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# Synthesis of the Pterocarpan Cabenegrin A-II

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## *Abstract*

Cabenegrin A-II is a molecule found in small quantities in the South American plant commonly called Portuguese Snake Herb, and is useful as a snake and spider anti-venom. Cabenegrin A-II occurs in these plants as a mixture of compounds. It belongs to the biologically active class of compounds known as pterocarpans. Synthetic methods for preparing cabenegrin A-II and other pterocarpans are highly valued. This research develops a novel route to construct the core portion of cabenegrin A-II, which is common to all pterocarpans. This method will allow us to synthesize both ends of the molecule separately. The fragments are then bonded together while the pterocarpan core is synthesized. The strategy used in these experiments will be useful for constructing other biologically important pterocarpans.

## *Introduction*

Cabenegrin A-II is a naturally occurring compound that acts as an antidote for snake and spider venom (Da Silva, 1997). South American plantation workers use an alcohol extract from a South American plant that contains this compound as an antidote for the venom of *Bothrops atrox*, the snake commonly known as Fer-de-Lance. Cabenegrin A-I and cabenegrin A-II were isolated from a local antidote for *Bothrops atrox* venom (Nakagawa, 1982) and (Nakaneshi, 2006). Research performed by Da Silvis and coworkers suggest that the identity of this plant is *Harpalyce brsiliana*, known also as Erva' de Cobra, or Portuguese snake herb. They report isolating both cabenegrin A-I and cabenegrin A-II from this plant (Da Silva, 1997).

Acquiring the molecule from natural sources is problematic, and the compound exists in very small, impure amounts. This molecule is a member of a class of molecules known as pterocarpans, which contain a common core substructure of four fused rings. In previous laboratory syntheses the core portion of cabenegrin A-II is synthesized first and the substituents added later (Ishiguro, 1982). These processes have consisted of lengthy linear syntheses with low overall yields. There is no industrially acceptable process to date. Our research focuses on a method to improve the synthesis of cabenegrin A-II. In the study detailed below, we synthesize two halves of the molecule from 1,3-dimethyoxybenzene, succinic anhydride, and seasmol. The two fragments are bonded together and cyclized to form the pterocarpan core in two or three quick steps.

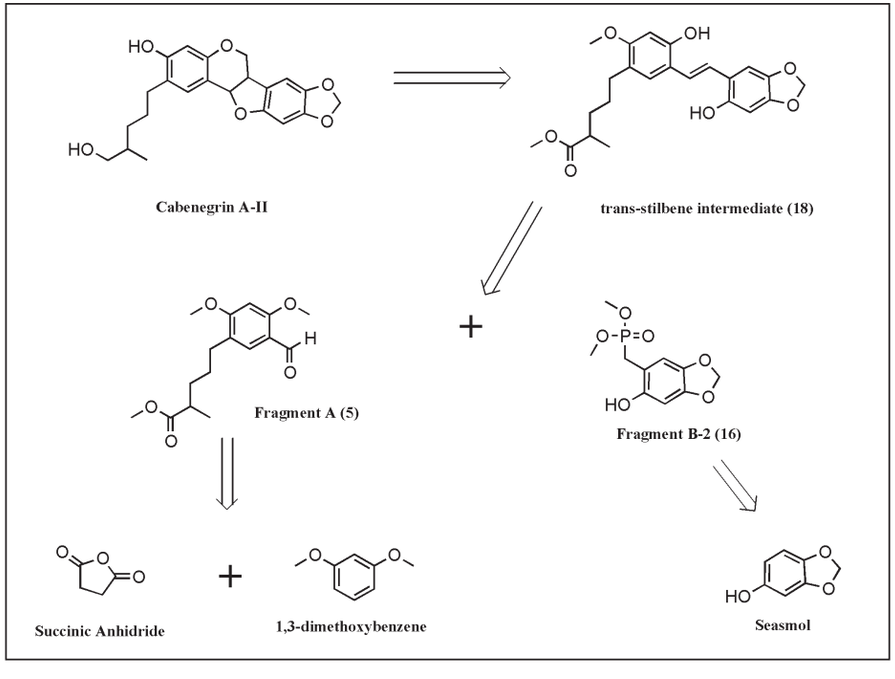
This research will provide an industrially acceptable process for the synthesis of cabenegrin A-II and other biologically active pterocarpans. We can decrease waste, production time, and financial risk by reducing the number of successive reactions as we reduce the complexity of the intermediate compounds.

