An Attempted Synthesis of a Menadione Derivative via a Diels-Alder Reaction

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Southern Scholars Honors Program
Senior Project Proposal Information Sheet

Name: Charity Peñaloza Date: 1-12-09
Major: Biochemistry

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Under the guidance of a faculty advisor, the Senior Project should be an original work, should use primary sources when applicable, should have a table of contents and works cited page, should give convincing evidence to support a strong thesis, and should use the methods and writing style appropriate to the discipline.

The completed project, to be turned in in duplicate, must be approved by the Honors Committee in consultation with the student's supervising professor four weeks prior to the last day of class for the semester the project is turned in. Please include the advisor's name on the title page. The 2-3 hours of credit for this project is usually done as directed study or in a research class.

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Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake. Attach a separate sheet of paper.

Signature of faculty advisor: 

Expected date of completion: 4/1/09

NOTE: An advisor's final project approval does not guarantee that the Honors Faculty Committee will automatically approve the project. The Honors Faculty Committee has the final vote.

Approval to be signed by faculty advisor when the project is completed:

This project has been completed as planned (date): 4/2/09

This is an "A" project

This project is worth 2-3 hours of credit

Advisor's Final Signature: Charity Peñaloza Date: 4/2/09

Chair, Honors Committee:  

Date Approved: 1 May 09

Dear Advisor,

(1) Please write your final evaluation on the project on the reverse side of this page. Comment on the characteristics that make this "A" quality work.

(2) Please include a paragraph explaining your specific academic credentials for advising this Senior Project.
An Attempted Synthesis of a Menadione Derivative via a Diels-Alder Reaction

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Southern Adventist University
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April 2, 2008

In fulfillment of a Senior Research Project for Southern Scholars
under the supervision of Professor Brent Hamstra, Spring 2009
Abstract

With menadione and cyclopentadiene as starting materials, a menadione-cyclopentadiene adduct was synthesized via a Diels-Alder reaction. This reaction serves as the first step in a pathway to synthesize a menadione-safranal derivative that could potentially have antioxidant activity.

Introduction

Menadione, also known as 2-methylnapthalene-1,4-dione, is a yellow crystalline solid that is used in fungicides and as a vitamin K supplement in medicine and animal feed.\(^1\) Menadione is known as synthetic vitamin K3 as well. Its derivatives have been shown to possibly aid in cancer treatment and are used to understand how cell mechanisms work. The function of menadione has been studied in many different cases. One study has shown that derivatives of menadione could possibly be “potent reversible and uncompetitive inhibitors” of glutathione reductases. These reductases have been shown to have “anticancer and antimalarial activity” and could possibly “contribute to the reversal of drug resistance.”\(^2\) Glutathione is a strong anti-oxidant and plays a major role in homeostasis. Oxidized glutathione is currently being studied in relation to breast cancer by the U.S National Institutes of Health to see if it “stimulates the immune system in different ways” to inhibit tumor cell growth.\(^3\)

Currently, menadione is being tested as a topical lotion to treat epidermal-growth-factor-receptor (EGFR) inhibitor associated rash. The rash is a side effect of EGFR inhibitors being used to treat non-small cell lung cancer (NSCLC), pancreatic cancer, colorectal, head, and neck cancer.\(^4\)

Menadione has also been found to induce oxidative stress. According to the German Research Center for Environmental Health, oxidative stress occurs when “the equilibrium in the
organism moves towards oxidative processes.” Oxidative stress is involved with the aging of body cells, and it also induces cell death. The buildup of reactive oxygen species and the oxidation of biomolecules causes many diseases.\(^5\) This may be significant in “inducing growth inhibition” of cancer cell lines.\(^6\) In research done at Anjou University in South Korea, studies have found that menadione-induced cell death in cultured C2 myoblasts.\(^7\) Menadione also inhibits intestinal calcium absorption by playing a role in mitochondrial dysfunction.\(^8\)

Menadione itself is toxic and causes injury to cells. It is lipid soluble and “promotes the hepatic biosynthesis of blood clotting factors.” Menadione is carcinogenic. However, cells treated with menadione and metabolic activation factors could “cause DNA damage and repair” without carcinogenicity.\(^9\) This may be key in finding alternative treatments for cancer.

