Search for Bioactive Alkaloids in *Hymenocallis* Species

Nury Rivero¹,², Matilde Gómez² and J. D. Medina²

¹Facultad de Farmacia, Universidad Central de Venezuela, Caracas Venezuela; ²Centro de Química, Instituto Venezolano de Investigaciones Científicas, Caracas Venezuela

Abstract

Extracts from five species of *Hymenocallis* Salisbury (Amaryllidaceae) were examined by means of gas chromatography-mass spectrometry (GC-EIMS), for sources of bioactive alkaloids; especially of the isocarbostyril type such as the antitumor compound narciclasine. Ten different alkaloids were identified, all of them exhibiting skeletons of the lycorine, galanthamine, and pyrrolophenanthridine types considered typical for the family. The detection of 1- O-acetyl-pseudolycorine and 4,5-dehydro-anhydrolycorine constitutes the first report of these alkaloids for the genus. In addition, the identification of anhydropseudolycorine and 4,5-dehydro-anhydropseudolycorine from several species constitutes the first report of these alkaloids as naturally occurring compounds. Neither of the species contains isocarbostyrils and, due to the low alkaloid concentrations observed, they show no promise as a source of Amaryllidaceae alkaloids.

Keywords: Amaryllidaceae alkaloids, antitumor, *Hymenocallis* species, isocarbostyrils.

Introduction

*Hymenocallis* Salisbury (Amaryllidaceae) has been chemically studied as a genus since 1920, when the isolation of lycorine (1; Fig. 1) from *H. littoralis* Salisb. (Amaryllidaceae) was first reported by Gorter (1920). This alkaloid is widely distributed within the Amaryllidaceae. It was first isolated from bulbs of *Narcissus pseudonarcissus* L. (Amaryllidaceae) in 1887, and its biological activity was demonstrated as early as 1898, when it was shown that lycorine produces an emetic effect (Morishima, 1898). This finding stimulated the pharmacological study of extracts and pure compounds isolated from plants of this family. Since then, and only to mention a few of the activities reported for lycorine, this alkaloid has been shown active as an antifungal (Cook & Loudon, 1952), antiviral (Dabire & Murav’eva, 1982), and as an antifeedant (Singh & Pant, 1980). It has also been shown to have activity against tumors with P388-PS system and protein synthesis inhibitor related to ADN and ARN (Suffnees & Cordell, 1985) and sarcomas (Pettit et al., 1986).

Despite the variety of activities reported for *Hymenocallis* alkaloids, they generated little scientific interest until 1993 when *Pancratium littorale* Jacq. (Amaryllidaceae), from which an antineoplastic alkaloid with an isocarbostyril-type structure (pancratistatin, 2) had been isolated, was reclassified as *Hymenocallis littoralis* Salisb. (Amaryllidaceae) (Pettit et al., 1993). Pancratistatin has proven active against the P388-PS system (Suffnees & Cordell, 1985; Pettit et al., 1986; 1993), sarcomas M5076 (Martin, 1987; Pettit et al., 1995), melanomas and viruses (Lewis, 1997; Pettit et al., 1995). In addition, narciclasin (3) and 7-deoxy-narciclasin (4) were isolated from the same plant, showing various interesting activities (Suffnees & Cordell, 1985; Martin, 1987; Pettit et al., 1993). These events induced a reinvestigation of many *Hymenocallis* species because these alkaloids are neutral (lactams), and they might have been overlooked in the search for basic compounds.

Capillary gas chromatography coupled with a mass detector (GC-MS) has been successfully used to determine Amaryllidaceae alkaloids as trimethylsilyl ethers (Onyiriuka & Jackson, 1977), *N*-oxide derivatives (Kobayashi et al., 1991), and, recently, extracts without derivatization (Kreh et al., 1995; Bastos et al., 1996). This tool provides a very rapid method of examining the alkaloid content of the plants of this family with very accurate identification through the fragmentation of the alkaloids. This paper reports the search for...
new bioactive alkaloids, especially the isocarbostyril type, through the analysis of the extracts from five *Hymenocallis* species: (1) *H. bolivariana* Traub., (2) *H. guianensis* (Ker Gawler) Herb., (3) *H. lobata* Klotzsch, (4) *H. tubiflora* Salisb. [syn. *H. guianensis* var. *tubiflora* (Salisb.) Herb. (two populations)] and (5) *H. venezuelensis* Traub.