There are many examples of biologically active pterocarpans. Some act as anti-fungal agents, such as glyceillin-I, glyceollin-II and glyceolin-III. Antiviral properties are reported as well (Bartz, 1992). When the methods developed by this research are established, not only will the synthesis of pterocarpans become more efficient, enhancement of the ability to synthesize other related molecules is also likely.

## *Research*

The research portion of the paper includes figures and schemes. Each figure shows the molecular structure of a compound. Schemes illustrate changes in molecular structure during a series of reactions. In figures and schemes each unique compound is given a boldface number to identify it. Our synthetic strategy for synthesizing cabenegrin A-II is described in Scheme 1 in retrosynthetic format. This type of analysis looks at synthesizing a complicated molecule such as cabenegrin A-II in reverse, moving from the complex target to simple starting materials. Cabenegrin A-II is synthesized from *trans*-stilbene intermediate **18** by cyclizing the pterocarpan core in several steps. Compound **18** is synthesized using a Wittig reaction to combine **5** and **16**. Compound **16** is synthesized from commercially available seasmol using a two-step process, while **5** is synthesized from 1,3-dimethoxybenzene and succinic anhydride (both commercially available materials) in a five-step process.

**Scheme 1**
***Retrosynthetic Analysis***



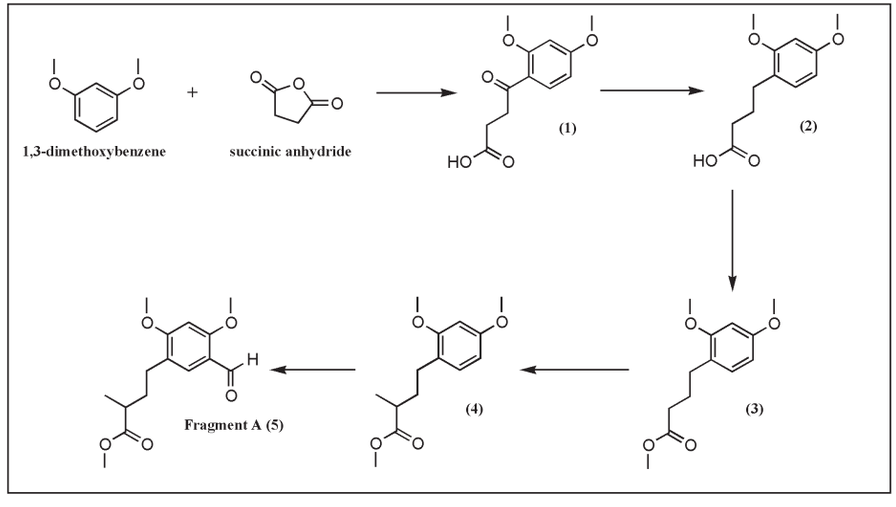

## Synthesis of Fragment A

The synthesis of Fragment A (**5**) is described in the forward direction in Scheme 2. A Friedel-Crafts acylation with succinic anhydride and 1,3-dimethoxybenzene produces the carboxylic acid **1**. The newly formed carbon-carbon bond forms selectively between the fourth carbon of 1,3-dimethoxybenzene and one of the carbonyl carbons of the anhydride ring, producing **1**. The carbonyl group (C=O) of



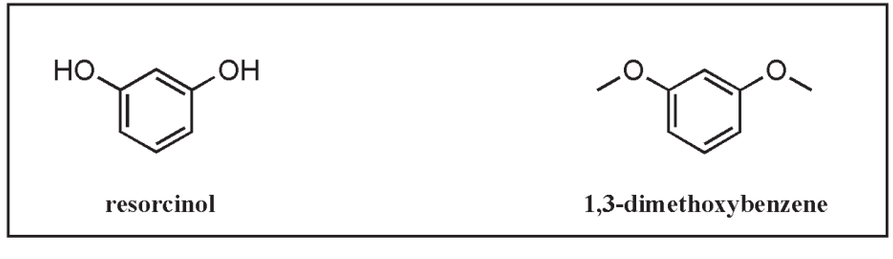
**1** is reduced using a Clemmensen reduction employing amalgamated zinc, hydrochloric acid (HCl), ether, and heat, yielding carboxylic acid **2**. The carboxylic acid **2** is next esterified using oxalylchloride and methanol to form **3**. The alpha carbon of **3** is methylated using lithium diisopropylamide (LDA) and iodomethane producing **4**. The final step in Scheme 2 is a Vilsmeier formylation to form the aldehyde **5**.

