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Isolation and identification macrocyclic spermine alkaloid(protoverbine) from verbascum speciosum

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Abstract

The 17-membered macrocyclic spermine alkaloid protoverbine (8-phenyl-1,5,9,13-tetra azacycloheptadecan-6-one) was isolated from the aerial part of *verbascum speciosum*. The natural polyamines are ubiquitous bases reported to have several important functions in animals, plants and microorganisms. They are involved in processes of transcription and translation in protein synthesis and influence growth and development (cell division, differentiation, embryogenensis, etc.). In plants, their involvement in organ development, flowering, fruit ripening, senescence and stress responses is reported. The structure of the protoverbine was established by the study of their spectral such as ^{1}HNMR,^{13}C-NMR, IR, ESI-MS analyses and chemical properties. To the best of our knowledge, this is the first report of protoverbine from this species.

Keywords: *Verbascum speciosum*, alkaloid, separation, protoverbine, macrocyclic spermine.

Introduction

Verbascum speciosum is a species of flowering plant in the figwort family known by the common name in Iran, Gole Mahur (Figure 1). It is native to Eastern Europe and Western Asia, known in many other regions as a roadside weed. It is a biennial herb forming a rosette of large leaves and an erect stem well exceeding one meter in maximum height. The leaves are 30 to 40 centimeters long and have smooth edges and pointed tips. The plant blooms in a large panicle with many branches lined with flowers. Each flower has a corolla measuring 2 to 3 centimeters wide with five yellow petals. There are five stamens coated in long white hairs at the center. The fruit is a capsule up to 7 millimeters in length containing many seeds [1].

In the past few years, a number of macrocyclic alkaloids structurally derived from spermine have been found to occur in certain species of the families Acanthaceae, Ephedraceae, Fabaceae, Flacourtiaceae and Scrophulariaceae [2 -7].

To date, various alkaloids have been isolated from the aerial parts of some *verbascum* species, protoverbine, protomethine [8], verbaskine [9], anabasine, plantagonine, acetamide [10], verbacine, verballocine, verbasitie, verbascenine, verballoscenine, verbaskin[11], verbametrine, isovebametrine [12],

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verbamedine, verdoline and verbasine [13].

Figure 1. *verbascum speciosum*

Since there are no reports on the isolated alkaloids of *Verbascum speciosum*, this paper has introduced macrocyclic spermine alkaloid, protoverbine, isolated for the first time from this species. . Polyamines ubiquitously occur in all organisms and many fundamental cellular processes like replication, transcription, membrane stabilization, and modulation of enzyme activities [14,15]. In plants, polyamines play an important role in development as well as responses to biotic and abiotic stress [16,17]. The polyamine spermine is a major natural intracellular compound capable of protecting DNA from free radical attack [18]. The structural elucidation of this alkaloid by means of spectroscopy analysis is described.

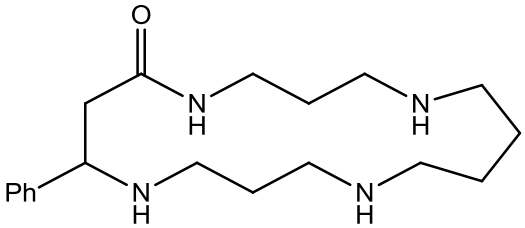

Figure 2. structural of protoverbine

Experimental

General

The NMR spectra were recorded on a FT- NMR Ultera shield BRUKER 400MHz spectrometer with TMS as the internal standard. IR spectra were obtained on an Avatar 370 FTIR Thermo Nicolet and EI-MS spectra were obtained on a HP5973 Series Mass spectrometer. MPLC was performed on a Büchi 861equipment (Switzerland) comprised of a specification pump module C-601 equipped with a UV-Detector C-640 set at a wavelength of 254 nm, a model 7725i sample injector equipped with a 5 µL loop and a Büchi software package for data collection. Column that used in

this assay was Glass Column C-690(4.6×250mm) and fraction collecter was C-660. The mobile phase was 10:1 (v/v) dichloromethane–methanol. All analyses were performed at a flow-rate of 0.5 mL/min with detection at 254nm. The mobile phase was filtered through a 0.45 µm filter and degassed. Separations were performed at room temperature.

Plant material

Leaves of *Verbascum speciosum* were collected at the flowering stage from Birjand, Iran, in June 2010 and identified at the Research Center for Plant Sciences at Ferdowsi University of Mashhad, Iran. A voucher specimen has been deposited in the Environmental Department of Bojnourd Herbarium (EDBH:00102).