**Materials and Methods**

**General**

Samples were dried in a 6-l Labconco Lyph-Lock Benchtop Freeze Dry System (model 77520). GC-MS was performed in a Hewlett-Packard model HP5973 instrument, with an HP-5MS [film 0.25µm, poly(5%-diphenyl-95%-dimethylsiloxane)], 30 m capillary column, using software G1034C, version C03.00 (HP 1989–1994). Temperature programming: 200–250°C (4°C/min), hold 5 min, 250–280°C (4°C/min), hold 1 min. Injector temperature: 250°C, split mode 50:1, pressure 23 psi, flow 75 ml/min. Helium as carrier gas, 1.5 ml/min, at constant pressure (23 psi). Transfer line temperature: 280°C. Source temperature: 250°C. Analyzer temperature: 150°C. Lycorine, isolated from *H. caribaea*, was used as standard at a concentration of 1 mg/ml and its spectrum compared with the databases of the instrument (Wiley 275.L library and NIST, version 1.1a). Samples were injected as methanol and/or *n*-butanol solutions.
Plant material

The bulbs of the five species studied were collected in 1992 in different regions of Venezuela. They were taxonomically identified by Dr. M. Reymúndez (Universidad Central de Venezuela, Caracas, Venezuela). Bulbs were kept frozen until used. Names were checked with the International Plant Name Index (IPNI) and W3Tropicos.

*H. bolivariana* was collected in a shaded site along the Orituco River near the town of Calabozo in the state of Guárico. A voucher specimen was filed with the National Herbarium (no. 113 1992, M. Reymúndez).

*H. venezuelensis* was collected to the south of the Orituco River in a flooded savanna exposed to direct sunlight. Voucher specimens were filed with the National Herbarium (nos. 106–112 1992, M. Reymúndez).

*H. guianensis* was collected from the understory of the gallery forest alongside the rivers in the vicinity of the road that connects the towns of Tumeremo and Bochinche in the state of Bolívar. Voucher specimens were filed with the National Herbarium (nos. 132–33, 276 1992, M. Reymúndez).

*H. lobata* was collected in a partially flooded sandy savanna, south of the Orinoco River, along the road connecting Caicara with Maripa in the state of Bolívar. Voucher specimens were filed with the National Herbarium (nos. 127, 228–232 1992, M. Reymúndez).

*H. tubiflora* was collected from two populations: one from Caripe del Guácharo (*H. tubiflora* Caripe), in the state of Monagas (no. 144 1992, M. Reymúndez), and the other, *H. tubiflora* Guaraunos (no. 142 1992, M. Reymúndez), from the understory of the gallery forest alongside the rivers near the road that connects Guaraunos with Ajíes in the state of Sucre. These, too, were filed with the National Herbarium.

Extraction of alkaloidal fractions

Alkaloids were extracted from the five species using the method reported by Pettit et al. (1993), with some minor modifications. The plant material, freeze-dried and powdered, was macerated with ethanol 95% for 15 days at room temperature. The extract was filtered through a cheesecloth and concentrated under vacuum. The dried extract was partitioned with water:chloroform (1:1). The CHCl₃ extract was dried under vacuum and then extracted with 10% aqueous HCl. The acidic solution was back-washed with CHCl₃, then basified with sodium bicarbonate and extracted with chloroform and, successively, with *n*-butanol to obtain fractions B and C (basic alkaloids). The aqueous phase obtained from the partition water:chloroform was extracted with *n*-butanol, and the organic phase was dried and evaporated under vacuum. The dry extract was dissolved in methanol. Acetone was then added until a 1:5 relation was obtained, and the solution was then placed in a refrigerator overnight. A precipitate resulted, and the supernatant was completely evaporated to give a fraction D (neutral alkaloids). During the GC-MS runs, it became evident that the alkaloid content of the fractions was very small, with yields of little significance.

Anhydro-pseudolycorine (8)

The observed retention time for this alkaloid under the experimental conditions was 11.90–11.94 min (anhydro-
Bioactive alkaloids in *Hymenocallis*

Corine's retention time under the same conditions was 10.41–10.48 min, which, together with the major peaks shifted by 2 atomic mass units from those of anhydrolycorine, is indicative of the polar substituted (OH) generated by the opening of the dioxygenymethylene ring. Mass spectrum (% relative abundance): *m/z* 253 (53), 252 (100), 237 (19), 209 (15).

4,5-dehydro-anhydro-pseudolycorine (10)

Under experimental conditions, the retention time observed for this alkaloid was 13.78–13.80 min (for 4,5-dehydro-anhydro-lycorine it was 11.99–12.04 min). An argument similar to the one mentioned above supports the structure assigned. Mass spectrum (% relative abundance): *m/z* 251 (75), 250 (100), 235 (15), 222 (4), 207 (21), 191 (8), 178 (15).

**Results**

A total of 17 extracts obtained from the plant material were examined by the GC-MS technique. *H. bolivariana* did not yield a fraction D. Due to the nature of the extraction, fraction D cannot be considered exclusively as alkaloids. Table 1 shows the alkaloids identified from the extracts of the five species. The alkaloid content in all fractions was very small. Ten different alkaloids were identified by means of their fragmentation patterns, exhibiting skeletons typical for the family, of the type lycorine, galanthamine, and pyrrolophenanthridine.

