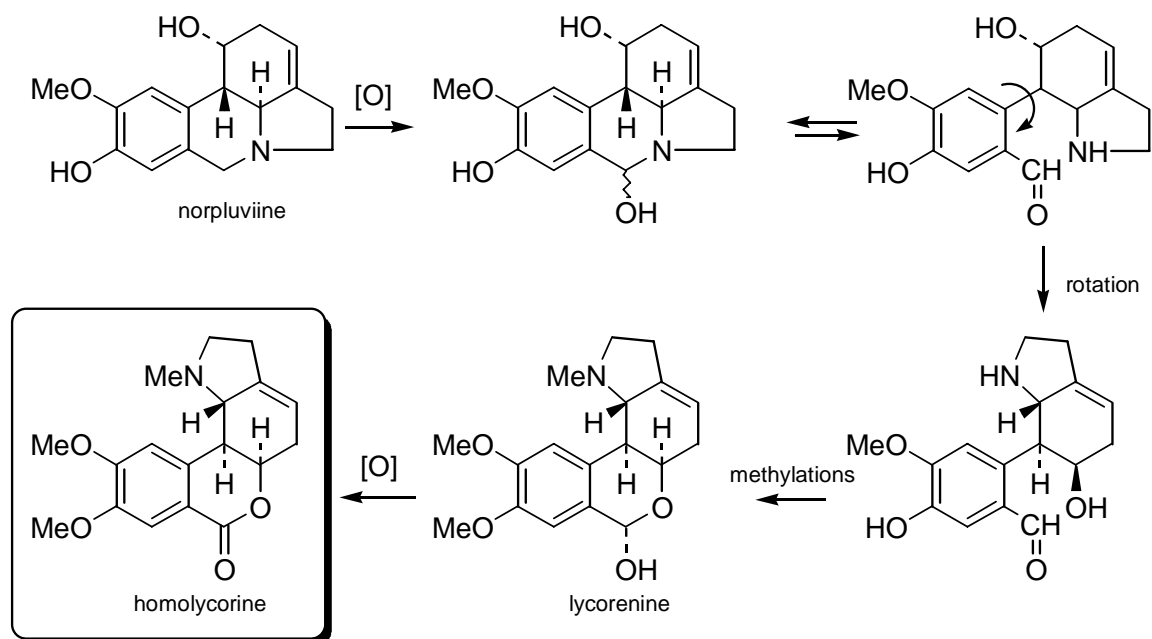


Figure 7. Conversion of norpluviine to homolycorine type alkaloids.

of these processes has been elucidated in subsequent experiments [33], and the results show that hydrogen addition and removal take place on the re-face of the molecules concerned [34], the initially introduced hydrogen being the one later removed [35]. It is noteworthy that norpluviine, unlike pluviine, is converted in *Narcissus* 'King Alfred' primarily to alkaloids of the homolycorine type. Benzylic oxidation at position 6 followed by a ring opening forms an amino aldehyde; the formation of hemiacetal and subsequent methylation provides lycorenine [30], which after oxidation gives homolycorine, as shown in Fig. 7.

1.4.2. Crinine, haemanthamine, tazettine, narciclasine and montanine types

This group includes the alkaloids derived from 5,10b-ethanophenanthridine (crinine and haemanthamine types), 2-benzopyrano[3,4-*c*]indole (tazettine type), phenanthridine (narciclasine type) and 5,11-

methanomorphanthridine (montanine type) skeletons, originating from a *para-para'* phenol oxidative coupling (Fig. 8).

Results of experiments with labelled crinine, and less conclusively with oxovittatine, indicate that the two naturally occurring enantiomeric series, represented in Fig. 8 by crinine and vittatine, are not interchangeable in *Nerine bowdenii* [36].

Incorporation of *O*-methylnorbelladine, labelled in the methoxy carbon and also in positions [3,5-³H], into the alkaloid haemanthamine was without loss of tritium, half of which was at C-2. Consideration of the possible mechanisms involved in relation to tritium retention led to the suggestion that the tritium which is expected at C-4 of haemanthamine might not be stereospecific [37]. The conversion of *O*-methylnorbelladine into haemanthamine involves loss of the *pro-R* hydrogen from the C- β of the tyramine moiety, as well as a further entry of a hydroxyl group at this site [38]. The subsequent benzylic oxidation results in an epimeric mixture that even HPLC cannot separate. The epimeric forms were proposed to be interchangeable. The biosynthetic conversion of the 5,10b-ethanophenanthridine alkaloids to the 2-benzopyrano[3,4-*c*]indole was demonstrated by feeding tritium-labelled alkaloids to *Sprekelia formosissima*. It was shown that this plant converts haemanthamine to haemanthidine/epihaemanthamine and subsequently to pretazettine in an essentially irreversible manner [39]. This transformation was considered to proceed through an intermediate but it has never been detected by spectral methods [40] (Fig. 9).

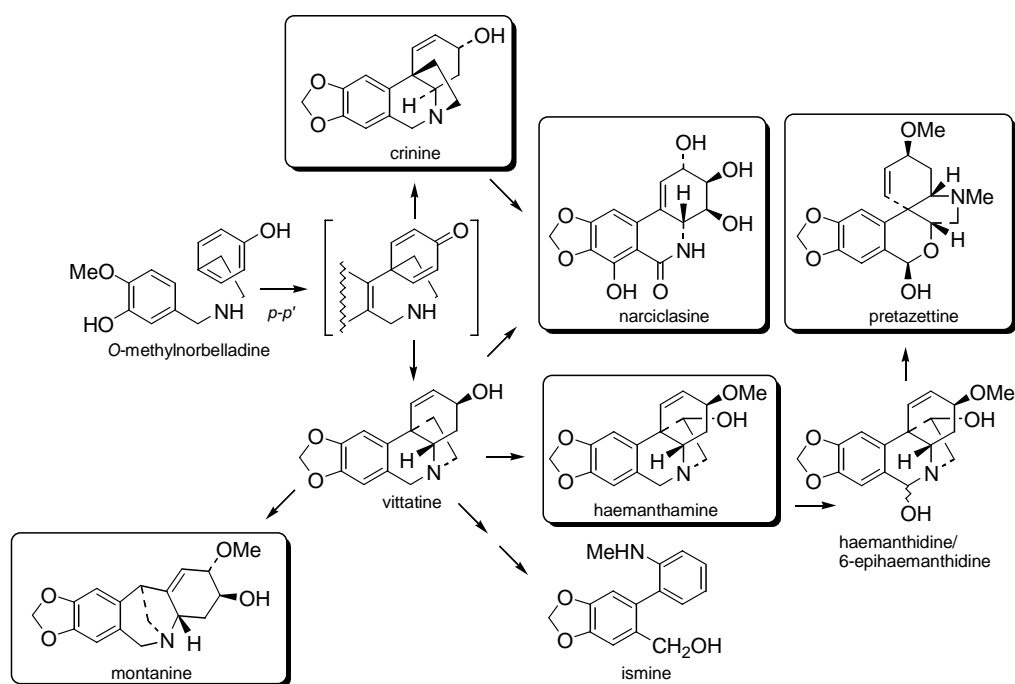


Figure 8. Alkaloids proceeding from a *para-para'* coupling.

