CHAPTER 5

ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF GALANTHAMINE ISOLATED FROM *SCADOXUS MULTIFLORUS*

*Acetylcholinesterase inhibitory activity of galanthamine isolated from *Scadoxus multiflorus*

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5.1. Abstract

Scadoxus multiflorus is used in traditional healing practises of South Africa, especially in wound treatment. A number of Amaryllidaceae species are rich in alkaloids. This group of compounds is known for a number of biological activities including acetylcholinesterase inhibitory activity. This activity is of interest for developing drugs for the treatment of Alzheimer's disease. The acetylcholinesterase inhibitory activity-guided fractionation of ethanolic extracts from the dried outer bulb scales of *S. multiflorus* led to the isolation of galanthamine. Centrifigal partition chromatography was mainly used as the fractionation tool. Galanthamine isolated from *S. multiflorus* showed acetylcholinesterase inhibitory activity at 0.1 mg/ml.

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5.2. Introduction

A number of South African Amaryllidaceae species have been used in traditional healing practices (Watt & Breyer-Brandwijk, 1962; Van Wyk *et al.*, 1997). The family is generally known to be rich in alkaloids. These have shown a wide range of activities (Campbell *et al.*, 1998). However, such alkaloids are deemed to be highly toxic (Watt & Breyer-Brandwijk, 1962; Bruneton, 1995). Bruneton (1995) stated that plants containing such alkaloids have limited medicinal value and their indiscriminate use is potentially lethal. *Scadoxus multiflorus* (Martyn) Raf. (Amaryllidaceae) is medicinally used in wound therapy, against colds and asthma as well (Watt & Breyer-Brandwijk, 1962, Van Wyk *et al.*, 1997).

Bulbs of *S. multiflorus* are regarded as highly poisonous and alkaloids have been isolated from the bulbs of the plant (Van Wyk *et al*, 1997). Galanthamine and other alkaloids like haemanthamine, haemultine and lycorine were isolated from the bulbs of *S. multiflorus* by Von Jaspersen-Schib in 1970 (Von Jaspersen-Schib, 1970; Hutchings *et al.*, 1996; Van Wyk *et al.*, 1997). Galanthamine, an alkaloid typically found in this family has recently been approved for the treatment of Alzheimer's disease (Bastida *et al.*, 1987, Selles *et al.*, 1997).

In this paper we report the acetylcholinesterase inhibitory effect of extracts of the bulbs of *S. multiflorus*. Bioassay-guided fractionation resulted in the isolation of galanthamine as the major active compound in the bulb.

5.3. Material and methods

Plant material

Bulb material of *S. multiflorus* were obtained from Wiljes & Zonnen BV, Hillegom, a bulb selling company in The Netherlands.

Chemicals

Acetylthiocholine iodide (ACTI), bovine serum albumin (BSA), 5,5'dithiobis-[2]-nitrobenzoic acid (DTNB) and acetylcholine esterase type VI-S from electric eel-lyophillised powder were obtained from Sigma (St Louis, MO, USA). All organic solvents were of analytical grade and purchased from J.T. Baker (Deventer, The Netherlands). The buffer used throughout the experiments was 50 mM Tris-HCl, pH 8.0. The acetylcholinesterase (AChE) source was electric eel lyophilised VI-S powder of 480 U/mg containing 530 protein units prepared to 1130 U/ml stock solution using buffer and were obtained from Sigma (St Louis, MO, USA). This was kept at –80 °C after the dilution to 1/5000 using 0.1% BSA. [DTNB (3 mM) was dissolved in buffer

containing 0.1 M NaCl and 0.02 M $MgCl_2.6H_20$ and 15 mM ACTI dissolved in "millipore" water].

Extract preparation

The air dried macerated bulb scales were shaken in methanol for 48 hours, concentrated to dryness and dissolved in heptane/ethyl acetate/methanol/water 6/1/6/1(v/v/v/v). The resulting extract was then subjected to centrifugal partitioning chromatography.

Centrifugal Partitioning Chromatography (CPC)

The CPC experiments were performed using a modular Sanki (Kyoto, Japan), type LLN apparatus. This apparatus consists of a centrifuge (NMF model), a power supply (SPL model) and a constant flow pump (LBP-V). To monitor the chromatograms, a Panasonic Pen recorder (VP 67222A) was used. This was connected to the UV detector (IS 200, Linear Instruments, Reno, NV, USA). Fractions were collected using a LKB2211 Superrac fraction collector. The total internal volume was 125 ml and the pressure limited to 60 bar. Fractions were collected after every 4 min and the flow rate was 2 ml/min. A maximum of six partition catridges was used and the void volume was rejected in all experiments.

The first CPC separation step was done using the solvent system, heptane/ethyl acetate/methanol/water 6/1/6/1 (v/v/v/v) (Ingkaninan *et al.*, 1999). The extract was injected into the CPC system (maximum of 4 ml) and the first 15 fractions were eluted using ascending mode and then the mode was reversed to descending to collect the rest of the fractions.

