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Exploring Cycloaddition Reactions for the Synthesis of Novel Organic Compounds, Including Microwave Promotion

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EXPLORING CYCLOADDITION REACTIONS FOR THE SYNTHESIS OF NOVEL ORGANIC COMPOUNDS, INCLUDING MICROWAVE PROMOTION

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ABSTRACT

The work presented here focuses on using the Diels-Alder reaction as the first step in "catch-and-release" strategies for isolating useful organic compounds, especially from natural product extracts. The purpose is to discover better isolation methods than those currently available. The proposed method uses a dienophile attached to a polymeric resin, allowing separation of the adduct from the filtrate by simply rinsing the polymer-bound adduct with solvents to remove extraneous compounds.

Different dienophiles were tested, including 4-phenyl-1,2,4-triazoline-3,5-dione, dimethyl acetylenedicarboxylate, and diphenyl acetylene. These were reacted with the furan ring of salvinorin A, an extract obtained from a natural product, which acted as the diene. These were tested to find dienophiles that could either potentially be fixed to a polymeric resin or provide some insight as to what conditions salvinorin A might react under. These were reacted in a microwave instrument and characterized with thin layer chromatography and infrared spectrometry. Initial results suggest new Diels-Alder adducts based on the salvinorin A skeleton, and one reaction that proceeds without heat. Finally, one polymer-bound dienophile, diethy diazodicarboxylate, was reacted with salvinorin A to produce a polymer-bound Diels-Alder adduct.

Keywords: Diels-Alder, salvinorin A, natural products, polymer bound, solid support, diethyl azodicarboxylate, DEAD, microwave

INTRODUCTION

Compounds derived from plants, animals, and microbes, called natural products, have been used to treat disease since the beginning of human medicine (Koehn, 2013). Consequently, “natural products play a dominant role in the discovery of leads for the development of drugs for the treatment of human diseases” (Newman and Cragg, p. 330). Discovery of natural products extracts can be bottlenecked by the extensive extraction procedures required to isolate even small quantities of the compound from large amounts of crude extract (Odendaal, Trader, and Carlson 2011). Additionally, current methods select compounds based on properties such as size, polarity, and charge (Trader and Carlson, 2011). New techniques that facilitate easy isolation or select compounds based on other properties such as functional groups would be beneficial to natural products chemistry.

The method being explored in this project is attaching a natural product to a polymeric resin via a Diels-Alder reaction. The Diels-Alder reaction is a very important synthetic reaction. It was developed by Otto Diels and Kurt Alder in 1928 (Diels and Alder, 1928), and they were awarded the Nobel Prize in Chemistry in 1950 (Nicolaou et al., 2002). It is a [4+2] cycloaddition between a conjugated diene and a compound containing a double bond, called a dienophile, resulting in six-membered rings with a double bond. It has been important in synthesis of compounds such as morphine (Gates and Tschudi, 1956), cholesterol and cortisone (Woodward et. al., 1952), and Taxol (Nicolaou et al., 1994). It is also reversible, normally called retro-Diels-Alder reactions. While it has been widely researched, there is still plenty of room for discovery.

So far, I have used only pure salvinorin A (Fig. 1), a compound extracted from the species of mint plant *salvia divinorum*, to test this method. Salvinorin A contains a furan ring as part of its structure, which is known to undergo Diels-Alder reactions (Lozama et al., 2011). Initially, without any polymer-bound reagents available, I tested reactions of a few dienophiles with salvinorin A that could either potentially be fixed to a polymeric resin or provide some insight as to what conditions salvinorin A might react under. Eventually, a commercially available polymer-bound reagent was located and tested.

