Alkaloids of *Crinum* x *powellii* "Album" (Amaryllidaceae) and their Topoisomerase Inhibitory Activity

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The alkaloids lycorine, 1-*O*-acetyllycorine and ismine were isolated from the basic dichloromethane-soluble fraction of *Crinum* x *powellii* "Album" bulbs. The alkaloid structures were established by physical and spectroscopic analyses, including 1D NMR techniques and GC-MS analysis. The three alkaloids are reported for the first time for this hybrid.

Additionally, the three alkaloids isolated were tested against a mechanism-based bioassay utilizing genetically engineered mutants of the yeast *Saccharomyces cerevisiae* strains RAD+, RAD52Y and RS321 where lycorine was the only alkaloid that displayed moderate topoisomerase I inhibitory activity.

Key words: Amaryllidaceae-Type Alkaloids, Mutant Yeast Assay, *Saccharomyces cerevisiae*

Introduction

Plants produce many secondary metabolites with an enormous range of biological activities and chemical structures which make them an important source of chemodiversity for the discovery of new therapeutic agents (Verpoorte, 1998). However, given the rapid tropical rainforests destruction and global climate variation, the potential for drug discovery from plant origin will be dramatically reduced in the next years, before an extensive study of such biodiversity has been conducted (Baker *et al.,* 1995).

Amaryllidaceae species are an exclusive source of Amaryllidaceae-type alkaloids (Ghosal *et al.,* 1985; Bastida *et al.,* 1998). Phytochemical investigation within the genus *Crinum*, a true representative of the Amaryllidaceae family, led to the isolation of alkaloids with a wide range of biological and pharmacological activities; for instance, 1-*O*-acetyllycorine and lycorine exhibited significant and weak acetylcholinesterase inhibition, respectively (Elgorashi *et al.,* 2004); lycorine is well known as a protein biosynthesis inhibitor in eukaryotic cells (Yui *et al.,* 2001) and can also suppress leukemia growth and reduce cell survival acting as an anti-leukemia agent (Liu *et al.,* 2004).

The *in vitro* mechanism-based assay employed in this study for potential anticancer agents is supported on the differential response of DNA repair-deficient and repair-proficient yeast strains to the test sample. The assay is carried out by measuring the growth inhibition of repair-deficient yeast, usually *rad*52, in comparison with the wild-type yeast RAD+. Then, a mutant strain lacking one of the repair mechanisms will be more sensitive than the wild-type yeast to agents that cause DNA damage, allowing these agents to be selectively detected (Gunatilaka *et al.,* 1994).

Although, phytochemical and biological studies on several *Crinum* species have been reported, the chemical constituents and biological properties of *Crinum* x *powellii* "Album" are left uncharacterized; even though, it was found that *Crinum* x *powellii* Baker displayed acetylcholinesterase (AChE) inhibitory activity (Rhee *et al.,* 2001). The main objective of this study was to isolate several alkaloids from *Crinum* x *powellii* "Album" (Amaryllidaceae) and investigate their DNA damaging activity through an *in vitro* mechanism-based bioassay utilizing genetically engineered mutants of the yeast *Saccharomyces cerevisiae.*

Experimental

Materials

In this research TLC silica gel plates F_{254} 0.20 mm and silica gel 70–230 and 230–400 mesh for column chromatography were used (Merck KGaA, Darmstadt, Germany). All solvents used for TLC, column separations and biological assay were analytical grade (Mallinckrodt, Phillipsburg,

NJ, USA). Nystatin was from Sigma (St. Louis, MO, USA).

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-300 spectrometer by using tetramethylsilane as internal standard. The alkaloids were also investigated by GC-MS on an Agilent gas chromatograph 6879N linked to an Agilent 5973N mass spectrometer system. The infrared spectra were recorded in a Nicolet Avatar spectrophotometer. TLC plates were monitored under UV light at 254 and 366 nm and by spraying with Dragendorff's reagent.

Plant material

Bulbs of *Crinum* x *powellii* "Album" were collected in Risaralda Department (coordinates: N 4° 42′ 35.923″, W 75° 36′ 23.658″), voucher No 137.806 HUA (Herbarium University of Antioquia, Medellín, Colombia). Fresh bulbs (5.60 kg) were washed, shopped and oven-dried at 50 °C and extracted with methanol at room temperature to render 641.1 g of a gummy brown extract.