The resulting fractions were still very complex and contained components with a wide polarity range. Therefore, a second CPC fractionation was performed with a different solvent system. A ternary diagram approach for solvent selection was used as described by Foucault and Menet & Rolet-Menet (Foucault, 1994; Menet & Rolet-Menet, 1999). Methanol was selected as the best solvent and the ethyl acetate and water proportions determined. The best results were obtained using ethyl acetate/methanol/water 5.5/1.5/3 (v/v/v). This was used as a solvent system at the second separation step.

TLC analysis

The different fractions obtained were checked for similarity using TLC on Silica gel F_{254} (Merck, Darmstad) with chloroform/methanol 9/1 (v/v) as an eluting solvent. Dried plates were observed and marked under UV light at 254 and 366 nm. These plates were then sprayed with Dragendorff's reagent for the visual detection of alkaloids (Stahl, 1967). Similar fractions from the TLC analysis were pooled together. The resultant fractions were tested for their acetylcholinesterase inhibitory activity.

Acetylcholinesterase inhibitory activity (AChE)

A microtitre plate assay was used to detect the AChE inhibitory activity. This was modified from the method by Ellman (Ellman *et al.*, 1961; Ingkaninan *et al.*, 1999). For the assay, the content of the well consisted of 125 µl of 3 mM DTNB, 25 µl of 15 mM ATCI, 50 µl of buffer and finally 25 µl of extract dissolved to 1mg/ml in buffer containing less than 10 % MeOH.

The main active fraction was further purified by preparative TLC (Silica gel $60F_{254}$) (Merck, Darmstad) with chloroform/methanol (9/1), (v/v). The distinct separated chromatographs were scraped and extracted in methanol. The resulting eluate was concentrated to dryness and tested for AChE activity. The most active fractions were analysed with nuclear magnetic resonance (nmr) for structure elucidation.

5.4. Results and discussion

The crude ethanolic extract from *S. multiflorus* showed an inhibitory activity on AChE. The AChE activity was observed to be increasing with the increasing number of fractionation steps. The alkaloid-containing fraction showed more activity and was hence subjected to further purification by preparative TLC. After the final purification step, only the alkaloid-containing subfraction showed activity.

The isolated alkaloid was analysed by nmr spectroscopy (^{1}H). The structure of the active compound was identified as galanthamine (Figure 5.1).

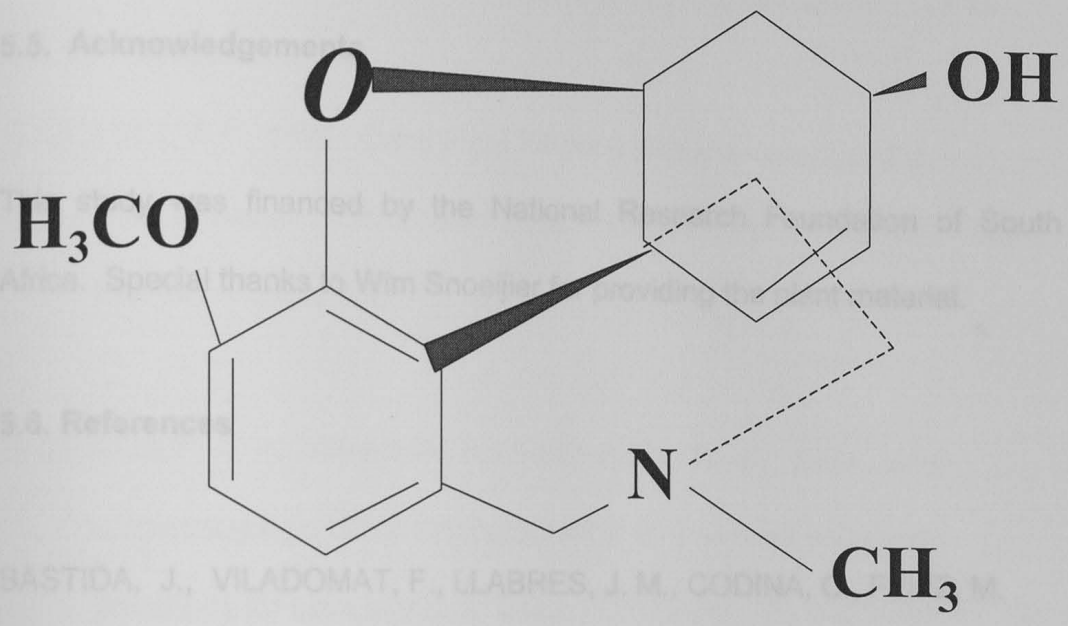

Figure 5.1. Galanthamine

The strong AChE activity found in this extract could thus be attributed to galanthamine isolated using bioassay-guided fractionation. This finding is in agreement with earlier reports on the occurrence of this compound in this plant. The high analgesic activity comparable to morphine of galanthamine could be the key factor in the verification of the reported traditional uses of this plant.

Ethanolic bulb extracts from *Narcissus tarzertta* showed inhibition of abdominal constrictions on mice (Caciki *et al.*, 1997) and AChE inhibitory activity (Hazenkamp *et al.*, 1999). Fractionation of *Narcissus confuss* also led

to the isolation of galanthamine, N-formylnorgalanthamine, haemanthamine and tazettine.

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5.6. References

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