**Discussion**

The use of the combined GC-MS technique for the examination of the five species of *Hymenocallis* allowed for the determination of the structures of the main constituents of the alkaloidal fractions. There are ample studies in the literature that demonstrate the power of this technique for the determination of structures of Amaryllidaceae alkaloids, especially for those for which skeletal features have been determined. For the alkaloids detected with the skeleton of the lycorine type (lycorine, 1, 1-O-acetyl-pseudolycorine, 11, and pseudolycorine, 12), the findings by Kinstle et al. (1966) and Llabrés et al. (1986) establish the principal fragments one should expect from this type of alkaloid. The loss of the C₆-C₇ bridge with their substituents gives, in general, the base peak of the spectrum. The spectra obtained for these two alkaloids were in close agreement with those reported in the literature (*The Wiley Registry of Mass Spectral Data, 6th ed.;* Llabrés et al., 1986).

Those alkaloids with a galanthamine-type skeleton (lycoramine, 5, N-demethyl-galanthamine, 6, and an isomer of galanthamine) are without doubt defined by the presence of a very intense molecular ion, the (M–1) fragment as the base peak (or vice versa), and a major peak that represents the loss of ring B from the (M–1) fragment (Razakov et al., 1969; Kreh et al., 1995; Bastidas et al., 1987). The alkaloid mentioned as an isomer of galanthamine gives all the fragments typical of that alkaloid reported in the literature, but their relative abundances are much smaller. For that reason, it was assumed that it is not galanthamine itself.

Finally, the alkaloids with a pyrrolophenanthridine-type skeleton (anhydrolycorine, 7, anhydro-pseudolycorine, 8, 4,5-dehydro-anhydro-lycorine, 9, and 4,5-dehydro-anhydro-pseudolycorine, 10) due to their extended aromaticity present the base peak at (M–1), with a few peaks in their spectra shifted according to the substituents present. In these cases, comparison of the spectra and retention times of the new alkaloids with the ones of their reported homologs (anhydrolycorine, *The Wiley Registry of Mass Spectral Data, 6th ed.,* and 4,5-dehydro-anhydro-lycorine, Ghosal et al., 1986) allowed for a full identification.

Furthermore, considering the characteristics of the chromatography column [nonpolar, poly(5%-diphenyl-95%-]

---

**Table 1.** Alkaloids identified for each species, listed in elution order.

<table>
<thead>
<tr>
<th>Alkaloid</th>
<th>Species 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lycoramine (5)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>N’-Demethyl-galanthamine (6)</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galanthamine isomer</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anhydrolycorine (7)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Anhydro-pseudolycorine (8)</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4,5-Dehydro-anhydrolycorine (9)</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4,5-Dehydro-anhydro-pseudolycorine (10)</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lycorine (1)</td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1-O-Acetyl-pseudolycorine (11)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pseudolycorine (12)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

*1, H. bolivariana; 2, H. guianensis; 3, H. lobata; 4, H. tubiflora (Caripe); 5, H. tubiflora (Guaraunos); 6, H. venezuelensis.*
dimethylsiloxane), one should expect the compounds to elute from the column in an order that follows their boiling (melting) points, with allowance for structural features that confer more or less polarity to the compounds. Table 2 shows a very good correlation in this respect, giving additional support to the proposed structures.

In conclusion, research of the five *Hymenocallis* species revealed the presence of two new natural compounds, namely anhydro-pseudolycorine (8) from *H. guianensis*, *H. lobata*, and *H. tubiflora* (Guaraunos), and 4,5-dehydro-anhydro-pseudolycorine (10) from *H. lobata*. In addition, we obtained two alkaloids that had not been previously reported for species of *Hymenocallis*, 4,5-dehydro-anhyrolycorine (9) (Ghosal et al., 1986) from *H. tubiflora* (both populations) and *H. venezuelensis* and 1-O-acetyl-pseudolycorine (11) (Llabrés et al., 1986) from *H. tubiflora* (Guaraunos). Despite a careful search, none of the species showed the presence of isocarbostyril-type alkaloids. Taking into account the low concentrations of alkaloids observed, these species show no promise as a source of Amaryllidaceae alkaloids.

Acknowledgments

We wish to thank the Instituto Venezolano de Investigaciones Científicas and the Universidad Central de Venezuela for financial support as well as Dr. M. Reymundez for her help in collecting and identifying the plant material. We are grateful to Dr. Werner Wilbert, of the Department of Anthropology of IVIC, for his kind review of the manuscript.

References