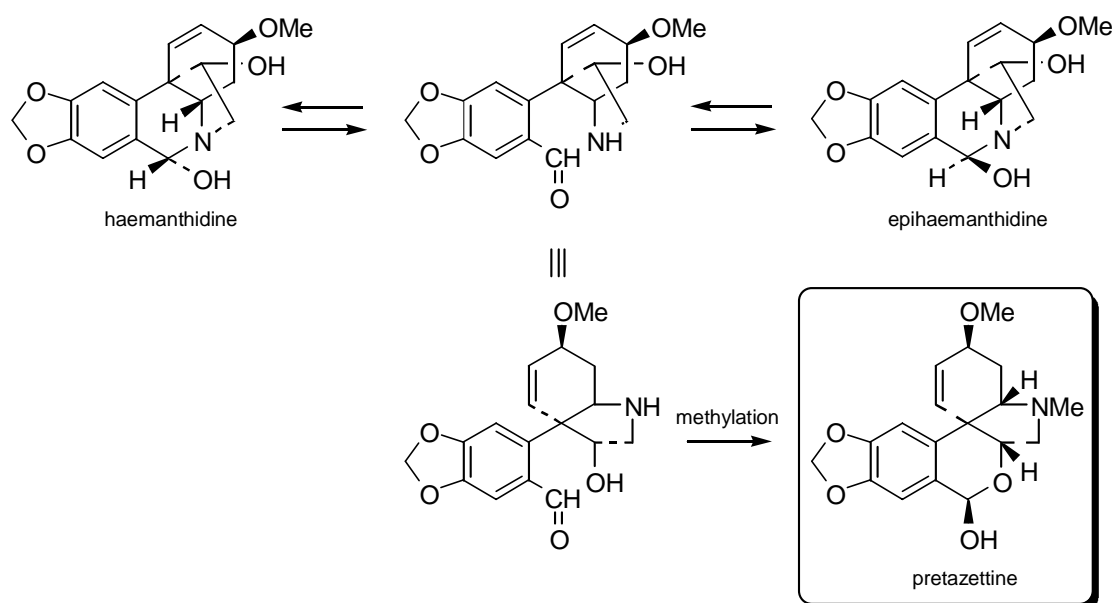


Figure 9. Biosynthesis of pretazettine.

It has also been proved that the alkaloid narciclasine proceeds from the pathway of the biosynthesis of crinine and haemanthamine type alkaloids and not through norpluviine and lycorine derivatives. In fact, in view of its structural affinity to both haemanthamine and lycorine, narciclasine could be derived by either pathway. When *O*-methylnorbelladine labelled in the methoxy carbon and in both protons of position 3 and 5 of the tyramine aromatic ring, was administered to *Narcissus* plants, all four alkaloids incorporated activity. The isotopic ratio [$^3\text{H}:$ ^{14}C] for norpluviine and lycorine was, as expected, 50% that of the precursor, because of its *ortho-para'* coupling. On the contrary, in haemanthamine the ratio was unchanged. These results show clearly that the methoxy group of *O*-methylnorbelladine is completely retained in the alkaloids mentioned, providing a satisfactory internal standard and also, the degree of tritium retention is a reliable guide to the direction of phenol coupling. Narciclasine showed an isotopic ratio (75%) higher than that of lycorine or norpluviine though lower than that of haemanthamine. However, the fact that more than 50% of tritium is retained suggests that *O*-methylnorbelladine is incorporated into narciclasine via *para-para'* phenol oxidative coupling.

O-methylnorbelladine and vittatine are implicated as intermediates in the biosynthesis of narciclasine [41-43], and the loss of the ethane bridge from the latter could occur by a retro-Prins reaction on 11-hydroxyvittatine. Strong support for this pathway was obtained by labelling studies. 11-Hydroxyvittatine has also been proposed as an intermediate in the biosynthesis of haemanthamine and montanine (a 5,11-methanomorphanthridine alkaloid) following the observed specific incorporation of vittatine into the two alkaloids in *Rhodophiala bifida* [36] (Fig. 10).

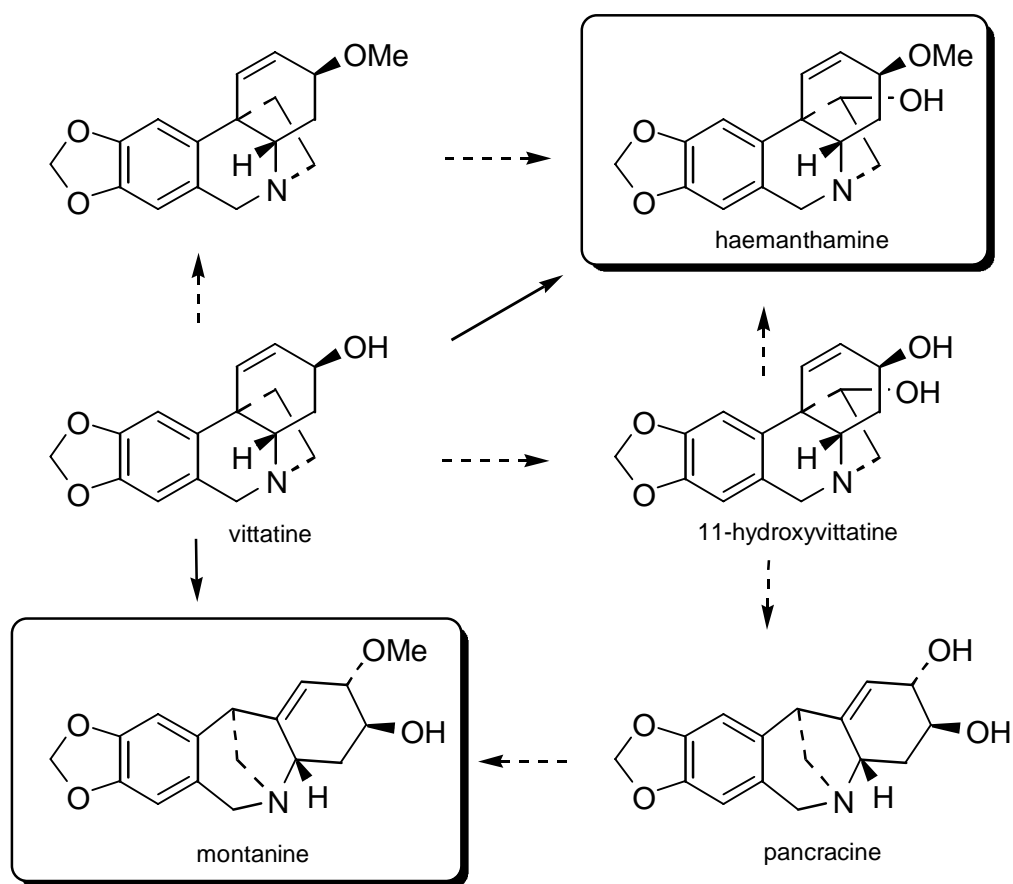


Figure 10. Proposed biosynthetic pathways to haemanthamine and montanine.