In addition, menadione exhibits antiestrogenic activity in an \emph{in vitro} assay. This has been achieved by the suppression of hepatic estrogen response genes. This may also have the same function in vivo.\(^10\)

Menadione has many different activities and is involved in inducing or inhibiting many different cell functions. Studies affirm menadione’s versatility. If menadione alone can achieve these different functions in the cell, then derivatives of menadione also have the same potential. These derivatives may have great effects as anti-cancer agents.

The synthesis of a menadione derivative could be significant because many are interested in its function as a means of treating cancer. It is known that there are two different methods of adding substituents to menadione to form menadione derivatives. Substituents may be added via radical decarboxylation and via a Diels-Alder reaction. The Diels-Alder method was chosen to “activate the bridgehead hydrogen for alkylation” in order to add safranal that will yield the menadione derivative ultimately.\(^11\)
The target compound is an intermediate to the following menadione derivative

The article entitled "Improved Synthesis of Vitamin K" by Ya-Fei Ji, Ahi-Min Zong, and Xian-Yong Wei in 2003 was used as a model and followed closely for the beginning reactions in the synthesis of the menadione derivative.\textsuperscript{11}

Menadione was sought to be combined with safranal due to its anti-oxidant activity. Safranal is derived from saffron which is a spice from the stigmas of crocus flowers (\textit{Crocus sativus}). It has been found that safranal inhibits cell growth of human tumor cells. Studies were done on HeLa cells which exhibited 50\% growth inhibition.\textsuperscript{12} According to the Centre for Cancer Education, HeLa cells are a line of human epithelial cells derived from the cervical carcinoma of Henrietta Lacks. These cells proliferate abnormally rapidly.\textsuperscript{13} Safranal has a high radical scavenging activity which means that it is highly involved in aging processes, anti-inflammatory, anti-cancer, and wound-healing activity.\textsuperscript{14} With anti-cancer activity or inhibition of certain cell mechanisms in each piece of the menadione derivative, there may be added anti-cancer activity with the pieces together in one compound.

\textit{Scheme 1. Proposed Reaction}
**Experimental Details**

In the first trials, the synthesis of the intermediate product (also known as the menadione-cyclopentadiene adduct) was attempted. 3.0408 g (0.0177 mol) of menadione was dissolved in 30 mL of glacial acetic acid in a beaker. Seven microspatula scoops of a Lewis Acid catalyst AlCl₃ (purple/white solid) were added to the dark, transparent yellow solution. After the addition of the catalyst, the solution became cloudy and turned dark yellow as the AlCl₃ dissolved. 4 mL (0.049 mol) of excess cyclopentadiene (cracked from dicyclopentadiene a few months prior) was then added to the solution.

The solution was left to stir with a magnetic stir bar in a 150 mL beaker for three days. The beaker was covered with a watch glass to prevent evaporation of solvent. After one day of stirring, the solution became a dark yellow-brown opaque solution. After the 3rd day, the solution was completely brown and cloudy. When taken off the magnetic stirrer, the solution separated with a darker brown layer on top of the cloudy brown layer.

Half of the organic intermediate product (around 17 mL) was then isolated using a separatory funnel, ~25 mL of reverse osmosis-purified water, and approximately 40 mL diethyl ether. Only half was separated out because this method was not performed in the literature. This route was a faster way to rid the product of the acetic acid solvent, which has a high boiling point and is hard to evaporate. The organic layer was washed three times with water and the yellow aqueous layer was extracted. Sodium sulfate was then added to the organic solution to get rid of excess water. Afterward, the brown solution was collected in a 50 mL round-bottom flask. The diethyl ether was then removed by rotary evaporation with Rotovapor Büchi RE111.
After evaporation of the solvent, the brown solid was recrystallized using methanol as the solvent. It was then vacuum filtered for 3 hours. Its melting point was 96-98°C as measured by a MELTEMP melting point device.

IR spectroscopy of the product dissolved in methylene chloride was performed using Nicolet IR200 FT-IR instrument and direct path methodology. The peaks were found at 1666 cm⁻¹, 1596 cm⁻¹, 1596 cm⁻¹, 1301 cm⁻¹ (Figure 3). The spectrum was very close to the literature observations but also similar to the menadione spectrum exhibiting peaks at 1664 cm⁻¹, 1593 cm⁻¹, and 1301 cm⁻¹ (Figure 1). The spectrum of cyclopentadiene was also measured (Figure 2).