**Scheme 2**
***Synthesis of Fragment A (5)***



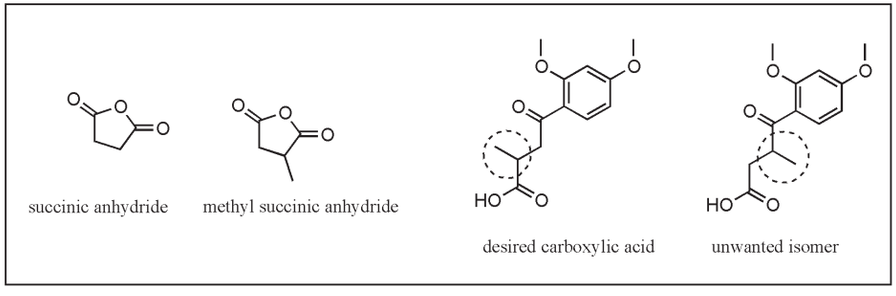

The synthesis of **5** originally started with resorcinol rather than 1,3-dimethoxybenzene (Figure 1). We tried both dichloromethane and nitrobenzene as solvents, but resorcinol is not very soluble in either solvent. Nitrobenzene is a better solvent than dichloromethane, but it is hard to get out of the end product and yields are low. For these reasons, nitrobenzene did not look promising as a solvent. Therefore, its use was discontinued. Several reactions using dichloromethane and resorcinol were explored with limited success. Finally we replaced resorcinol with a 1,3-dimethoxybenzene. This eliminated the solubility issues with resorcinol.

**Figure 1**
***Structures of resorcinol and 1,3-dimethoxybenzene***



The synthesis of Fragment A initially used methyl succinic anhydride in place of succinic anhydride (Figure 2). The yields from these reactions with dichloromethane as the solvent were still relatively low. The reactions continued to produce many impurities that were hard to separate from the desired product. Thin layer chromatography suggested the impurities were not starting material, and a workup to remove the impurities was elusive.

**Figure 2**
***Structural Isomers Using Methyl Succinic Anhydride***




Recrystallization was explored extensively, but no suitable solvent system was found. Using column chromatography, we purified a very small portion of the overall yield. Analysis of the fractions with proton nuclear magnetic resonance spectroscopy ($^1$H NMR) suggested that the methyl succinic anhydride was not binding to the benzene ring selectively. The reaction was producing two structural isomers, as shown in Figure 2. The first was the desired product with the methyl group on the alpha carbon. The other had the methyl group on the beta carbon. These compounds have similar properties, making separation difficult. Selectivity was very close to 50:50 and much starting material was lost to the undesired isomer regardless of purity after separation.

To solve this problem methyl succinic anhydride was replaced by succinic anhydride (Figure 2). Analysis of a small reaction revealed the expected product. The product was impure, but column chromatography successfully removed most of the impurities.

A one-gram batch of **1** was prepared. During this reaction it was necessary to heat the dichloromethane to 30ºC to dissolve the succinic anhydride. The product was very impure, and the yield was significantly reduced. The reaction was performed again and during the workup I failed to adjust the pH of the solution to basic before extraction. Pure product precipitated out with a yield of 98%.

The research continued until we produced compound **5**. All went well until we attempted a six-gram batch of compound **1**. During this reaction solubilizing the succinic anhydride was more difficult. Large amounts of dichloromethane were required to dissolve the succinic anhydride. The yield of the six-gram batch was only 60%. The yield decreased dramatically as the scale went up. The workup became physically difficult and used a lot of solvent.

