THE SYNTHESIS OF ALKALOIDS FROM *CRINUM DELAGOENSE*, COMPOUNDS WITH POTENTIAL TUMOR SUPPRESSING ABILITY

by

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THE SYNTHESIS OF ALKALOIDS FROM *CRINUM DELAGOENSE*, COMPOUNDS WITH POTENTIAL TUMOR SUPPRESSING ABILITY

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ABSTRACT

Crinum delagoense, a plant native to South Africa, contains six different alkaloids (Nair, Campbell et al. 1997). Two of these alkaloids, delagoensine and delagoenine, had not been identified or characterized prior to the point of their initial isolation in 1997 and have since not been synthesized in a laboratory setting using a dedicated synthetic route. Due to their potential to combat certain types of human cancer, these two alkaloids are worthy targets for total synthesis. The purpose of this study is to synthesize delagoensine and delagoenine by modifying a previous route used in the synthesis of a related alkaloid system.

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INTRODUCTION

Alkaloids are a class of nitrogen-containing organic compounds (Bhusal, Uti et al. 2023). Alkaloids are basic in nature—they have a tendency to receive or "steal" a proton from solution or donate a pair of non-bonding electrons to a nearby compound. Alkaloids are heterocyclic, meaning that they contain ring structures composed of several different atoms. In the case of alkaloids, these atoms are carbon and nitrogen. These compounds typically originate in plants, and they are often physiologically active in that their consumption or entrance into the body via oral, respiratory, or IV intake may cause corporal responses. Examples of common types of alkaloids include morphine, nicotine, and quinine, each pictured below in Figure 1.

It can be clearly seen that each of these compounds contains ring-like structures and within each of them is a nitrogen. While each of these compounds is physiologically active, they each generate a different response in the human body. Morphine is a narcotic analgesic originating from *Papaver somniferum*, more commonly known as the poppy plant. It is a type of pain medication often seen in the hospital setting administered to patients after surgery. Nicotine comes from *Nicotiana tabacum*, or the tobacco plant, and is a highly addictive stimulant that is often found in smoking products. Quinine, a lesser-known alkaloid, is extracted from the bark of the cinchona tree (*Cinchona officinalis*) and has been used for centuries in the treatment of malaria, particularly in Africa and islands in the Caribbean.

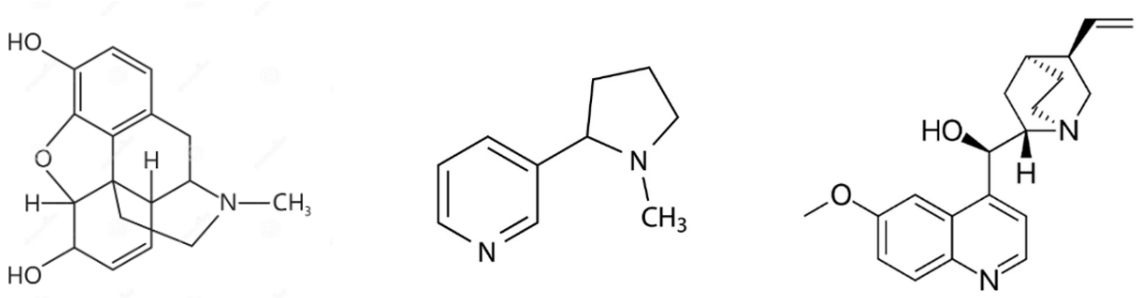


Figure 1. Chemical structures of morphine, nicotine, and quinine, respectively.

Crinum delagoense is a plant native to the Gauteng and KwaZulu-Natal Provinces of South Africa. It is often found in grasslands and deciduous woodlands, particularly on sandy soils. Aqueous extracts of this plant have been used for many years by the Zulu and Xhosa tribes in this region for medicinal purposes. For example, the Zulu people employ these extracts to treat urinary tract infections and swelling of the body (Watt and Breyer-Brandwijk 1962). We see once again that these alkaloids have significant medicinal properties just like morphine and quinine. Additionally, reports from the late 20th century showed that a hot water extract of *C. delagoense*, along with four other species, may be a cure for a type of human cancer. Thus, the synthesis of these alkaloids is worth an investigation.

Due to these reports, a group of researchers at the University of Cape Town in Cape Town, South Africa, set out in 1997 to isolate and characterize the alkaloids found in *C. delagoense*. Of the six that they found, four had been previously characterized—lycorine, 6-hydroxycrinamine, hamayne, and criwelline—pictured below in Figure 2. These compounds, along with others with similar chemical makeup, have been more recently tested for other physiological properties such as acetylcholinesterase inhibition (Moodley, Crouch et al. 2020).