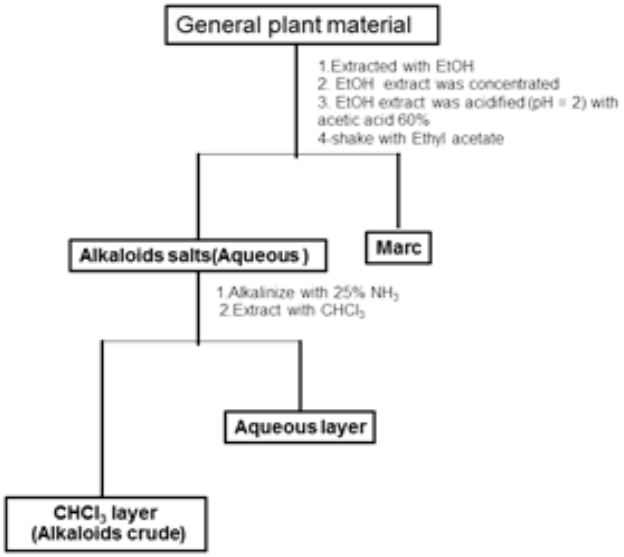

Figure 3. Scheme the extraction procedure

Extraction and isolation procedure

Alkaloids of the air-dried powdered aerial parts of *verbascum speciosum* were extracted following a classical protocol (Figure 2). Briefly, the plant material (808g) was macerated with EtOH (2.0 L) four times and filtered. The procedure was repeated until the negative test against Dragendroff's reagent. The EtOH extracts were concentrated to give 244.25g of crude extract (yield: 30.22%). The ethanol extract was acidified (pH = 2) with aqueous acetic acid 60% and the final volume was adjusted to 400 mL. The aqueous acidic solution was then extracted with ethyl acetate (3×200 mL) to remove neutral components.

After the removal of neutral components, the aqueous layer was basified with 25% NH_3 on the ice chest (pH 10-12) and extracted with $CHCl_3$(300 mL×10). $CHCl_3$ was removed under reduced pressure to give a crude alkaloidal fraction. The alkaloid extract (2g) which was subjected to a Medium-Pressure Liquid Chromatographies (MPLC) was performed using a Büchi 861 apparatus with RP18 and silica gel (70-230

mesh). For MPLC analysis, the crude extract was dissolved in CH_2Cl_2 / MeOH (10:1) and 10 µL were injected onto a column chromatography on silica gel (70-230 mesh), eluted with CH_2Cl_2-MeOH (10:1) isocratic to give one fraction.

Characteristics of protoverbine alkaloid

Solid (41mg), ^{1}H-NMR (400MHz, $CDCl_3$) δ:7.33-7.26 (m, 5H), 3.67-3.64 (m, 1H), 3.48-3.45(m, 1H), 3.11-3.08 (m, 2H), 2.90-2.51 (m, 11H), 1.74-1.70 (m, 2H), 1.57-1.53 (m, 2H), 1.44-1.41 (m, 4H).^{13}C-NMR (100MHz, $CDCl_3$) δ:171.5 ,128.4 127.6, 126.8, 62.6, 47.4, 46.8, 46.1, 39.3, 39.1, 37.5, 31.5, 25.6, 25.2, FT-IR ν_{max} cm^{-1}: 3342, 1300, 1100, 1660 EIMS 70eV, m/z: 332.

Results and discussion

The alkaloids of *verbascum speciosum* were exhaustively extracted corresponding with Figure 2. The curde alkaloids were subjected to MPLC and eluted with solvent (CH_2Cl_2/MeOH) (10:1). A single MPLC separation step performed on the alkaloid extract gave one fraction. The structure elucidation of this compound was established by spectroscopic methods, including EI-MS, IR, ^{1}H-NMR and ^{13}C-NMR experiments.

Macrocyclic spermine alkaloid was isolated as a white to yellow needle crystal (m.p=187°C). The molecular formula $C_{19}H_{32}N_4O$ was assigned to protoverbine based on mass spectrometry evidence EI-MS (positive ion) m/z 332[M$^+$]. IR spectrum showed bands at 3342cm^{-1} (N-H stretching vibration), 1300-1100 cm^{-1} (C-N stretching) and 1660cm^{-1} (carbonyl group C=O).

The ^{1}H-NMR spectrum of protoverbine showed ten signals, a multiplate at δ_H 7.33-7.26 in the aromatic region, eight methylene multiplates (δ_H 1.41-3.48) and one methine multiplate (δ_H 3.65). The 14 carbon signals present in the ^{13}C-NMR spectrum were assigned. The ^{13}C-NMR spectrum revealed the presence of aromatic carbon at (δ_C 126.8-128.4), aliphatic carbon at (δ_C 25.2-62.6) and carbonyl carbon at (δ_C 171.5).

Conclusion

In summary, macrocyclic spermine alkaloid was isolated from the leaves of *verbascum speciosum* with MPLC. As expected, protoverbine is the largest component of *verbascum speciosum* alkaloid isolated for the first time from this plant.

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