Because of these solubility issues we again investigated this reaction using nitrobenzene, succinic anhydride, and the revised, acidic, workup. This produced



a lot of solid product with the familiar impurities. Several unsuccessful attempts to purify the solid were made. Then the breakthrough came. The contaminated solid was dissolved in sodium hydroxide solution and washed with ether. The aqueous layer containing the product was acidified and the product precipitated. After filtering and drying, the product was free from the organic impurities.

The need to remove the impurities led to the current procedure where product is extracted from the nitrobenzene with sodium hydroxide solution. This is washed with ether, acidified with HCl, and then extracted with dichloromethane. When the solvent is evaporated we end up with an extremely pure product. This is a good method that can produce yields as high as 98% and is a scalable procedure.

The next reaction, a Clemmensen reduction of **1** to produce **2**, worked the first time and always works well. The most toxic substance in our procedure, zinc/mercury amalgam, is used in this reaction. Methods to remove mercury from the synthesis may be explored in the future.

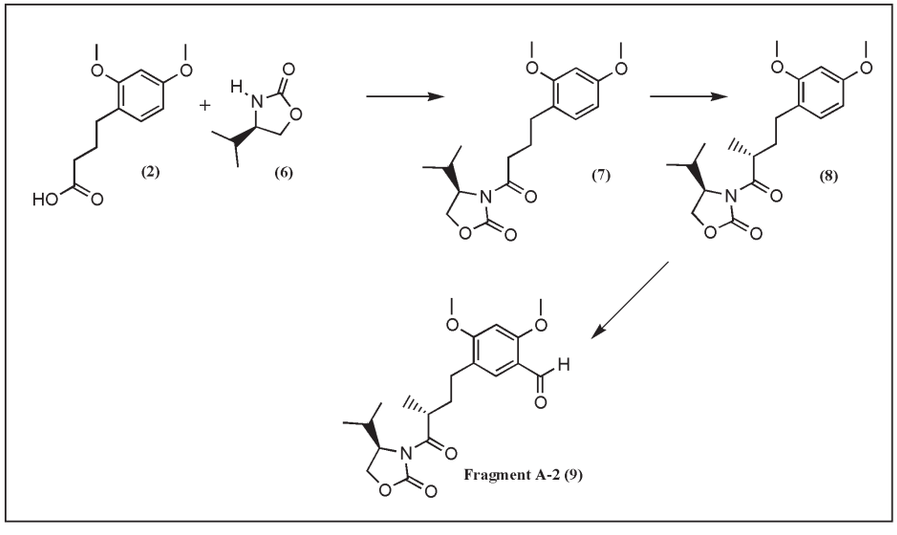
The esterification to produce **3** from **2** is a difficult reaction. The current procedure for the production of this intermediate is sometimes unpredictable. This reaction needs more experimentation and may be replaced in the future. Even though this reaction has problems, it can and does produce useful amounts of product. This reaction uses oxalyl chloride to form an acid chloride. Methanol then displaces the chloride, giving the ester. This nucleophilic substitution gives good yields with reasonably pure product. However, as stated before, this reaction can occasionally produce unexpected results. The ester is a protecting group used to prevent side reactions involving the carboxyl group. It will be converted into the hydroxyl group at the end of the synthesis. We are exploring other protective groups that may improve the synthesis.

The current methylation procedure to produce **4** from **3** is problematic. This reaction uses the base LDA to deprotonate **3**, and methyl iodide to install the methyl group. The reaction is procedurally difficult, requiring a reaction temperature of -78 ºC and a nitrogen atmosphere. It also produces a dimethylation by-product. Many variations in conditions have been explored. The first few attempts at this reaction suggested that it was not proceeding to completion. The reaction time was increased to three days, but thin layer chromatography (TLC) revealed that several compounds were present. Running the reaction for a longer time had little effect. A sample was analyzed by Gas Chromatography/Mass Spectrometry, which revealed fragmentation patterns consistent with dimethylation. The amount of LDA was lowered to a 1:1 molar ratio, but this had little effect on the product. This reaction is productive, but it is operationally difficult and the yields are approximately 50% after column chromatography. The methylation of **4** remains the biggest problem in Scheme 2. We are hopeful that by adjusting the reaction conditions the results can be improved.