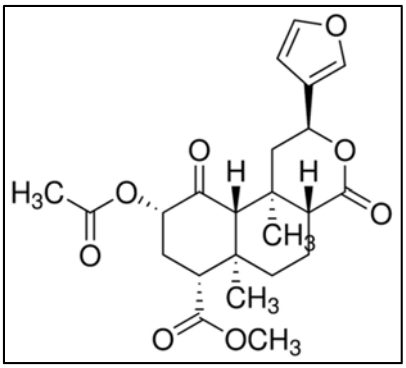

Fig. 1: Salvinorin A

I used pure salvinorin A as the first stage to test the use of Diels-Alder reactions on solid supports to isolate a target compound. Isolation from crude product was not attempted because salvinorin A was provided to us as a pure sample, and the plant it is extracted from is not readily available. If crude product were available, the insoluble resin would immobilize the salvinorin A, allowing unwanted compounds and materials to be washed away with solvents. Reversing the Diels-Alder reaction would then be used to recover salvinorin A.

REVIEW OF LITERATURE

The furan ring of salvinorin A was first utilized in Diels-Alder reactions by Lozama et al. (2011). They found that prolonged exposure to heat would cause the retro Diels-Alder reaction, and that the best initial yields of Diels-Alder adducts were obtained when microwave-assisted methods were used. This is useful information for my project because I would not want to cause the retro Diels-Alder until after the extraneous materials are washed away. This suggests I should use microwave irradiation initially, and prolonged heat to reverse the Diels-Alder reaction. The microwave instrument used by this group is identical to the one owned by ONU, allowing use of methods based on those in this paper.

Tidgewell et al. (2004) illustrate typical extraction methods used to obtain natural products, where they describe the extraction of salvinorin A from the mint plant *salvia divinorum.* The leaves were ground into a powder, soaked in solvent, concentrated and subjected to column chromatography. Certain fractions were concentrated, stirred with additional reagents, and washed. This mixture was dried and the solvent removed. This was subjected to column chromatography again, and certain fractions were combined and their solvents evaporated.

Similar procedures were used by Nakajima et al. (2010), who isolated a compound from metabolites of a fungus. Metabolites were extracted from the culture filtrate with solvent, dehydrated, and concentrated to dryness. They were subjected to column chromatography with 32 L of solvent. One fraction was concentrated to dryness, and further purification was accomplished by flash chromatography and high pressure liquid chromatography (HPLC). (Interestingly, this compound also contains a furan ring, and could be a candidate for the same extraction method.)

As these examples demonstrate, extractions are usually several steps long, involving multiple solvent extractions, concentrations, and elutions with column chromatography. Development of simpler extraction methods that are faster, have fewer steps, and require fewer materials would be greatly beneficial in making these compounds available for scientific research.

A possible alternative method would be using reagents that are immobilized on insoluble polymeric resins. Odendall, Trader, and Carlson (2011) developed a method which used solid supports for chemoselective enrichment of alcohol-containing compounds. The activated resin was successfully used to separate these compounds from a biological matrix. After extensive washing of the resin, the compounds of interest were then cleaved form the resin, which was regenerated for reuse. Similar techniques were used by Trader and Carlson (2011) to identify a resin that was completely chemoselective for carboxylic acids, which was also successfully used in isolating these compounds from a biological source. The most important aspect of these methods is that the resins are chemoselective; that is, they react with a single functional group. This is useful for isolating natural products extracts based on their functional group composition rather than solubility, charge state, or size. Additionally, the resins can be reused, which creates less waste.

My project explores the possibility of using a Diels-Alder reaction in the same manner to capture and isolate certain extracts. Many polymer supported Diels-Alder reactions have been reported (Yli-Kauhaluoma, 2001). A possible resin candidate was found in polymer-bound diethyl azodicarboxylate (DEAD; Arnold & Vederas, 1988; Fig. 2). While not developed

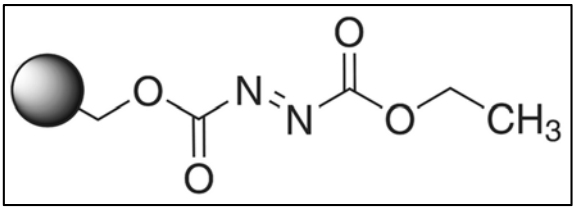

Fig. 2: Polymer-Bound DEAD

for Diels-Alder reactions on solid supports, DEAD (without the solid support) is known to act as a dienophile (Okamoto et al., 2008), and therefore could potentially work well for isolation via a Diels-Alder reaction.

