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Piperidine alkaloids from *Lobelia polyphylla* Hook. & Arn. (Campanulaceae)

[Alcaloides piperidínicos de *Lobelia polyphylla* Hook. & Arn. (Campanulaceae)]

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Abstract: The piperidine alkaloid composition from young stems of *Lobelia polyphylla* Hook & Arn. was determined by gas chromatography mass spectrometry (GC-MS). The tentative structures, without the stereochemistry, were obtained by the analysis of the fragmentation patterns of the mass spectra of each compound. The stems contained a mixture of lobeline (**1**), norlobelanidine (**2**), 1-(1-(2-hydroxy-2-phenylethyl)-1-methylpiperidin) butane-2-ol (**3**), 8-propyl-10-phenyl lobelionol (**4**), 1-(6-(2-hydroxy-2-phenylethyl)-1-methylpiperidin) butane-2-one (**5**), 1-(6-(2-hidroxypentyl)-1-ethylpiperidin) butane-2-one (**6**) and 1-methyl-2-piperidinemethanol (**7**). The role of these alkaloids in the toxic, narcotic and hallucinogenic effects, produced after smoking the aerial parts of this species is discussed.

Keywords: *Lobelia polyphylla*; piperidine alkaloids; GC-MS analysis.

Resumen: La composición de alcaloides piperidínicos de tallos jóvenes de *Lobelia polyphylla* Hook & Arn. se determinó por cromatografía de gases acoplada a espectrometría de masas (CG-EM). Las estructuras tentativas sin incluir la estereoquímica, se obtuvieron mediante el análisis de los patrones de fragmentación de los espectros de masas de cada compuesto. Los tallos contienen una mezcla de lobelina (**1**), norlobelanidina (**2**), 1-(1-(2-hidroxi-2-feniletil)-1-metilpiperidin) butano-2-ol (**3**), 8-propil-10-fenil lobelionol (**4**), 1-(6-(2-hidroxi-2-feniletil)-1-metilpiperidin) butano-2-ona (**5**), 1-(6-(2-hidroxypentyl)-1-etilpiperidin) butano-2-ona (**6**) y 1-metil-2-piperidinmetanol (**7**). Se discute el posible papel de estos alcaloides en los efectos tóxicos, estupefacientes y alucinógenos, producidos después de haber fumado la parte aérea de esta especie.

Palabras clave: *Lobelia polyphylla*; alcaloides piperidínicos; análisis por CG-EM.

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INTRODUCTION

Species of *Lobelia* (Campanulaceae; Lobelioideae) have been used in folk medicine throughout the world to treat various diseases. It has been shown that *Lobelia* species can be characterized by their levels of an alkaloid-rich latex exudate from which piperidine alkaloids have been isolated, such as lobeline (**1**) and norlobelanidine (**2**) (Williams *et al.,* 1987; Shibano *et al.,* 2001; Felpin and Lebreton, 2004; Kesting *et al.,* 2009; Kuo *et al.,* 2011).

The *Lobelia* piperidine alkaloids normally include substitutions at C-2 and/or C-6 in piperidine or N-methylpiperidine rings with functionalized chains that usually contain –C=O, –OH and phenyl groups (Figure 1). For example, *Lobelia inflata* commonly named "Indian Tobacco" contains a mixture of 20 piperidine alkaloids, with lobeline (**1**) as the principal component (Felpin and Lebreton, 2004).

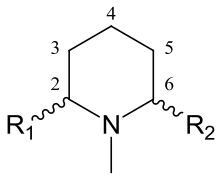


Figure 1
General structure of piperidine alkaloids from *Lobelia*

The genus *Lobelia* L. includes over 400 species with cosmopolitan distribution (Antonelli, 2008) and is represented in Chile by 4 species: *Lobelia tupa* L., *Lobelia bridgesii* Hook & Arn., *Lobelia excelsa* Bonpl. and *Lobelia polyphylla* Hook & Arn. (Riedemann and Aldunate, 2001).