The next step is the Vilsmeier formylation to produce **5** from **4**. This reaction, which uses phosphorusoxychloride and dimethyl formamide, is a good procedure. It worked well the first time it was performed.

Scheme 2 does not provide control of stereochemistry. Scheme 3 explores a way to control stereochemistry. Many pterocarpans, including cabenegrin A-II, are found as mixtures of stereoisomers. Stereoisomers are molecules with the same atom connectivity, but different orientations in space, as shown in Figure 3. Their shape differs in a manner similar to left and right hands.

**Scheme 3**
***Control of Stereochemistry in the Synthesis of Fragment A-2***

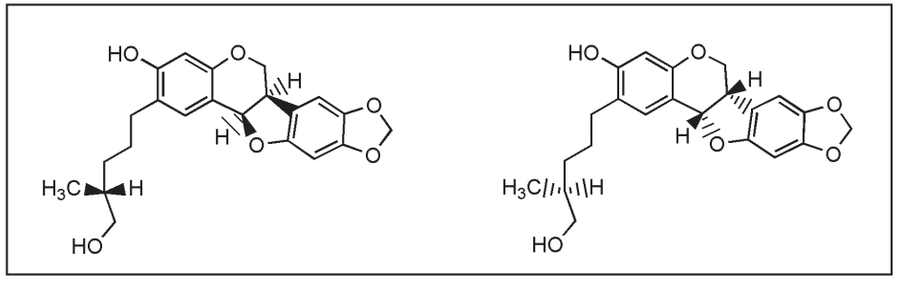



The stereochemistry of cabenegrin A-II does not have an effect on its effectiveness as snake venom antidote. However, molecular shape does affect some biochemical processes. The incorrect stereoisomer will not react with the intended substrate. Therefore, control of stereochemistry may become important in future applications using this synthetic method. Our research explores controlling stereochemistry.

Scheme 3, which is an attempt to control the stereochemistry of the methylation, is currently under development. Steric hindrance provided by an optically pure oxazolidinone is used to control stereochemistry (Evans, 1982). The large substituent will only allow nucleophilic attack from one direction.

The synthesis of **6** begins when L-valine is converted into L-valinol using $LiAlH_4$ (Evans, 1981). This reaction has been performed several times and the results are encouraging, although analysis suggests the reaction is not proceeding to

**Figure 3**
***Stereoisomers of Cabenegrin A-II***

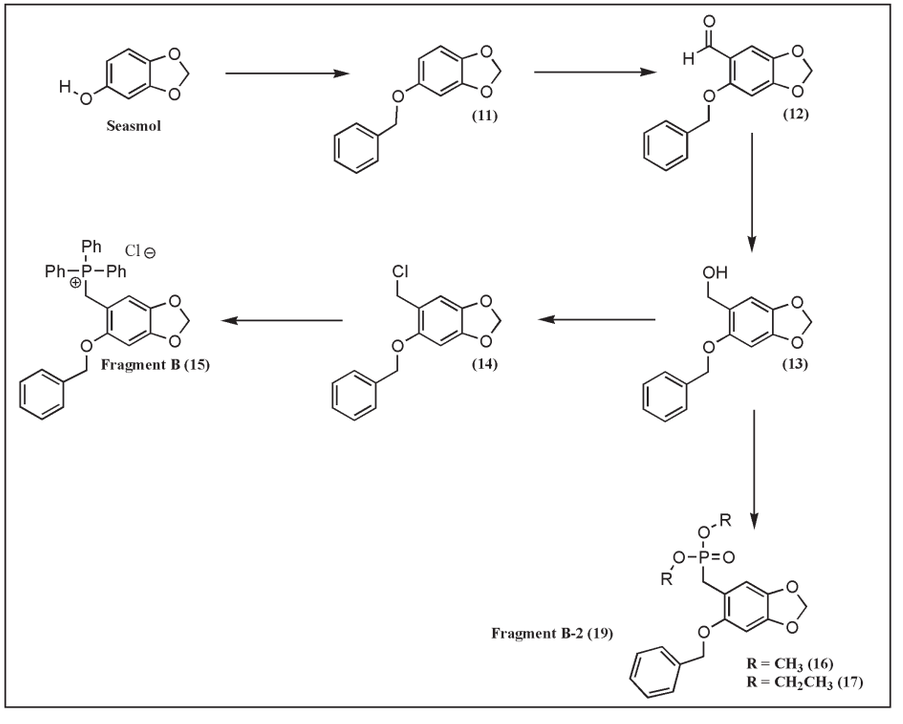




completion and therefore produces very low yields. In an attempt to improve yields we are lengthening the reaction time. The valinol is then converted to oxazolidinone **6** using carbonyl diimidazole to donate the carbonyl group. This reaction was performed several times. The last attempt produced a yield of 84%.