Fuganti and Mazza [42,43] concluded that in the late stages of narciclasine biosynthesis, the two-carbon bridge is lost from the oxocrinine skeleton, passing through intermediates bearing a pseudoaxial hydroxy-group at C-3 position and further hydrogen removal from this position does not occur. Noroxomaritidine was also implicated in the biosynthesis of narciclasine and further experiments [44] showed that it is also a precursor for ismine.

The alkaloid ismine has also been shown [45] to be a transformation product of the crinine-haemanthamine series. The precursor, oxocrinine labelled with tritium in the positions 2 and 4, was administered to *Sprekelia formosissima* plants and the radioactive ismine isolated was shown to be specifically labelled at the expected positions.

1.4.3. Galanthamine type

This type of alkaloids have a dibenzofuran nucleus (galanthamine type) and are obtained from a *para-ortho*' phenol oxidative coupling.

The initial studies of this pathway suggested that the *para-ortho'* coupling does not proceed from *O*-methylnorbelladine but from *N,O*-dimethylnorbelladine to finally give galanthamine [46]. *N,O*-dimethylnorbelladine was first isolated from *Pancreatum maritimum* [47] a species that also contains galanthamine.

However, the most recent study seems to contradict the evidence set forth here. Experiments carried out with application of ^{13}C -labelled *O*-methylnorbelladine to organs of field grown *Leucojum aestivum* have shown that the biosynthesis of galanthamine involves the phenol oxidative coupling of *O*-methylnorbelladine to a postulated dienone, which undergoes spontaneous closure of the ether bridge to yield *N*-demethylnarwedine, giving norgalanthamine after stereoselective reduction. Furthermore, it was shown that norgalanthamine is *N*-methylated to galanthamine in the final step of biosynthesis [18] (Fig. 11). In contrast with the literature, *N,O*-dimethylnorbelladine was metabolized to a lesser extent in *L. aestivum* and incorporated into galanthamine as well as norgalanthamine at about 1/3 of the rate of *O*-methylnorbelladine.

According to Eichhorn *et al.* [18], narwedine is not the direct precursor of galanthamine, and could possibly exist in equilibrium with galanthamine, a reaction catalyzed by a hypothetically reversible oxido-reductase.

Chlidanthine, by analogy with the known conversion of codeine to morphine, might be expected to arise from galanthamine by *O*-demethylation. This was shown to be true when both galanthamine and narwedine, with tritium labels, were incorporated into chlidanthine [48].

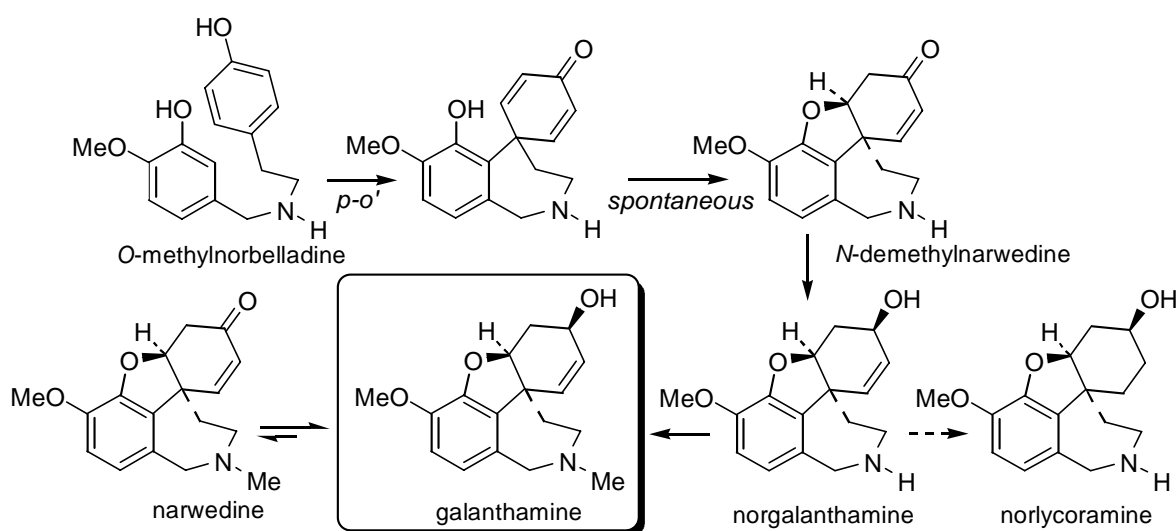


Figure 11. Biosynthesis of galanthamine and derivatives.

2. NMR studies

In a discussion of Proton Nuclear Magnetic Resonance (^1H NMR) and Carbon Nuclear Magnetic Resonance (^{13}C NMR), the most significant characteristics of each of Amaryllidaceae alkaloid-type are outlined, indicating the keys for their identification.

2.1. Proton nuclear magnetic resonance

^1H NMR spectroscopy gives the most extensive and important information about the different types of Amaryllidaceae alkaloids. In the last 25 years, the routine use of 2D NMR techniques has facilitated the structural assignments and the settling of their stereochemistry.

2.1.1. Lycorine type

This group has been subjected to several ^1H NMR studies and lycorine, as well as its main derivatives, has been completely assigned. The general characteristics of the ^1H NMR spectra are:

- a. Two singlets for the *para*-oriented aromatic protons, together with a unique olefinic proton.
- b. Two doublets as an AB system corresponding to the benzylic protons of C- 6. The deshielding observed in the β -protons of positions 6 and 12 in relation to their α -homologues is due to the effect of the *cis*-lone pair of the nitrogen atom.
- c. Like almost all other lycorine type examples, the alkaloids isolated from the *Narcissus* genus show a *trans* B/C ring junction, the coupling constant being $J_{4a,10b} \sim 11$ Hz. Only kirkine shows a *cis* B/C ring junction, with a smaller coupling constant $J_{4a,10b}$ 8 Hz.

In the plant, the alkaloid lycorine is particularly vulnerable to oxidation processes, giving several ring-C aromatized products.