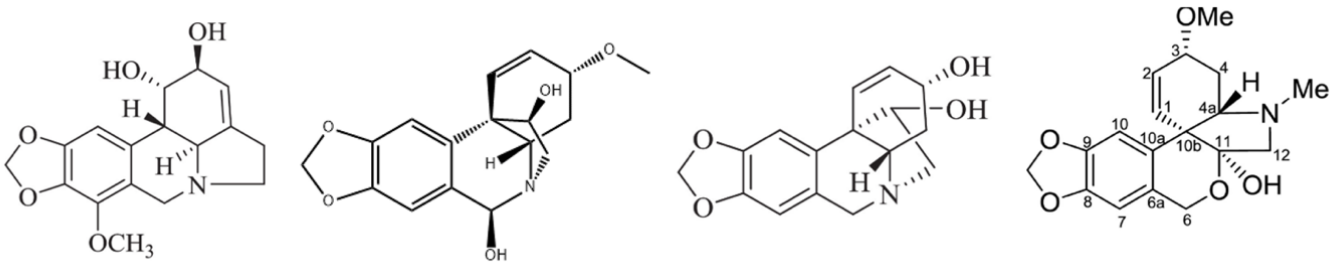
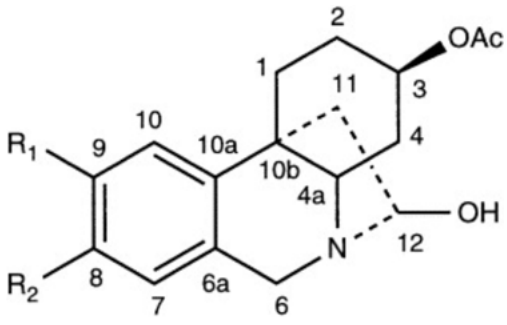


Figure 2. Four of six alkaloids isolated from *C. delagoense*: lycorine, 6-hydozycrinamine, hamayne, and criwelline, respectively.

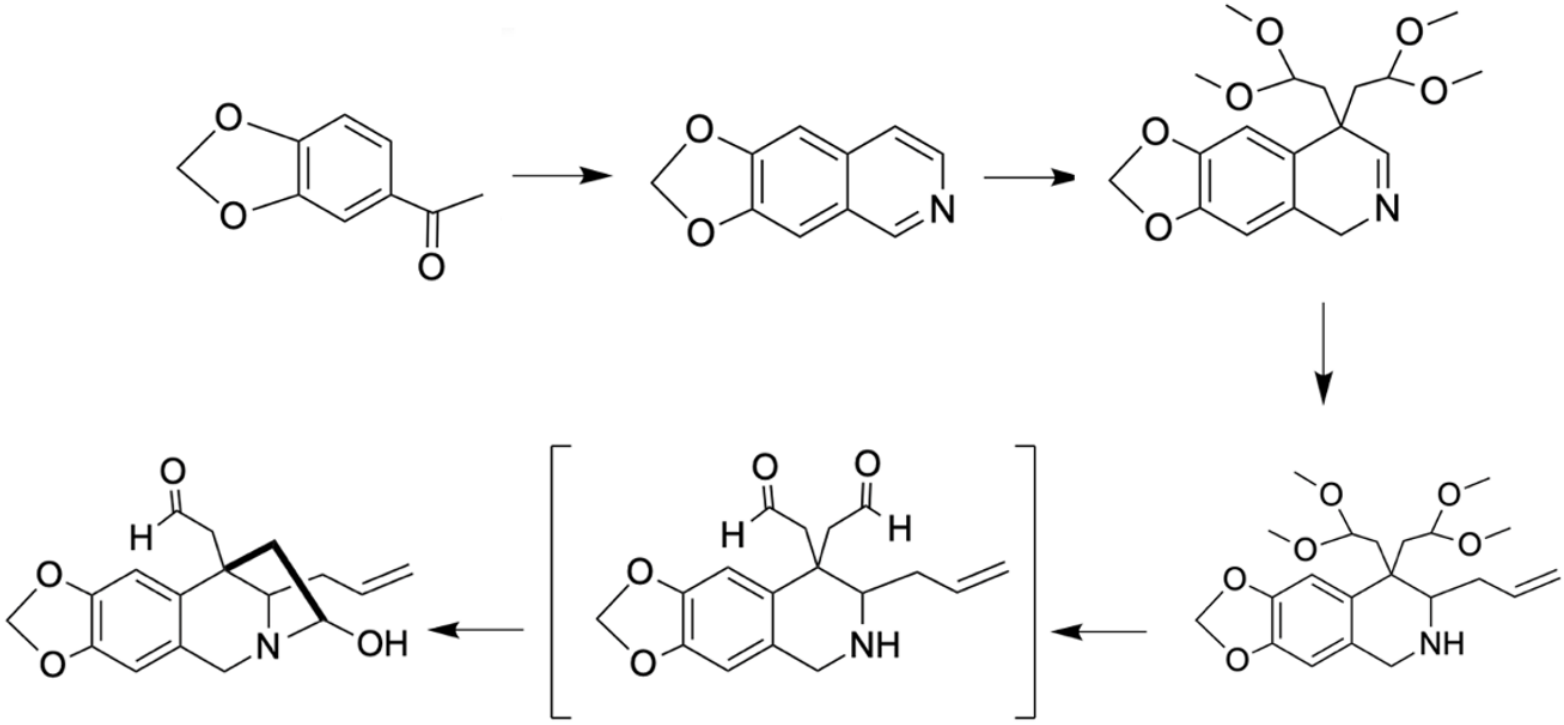
The two alkaloids from *C. delagoense* that had not been previously characterized were named “delagoensine” and “delagoenine.” The difference between these two compounds is simply the nature of a particular functional group on one end of the molecule. Delagoensine contains a methylenedioxy group while delagoenine contains two separate methoxy groups, making the overall structure a dimethoxy compound. The structures of both of these compounds can be seen in Figure 3 below (Nair, Campbell et al. 1997). Since their initial characterization, there have been no research studies published in the literature that show an attempt at their synthesis.



