Tropane Alkaloids from *Latua pubiflora*

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Four known tropane alkaloids were isolated from the leaves of the endemic Chilean plant *Latua pubiflora* (Solanaceae). For the first time; 3α-cinnamoyloxitropane and apoatropine are reported in this plant. Scopolamine and hyoscyamine were previously reported.

Key words: Latua pubiflora, Solanaceae, Tropane Alkaloids

Introduction

Latua is a monotypic genus endemic to the coastal mountains of Southern Chile, apparently nowhere abundant. It prefers extreme wet climate from Valdivia to Chiloé range.

L. pubiflora (Griseb.) Phil. is a shrub or a small tree up to 6 m in height, with a few thorny branches. This plant presents very showy large bisexual flowers of a tubular shape, somewhat inflated in the center and of a dark violaceous to red violaceous (Rodríguez *et al.*, 1995) and locally known as "palo de brujo" or "palo malo".

This plant is very toxic, causing hallucinations and delirium, which can even lead to madness. Its was used by the Mapuche *machis* in religious rituals (Donoso and Ramírez, 1994). Previous chemical work on aerial parts of *Latua* has shown that this plant accumulates a number of tropane-derived alkaloids, mainly scopolamine and atropine (Silva and Mancinelli, 1959; Bodendorf and Kummer, 1962; Plowman *et al.*, 1971). Further investigation of *L. pubiflora* was undertaken in our laboratory using techniques of capillary gas chromatography (GC) and GC coupled with mass spectrometry (GC-MS) which has been used successfully for the identification of tropane alkaloids (Christen *et al.*, 1993), This is the first report of the isolation of the minor alkaloids detected.

Material and Methods

Plant material

Aerial parts of *Latua pubiflora* (Griseb.) Phil. was collected in December 1990 in Valdivia, Chile and identified by Mrs. Ida Latorre. A voucher specimens is kept at the Herbarium of the Escuela de Química y Farmacia (SQF 18652), Universidad de Chile.

Extraction and isolation

The dried and pulverized aerial plant material (1.05 kg) was exhaustively extracted with *n*-hexane (3 l). The dried defatted plant material was extracted at room temperature for 2 h with MeOH (5×4.0 l) and the extracts concentrated to dryness. The residue was treated with 0.5 M aq. HCl (5×1.2 l), filtered and the filtrate washed exhaustively with Et_2O (6×300 ml) to remove non-basic material. The acidic layer was basified with NH_4OH (pH = 11), extracted with CH_2Cl_2 (6×500 ml) and dried (Na_2SO_4). Removal of solvent yielded a residue (0.25 g). The basic material was subjected to repeated CC on silica gel F_{254} (5–40 μm) and aluminum oxide with *n*-hexane/EtOAc gradient (0, 2, 5, 10, 20, 50, 100 % EtOAc v/v) and EtOAc/MeOH gradient (0, 10, 50, 100 % MeOH), respectively, to afford a mixture of four alkaloids (0.12 g). This last was subject to capillary gas chromatography-mass spectrometry.

Capillary gas chromatography spectrometry

Mass spectra were obtained on a Hewlett Packard model 5972 mass selective detector (MSD) interfaced with a Hewlett Packard 5890 Series II gas chromatograph (GC). The MSD operated under electron ionization (EI) conditions at 70 eV, a secondary electron multiplier value of 1766 and at 1.2 sans/s. The GC was fitted with a 30 m × 0.25 mm *i.d.* fused silica capillary column coated with 0.25 μm DB-1 (J & W Scientific CA, USA). A pressure programmed constant linear velocity of 34.0 cm/s helium (99.999 % UHP) was used. The injection port and MSD were maintained at 250 and 280 °C, respectively. Samples were injected in the split mode (20:1) using a Hewlett Packard model 7673A auto injector (2 μl injection). The oven temperature was programmed as follows: initial temperature, 90 °C; initial hold, 1.0 min; program rate, 6.0 °C/min; final temperature, 300 °C; final hold, 4.0 min.