2.1.2. Homolycorine type

This group includes lactone, hemiacetal or the more unusual cyclic ether alkaloids. The general traits for this type of compounds could be summarized as follows:

- a. Two singlets for the *para*-oriented aromatic protons. In lactone alkaloids, the deshielding of H-7 is caused by the *peri*-carbonyl group.
- b. The hemiacetal alkaloids always show the substituent at C-6 in α -disposition.
- c. The majority of compounds belong to a single enantiomeric series containing a *cis* B/C ring junction, which is congruent with the small size of the coupling constant $J_{1,10b}$. In the *Narcissus* genus no exception to this rule has been observed.
- d. The large coupling constant between H-4a and H-10b ($J_{4a,10b} \sim 10$ Hz) is only consistent with a *trans*-diaxial relationship.
- e. In general, ring C presents a vinylic proton. If position 2 is substituted by an OH, OMe or OAc group, it always displays an α -disposition.
- f. The singlet corresponding to the *N*-methyl group is in the range of δ 2.0-2.2 ppm, its absence being very unusual.
- g. The H-12 α is more deshielded than H-12 β as a consequence of the *cis*-lone pair of the nitrogen atom.

Homolycorine type alkaloids with a saturated ring C have been studied by Jeff and co-workers [49]. They describe empirical correlations of *N*-methyl chemical shifts with stereochemical assignments of the B/C and C/D ring junction.

2.1.3. Haemanthamine and crinine types

The absolute configuration of these alkaloids is determined through the circular dichroism spectrum. The alkaloids of the *Narcissus* genus are exclusively of the haemanthamine type, while in genera such as *Brunsvigia*, *Boophane* etc., the crinine type alkaloids are predominant. It is also noteworthy 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. Thus, haemanthamine type alkaloids show the following characteristics:

- a. Two singlets for the *para*-oriented aromatic protons, although of course only one for crinine type alkaloids substituted at C-7.
- b. 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 ($J_{1,3} \sim 1-2$ Hz) and H-2 shows a smaller coupling with H-3 ($J_{2,3} \sim 0-1.5$ 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 ($J_{2,3} \sim 5$ Hz) is shown, the coupling between H-1 and H-3 not being detectable. This rule is also applicable to the crinane type alkaloids.

- c. In the haemanthamine series there is frequently an additional W coupling of H-2 with the equatorial H-4 β , while the proton H-4 α shows a large coupling with H-4a ($J_{4\alpha,4a} \sim 13$ Hz) due to their *trans*-diaxial disposition. The same is applicable in the crinane series.
- d. Two doublets for an AB system corresponding to the benzylic protons of position C-6.
- e. The pairs 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.
- f. Also in relation with position C-6, it is interesting to note that ismine, a catabolic product from the haemanthamine series, shows a restricted rotation around the biaryllic bond, which makes the methylenic protons at the benzylic position magnetically non-equivalent.

2.1.4. Tazettine type

Although tazettine is one of the most widely reported alkaloids in the Amaryllidaceae family, it was found to be an extraction artifact from pretazettine [50].

The presence of an *N*-methyl group (2.4-2.5 ppm) in tazettine type alkaloids immediately distinguishes them from the haemanthamine or crinine types, from which they proceed biosynthetically. Moreover, the ^1H NMR spectrum always shows the signal corresponding to the methylenedioxy group.

2.1.5. Montanine type

The absolute configuration of Montanine-type alkaloids is determined through the circular dichroism spectrum. Their ^1H NMR data are very similar to those of alkaloids with a lycorine skeleton, but Montanine-type alkaloids can be distinguished by the analysis of a COSY spectrum. The signals attributable to the H-4 hydrogens (the most upfield signals) show correlation with those corresponding to H-3 and H-4a, while in a lycorine skeleton the most upfield signals correspond to the H-11 hydrogens.

2.1.6. Narciclasine type

The narciclasine-type alkaloids present the highest degree of oxidation. The absolute configuration of the most studied alkaloid of this group pancratistatin, was determined by X-ray diffraction [51]. The main ^1H NMR characteristics of the narciclasine-type alkaloids are:

- The only aromatic hydrogen appears as a singlet with a chemical shift higher than 7 ppm.
- Those alkaloids with a hydrogenated double bond C-1/C-10b possess a *trans* stereochemistry for the B-C ring junction and, consequently, a large coupling constant value for J_{4a-10b} .
- The hydrogen attached to the nitrogen atom appears as a broad singlet with a chemical shift around 5 ppm, which disappears on the addition of D_2O .

2.1.7. Galanthamine type

Among the Amaryllidaceae alkaloids, only the galanthamine type shows an *ortho*-coupling constant between both aromatic protons of ring A. The general characteristics of their ^1H NMR spectra are:

- Two doublets for the two *ortho*-oriented aromatic protons with a coupling constant of $J_{7,8} \sim 8$ Hz.
- 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 coupling constant $J_{3,4}$ is about 5 Hz, the substituent is pseudoaxial, while if it is ~ 0 Hz this indicates that the substituent at C-3 is pseudo-equatorial.
- Two doublets corresponding to the AB system of the C-6 benzylic protons.
- The existence of the furan ring results in a deshielding effect in H-1.
- This type of alkaloids often shows an *N*-methyl group but occasionally an *N*-formyl group has been reported.

2.2. Carbon¹³ nuclear magnetic resonance

^{13}C NMR spectroscopy has been extensively used for determining the carbon framework of Amaryllidaceae alkaloids, and there are several major contributions [52-54]. The assignments are made on the basis of chemical shifts and multiplicities of the signals (by DEPT experiment). The use of 2D

NMR techniques such as HMQC and HMBC allow the assignments to be corroborated.

The ^{13}C NMR spectra of Amaryllidaceae 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 40-46 ppm.

The effect of the substituent (OH, OMe, OAc) on the carbon resonances is of considerable importance in localizing the position of the functional groups.

The analysis of the spectra allows conclusions to be drawn about the following aspects:

- The number of methine olefinic carbons.
- The presence and nature of the nitrogen substituent.
- The existence of a lactonic carbonyl group.
- The presence of a quaternary carbon signal assignable to C-10b in the chemical shift range of 42-50 ppm.

3. Biological and pharmacological activities

This section covers the pharmacological and/or biological properties of the most representative Amaryllidaceae alkaloids. Until now only galathamine is being marketed, but the significant activities of other alkaloids in the family demonstrated in recent years could favour their therapeutic use in the near future.

3.1. Lycorine type

The most characteristic and common Amaryllidaceae alkaloid is lycorine, reported to be a powerful inhibitor of ascorbic acid (L-Asc) biosynthesis [55,56], and thus a useful tool in studying Asc-dependent metabolic reactions in L-Asc-synthesising organisms [57,58]. Specifically, lycorine is a powerful inhibitor of the activity of L-galactono- γ -lactone dehydrogenase, the terminal enzyme of L-Asc biosynthesis [59-62], which is thought to be localised in the mitochondrial membrane [63,64]. Galanthine also has a high capacity to inhibit ascorbic acid biosynthesis [56].