Compound isolation

The methanolic extract (200.00 g) from the bulbs of *Crinum* x *powellii* "Album" was dissolved in aqueous hydrochloric acid (HCl), following the procedure described by Viladomat *et al.,* (1996). The acidic aqueous phase was extracted with dichloromethane (CH_2Cl_2) and then the acidic aqueous phase was made basic (pH 8.13) with 28% ammonium hydroxide (NH_4OH) followed by extraction with CH_2Cl_2 to furnish the acidic-CH_2Cl_2- (3691.0 mg), the basic-CH_2Cl_2-soluble fractions (6747.8 mg) and the aqueous layer, respectively. From the basic-CH_2Cl_2-soluble fraction a solid was obtained which was crystallized in hot methanol to furnish lycorine (**1**) (225.5 mg) and the mother liquid fraction (6234.0 mg). The mother liquid fraction was subjected to successive normal-phase silica gel column chromatography to afford 1-*O*-acetyllycorine (**2**) (19.3 mg) and ismine (**3**) (17.1 mg), respectively.

(–)-Lycorine (**1**): Colorless needles, m.p. 219.5–221.4 °C. – IR (KBr): ν_{max} = 3350, 3340, 3070, 3000, 2970, 2770, 1500, 1490, 1250, 1050, 950 cm^{-1}. – EI-MS: *m/z* = 287 [M^+] (Bastida *et al.,* 1998).

1-O-Acetyllycorine (**2**): Colorless needles, m.p. 170–172 °C. – IR (KBr): ν_{max} = 3600, 3100, 2950, 2870, 1740, 1510, 1495, 1240, 1050, 980 cm^{-1}. – EI-MS: *m/z* = 329 [M^+] (Bastida *et al.,* 1998).

Ismine (**3**): Colorless needles, m.p. 92–94 °C. – IR (KBr): ν_{max} = 3430, 3360, 3199, 3062, 2897, 2817, 1600, 1514, 1428, 1345, 1239, 1040, 930 cm^{-1}. – EI-MS: *m/z* = 257 [M^+] (Cabezas *et al.,* 2003).

Yeast bioassay

The yeast mutant bioassay was carried out using the following *Saccharomyces cerevisiae* strains: RJ03 (RAD+), RAD52Y and RS321. All strains were kindly donated by Dr. D. G. I. Kingston (Virginia Polytechnic Institute and State University, USA). Cultures were grown on YEPD broth and a portion of them was resuspended in 0.9% saline solution to obtain a 25% transmittance at 600 nm and worked out according to the agar well diffusion method described by Ríos *et al.* (1988). Plates were prepared by adding 1 ml of the respective yeast strain as inoculum, after that 20 ml of the agar were transferred to each Petri dish with homogenization. After solidification wells of 6.0 mm diameter were cut in the agar layer to deliver the plant extracts and controls. The isolated alkaloids were dissolved in methanol and tested at concentrations of 1000, 500, 250, 125 and 62.5 μg/ml. Nystatin at 30, 40 and 150 μg/ml was used as positive control for *S. cerevisiae* strains RAD+, RS321 and RAD52Y, respectively. After that, the plates were incubated at 30 °C for 36 h, and the inhibition zones were measured in millimeters. All determinations were performed in triplicate with two replicates.

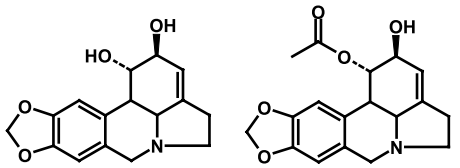
Data analysis

Activity was determined from a dose to response curve and is reported as IC_{12} (μg/ml) values, which is the concentration required for a pure compound to produce an inhibition zone of 12 mm in diameter, around each 6.0 mm diameter well (Gunatilaka and Kingston, 1998).

In this bioassay an extract is considered active if it displays selective activity against one or more repair-deficient yeasts; for instance, an agent that displays greater activity against RS321 rather than to RAD52Y, with an IC_{12} less than one-third between both yeast strains and in general exhibits an IC_{12} less than 2000, most probably mediates its inhibitory activity through topoisomerase II. Conversely, greater activity against RAD52Y implies an inhibitory DNA topoisomerase I mechanism (Zhou *et al.,* 2000; Gunatilaka *et al.,* 1994).