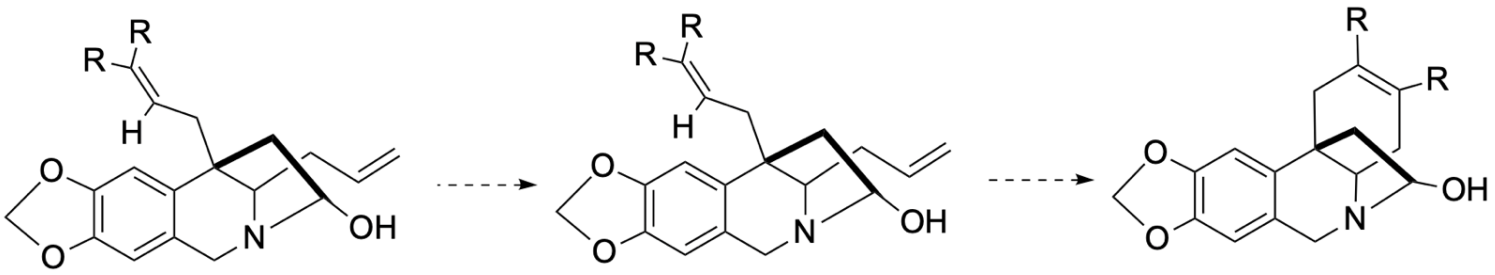
1: $R_1 + R_2 = OCH_2O$
2: $R_1 = R_2 = OMe$

Figure 3. General structures for delagoensine and delagoenine, the two previously uncharacterized alkaloids from *C. delagoense*

The synthetic route attempted in this study began with piperonal and continued through four steps to an isoquinoline intermediate. An acetal protecting group would be added using a reductive alkylation sequence followed by the addition of an allyl group via a Grignard reagent. Hydrolysis of the acetal to the aldehyde was expected to lead to a spontaneous cyclization thus forming one of the two new rings in the final product as indicated in Scheme 1. Due to time constraints, the Wittig reaction and olefin metathesis that would lead to the closure of the second ring and the formation of delagoensine/delagoenine depicted in Scheme 2 were not attempted.



Scheme 1. Steps of the synthetic route towards delagoensine partially completed.



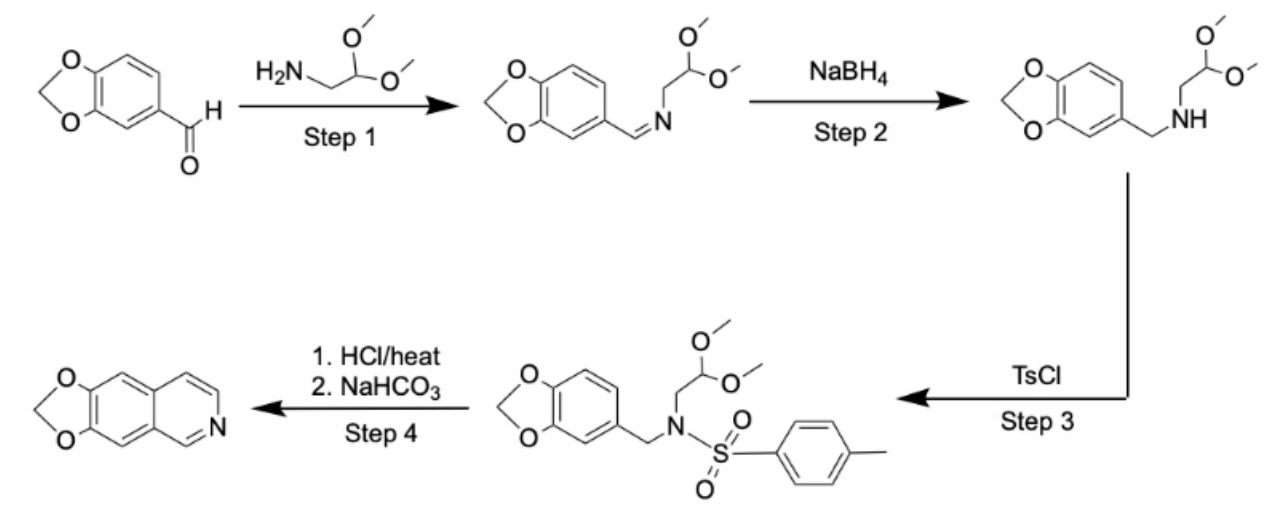
Scheme 2. Final steps (Wittig reaction and olefin metathesis) in synthesizing delagoensine that were not attempted.

The remainder of this paper will discuss the specific details of this synthetic route—the reagents and procedures used, what was found to be successful, and what may need modification for future researchers.

RESULTS AND DISCUSSION

In order to begin the synthetic route as previously described, a significant amount of 6,7-methylenedioxyisoquinoline had to be synthesized using a combination of various procedures found in the literature that had previously been accomplished in Dr. Minter's lab (Kornblum, Powers et al. 1957, Birch, Jackson et al. 1972, Miller and Frincke 1980). It is worth clarifying that in the overall synthetic route to delagoensine, which contains the methylenedioxy group, the required starting material is 6,7-methylenedioxyisoquinoline. However, the starting material for delagoenine contains two methoxy groups instead of one methylenedioxy group. Fortunately, the synthesis shown in Scheme 3 can be used to prepare both molecules by simply changing the aldehyde from piperonal to 3,4-dimethoxybenzaldehyde and using the same four steps.