Lycorine is a powerful inhibitor of cell growth, cell division and organogenesis in higher plants, algae, and yeasts, inhibiting the cell cycle during interphase, which seems to be related with the L-Asc levels [57,65-69]. In plants, it also inhibits cyanide-insensitive respiration, peroxidase activity

and protein synthesis [70-72]. The effects of lycorine on L-Asc biosynthesis have been reported to occur at concentrations below those at which protein synthesis is affected, but it seems difficult to completely rule out non-specific effects of this alkaloid since it has been reported that, at least in yeasts, lycorine is able to interact directly with mitochondrial DNA. Thus, differing sensitivity to the alkaloid among cells devoid of mitochondrial DNA (ρ^0) and cells with mitochondrial DNA either ρ^+ or ρ^- has been found in yeasts [59,67,73,74], ρ^0 cells being resistant to high concentrations of the drug [69,75-77]. Some strains can even adapt to the presence of lycorine, because they are able to degrade the alkaloid and use its biotransformation products as growth stimulating factors [77]. In contrast, lycorine-1-*O*- β -D-glucoside promotes cell growth, seed germination, and rate of development of root and root hairs in higher plants. The glucosyloxy derivatives of lycorine and pseudolycorine and their aglicones form stable complexes with phytosterols and also with divalent metal ions and are able to translocate them from the rhizosphere to the aerial part [78]. Palmilycorine and some acylglucosyloxy conjugates of lycorine, in turn, are frequently encountered among the phytosterols exhibiting membrane-stabilizing action. Plants also use lycorine-1-*O*- β -D-glucoside and acylglucosyloxy conjugates of lycorine to recognize and reject microorganisms and parasites [79].

The antitumor activity of lycorine in animals [80,81] has been demonstrated by the inhibition of *in vivo* and *in vitro* growth of diverse tumor cells, such as BL6 mouse melanoma, Lewis lung carcinoma, murine ascite or HeLa cells [3,79,82-86]. It induces flat morphology in K-ras-NRK cells (transformed fibroblasts) [87], and reduces the cellular activity in femoral bone marrow tissue that results in granulocytic leucopenia and a decrease in the number of erythrocytes. This alkaloid's mechanism of action is thought to be through inhibition of protein synthesis at the ribosomal level, even though the cytotoxic effects of calprotectin can also be suppressed using lycorine [80,81,88-90]. Lycorine also inhibits murine macrophage production of Tumor Necrosis Factor alpha (TNF- α) [91], and shows inhibitory effects on nitric oxide production and induction of inducible nitric oxide synthase (NOS) in lipopolysaccharide-activated macrophages [92]. The molecular mechanism of lycorine against leukaemia (human cell line HL-60) shows that it can suppress cell growth and reduce cell survival by arresting the cell cycle at the G₂/M phase and inducing apoptosis of tumor cells [93]. Recent studies show that the TNF- α signal transduction pathway and p21-mediated cell-cycle inhibition are involved in the apoptosis of HL-60 cells induced by lycorine [94]. The effects of lycorine on the human multiple myeloma cell line KM3, and the possible mechanisms of these effects have also been studied [95]. The growth rates of the KM3 cells exposed to lycorine clearly slowed down. Cell

fluorescent apoptotic morphological changes, DNA degradation fragments, and a sub-G₁ peak were detected, indicating the occurrence of cell apoptosis after lycorine treatment. Furthermore, the release of mitochondrial cytochrome c, the augmentation of Bax with the attenuation of Bcl-2, and the activation of Caspase-9, -8, and -3 were also observed, suggesting that the mitochondrial pathway and the death receptor pathway were involved. The results also showed that lycorine was able to block the cell cycle at the G₀/G₁ phase through the downregulation of both cyclin D1 and CDK4. In short, lycorine can suppress the proliferation of KM3 cells and cell survival by arresting cell cycle progression as well as inducing cell apoptosis [96]. A recent paper describes the preparation of a mini-library comprised of synthetic and natural lycorane alkaloids and the investigation of apoptosis-inducing activity in human leukemia (Jurkat) cells. Further insights into the nature of this apoptosis-inducing pharmacophore are described, including the requirement of both free hydroxyl groups in ring-C [97]. Another recent study describes the induction of apoptosis in human leukemia cells by lycorine *via* an intrinsic mitochondria pathway, causing a rapid turnover of protein level of Mcl-1 before Caspases activation. Pronounced apoptosis accompanied by the down-regulation of Mcl-1 was also observed in blasts from patients with acute myeloid leukemia. Lycorine also displays pronounced cell growth inhibitory activities against both parental and multidrug resistant L5178 mouse lymphoma cell lines, but is almost inactive in inhibiting the glycoprotein responsible for the efflux-pump activity of tumor cells. Assays for interactions with tRNA revealed that the antiproliferative effects of lycorine result from their complex formation with tRNA [98]. Interaction of lycorine, pseudolycorine and 2-*O*-acetylpseudo-lycorine with DNA has been observed [99,100]. Most of the alkaloids that showed promising antiproliferative activities have also proved to be efficient apoptosis inducers [14].

Some other alkaloids of this series, such as caranine, galanthine, pseudolycorine and 2-*O*-acetylpseudo-lycorine, are also active against a variety of tumor cells [84,101,102]. Pseudolycorine inhibits the protein synthesis in tumor cells at the step of peptide bond formation, but it has a different binding site than lycorine [89,103]. Ungeremine, a natural metabolite of lycorine, is responsible, at least partially, for the growth-inhibitory and cytotoxic effects of lycorine, being active against leukemia [104,105]. Lycorine-1-*O*-β-D-glucoside, in turn, has the reverse effect of lycorine, and may produce mitogenic activity in animal cells [106].

A mini-panel of semi-synthetic analogs of lycorine was screened for cytochrome P450 3A4 (CYP3A4) inhibitory activity, the most potent of which (1-*O*-acetyl-2-*O*-*tert*-butyldimethylsilyllycorine) exhibited inhibition at a concentration as low as 0.21 μM. Elements of this unraveled novel

pharmacophore include bulky lipophilic substitution at C-2 in conjunction with a small hydrogen donor/acceptor bond at C-1, or bulky electron-rich substitution at C-1 in conjunction with a vicinal hydrogen donor/acceptor bond [107]. Two semisynthetic silylated lycorane analogs, accessed via a chemoselective silylation strategy from lycorine exhibited low micromolar activities [108].