Results and Discussion

From *Crinum* x *powellii* "Album" three alkaloids were isolated, and based on the interpretation of ^{1}H and ^{13}C NMR and mass spectra, their correlation with published spectral data and by comparison with authentic alkaloid samples the structure deduction for compounds **1**, **2** and **3** (Fig. 1) was possible.



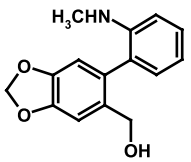


Fig. 1. Structure of alkaloids isolated from *Crinum* x *powellii* "Album".

The biological activity data for compounds **1**–**3** in the *in vitro* mechanism-based yeast mutant bioassay are displayed in Table I. The only compound that exhibited moderate but selective activity toward the repair-deficient RAD52Y (IC_{12} 218.7 μg/ml) compared to RS321 (IC_{12} 1542.7 μg/ml) was lycorine, indicating topoisomerase I inhibitory activity.

The fact, that lycorine (a phenanthridine alkaloid) showed significant DNA topoisomerase I inhibitory activity in this study, confirmed that plant-derived compounds are excellent sources for the discovery and development of new cancer chemotherapeutic agents with innovative mechanisms of DNA interaction. It is important to mention, that many cancer chemotherapies in current use today have their origins in plants; examples include: taxol, camptothecin, etoposide, among others.

Table I. IC_{12} values of alkaloids isolated from *Crinum* × *powellii* "Album" against *Saccharomyces cerevisiae* yeast mutant assay.

Alkaloid	IC_{12} [μg/ml] *S. cerevisiae* strains		
	RAD+	RAD52Y	RS321
Lycorine (**1**)	1542.7	218.7	1542.7
1-*O*-Acetyllycorine (**2**)	>2000	>2000	>2000
Ismine (**3**)	>2000	>2000	>2000

DNA synthesis is required for cancer cell growth; hence, DNA synthesis inhibitors may be considered as anticancer agents. Of the limited number of chemically active cancer chemotherapeutic compounds, DNA topoisomerases inhibitors are an invaluable subset in the artillery to develop novel anticancer agents (Yonezawa *et al.*, 2005). DNA topoisomerases are ubiquitous enzymes present in eukaryotic and prokaryotic cells; they are implicated within DNA topological changes, critical for many vital cellular processes such as replication, transcriptions, chromosome condensation. There are two classes of DNA topoisomerases: Topo I and Topo II.

Topo I acts by transiently breaking one of the two DNA strands. To date there are few compounds reported to inhibit Topo I activity, displaying different inhibitory degrees. Among them camptothecin and its semisynthetic analogues (topotecan, irinotecan, gimatecan, SV38, rubitecan, exetecan) are included (Di Francisco *et al.,* 2005; Kudoh *et al.,* 2005; Baka *et al.,* 2005; Kuo *et al.,* 2005). All these agents exert their cytotoxicity by stabilizing a ternary DNA-drug-enzyme complex which leads to DNA breaks and blocks the subsequent rejoining of the DNA (Etiévant *et al.,* 2003). On the other hand, different types of agents inhibit the catalytic activity of topoisomerase II, which breaks both DNA strands and requires ATP for full catalytic activity. Included in this group are aclarubicin, etoposide (Hajji *et al.,* 2005), bisdioxopiperazine derivatives, merbarone (Etiévant *et al.,* 2003), and doxorubicin (Yonezawa *et al.,* 2005).

In summary, in this work for the first time the isolation and structural elucidation of lycorine, 1-*O*-acetyllycorine and ismine from *Crinum* x *powellii* "Album" bulbs is reported and, under the yeast mutant assay, lycorine showed (IC_{12} 218.7 μg/ml) moderate topoisomerase I inhibitory activity. This finding opens the possibility to continue studies with this alkaloid or its semisynthetic derivatives and to explore it as a therapeutic agent.

In addition, this work demonstrates the ability of the *in vitro* mechanism-based yeast assay to

lead to the isolation of bioactive compounds with topoisomerases inhibitory activities and the potentiality of the Amaryllidaceae family as a source of novel anticancer agents.

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