A. Preparation of 6,7-methylenedioxyisoquinoline



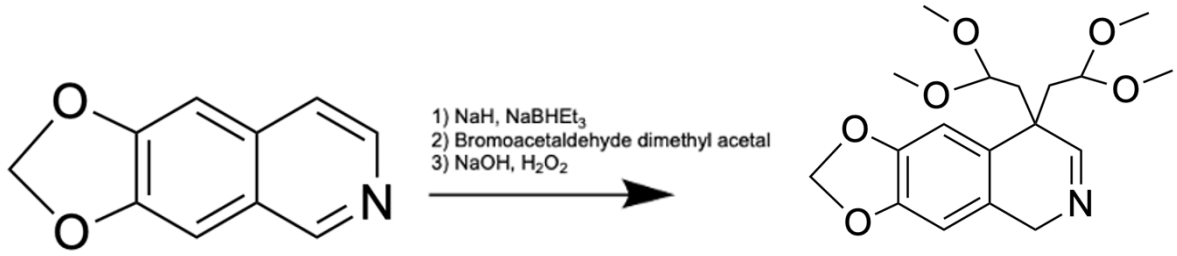
Scheme 3. Synthetic pathway to 6,7-dimethoxyisoquinoline, which was used to synthesize 6,7-methylenedioxyisoquinoline.

The first step in the synthesis of 6,7-methylenedioxyisoquinoline is the addition of aminoacetaldehyde dimethyl acetal to the starting material, piperonal, which is commercially

available. This leads to the formation of an imine intermediate in quantitative yield. Following the formation of the imine, sodium borohydride ($NaBH_4$), a strong reducing agent, was used to reduce to carbon-nitrogen double bond and form a secondary amine, also resulting in a quantitative yield. This secondary amine is a sufficiently strong nucleophile to attack the electrophile tosyl chloride (TsCl) used in step 3 to form an N-tosylated compound in 97.5% yield. The final step in this synthesis is a ring closure using acid and heat. This reaction proceeds through a carbocation intermediate, and both methoxy groups from aminoacetaldehyde dimethyl acetal as well as the tosyl group, are lost in the overall process. This results in the desired 6,7-methylenedioxyisoquinoline product, which will be used as depicted in Scheme 4 below.

The synthesis from piperonal to 6,7-methylenedioxyisoquinoline was repeated five times in order to accumulate a sufficient amount of material to proceed with the remainder of the overall synthetic route and avoid having to return to this procedure. To gain proficiency in essential laboratory techniques, this reaction was deliberately conducted several times on a small scale rather than once on a larger scale. Of these five attempts, three were successful. Step 4, the cyclization under acidic conditions, provided the greatest challenge. The proton NMR spectrum of the final product when the reaction failed showed several undesired peaks specifically in the range of 2 to 4.5 ppm. Since 6,7-methylenedioxyisoquinoline has no peaks upfield of 6 ppm in the proton NMR spectrum, the appearance of these peaks was interpreted as a failed reaction. The product mixture did contain 6,7-methylenedioxyisoquinoline, but there was also another compound in which the tosyl group was still present. Since the procedure to synthesize this product was almost always successful and separating the mixture would require development, we elected to re-run the reaction until we had accumulated a sufficient amount of material to proceed toward the steps shown earlier in Scheme 1.

B. Reductive alkylation of 6,7-methylenedioxyisoquinoline



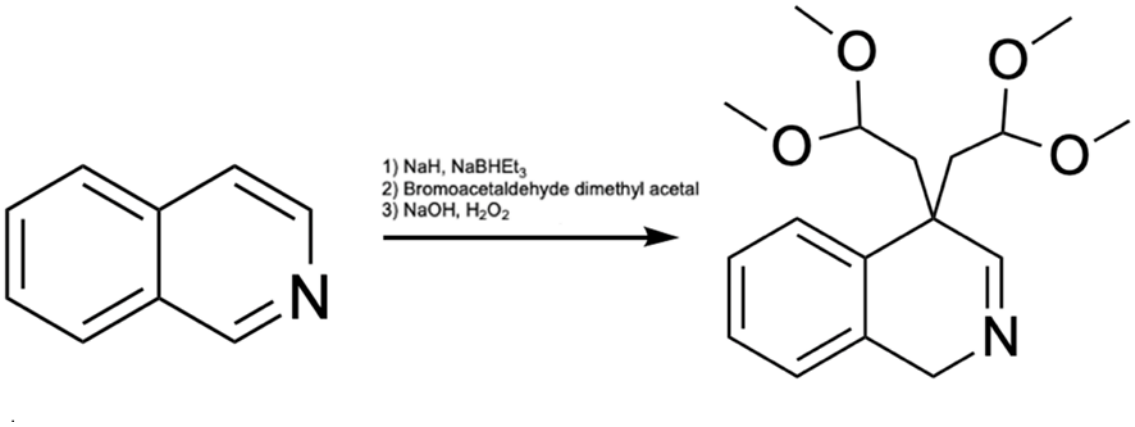
Scheme 4. Attempted reductive alkylation of 6,7-methylenedioxyisoquinoline.