Lycorine and pseudolycorine exert antiviral effects on several RNA and DNA-containing viruses [109]. Antiviral activity has been observed in tests with flaviviruses, and to a slightly lesser degree, bunyaviruses. Lycorine and pseudolycorine also show inhibitory activity against the Punta Toro and Rift Valley fever viruses, but with low selectivity [110,111]. Lycorine, in turn, acts as an anti-SARS-CoV (Severe Acute Respiratory Syndrome-associated Coronavirus) and shows pronounced activity against poliomyelitis, coxsackie and herpes type 1 [3,112]. It possesses high antiretroviral activity accompanied by low therapeutic indices [113]. The relationship between its structure and the mechanism of activity has been studied in the *Herpes simplex* virus, suggesting that alkaloids that may eventually prove to be antiviral agents have a hexahydroindole ring with two functional hydroxyl groups [114]. The activity was found to be due to the inhibition of multiplication, and not to the direct inactivation of extracellular viruses, and the mechanism of the antiviral effect was partially explained as a blocking of viral DNA polymerase activity [109,115-117].

Lycorine has appreciable inhibitory activity against acetylcholinesterase [118]. Cholinesterase activity appears to be associated with the two free hydroxyl groups present in some of the alkaloids of this structural type [119]. The higher acetylcholinesterase inhibitory activity of assoanine and oxoassoanine with respect to the other lycorine-type alkaloids could be explained by an aromatic ring C, which gives a certain planarity to those molecules [120]. Another alkaloid, galanthine, exhibits powerful cholinergic activity and has therefore attracted much interest in the treatment of myasthenia gravis, myopathy and diseases of the central nervous system [121]. Caranine, pseudolycorine, unginorine, and in particular, ungeremine, also show an inhibitory effect on acetylcholinesterase [120,122,123]. Recently, the synthesis of differentially functionalized analogs of lycorine, accessed via a concise chemoselective silylation strategy, has allowed two of the most potent inhibitors of acetylcholinesterase to be described. Important elements of this novel pharmacophore were elucidated through SAR studies [94].

Lycorine is analgesic, more so than aspirin, and hypotensive [124,125], as are caranine and galanthine. The analgesic activity exhibited by the Amaryllidaceae alkaloids is attributed to their similarity with the morphine

and codeine skeletons. Lycorine also has antiarrhythmic action, and lycorine hydrochloride is a strong broncholytic [126]. In fact, lycorine shows a relaxant effect on an isolated epinephrine-precontracted pulmonary artery and increases contractility and the rate of an isolated perfused heart. These effects are mediated by stimulation of β -adrenergic receptors [127].

Lycorine also has a strong inhibitory effect on parasite (*Encephalitozoon intestinalis*) development [128] and antifungal activity against *Candida albicans* [129]. Recently, several lycorine derivatives were examined for their activity against *Trypanosoma brucei* and *Plasmodium falciparum*. Among them, 2-*O*-acetyllycorine showed the most potent activity against parasitic *T. brucei*, while 1-*O*-(3*R*)hydroxybutanoyllycorine, 1,2-di-*O*-butanoyllycorine, and 1-*O*-propanoyllycorine showed significant activity against *P. falciparum* in an *in vitro* experiment [130], although the antimalarial activity of lycorine was already known [131-133]. Galanthine, in turn, shows mild *in vitro* activity against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* [134]. Additionally, lycorine has antifeedant [135], emetic [136], anti-inflammatory [137], antiplatelet [138] as well as antifertility [125] activities.

3.2. Homolycorine type

It is reported that some alkaloids of this series, such as homolycorine, 8-*O*-demethylhomolycorine, dubiusine, 9-*O*-demethyl-2 α -hydroxyhomolycorine, hippeastrine, lycorenine or *O*-methyllycorenine present cytotoxic effects against non-tumoral fibroblastic LMTK cells [84], also being moderately active in inhibiting the *in vivo* and *in vitro* growth of a variety of tumor cells, such as Molt 4 lymphoma, HepG2 human hepatoma, LNCaP human prostate cancer or HT [84,125,139]. Dubiusine, lycorenine, 8-*O*-demethylhomolycorine and 9-*O*-demethyl-2 α -hydroxyhomolycorine also show DNA binding activity comparable to that of vinblastine [99]. Homolycorine possesses high antiretroviral activity, accompanied by low therapeutic indices [113]. Hippeastrine, in turn, displays antiviral activity against *Herpes simplex* type 1 [114].

Dubiusine, homolycorine, 8-*O*-demethylhomolycorine and lycorenine have a hypotensive effect on the arterial pressure of normotensive rats [140]. Lycorenine also shows a vasodepressor action ascribed to the maintenance of its α -adrenergic blocking action, and produces bradycardia by modifying vagal activity [141]. Another feature of lycorenine is its analgesic activity [3].

Homolycorine and masonine are other inductors of delayed hypersensitivity in animals [142]. Hippeastrine, in turn, shows antifungal activity against *Candida albicans* and it also possesses a weak insect antifeedant activity [129].

3.3. Haemanthamine and crinine types

Haemanthamine, haemanthidine, crinamine, maritidine and papyramine display pronounced cell growth inhibitory activities against a variety of tumor cells, such as Rauscher viral leukaemia, Molt 4 lymphoma, BL6 mouse melanoma, HepG2 human hepatoma, HeLa, LNCaP human prostate cancer or HT [82-84,88,139,143,144]. Some of these alkaloids, namely crinamine, haemanthamine and papyramine, also present a cytotoxic effect against non-tumoral fibroblastic LMTK cells [84]. The mechanism of action of haemanthamine is thought to be through inhibition of protein synthesis, blocking the peptide bond formation step on the peptidyl transferase centre of the 60S ribosomal subunit [89,103]. Haemanthamine and haemanthidine also display the same pronounced cell growth inhibitory activities against both parental and multidrug resistant L5178 mouse lymphoma cell lines as described above for lycorine [98]. Crinamine, in turn, shows inhibitory effects on nitric oxide (NO) production and induction of inducible nitric oxide synthase (NOS) in lipopolysaccharide-activated macrophages [92]. Crinamine and haemanthamine are potent inducers of apoptosis in tumor cells at micromolar concentrations [145]. The pharmacophoric elements are the alpha-C-2 bridge as well as a small substituent (H, or OH) at C-11. Studies have also shown that α - or β -methoxy or the hydroxyl H-bond acceptor are all tolerated at C-3, and that a C-1/C-2 double bond modulates, but is not a requirement, for apoptosis-inducing activity [146].

The antimalarial activity against strains of chloroquine-sensitive *Plasmodium falciparum* observed in haemanthamine and haemanthidine can be attributed to the methylenedioxybenzene part of the molecule and the tertiary nitrogen without methyl [131]. Crinamine also exhibits moderate antimalarial activity [132,147]. Haemanthidine also works *in vitro* against *Trypanosoma brucei rhodesiense* and to a lesser extent against *Trypanosoma cruzi* [134]. Vittatine has antibacterial activity against the Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli* [129], and the alkaloid crinamine shows strong activity against *Bacillus subtilis* and *Staphylococcus aureus* [148].