METHODS

A quantity was salvinorin A was provided by Dr. Tom Prisinzano of Kansas University. All other reagents were from commercial suppliers and used without further purification. Microwave-promoted reactions were done using a Biotage Initiator model in 0.5-2.0 ml vials at normal absorbance levels. Thin-layer chromatography (TLC) was done on silica gel plates with the specified solvents, and spots on TLC plates were detected with ultraviolet light (UV) or iodine. The product, salvinorin A, and the dienophile were all placed on each TLC plate for comparison. Infrared (IR) spectra were obtained using either a Perkin Elmer Spectrum 2 or a Mattson Satellite instrument.

Reaction of salvinorin A and 4-Phenyl-1,2,4-triazoline-3,5-dione

135 mg (0.31 mmol) of salvinorin A and 55 mg (0.31 mmol) of red 4-Phenyl-1,2,4-triazoline-3,5-dione (PTAD) were added to 3 ml of toluene in a microwave vial. After dissolving by stirring, the vial was heated for 5 min at 135°C in the microwave. A pale yellow precipitate resulted, which was removed with filtration. I took IR spectra of both reactants and the precipitate. TLC plates (Table 1) were developed with the following solvents, and spots were detected with UV.

Table 1: Reaction 1 TLC

TLC #	Ratio ethyl acetate:heptane
1	80:20
2	20:80
3	50:50
4	80:20
5	70:30
6	60:40
7	75:25

Reaction of salvinorin A and PTAD (second run)

135 mg (0.31 mmol) of salvinorin A and 55 mg (0.31 mmol) of red PTAD were added to 3 ml of toluene in a microwave vial. The vial sat undisturbed while the microwave instrument was prepared. Before starting the microwave run, I observed that the solution in the vial had an identical appearance to that of the first run after removal from the microwave, so no microwave radiation was applied. The precipitate was removed by filtration. TLC were developed with this product with the solvent ratios in Table 2. Because the salvinorin A was not quickly detected with UV, I used iodine for detection of spots.

Table 2: Reaction 2 TLC

TLC #	Ratio ethyl acetate:heptane
12	80:20
13	80:20

Reaction of salvinorin A with dimethyl acetylenedicarboxylate

135 mg (0.31 mmol) of salvinorin A and 55 mg (0.31 mmol) of dimethyl acetylenedicarboxylate were added to 3 ml of toluene in a microwave vial. It was heated for 5 min at 135°C in the microwave. TLC 14 showed a majority of salvinorin A was unreacted, so the vial was placed back in the microwave for 30 min at 115°C. After cooling to room temperature and sitting undisturbed overnight, no crystals had precipitated, so the solvent was evaporated until a solid was formed. I removed the remaining solvent with a pipet. I obtained IR spectra of the reactants and products. TLC plates were developed with the solvents in Table 3 and detected with UV and iodine.

Table 3: Reaction 3 TLC

TLC #	Ratio ethyl acetate:heptane
14	80:20
15	80:20
16	80:20
17	1:1
18	2:3

Reaction of salvinorin A with diphenyl acetylene

67.5 mg (0.156 mmol) of salvinorin A and 27.8 mg (0.156 mmol) of diphenyl acetylene were added to 2 ml of 1,2-dichlorobenzene in a microwave vial. After stirring it was irradiated in the microwave at 180°C for 20 min. When left undisturbed for a period of days, crystals were formed. I compacted these by spinning the suspension in a centrifuge and removed the liquid by pipet. The solid was filtered and washed with dichlorobenzene. I obtained an IR spectrum of the solid and TLC plates were developed and detected with iodine (Table 4).

The supernatant was heated under nitrogen in an oil bath to evaporate the solvent. It was reduced to a brown oil-like substance, which I dissolved it in dichloromethane and transferred from a beaker to a vial and evaporated to an oil again, which then dried to brown crystals. I obtained an IR spectrum of this product. TLC plates of this solid were not developed due to time constraints.