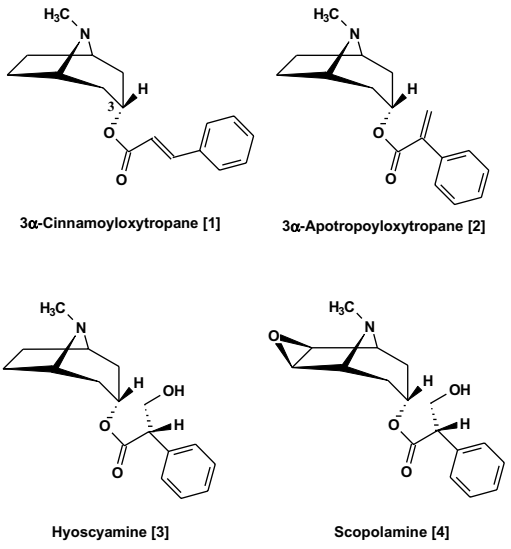

Fig. 1. Tropane alkaloids from *Latua pubiflora*.

Results

This work reports the detection and characterization of four tropane alkaloids from *L. pubiflora* via electron and chemical ionization gas chromatographic-mass spectrometric analyses and comparison of their R_f values and fragmentation patterns with those of authentic samples or from literature values. Previously, we have definitively established the basic mass spectral fragmentation pattern for 3α- or β-tropane esters without C2 substituents, that is, ions at *m/z* 82, 94, 124 and 140 (San Martín *et al.*, 1987; Casale and Moore, 1996a; 1996b). In this work all compounds displayed the basic tropane ester fragmentation pattern. Thus, the initial tentative identification of the individual components was simplified in that only the ester moiety was unknown.

Alkaloid **1** was identified by GC-MS as 3α-cinnamoyloxytropane. Its mass spectrum exhibited the fragmentation pattern of a 3-substituted tropane. The molecular ion peak at *m/z* 271, a base peak at *m/z* 124 and other ions corresponded to a 3-substituted tropane derivative with the molecular formula $C_{17}H_{21}NO_2$. The presence of ions relating to cinnamic acid at *m/z* 148 $[PhCH{=}CHCO_2H]^+$, 140 $[M\text{-}PhCH{=}CHCO]^+$, 131 $[PhCH{=}CHCO]^+$, 103 $[PhCH{=}CH]^+$, and 77 $[C_6H_5]^+$ was consistent with 3-cinnamoyloxytropane. The relative abundance of *m/z* 83 *vs. m/z* 82 was indicative of a 3α-substitution (Christen *et al.*, 1995). Comparison with reference material allowed assignment of the stereochemical orientation of the C-3 substituent as 3α (Casale and Moore, 1996a; 1996b; De la Fuente *et al.*, 1988). This is the first report of 3α-cinnamoyloxytropane identified in *Latua pubiflora*.

Alkaloid **2** was identified as 3α-apotropoyloxytropane (apoatropine) (Christen *et al.*, 1993; Muñoz *et al.*, 1996). Its mass spectrum exhibited the fragmentation pattern of a 3-substituted tropane. The molecular ion peak at *m/z* 259, together with

Alkaloid	GC tr	*m/z*[a]
3α-Cinnamoyloxytropane (**1**)	22.58	42, 55, 77, 82, 103, 124, 140, 148, 271
3α-Apotropoyloxytropane (**2**)	21.19	42, 55, 67, 82, 96, 124, 140, 259
Hyoscyamine (**3**)	24.76	42, 55, 82, 94, 103, 124, 140, 289
Scopolamine (**4**)	26.32	42, 68, 81, 94, 108, 138, 154, 303

Table I. GC-MS Fragmentation and retention times of a basic alkaloid fraction from *Latua pubiflora.*

[a] Selected ion of significant abundance.

signals at *m/z* 140, 124 (base peak), 96, 94, and 82 strongly suggestet the attachment of the ester function at C-3 and an esterifying acid C_9H_7O (Casale and Moore, 1996a; Christen *et al.*, 1995). This alkaloid was not previously identified in *Latua pubiflora.*

The alkaloids **3** and **4** were identified directly by comparison of their retention data and mass spectra with reference material.

Latua pubiflora is now understood to owe its pharmacological and toxicological properties to several extremely potent alkaloids: scopolamine and hyoscyamine. The main pharmacological effects of the naturally occurring alkaloids were already well recognized in the late nineteenth century: antispasmodic, antisecretory, and mydriatic effects. These substances have a marked effect on the central nervous system, producing delirium, hallucinations a trance-like state often resembling psychosis, for this reason drug of this plant have been widely used in the Mapuche tradition (Plowman *et al.*, 1971; Wolff, 1997).

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