The next step in the overall synthetic route towards delagoensine is the reductive alkylation of 6,7-methylenedioxyisoquinoline. Sodium triethylborohydride ($NaBHEt_3$) is a strong reducing agent, with the hydride ion acting as a nucleophile in step 1. In the mechanism of this reaction, bromoacetaldehyde dimethyl acetal is added to the enamine generated in step 1 from 6,7-methylenedioxyisoquinoline and $NaBHEt_3$. Sodium hydride (NaH) acts as a base that regenerates the enamine necessary to add the second equivalent of bromoacetaldehyde dimethyl acetal. In step 3, which is essentially a hydroboration workup, sodium hydroxide and hydrogen peroxide are used to destroy triethylborane forming ethanol and trisodium borate.

An NMR spectrum of the product of this step showed clearly that a reaction occurred. As previously mentioned, the proton spectrum of 6,7-methylenedioxyisoquinoline did not show any peaks upfield of 6 ppm; but the spectrum of the product showed several peaks attributable to the acetal function. However, we were unable to analyze all of the peaks including some that were very prominent and could not be attributed to the structure of the desired product.

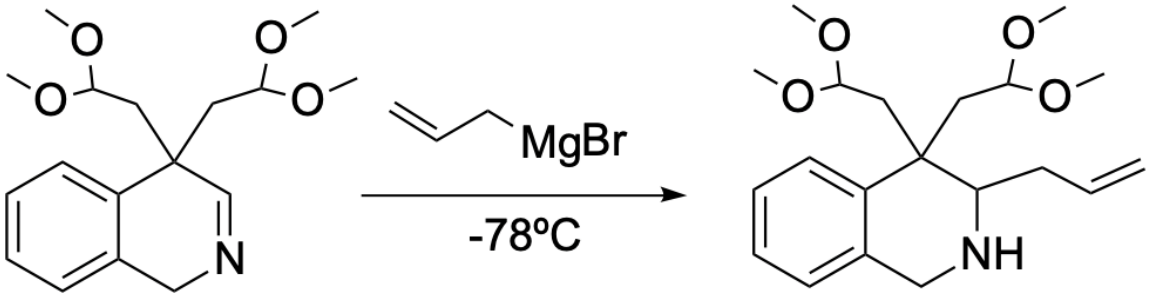
Despite several attempts at this reaction, it continued to produce mixtures. We attempted to allow the reaction to run for more time (24 hours, 48 hours, 72 hours), to reflux the reaction

mixture, and to add the reagents in a slightly different order and allow more time between the additions. None of these modifications were successful as each led to a product with an NMR spectrum containing unrecognizable peaks. Thus, we decided to use a simpler model system, isoquinoline, rather than the 6,7-methylenedioxyisoquinoline, as shown in Scheme 5 below. Utilizing isoquinoline allowed for a successful reaction using the original procedure and giving the desired product in 97.3% yield. This leaves the door open for future researchers in Dr. Minter's laboratory to explain why the presence of a methylenedioxy group apparently affects the efficacy of the procedure.



Scheme 5. Reductive alkylation of isoquinoline

C. Addition of an allyl group via a Grignard reagent

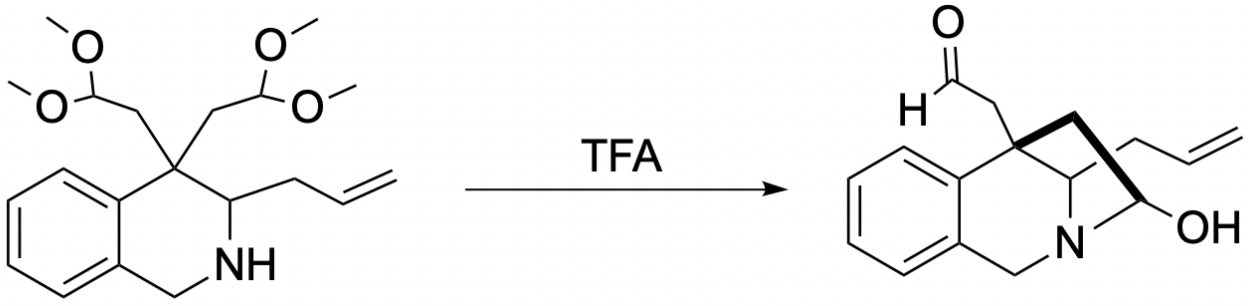


Scheme 6. Addition of an allyl group via a Grignard reagent.

The next step in the overall synthetic route to delagoensine is the addition of an allyl group via a Grignard reagent as shown in Scheme 6. This reaction was carried out at -78 ºC using a dry ice/acetone bath for the first several hours and continuing at room temperature overnight. The magnesium in the Grignard reagent creates a very nucleophilic carbon center that can then be used in a nucleophilic attack at carbon of the carbon-nitrogen double bond. The workup is completed using saturated ammonium chloride. A strong acidic workup was avoided in order to prevent accompanying hydrolysis of the acetal. We wanted to avoid taking too many steps forward, i.e., adding the allyl group and hydrolyzing the acetal, without first checking the purity of the Grignard reaction product.