According to the ethno-pharmacological Mapuche tradition, Chilean *Lobelia* species commonly known as "Tupa", "Trupa" or "devil Tobacco" contains a venomous and caustic latex exudate that ingested produces intestinal irritation, vomits and delirium. Topically, is caustic for the skin and particularly to the eyes, but useful for toothache (Wilhelm de Mösbach, 1992). In addition, dried leaves and young stems were smoked by Mapuche people for "unholy" proposes, as a narcotic and presumed hallucinogen. The hallucinogen effects have not been proved, but Schultes considers *Lobelia* species to be definitely psychoactive (Lammers, 2010). Although the risk of using *L. tupa* and the other species is evident, the plant continues to be utilized as a recreational drug and even promoted on the Internet (**https://lisergia.org/temas/guia-de-plantas-psicoactivas-de-chile-revista-canamo.4776/**). This promotion may occur, at least in part, because the content and composition and biological properties of the alkaloids from leaves and young stems from Chilean *Lobelia* have not been fully studied and the danger of smoking these species is unknown.

Among the Chilean species of *Lobelia*, *L. polyphylla* is a rare endemic shrub between 0.6 to 2 m high, with purple-wine color flowers, is found in Central Chile growing in the sandy rocky coast between Coquimbo and Valparaíso (29°-33° S) (Lammers, 2010).

A previous study of the alkaloid content from *L. polyphylla* informs only the presence of norlobelanidine (**2**) (Weinges *et al.*, 1972).

In this study, gas chromatography mass spectrometry (GC-MS) was used to determinate the chemical composition of alkaloids from the young stems of *L. polyphylla*. The alkaloid mixture is probably involved in the toxic, narcotic and hallucinogenic effects, produced after smoking or consumed the aerial parts of this species.

MATERIAL AND METHODS

Plant material

Representative samples of stems of *L. polyphylla* Hook & Arn were collected during the flowering season at Huaquén del Mar, (V Region, Chile) (176 km west of Santiago,32° 18' 55" S, 71° 28' 18" W; 27 m above sea level) in September 2012. Voucher specimens were deposited in the Herbarium of the Laboratory of Chemical Ecology, Universidad de Santiago de Chile, Santiago, Chile.

Extraction of the alkaloid fraction

Oven dried and milled plant material (65 g) was extracted with methanol using a Soxhlet during 36 h. The extract was filtered through a frit funnel and was evaporated under reduced pressure on rotary evaporator. The syrupy residue was agitated with 200 ml 5% HCl for 1 h, allowed to stand for 24 h at 35º C, and filtered through paper. The clear filtrate was washed with CH_2Cl_2 (3x50 mL). The aqueous phase was adjusted to pH 10 with NH_4OH and extracted with CH_2Cl_2 (5x50 mL) until the extracts gave negative Dragendorff reaction. Finally, the organic extract was dried with anhydrous Na_2SO_4 and

evaporation of the solvent yielded an extract potentially containing alkaloids (0.020 g).

GC-MS analysis of the alkaloid fraction

Analyses of the basic extract, were conducted on a GC-MS Thermo Scientific (trace GC ultra, MS ISQ) apparatus with a Rtx 5MS fused silica capillary column (60 m x 0.25 mm x 0.25 µm film thickness). The operating temperatures used were: injector 250° C, detector 300° C and column temperature program: 100º C increase to 160º C at 20º C/min and then 160º C for 2 min. Then increase to 238º C at 3º C/min. and hold for 2 min. Finally, increase to 300º C at 30º C/min and hold for 10 min. Helium at 1.3 mL min^{-1} was used as carrier gas. Compounds in the chromatograms were identified by comparison of their mass spectra with those in the NIST08 library database and by study of their fragmentation pattern from mass spectrum obtained.

RESULTS AND DISCUSSION

The methanol extract of aerial parts of *Lobelia polyphylla* subjected to acid-base treatment yielded a basic extract (20.30 mg; 0.03 %). TLC analysis was positive to Dragendorff reaction showing the presence of a complex mixture of alkaloids.