Table 4: Reaction 4 TLC

TLC #	Ratio ethyl acetate:heptane
19	80:20
20	70:30
16	80:20
17	1:1
18	2:3

Reaction of salvinorin A with diethyl azodicarboxylate on polystyrene

118.0 mg (1.2 mmol/g loading) of yellow diethyl azodicarboxylate on polystyrene (polymer-bound DEAD) were added to 5 ml of tetrahydrofuran (THF) in a round bottom flask and allowed to sit for 15 min to swell. 67.5 mg (0.156 mmol) salvinorin A was then added. I heated it in an oil bath, and reflux began at about 73°C. It was heated for a total of 95 min, during which the temperature was between 60 and 85°C. The solid was filtered and washed with 8 ml of dichloromethane to give a pale yellow solid. I obtained IR spectra of polymer-bound DEAD and the product. TLC plates were not developed because the polymer is insoluble.

RESULTS

For all solvent combinations tested in each reaction, the product and salvinorin A spots on TLC plates had different R_f values but were not separated significantly enough to consider column chromatography. The major IR peaks (cm^{-1}) of the spectrum of salvinorin A are given below for reference, and the other reactants and products are given below the description of each reaction

Salvinorin A IR peaks: 1725.70, 1265.48, 1226.42, 1200.98, 1156.65, 1142.86, 1052.42, 1019.54, 1003.94, 984.67, 952.05, 875.22, 781.66, 600.16, 460.60

In both reactions of salvinorin A and PTAD, the characteristic red color of PTAD disappeared, providing initial indication the reaction proceeded. From TLC, I believe the product is quite impure and may contain side products. IR showed some differentiation from reactants. Additionally, the IR of the product from the reaction that was heated is essentially identical to that which was not heated.

PTAD IR peaks: 1745.34, 1499.81, 1395.81, 1172.94, 897.55, 721.96, 675.61, 612.77, 568.38, 492.27

Product (heated) IR peaks: 1719.5, 1419.87, 1233.49, 1161.41, 1050.89, 1004.84, 765.75, 690.25

Product (no heat) IR peaks: 1717.04, 1421. 42. 1232.60, 1161.35, 1050.95, 1004.61, 766.91, 690.27

In the reaction of salvinorin A with dimethyl acetylenedicarboxylate, the IR spectrum of the product showed no evidence of dimethyl acetylenedicarboxylate, but was difficult to differentiate from salvinorin A except in the intensities of a few peaks, especially the much more intense peaks at 1265.42 and 1420.90 cm^{-1} in the product.

Dimethyl acetylenedicarboxylate IR peaks: 1717.44, 1435.72, 1241.41, 1038.05, 892.68, 745.97, 677.88, 563.36

Product IR peaks: 1721.77, 1420.90, 1265.42, 1226.08, 1201.25, 1156.83, 1142.03, 1051.26, 951.17, 874.87, 600.02

In the reaction of salvinorin A with diphenyl acetylene, I determined from the TLC and IR that the solid that crystalized after the second microwave irradiation was almost entirely salvinorin A. 25.8 mg of this solid was collected. In the IR of the second solid (recovered from the supernatant), nearly every peak in the product could be matched with a peak in either salvinorin A or diphenyl acetylene dicarboxylate. As mentioned previously, TLC plates were not developed. 43.8 mg of this solid was collected.

Diphenyl acetylene IR peaks: 1598.73, 1491.55, 1441.74, 1069.34, 916.66, 753.24, 687.01, 534.91, 507.21

Product (from supernatant) IR peaks: 1726.91, 1492.46, 1442.22, 1226.93, 1156.79, 1069.94, 1025.21, 917.17, 753.63, 687.54, 535.29, 507.75

In the reaction of salvinorin A with diethyl azodicarboxylate on polystyrene, the IR spectra of polymer DEAD and the product are very similar except for one peak. This peak is at 1775.07 cm^{-1} in polymer DEAD, and at 1723.94 cm^{-1} in the product. 131.9 mg of solid was collected.