Like lycorine, haemanthidine has stronger analgesic and anti-inflammatory activity than aspirin [118,137], and vittatine has been found to potentiate the analgesic effect of morphine [149]. Moreover, some alkaloids of this series, such as haemanthamine or papyramine have a hypotensive effect [140,150], and haemanthamine strong antiretroviral activity [113].

3.4. Tazettine type

Tazettine is mildly active against certain tumor cell lines [88,139,151], with a slight cytotoxicity when tested on fibroblastic LMTK cell lines [84]. Tazettine also displays weak hypotensive and antimalarial activities and interacts with DNA [99,138,140]. Its chemically labile precursor, pretazettine, is far more interesting owing to its antiviral and anticancer activities. In fact, when pretazettine is stereochemically rearranged to tazettine, the biological activity of the precursor is to a large extent inactivated [152,153].

Pretazettine shows cytotoxicity against fibroblastic LMTK cell lines and inhibits HeLa cell growth, being therapeutically effective against advanced Rauscher leukaemia, Ehrlich ascites carcinoma, spontaneous AKR lymphocytic leukaemia and Lewis lung carcinoma [151,154-159]. It is one of the most active of the Amaryllidaceae alkaloids against Molt4 lymphoid cells [84], and is used in combination with DNA-binding and alkylating agents in treating the Rauscher leukaemia virus [151,154]. In fact, pretazettine strongly inhibits the activity of reverse transcriptase from various oncogenic viruses by binding to the enzyme [3]. It inhibits both the growth of the Rauscher virus and cellular protein synthesis in eukaryotic cells by a mechanism that does not affect DNA and RNA synthesis, even though it has a pronounced DNA binding activity [88,89,99,101,111,156,160]. Pretazettine on human MDR1-gene-transfected L5158 mouse lymphoma significantly increased the intracellular concentration of Rh-123 and enhanced the antiproliferative activity of doxorubicin in the L5178 MDR cell line [161]. This alkaloid has also been shown to be active against selected RNA-containing flavoviruses (Japanese encephalitis, yellow fever and dengue) and bunyaviruses (Punta Toro and Rift Valley fever) in organ culture [111]. It also possesses pronounced activity against *Herpes simplex* type 1 virus [114]. This activity may reflect a general ability to inhibit protein synthesis during viral replication [162].

3.5. Narciclasine type

Narciclasine, an antimetabolic and antitumoral alkaloid [163], affects cell division at the metaphase stage and inhibits protein synthesis in eukaryotic ribosomes by directly interacting with the 60s subunit and inhibiting peptide bond formation by preventing binding of the 3' terminal end of the donor substrate to the peptidyl transferase center [89,103,164-166]. It also retards DNA synthesis [167] and inhibits calprotectin-induced cytotoxicity at a more than 10-fold lower concentration than lycorine [90]. The peculiar effects of

narciclasine seem to arise from the functional groups and conformational freedom of its C-ring [168], with the 7-hydroxyl group believed to be important in its biological activity [169]. This alkaloid, related to pancratistatin [167], is one of the most important antineoplastic Amaryllidaceae alkaloids [80] and shows some promise as an anticancer agent. It inhibits HeLa cell growth, has antileukaemic properties and is active against a variety of tumor cells, such as human and murine lymphocytic leukaemia, larynx and cervix carcinomas and Ehrlich tumor cells [115,167,170-172]. One hemisynthetic derivative of narciclasine demonstrated higher *in vivo* antitumor activity in human orthotopic glioma models in mice than narciclasine in nontoxic doses [173], by both the i.v and oral routes. No effect has been observed on solid tumors. Narciclasine-4-*O*- β -D-glucopiranoside shows a very similar cytotoxic and antitumoral activity to narciclasine [174]. The anticancer activity and preclinical studies of narciclasine and its congeners has been gathered by Kornienko and Evidente in a recent review [175]. Melanomas display poor response rates to adjuvant therapies because of their intrinsic resistance to proapoptotic stimuli. Such resistance can be overcome, at least partly, through the targeting of the eEF1A elongation factor with narciclasine [176]. This alkaloid directly binds to human recombinant and yeast-purified eEF1A in a nanomolar range, but not to actin or elongation factor 2. Thus, eEF1A is a potential target to combat melanomas regardless of their apoptosis-sensitivity, which has renewed interest in the pleiotropic cytostatic activity of narciclasine. Apoptosis in Jurkat cells was triggered by narciclasine, narciclasine tetraacetate, C-10b-R-hydroxypancratistatin, *cis*-dihydronarciclasine and *trans*-dihydronarciclasine [177].

The effect of pancratistatin treatment on cancerous and normal cells has also been reported [178]. The results indicated that pancratistatin selectively induced apoptosis in cancer cells, and the mitochondria may be the site of action. To further explore the structure-activity relationship of pancratistatin-related compounds, the anticancer efficacy and specificity of two related natural alkaloids were investigated. Both of these compounds lack the polyhydroxylated lycorane element of pancratistatin, instead having a methoxy-substituted crinane skeleton. These results indicated that the phenanthridone skeleton in natural Amaryllidaceae alkaloids may be a significant common element for selectivity against cancer cells. The synergy of pancratistatin and tamoxifen on breast cancer cells in inducing apoptosis by targeting mitochondria has been also reported [179]. The 3,4-*O*-cyclic phosphate salt of pancratistatin is a novel, water soluble synthetic derivative of pancratistatin that *in vivo* caused statistically significant tumor growth delays at its maximum-tolerated dose. Significant vascular shutdown and tumor necrosis were also observed [180],

offering a way forward for improved clinical treatment by greatly enhancing solubility without loss of antitumor activity.

Narciclasine has a prophylactic effect on the adjuvant arthritis model in rats, significantly suppressing the degree of swelling of adjuvant-treated as well as untreated feet [90]. This alkaloid is also active against *Corynebacterium fascians*, inhibits the pathogenic yeast *Cryptococcus neoformans*, and modifications like 2,3,4,7-tetra-*O*-acetylnarciclasine inhibit the growth of the pathogenic bacterium *Neisseria gonorrhoeae* [181]. Antiviral activity has been observed against RNA-containing flaviviruses and bunyaviruses [111].

At the plant level, 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 [182]. Thus, indole-3-acetic acid cannot overcome the inhibition of elongation of wheat coleoptile sections caused by narciclasine. Additionally, narciclasine suppresses the gibberellin-induced α -amylase production in barley seeds and cytokinin-induced expansion and greening of excised radish cotyledons [183]. Like lycorine, narciclasine also inhibits ascorbic acid biosynthesis [184]. Narciclasine, present in daffodil mucilage, can delay tepal senescence in cut *Iris* flowers by attenuation of protease activity, which, in turn, is apparently related with the inhibition of the protein synthesis involved in senescence [185]. At the organelle level, narciclasine inhibits both isocitrate lyase (ICL) activity in glyoxysomes and hydroxypyruvate reductase (HPR) activity in peroxisomes. It also blocks the formation of chloroplasts, markedly reducing the chlorophyll content of light-grown wheat seedlings, probably due to the inhibition of the formation of 5-aminolevulinic acid, an essential chlorophyll precursor [186]. The formation of light harvesting chlorophyll a/b binding protein (LHCP) is also inhibited by this alkaloid [187].