The GC-MS analysis of the basic extract showed the presence of around 100 compounds, from which seven were principal, accounting for 67.52 % of the extract. Their tentative structures were obtained by the analysis of the fragmentation patterns of the mass spectra of each compound.

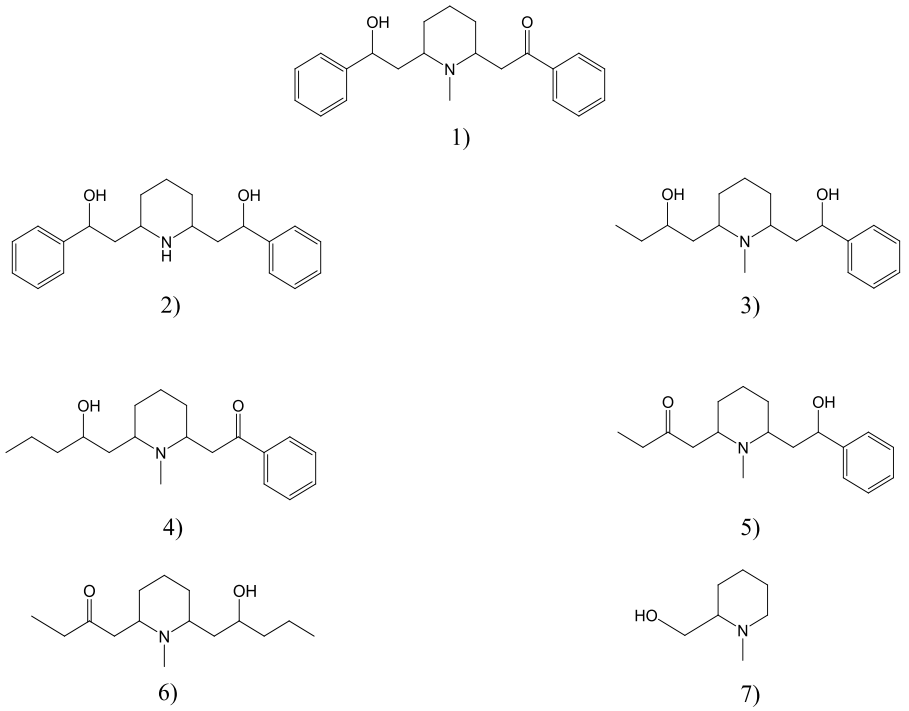


Figure 2
Structures of alkaloids from *L. polyphylla*

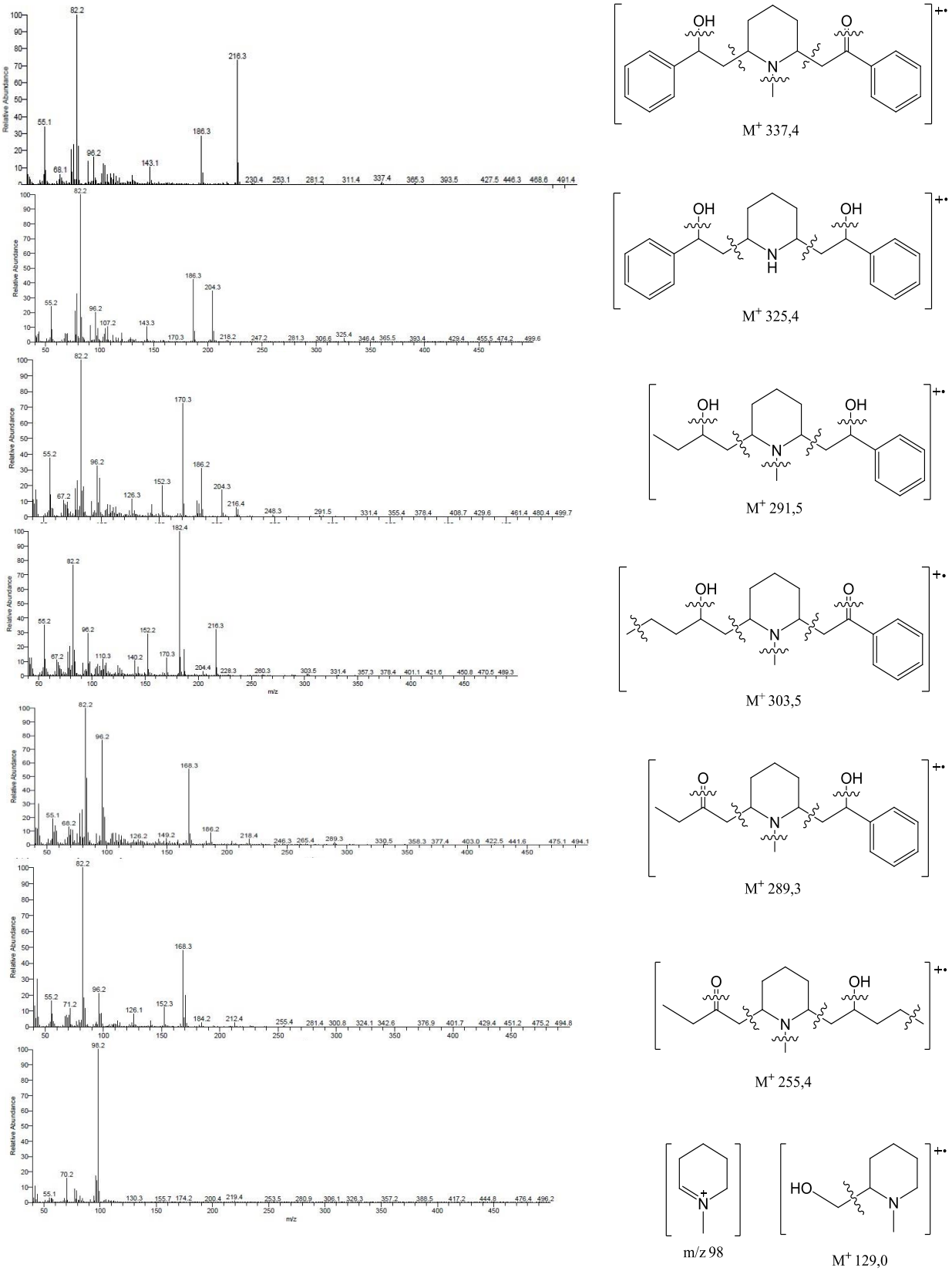
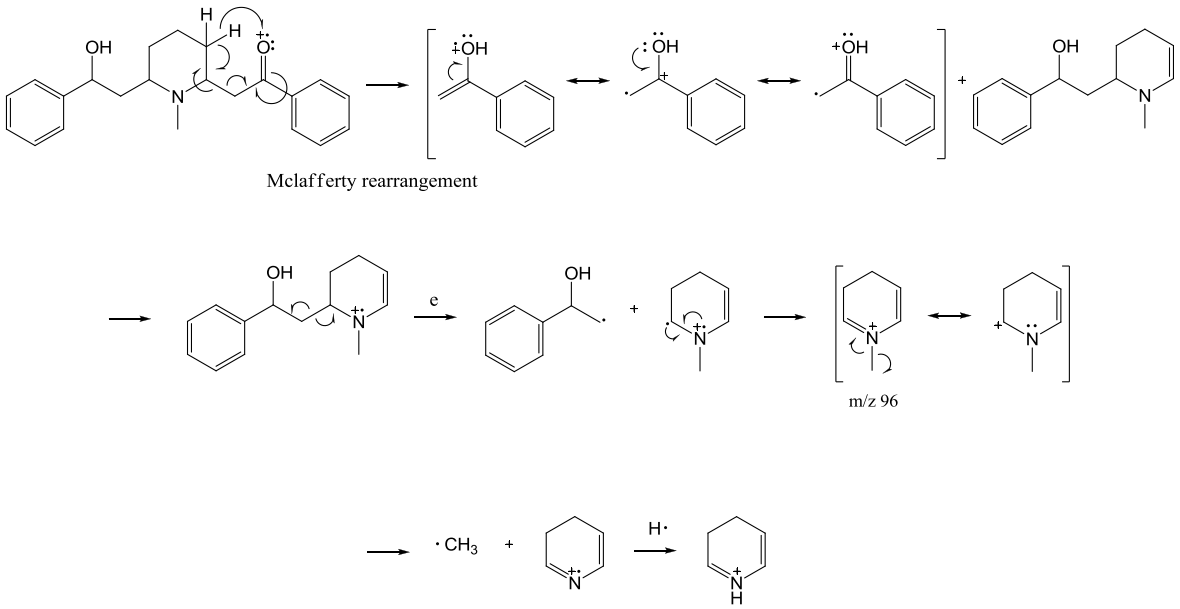