Gas Chromatography-Mass Spectroscopy was performed using the Agilent Technology 6890N GC System on this product, and it was found that there was a large peak for the molecular weight of menadione 172 g. There were also significant peaks that showed up later giving peaks at 416.4 g and 430. 4 g (Figures 6-8). A small peak was observed at the targeted molecular weight 238 g for the menadione-cyclopentadiene adduct (Figure 8). However, its magnitude was very small.

The reaction was then attempted again using freshly cracked cyclopentadiene. An apparatus was set up to collect cyclopentadiene (a colorless semi-transparent liquid) from dicyclopentadiene (a dark, brown/orange liquid). 3.0260 g (0.0176 mol) of menadione was combined with 4 mL (0.049 mol) cold excess cyclopentadiene and 30 mL glacial acetic acid was the solvent. The same protocol was performed as in the previous trial. The reaction was left to react for two weeks.

The product was a lot browner than the previous reaction, almost black. It was recrystallized with methanol and the resulting crystals were light brown and needles. Some yellow/brown flat flakes (thin) were also observed in the dried product. The product was
recrystallized again and a smaller amount of brown solid resulted. Its melting point was found to be 95-98°C. Activated charcoal was used in the recrystallization to rid the mixture of more impurities. Very little product was recovered, however. The charcoal and undissolved solid during recrystallization was filtered with a funnel and filter paper. The IR spectra exhibited peaks at 1665 cm\(^{-1}\) and 2360 cm\(^{-1}\). GC-MS analysis was performed, and it showed a large peak at 172 g, the molecular weight of menadione (Figure 6 and 11). There were no peaks for products of higher molecular weight near the targeted molecular weight. Figures 5 and 9 illustrate the peaks of greatest magnitude in two different reaction trials. Figures 10 and 11 indicate which compound fragments were predominant.

A third trial was attempted using 3.2204 g (0.0187 mol) menadione and 4 mL excess cyclopentadiene. The same protocol was again performed, however, this time the mixture reacted for three days. Yellow and brown solids were separated out during recrystallization. The yellow solid had a melting point of 58-60°C while the brown/black solid had a melting point of 50-55°C.

A fourth trial was attempted this time using a newer bottle of catalyst AlCl\(_3\). 3.018 g (0.0175 mol) menadione was added to ~5 mL (0.0607 mol) of cyclopentadiene. The newer catalyst was a lighter yellow, powdery solid. The same protocol was followed. After the addition of AlCl\(_3\), the solution turned green. The addition of the catalyst prompted an exothermic reaction. The solution became darker green and opaque. It then became black.

The reaction mixture was performed under the hood and left to stir overnight with a magnetic stir bar. The black solution then began to have a hint of dark purple. The reaction was left to react for three days. The dark purple/black solution had clumps of dark solid granules. The beaker was placed over the magnetic stirrer to dissolve the solid. It became a liquid
consistency. It was then poured into a separatory funnel for organic extraction and to wash away the acetic acid. Upon addition of reverse osmosis-purified water, a purple/black solid precipitated. The solid was then removed from the separatory funnel for recrystallization. Diethyl ether was added to the separatory funnel to salvage any other product in the solution; however, not much was recovered.

An attempt was made to recrystallize the black solid in methanol, but it did not dissolve very well. Instead, it became a hard, tar like substance. Methylene chloride was then added to the mixture. It did not dissolve the solid right away. The solid softened mostly and became a dark purple/black, oily liquid in methylene chloride. The solid did not behave like the product of previous reactions.

Results and Discussion

Table 1. Characterization of starting materials and product

<table>
<thead>
<tr>
<th>Compound</th>
<th>Melting Point°C</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Menadione</td>
<td>105-107</td>
<td>Yellow powder</td>
</tr>
<tr>
<td>Cyclopentadiene</td>
<td>-97.2</td>
<td>Clear liquid</td>
</tr>
<tr>
<td>Menadione-cyclopentadiene (Trial 1)</td>
<td>96-98</td>
<td>Brown solid</td>
</tr>
<tr>
<td>Menadione-cyclopentadiene (Trial 2)</td>
<td>95-98</td>
<td>Brown solid</td>
</tr>
</tbody>
</table>

The intermediate product was reported to be a white solid\textsuperscript{11}. The Diels-Alder reaction did not occur in the first trial because the cyclopentadiene that had been shelved for a few months dimerized. This was concluded after a Diels-Alder test reaction was performed with the older cyclopentadiene and maleic anhydride. No solid was formed in the test reaction. Solid would have formed, however, with the monomer cyclopentadiene. In addition, the IR spectrum
of the older cyclopentadiene was similar to the spectrum of the dimer dicyclopentadiene. What may have occurred was some unknown side reaction between the dimer and the menadione.