Some alkaloids of this series, such as trisphaeridine, possess high antiretroviral activities, accompanied by low therapeutic indices [113]. Ismine, in turn, shows a significant hypotensive effect on the arterial pressure of normotensive rats [140] and is cytotoxic against Molt 4 lymphoid and LMTK fibroblastic cell lines [84].

3.6. Montanine type

There is little information about the montanine type alkaloids, only some data about pancracine, which shows antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [129], as well as weak activity against *Trypanosoma brucei rhodesiense*, *T. cruzi* and *Plasmodium falciparum* [188]. Montanine inhibited, in a dose-dependent manner, more

than 50% of the enzyme acetylcholinesterase at 1 mM concentration. With the concentrations 500 μ M and 100 μ M, 30-45% of inhibition was detected [189].

3.7. Galanthamine type

Galanthamine, originally isolated from *Galanthus nivalis* L. in the 1940s, is a long-acting, selective, reversible and competitive inhibitor of acetylcholinesterase. This enzyme is responsible for the degradation of acetylcholine at the neuromuscular junction, in peripheral and central cholinergic synapses and in parasympathetic target organs [190-192]. Galanthamine has the ability to cross the blood-brain barrier and act within the central nervous system [193,194]. It binds at the base of the active site gorge of acetylcholinesterase, interacting with both the choline-binding site and the acyl-binding pocket, having a number of moderate-to-weak interactions with the protein [195-197]. In addition, galanthamine stimulates pre- and postsynaptic nicotinic receptors which can, in turn, increase the release of neurotransmitters, thus directly stimulating neuronal function [192,198]. It is also suggested that the stimulation of nicotinic receptors protects against apoptosis induced by β -amyloid toxicity [192,199,200]. Its dual mode of action [195], coupled with the evidence that galanthamine has reduced side effects, make it a promising candidate for the treatment of nervous diseases, paralysis syndrome, schizophrenia and other forms of dementia, as well as Alzheimer's disease [192,195,196].

Other significant pharmacological actions of Galanthamine include an ability to amplify the nerve-muscle transfer [3], affecting membrane ionic processes [201]. It is also known to cause bradycardia or atrioventricular conduction disturbances [150], has long been used as a reversal agent in anaesthetic practice [18], inhibits traumatic shock and has been patented for use in the treatment of nicotine dependence. 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 [3,202-204]. It also acts as a hypotensive and has a weak antimalarial activity [138,140].

At present, Alzheimer's disease cannot be prevented or cured, so the symptomatic relief offered by AChEI therapy is the only approved therapeutic option. Due to the relative lack of alternative treatment, galanthamine is a reasonable approximation of the ideal concept of symptomatic Alzheimer's disease therapy [191,205]. Galanthamine hydrobromide (a third-generation cholinesterase inhibitor used against Alzheimer's disease) offers superior pharmacological profiles and increased tolerance compared to the original

acetylcholinesterase inhibitors, physostigmine or tacrine [193,206-209]. Galanthamine is effective and well tolerated, resulting in short-term improvements in cognition, function and daily life activities in patients with mild to moderate symptoms [198,210,211]. However, there is doubt about its long-term benefits [212] since persistent elevation of acetylcholine beyond 6 months may lead to over-stimulation of both nicotinic and muscarinic acetylcholine receptors, the former causing receptor desensitisation and the latter potentially causing an increased frequency of cholinergic side effects [192,198,213]. The safety profile of galanthamine as well as its clinical effectiveness will only be demonstrated after large-scale clinical trials [213-215].

The development of galanthamine into a widely used Alzheimer's drug can be divided into three main periods: 1- the early development in Eastern Europe for its use in the treatment of poliomyelitis; 2- the pre-clinical development in the 1980s; 3- the clinical development in the 1990s [213]. Galanthamine hydrobromide was first used by Bulgarian and Russian researchers in the 1950s and exploited for a variety of clinical purposes. It has been used clinically for postsurgery reversal of tubocurarine-induced muscle relaxation and for treating post-polio paralysis, *myasthenia gravis* and other neuromuscular diseases, as well as traumatic brain injuries [216,217]. As early as 1972, Soviet researchers demonstrated that galanthamine could reverse scopolamine-induced amnesia in mice, a finding that was demonstrated in man 4 years later. However, this compound was not applied to Alzheimer's disease until 1986, long after the widely accepted cholinergic hypothesis had been first postulated, when researchers in Western Europe switched their attention to galanthamine due to its ability to penetrate the blood-brain barrier and specifically to augment the central cholinergic function [213,218]. This led to clinical trials of galanthamine in the treatment of Alzheimer's disease. In 1996, Sanochemia Pharmazeutika in Austria first launched galanthamine as 'Nivalin[®]', but its strictly limited availability meant the international pharmaceutical community adopted a cautious approach [18,194], until Sanochemia Pharmazeutika developed a method to synthetically produce the compound in 1997 [219]. Later, galanthamine was co-developed by Shire Pharmaceuticals (Great Britain) and the Janssen Research Foundation (Belgium), who have launched galanthamine as 'Reminyl[®]' in many countries [192,213]. This renewed interest is reflected in the increasing number of scientific reviews dealing exclusively with galanthamine and its derivatives [220-223].

Sanguinine has a more potent acetylcholinesterase inhibitory activity than galanthamine due to an extra hydroxyl group available for potential interaction with acetylcholinesterase [120]. Sanguinine, in turn, is 10-fold

more selective than galanthamine for acetylcholinesterase (AChE) vs. butyrylcholinesterase (BuChE) [224]. The lack of AChE inhibitory activity of lycoramine and epinorlycoramine could be due to the occurrence of a double bond in ring C, which does not allow these compounds to have the same spatial configuration as the active alkaloids of this series [120].

Narwedine, the biogenic precursor of galanthamine, has been studied as a respiratory stimulator. It increases the amplitude and decreases the frequency of cardiac contractions and would therefore be of value in reducing blood loss during surgery [150]. It also inhibits the action of narcotics and hypnotics, and increases the analgesic effect of morphine [149], as well as the pharmacological effects of caffeine, carbazole, arecoline and nicotine [126].

3.8. Other alkaloids

Cherylline is a 4-arylisoquinoline derivative, a group with several potential medicinal properties [188], including a weak acetylcholinesterase inhibitory activity [118]. Mesembrenone, in turn, is mildly active against Molt 4 lymphoid and non-tumoral fibroblastic LMTK cells [84], has a moderate hypotensive effect on arterial pressure and interacts slightly with DNA [99,140].

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