This reaction was successful as indicated by the proton NMR spectrum. First, the peak from the imine hydrogen on the carbon-nitrogen double bond, which previously appeared at 4.8 ppm, disappeared. While that proton is still present in the product, the reduction of the carbon-nitrogen double bond and addition of an allyl group necessitates a change in chemical shift of that proton. Second, the appearance of peaks associated with a terminal alkene in the 5-6 ppm range points to the successful addition of the allyl group via the Grignard reagent.

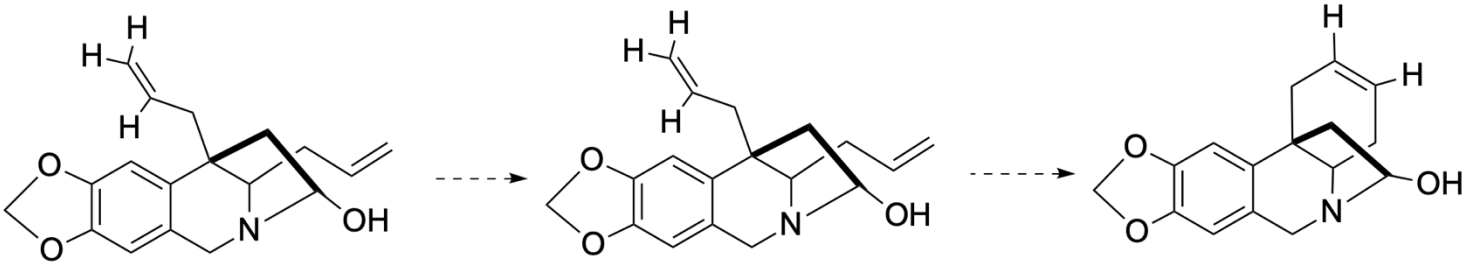
D. Hydrolysis of acetal group and spontaneous cyclization



Scheme 7. Hydrolysis of acetal group and spontaneous cyclization.

The next step in the overall synthetic route to delagoensine is the hydrolysis of the acetal protecting group, which should then lead to a spontaneous ring closure as shown in Scheme 7. If time had permitted, the Grignard product would have been purified at this stage. Nevertheless, the hydrolysis reaction using aqueous TFA was carried out with the hope that an NMR spectrum of the crude product would reveal evidence of the desired compound. Unfortunately, this was not the case. We anticipated seeing a triplet in the proton NMR spectrum with a chemical shift of approximately 9 ppm. This is where aldehyde hydrogens typically appear. However, this peak was not visible. There were several smaller peaks in the spectrum with a chemical shift close to 9 but none that appeared as a triplet or with an acceptable coupling constant. Therefore, we concluded that the reaction was unsuccessful.

E. Wittig reaction and olefin metathesis



Scheme 8. Projected final steps in completing the delagoensine ring system.

Due to time constraints, the reactions shown in Scheme 8 were not investigated. The repeated failure of the reductive alkylation of 6,7-methylenedioxyisoquinoline delayed the projected timeline beyond the due date for this thesis. However, the success of the reductive alkylation shown in Scheme 5 provides encouragement that the project has merit and will provide a good place for future researchers to start. The failure of the reductive alkylation to proceed as shown in Scheme 4 remains a mystery that will require an immediate solution. On

the other hand, the steps shown in Scheme 8 have already been accomplished in another study from this laboratory using a different but very similar substrate. Therefore, these are likely to proceed smoothly.

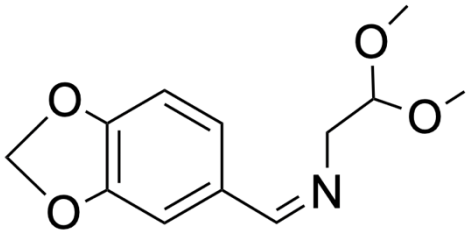
CONCLUSION

The pursuit of a total synthesis of delagoensine has served as a challenging chemical endeavor. Delagoensine, a structurally intricate alkaloid, presents a compelling synthetic target due to its unique polycyclic framework and potential biological significance. While the complete synthesis of delagoensine remains an ongoing effort, the progress achieved thus far has yielded valuable intermediates and confirmed the viability of key transformations within the synthetic pathway. Importantly, the work has contributed to a growing understanding of how to approach the construction of the delagoensine scaffold with both efficiency and selectivity. The reactions performed, including successes as well as the difficulty encountered in the reductive alkylation of 6,7-methylenedioxyisoquinoline, underscore the complexity of natural product synthesis and the precision required to manipulate such intricate molecular architectures.

EXPERIMENTAL PROCEDURES

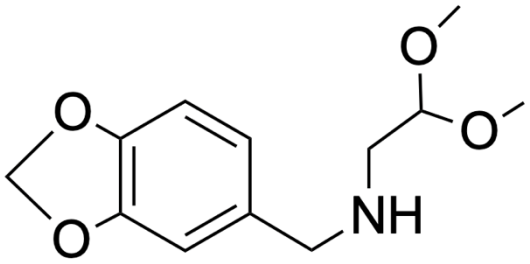
Preparation of 6,7-methylenedioxyisoquinoline.

a. Formation of the imine intermediate from piperonal.