Figure 3
Mass spectra of compounds 1 – 7



Proposed fragmentation patter of compound 1
Figure 4

Compound 1 (6.06%); RT: 44.68 min; MS: m/z 337.4 (1.46%) $[M]^+$, consistent with the molecular formula $C_{22}H_{27}NO_2$; the abundant ion at m/z 216 (73.06%) was formed by loss of a phenyl-2-hydroxyethyl unit (C_8H_9O, 121 Da); ion at m/z 96 (16.06%) was formed by loss of both side chains and corresponds to N-methylated 1, 3-dihydropyridine ion. Finally, ion at m/z 82.2 (100%) should be produced by the loose of a methyl group from the N-methylated 1, 3-dihydropyridine ion with H transfer. By the proposed fragmentation pattern, compound **1** (Figure 4), was identified as **lobeline** (**1**).

Compound 2 (22.95%); RT: 43.43 min; MS: m/z 325.4 (2.57) $[M]^+$, consistent with the molecular formula $C_{21}H_{27}NO_2$; the ion at m/z 204.3 (34.54%) was formed by loss of a phenyl-2-hydroxyethyl unit (C_8H_9O, 121 Da) and ion at m/z 186 (42.37%) was formed by loss of a phenyl-2-hydroxyethyl unit (C_8H_9O, 121 Da) and H_2O. Finally the base peak al m/z 82 (100%) was formed by loss of both side chains of one N-demethylated derivative of a *Lobelia* alkaloid. It was identified as **norlobelanidine (2).**

Compound 3 (8.10%); RT: 37.73 min; MS: m/z 291.5 (0.90) $[M]^+$, consistent with the molecular formula $C_{18}H_{29}NO_2$; the intense ion at m/z 170.3 (72.49) was formed by loss of a phenyl-2-hydroxyethyl unit (C_8H_9O, 121 Da) and ion at m/z 152.2 (20.19) was formed by loss of a phenyl-2-hydroxyethyl unit (C_8H_9O, 121 Da) and H2O. Finally, fragments at m/z 96.2 (32.37%) and 82.2 (100%) (Idem compound 1).] It was identified as **1-(1-(2-hydroxy-2-phenylethyl)-1-methylpiperidin) butane-2-ol (3).**

Compound 4 (6.49%); RT: 37.57 min; MS: m/z 303.5 (1.12) $[M]^+$, consistent with the molecular formula $C_{19}H_{29}NO_2$; the ion at m/z 216.3 (32.27 %) was formed by loss of a propyl-2-hydroxyethyl unit (C5H11O, 87 Da) and the base peak m/z 182.4 (100) was formed by the loose of a phenyl-2-hydroxyethyl unit (C_8H_9O, 121 Da). 152.2 (29.05 %) [M – CH_2COPh and –OH]$^+$; fragment at 82.2 (77.00%) (Idem compound 1); 55.2 (35.16 %) [-$CH_2CHCH_2CH_3$]$^+$. It was identified as **8-propyl-10-phenyl lobelionol (4).**