Polymer DEAD IR peaks: 1775.07, 1492.30, 1451.23, 1216.23, 1018.71, 757.22, 697.29, 537.94

Product IR peaks: 1723.94, 1492.34, 1451.29, 1220.57, 1045.34, 756.41, 697.06, 538.40

DISCUSSION

Current Findings

The primary reactions performed were exploring the variety of potential dieneophiles that participate in Diels-Alder reactions. All results are still preliminary. In all reactions, TLCs were performed with the intent of finding suitable solvent combinations for column chromatography. At this stage, however, no satisfactory conditions have been found for application to column chromatography. The R_f values, while not exactly the same, did not show enough separation of products and reactants to warrant column chromatography. Consequently, most products are still relatively impure, and IR spectra have only been somewhat helpful in determining structures and identities of products. This does not mean that reactions have not been beneficial or informative.

PTAD has a characteristic red color. In both reactions with PTAD, the red color disappeared, providing initial indication that the reaction proceeded. This product is different than the reactants, but may have significant impurities. The fact that the second run proceeded with no heat yet produced the same product (as shown by TLC and IR) is significant. Any reaction that proceeds at room temperature and without the input of external energy is favorable over one that requires some energy input. It is unknown what factors allowed this reaction to proceed at room temperature; this would be incentive to pursue this reaction.

The reaction of salvinorin A with dimethyl acetylenedicarboxylate is one that was done by Lozama et al (2011). They reported that it successfully created Diels-Alder adducts, which, under extended heat, could undergo a retro Diels-Alder. This was of particular interest because accomplishing the goal of our research requires a retro Diels-Alder reaction to remove the desired product from the compound. Initial results indicate I also produced an adduct, as the IR

of the product did not indicate the presence of dimethyl acetylenedicarboxylate. Further pursuit of this reaction would be with the intent of attaching dimethyl acetylenedicarboxylate to a polymer and to induce the retro reactions with extended heat.

The reaction of salvinorin A with diphenyl acetylene was also pursued with the potential of using one of the phenyl groups of the dienohpile to attach it to a polymer. The first solid collected from the reaction was determined to be mostly salvinorin A. The IR of the solid isolated from the supernatant had qualities of both salvinorin A and diphenyl acetylene; it is yet to be determined if this solid is the desired adduct, or just a mixture of the reactants.

The reaction of salvinorin A with polymer bound DEAD was not done in our microwave instrument because it was decided that our first attempt at a reaction with a polymer bound reagent would best be done with traditional heating methods. Initial results suggest the reaction was very successful. TLC was not an option because of the insoluble nature of the product and reactant, but the IR spectra obtained from this reaction are promising. They showed a peak that shifts from 1775 cm^{-1} in the reactant to 1723 cm^{-1} in the product. This may be a peak identifying carbonyls, and the shift could be the result of additional carbonyls added by salvinorin A.

Future Research

Future work on this project could include significant work to purify reaction products. This would include column chromatography once ideal conditions are found. Additionally, to date no NMR spectra have been run due to lack of column chromatography. Once column chromatography (or some other method of separation/purification) is accomplished, future research may obtain definite characterization.

So far, only one polymer-bound reagent has been tested because it was easily accessible through a commercial supplier (although quite expensive). The other option was to synthesize such reagents on my own, which time did not permit; however, potential dienophiles were explored in the initial reactions. It would be ideal to explore the synthesis option, including attaching these and other dienophiles to other polymers to customize the reaction to enhance yield. Using the polymer with impure samples to test selectivity has not yet been attempted. Finally, inducing the retro Diels-Alder to retrieve the isolated product has yet to be attempted.

Conclusion

Initial results suggest synthesis of new Diels-Alder adducts on the salvinorin A skeleton. Additionally, synthesis of a polymer bound Diels-Alder adduct from a natural products extract was accomplished. While there is much more work capable of being accomplished with this project, this work represents significant progress towards using a Diels-Alder reaction to isolate extracts from biological sources.

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