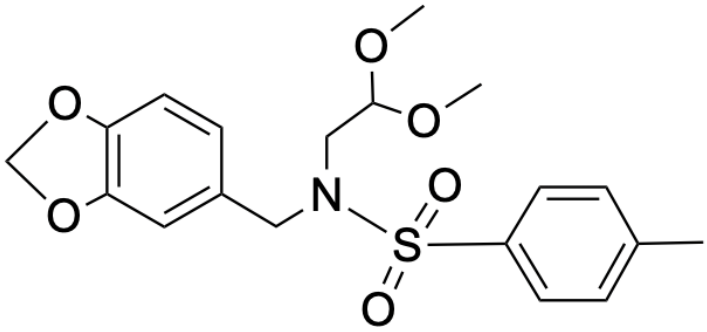
A mixture of 9.01 g (60.0 mmol) of piperonal and 7.0 g (66.5 mmol) of aminoacetaldehyde dimethyl acetal was heated at 70 ºC under nitrogen with magnetic stirring. After 5 hours of stirring, the reaction flask was cooled to room temperature and placed on a vacuum line at 0.5 mm Hg to remove residual volatiles. The crude product crystallized during this process and weighed 14.78 g, indicating a near quantitative yield.

b. Reduction of imine to a secondary amine.



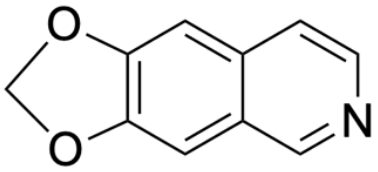
The imine from the previous step (14.78 g, 62.3 mmol) was dissolved in 70 mL of 90% ethanol and cooled to 0 ºC. Sodium borohydride (2.23 g, 58.8 mmol) was added in small portions over 30 minutes. After addition, the pale orange solution was allowed to stir under nitrogen for 18 hours. The reaction mixture was poured into 75 mL of water in an Erlenmeyer flask and stirred for about 45 minutes until hydrogen evolution stopped. The solution was added to a separatory funnel along with an additional 150 mL of water and extracted with dichloromethane (3 x 50 mL). After drying over Na_2SO_4, the solvent was removed by rotary evaporation and then placed on a vacuum line to remove residual volatiles. The crude product weighed 14.06 g (59.0 mmol).

c. Addition of the tosyl group.



The crude amine from the previous step (14.06 g, 59.0 mmol) was dissolved in 60 mL of dry pyridine and cooled to 0 °C. Tosyl chloride (13.31 g, 69.8 mmol) was added all at once and the ice bath was removed. After 48 hours of stirring at room temperature, the reaction mixture was poured into 400 mL of saturated sodium bicarbonate solution at 0 °C with rapid stirring. After evolution of CO_2 ceased (about 1 hour), the reaction mixture was transferred to a separatory funnel and extracted with dichloromethane (4 x 40 mL). The combined extract was washed successively with 5% KOH (1 x 50 mL) and brine (1 x 50 mL), dried over Na_2SO_4, rotary evaporated, and then pumped at 0.1 mm Hg to remove residual pyridine. The resulting brown solid (22.6 g, 57.5 mmol) was used for the next step without purification.

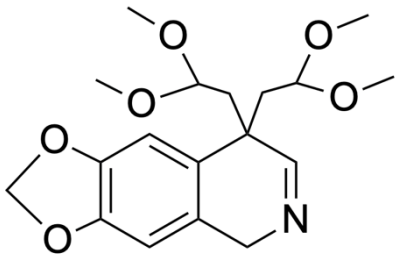
d. Removal of tosyl group via cyclization using acid and heat.



The crude tosylamide from the previous step (22.6 g, 57.5 mmol) was dissolved in 300 mL of dioxane and 82 mL of 6M HCl. After heating at reflux for five hours, the solution was concentrated using simple distillation to remove 150 mL of dioxane. The solution was cooled to 0 °C and neutralized by slow addition of sodium bicarbonate. When evolution of CO_2 had ceased (about 3 hours), the mixture was transferred to a separatory funnel and extracted with dichloromethane (1 x 80 mL, 2 x 60 mL). The combined extracts were dried over Na_2SO_4,

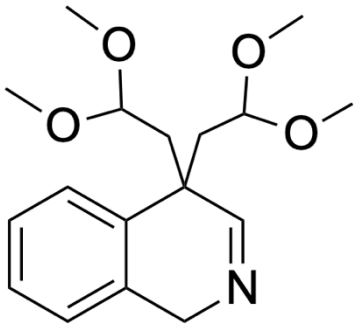
filtered, and concentrated by rotary evaporation to give a brown liquid. The liquid was pumped at 0.5 mm Hg to remove residual dioxane. The crude product was recrystallized and vacuum filtered from cyclohexane (about 65 mL). Not all of the product dissolved. Residual cyclohexane was removed at 0.5 mm Hg resulting in a pure pale-yellow product, MP 120 ºC.

Failed reductive alkylation of 6,7-methylenedioxyisoquinoline.