Compound 5 (7.81%); RT: 36.69 min; MS: m/z 289.3 (1.66%) $[M]^+$, consistent with the molecular formula $C_{18}H_{27}NO_2$; the ion at 218.4 (4.23%) was formed by lose of ethy-2-ketoethyl unit (C_4H_7O, 71 Da); the intense ion at 168.3 (55.44%) was formed by the loose of a phenyl-2-hydroxyethyl unit (C_8H_9O, 121 Da). Finally fragments at m/z 96.2 (76.59%) and m/z 82.2 (100%) (Idem compound 1). It was identified as **1-(6-(2-hidroxy-2-phenylethyl)-1-methylpiperidin) butane-2-one (5).**

Compound 6 (4.03%); RT: 25.89 min; MS: m/z 255.4 (0.77%) $[M]^+$ is consistent with the molecular formula $C_{15}H_{29}NO_2$; 212.4 (2.74) $[M - (CH_2)_2CH_3]^+$; 168.3 (48.16) $[M - CH_2CH(OH)(CH_2)_2CH_3]^+$; 96.2 (21.06) and 82.2 (100) (Idem compound 1); 55.2 (16.22%) [-C $(CH_2)_2CH_3]^+$. It was identified as **1-(6-(2-hidroxypentyl)-1-methylpiperidin) butane-2-one (6).**

Compound 7 (11.81%); RT: 22.94 min; MS: m/z 129.4 (0.28%) $[M]^+$is consistent with the molecular formula $C_7H_{15}NO$; the ion at m/z 98,2 (100%) was formed by de loose of a hydroxymethyl group (30 Da). It was identified as **1-methyl-2-piperidinemethanol (7).**