In the next reaction the oxazolidinone **6** reacts with **2** to give **7**. This will provide the steric hindrance that will allow us to selectively add the methyl group, yielding the single stereoisomer **8**. This synthesis was attempted once; analysis suggested it was successful (Kleschick, 1987). The size of the reaction was so small that determining a yield with the equipment available was not possible. This scheme is showing promise and may be successful at providing control over the chiral center. This may also provide a solution to the dimethylation problem.

**Scheme 4**
***Synthesis of Fragment B***




## Synthesis of Fragment B

The synthesis of Fragment B from seasmol is described in Scheme 4. Our first attempts used a Duff Formylation reaction to produce **12** directly from seasmol in yields of approximately 45%. Direct chloromethylation of seasmol was also unsuccessfully explored. In the current process, compound **11** is synthesized from seasmol by benzylation. Next, a Vilsmeier Formylation is used to convert **11** to **12** in an 87% yield. A reduction produces **13** from **12**. Sodium borohydride is used to reduce the carbonyl carbon to produce the alcohol **13**. This reaction works well

and in high yield. The next step is to produce **14**. This is done with thionylchloride and dichloromethane. The Wittig reagent **15** is synthesized from **14** using triphenylphosphine in dimethylformamide. $^{1}$H NMR analysis showed that compound **14** is unstable. We avoided it by producing Fragment B-2 from **13**, giving compounds **16** and **17**. Compound **13** is treated with iodine and trimethylphosphite to produce the Wittig reagent **16** (McKennon, 1993). We attempted this reaction twice. The first time we tried distillation to remove the excess trimethylphosphite. This was too much heat for the compound and it became a black tar-like mass. By more carefully controlling the temperature during distillation, the reaction was successful and the yield appeared to be good. The product was purified with column chromatography, but the yield was difficult to quantify because of the small amount of product. We also explored using triethylphosphite in place of trimethylphosphite giving a small amount of **17**. This was the molecule used in the first attempt at stilbene synthesis (McKennon, 1993). Removal of excess triethylphosphite was difficult. Because removal of excess trimethylphosphite was easier, the focus remained on compound **16**.

## Stilbene Syntheses

The stilbene synthesis is promising, but only a few reaction attempts have been made thus far. These reactions use sodium hydride with dimethylformamide as the solvent (Moody, 1990). A reaction using **15** successfully formed **18** as a mixture of trans and cis isomers. The next several reactions require the trans isomer; attempts to purify **18** were unsuccessful. No further experiments have yet taken place.

The synthesis was more successful using **16**. Tetrahydrofuran (THF) was used as the solvent, and sodium hydride was the second reagent. This reaction looks very promising and it was the first to produce a nice dry solid. Future work will follow. It is clear that this research will produce a useful method to synthesize cabenegrin A-II.

## *Conclusion*

The results of this research are significant in several ways. First, they offer the promise of greater efficiency in the synthesis of cabenegrin A-II. Having a better synthesis of this valuable molecule will improve its accessibility, which is presently limited. Second, the lessons learned from both successful and disappointing reactions help us better understand the chemical behavior of these types of molecules. Finally, this research advances the larger goal of developing a new general strategy for the synthesis of pterocarpans. This new strategy seeks to form the fully substituted core of pterocarpans near the end of the synthesis rather than at the beginning. The synthesis of cabenegrin A-II is a proof of concept case study for this strategy. Thus, the new knowledge generated in the present study may prove valuable in future syntheses of other pterocarpans. Work is continuing on completing the synthesis of cabenegrin A-II.

## *Acknowledgements*

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