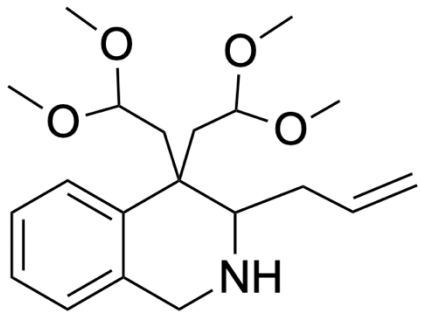
NaH dispersed in mineral oil (0.21 g of 60% dispersion, 5.10 mmol) was placed in a 3-neck, 50 mL RBF under a nitrogen atmosphere and rinsed with two 4.0 mL portions of hexane. A solution containing 0.52 g (3.01 mmol) of 6,7-methylenedioxyisoquinoline in approximately 4 mL of dry THF was added via syringe. To this mixture was added 4.0 mL of 1.0 M $NaBHEt_3$ (4.0 mmol) dropwise over two minutes at room temperature. The reaction mixture became a dark brown color immediately. After 15 minutes, 1.02 mL (6.8 mmol) of bromoacetaldehyde dimethyl acetal was added dropwise. Unexpectedly, gas evolution and a color change were not observed. The mixture was allowed to stir for approximately 16 hours after which it became a brownish yellow color. The mixture was cooled to 0 °C and 20 mL of 0.5 M NaOH was added slowly. This was followed by the addition of 4.0 mL of 35% H_2O_2 and the ice bath was removed. After two hours of rapid stirring, the mixture was poured into a separatory funnel containing 30 mL of water and 20 mL of brine and extracted with dichloromethane (1 x 30 mL, 2 x 15 mL). Extracts were dried over Na_2SO_4 and concentrated by rotary evaporation. The sample was placed on a vacuum line to remove residual solvent. The proton NMR spectrum indicated an intractable mixture of compounds including the starting isoquinoline.

Successful alkylation of isoquinoline



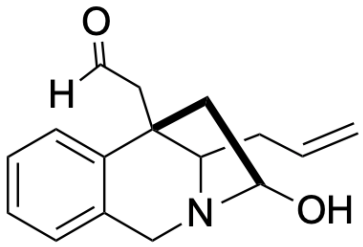
NaH dispersed in mineral oil (0.60 g of 60% dispersion, 15.0 mmol, 50% excess) was placed in a 3-neck, 250 mL RBF under a nitrogen atmosphere and rinsed with three 5.0 mL portions of hexane. $NaBHEt_3$ (12 mL of 1.0 M solution in THF, 12.0 mmol, 20% excess) was added dropwise at room temperature. Isoquinoline (1.20 mL, 10.1 mmol) was then added. After 3-4 drops were added, the reaction mixture turned a greenish yellow. Immediately after full addition, the reaction mixture was a dark maroon. Two to three minutes after full addition, the reaction was a burnt orange/light brown color. After approximately 30 minutes of stirring at room temperature, 2.60 mL (22.0 mmol, 10% excess) of bromoacetaldehyde dimethyl acetal was added dropwise. The reaction mixture turned a dark forest green color and bubbles of hydrogen gas were immediately observed. The suspension was allowed to stir for approximately 60 hours after which the reaction mixture was a tan color. The mixture was cooled to 0 °C and 78 mL of 0.5 M NaOH was added slowly followed by 15 mL of 35% H_2O_2. The reaction mixture was pale yellow after 2.5 hours of stirring. The mixture was then poured into a 1 L separatory funnel containing 90 mL of water and 60 mL of brine and extracted with dichloromethane (1 x 90 mL, 2 x 45 mL). Extracts were dried over Na_2SO_4 and concentrated by rotary evaporation. The sample was placed on a vacuum line to remove residual solvent. The sample weighed 2.88 g (9.86 mmol). The proton NMR spectrum showed that this reaction led to the successful addition of the dimethyl acetal groups, and a triplet at approximately 7.70 ppm indicated that the proton on the imine carbon was present.

Addition of the allyl group via a Grignard reagent



The imine formed from the previous reaction (2.88 g, 9.86 mmol) was dissolved in 40 mL of dry THF. The solution was cooled to -78 °C and a 100% excess of 1 M allylmagnesium bromide in diethyl ether (20.2 mL, 20.2 mmol) was added slowly under a nitrogen atmosphere. The reaction mixture stirred for two hours, then the dry ice bath was removed and the reaction continued to stir overnight. The reaction was quenched using 25 mL of saturated NH_4Cl. The mixture stirred for approximately 12 hours before being added to a 250 mL separatory funnel. The aqueous layer was removed, and the organic layer was washed with 25 mL of brine. The organic layer was dried with Na_2SO_4 and solvents were removed via rotary evaporation. Residual solvent was removed on a vacuum line. The product weighed 2.77 g (8.31 mmol).

Hydrolysis of the acetal and spontaneous ring closing



A solution containing 2.77 g (8.31 mmol) of the crude product from the previous step in 35 mL of chloroform was cooled to 0 °C in an ice bath under nitrogen. A solution containing 17.5 mL of TFA and 17.5 mL of water was added dropwise during 30 minutes using a dropping funnel. The reaction mixture was then allowed to warm to room temperature and stir overnight after which it was cooled to 0 °C and neutralized by slow addition of saturated $NaHCO_3$ with rapid stirring. The chloroform layer was separated and the aqueous layer was extracted with dichloromethane (3 x 25 mL). The combined dichloromethane and

chloroform layers were washed with 50 mL of brine, dried over Na_2SO_4 and rotary evaporated. Residual solvents were removed on a vacuum line.

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