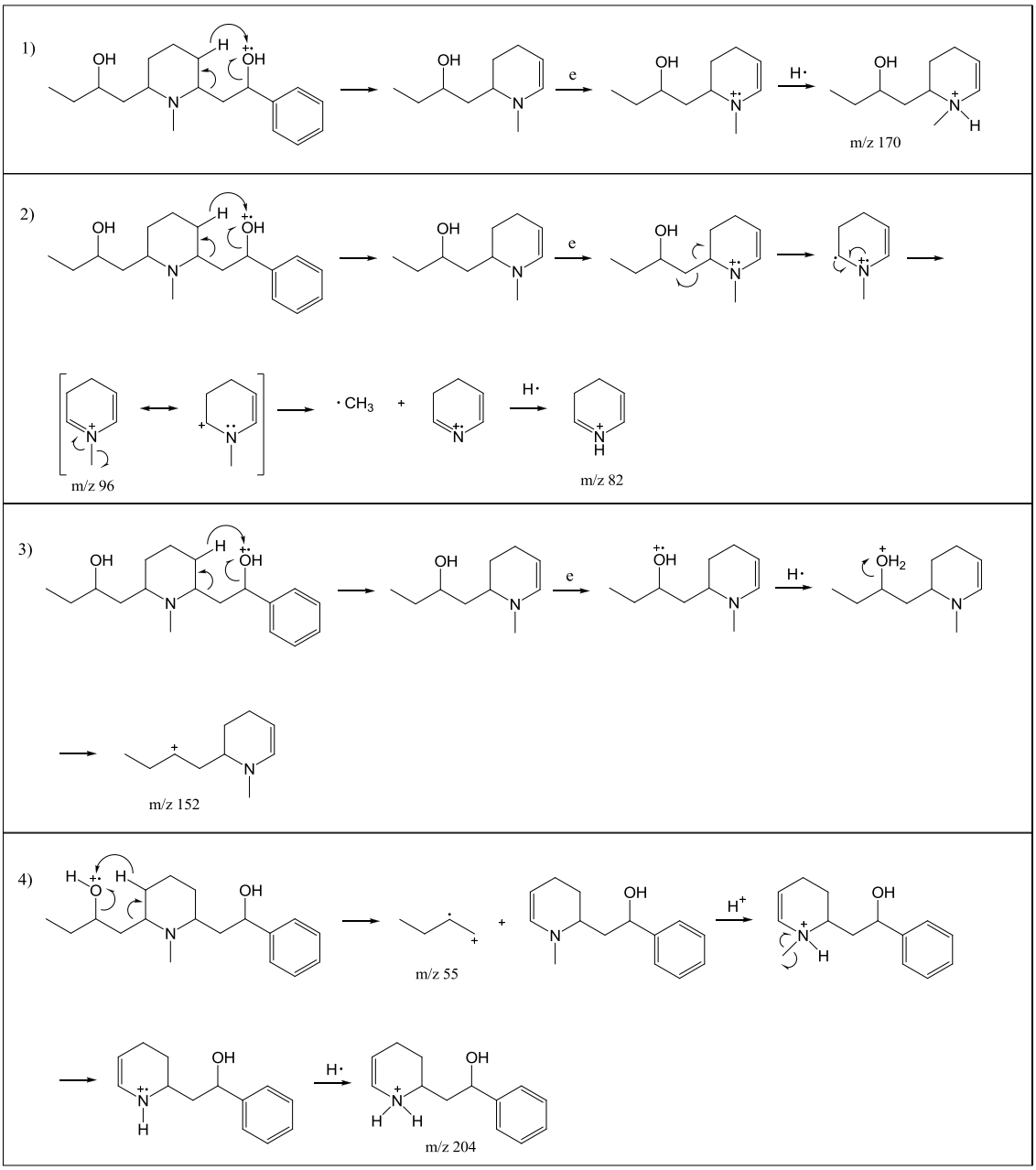


Figure 5
Fragmentation patter of compound 3

The fragmentation patterns of each compound were analyzed and the structures were obtained following the same method used for compound **1** and **3** (Figure 4 and 5).

Lobeline (**1**) is the most common alkaloid of Lobelia species and consequently has been the subject of several studies. Biological Investigations over the last years have shown that many of the ethnomedicinal properties of Lobelia have a scientific basis (Felpin and Lebreton, 2004). Example of these is the respiratory stimulant effect and as a treatment for CNS disorders, binding to the nicotinic acetylcholine receptors (nAChR) (Arihan *et al.*, 2009). Surprisingly the related alkaloids lobelanine (**8**) and lobelanidine (**9**) showed only modest affinity for nAChR. In addition, lobeline (**1**) is a powerful respiratory stimulant, while isolobelanine (**10**), is an emetic and respiratory relaxant (**http://www.altnature.com/gallery/lobelia.htm**; **http://www.healthy.net/asp/templates/article.asp?PageType=article&ID=1409**). These facts show that it is necessary to know the alkaloid composition of *Lobelia* species and their individual biological properties, in order to correlate these results with the paradoxical ethnomedicinal effects associated with some of the species (Lammers, 2010).

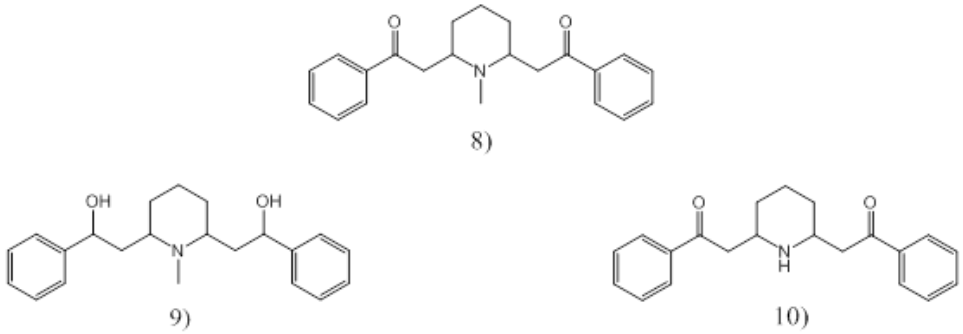


Figure 6
Structures of lobelanine (8), lobelanidine (9) and isolobelanine (10)

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