The targeted Diels-Alder reaction was only possible with the monomer cyclopentadiene obtained from freshly cracked dicyclopentadiene. It too produced a brown solid but different than the previous collected product. The solid produced in this experiment was brown possibly due to some impurities and poor technique in recrystallization. All the impurities may not have been completely removed as the recrystallized product was washed and filtered. Though its melting point was in agreement with the literature value of 97-98°C (See Table 1 above), the color differed. Also, the mass spectrum did not show a large peak at the desired molecular weight of 238.27 g.

However, peaks were observed at molecular weights of 416 g and 430 g for the first trial product. These fragments indicate some other reaction may have occurred. The dimer may have reacted somehow producing some adduct of the dimer and menadione. The magnitude of the peaks at 172 g (Figures 6 and 11) was great, possibly indicating unreacted menadione in the products or the menadione component of the fragmented product. The GC-MS data for the trial with the newly cracked cyclopentadiene indicated that there were no significant peaks at the targeted molecular weight. The high heat of the GC-MS also could have induced a reverse Diels-Alder reaction, resulting in the absence of higher molecular weight compounds.

The IR data indicate that menadione, the starting material, is comparable to the product. Their fingerprint regions do share similar peaks. There are no sp³ C-H peaks in the menadione spectrum but there are both sp² and sp³ C-H peaks in the product spectrum (Figure 3) of Trial 1 indicating C-H double bonds and C-H single bonds as part of an adduct of the dimer and menadione.
It was realized that a catalyst was necessary to mediate the Diels-Alder reaction. This is due to the high activation energy required to attach cyclopentadiene onto menadione to form a multi-cyclic compound. The carbonyl groups on menadione are electron-withdrawing groups making it a good, reactive dienophile or “lover of dienes.” However the methyl group on menadione, an electron-donating group, could lessen the reactivity of menadione. It was also discovered that a new bottle of catalyst prompted a different product and reaction than the older catalyst. The newer AlCl$_3$ was better able to decrease the activation energy needed to increase the rate of the Diels-Alder reaction. A better catalyst greatly influences the rate of reaction.

A different separation technique was used to isolate the organic product because it took over three hours to remove the acetic acid solvent. Evaporating acetic acid takes an inordinate amount of time, so separating the organic layer first with diethyl ether is more efficient. There are many factors that influence this reaction.

**Conclusion**

The intermediate product formed is only a precursor to the desired target menadione derivative. In succeeding experiments, adding safranal to the adduct can be attempted. The accuracy of the melting point shows that the synthesized product is very similar to the literature value except in appearance. However, analysis of the GC-MS spectra shows that there may also be another reaction that may have occurred.

In future work, the purity of the starting materials should be tested first. Due to the minimal product yield after recrystallization, the experiment could be performed on a bigger scale. More research time is necessary to make definitive conclusions about the product.
Acknowledgments

Dr. Brent Hamstra and Dr. Loren Barnhurst are greatly appreciated for their guidance in the experiment. This research was supported by Southern Adventist University.

References

13 University of Newcastle upon Tyne. Department of Medical Oncology. Center for Cancer Education Online Medical Dictionary. 2000.
Fig 1. Menadione
Fig 2. Cyclooctadiene

Wave Numbers (cm⁻¹)

% Transmittance
Fig. 4. Menadione-cyclopentadiene Product adduct

Trial 2

Wavenumbers (cm⁻¹)
Figure 5

File: C:\msdchem\1\DATA\Penaloza\MENADIONECYCLOPENTADIENE.D\Snapshot\BROWNPRODUCT.D
Instrument: Instrument #1
Sample Name:
Misc Info: 3rd trial menadione and cyclopentadiene

TIC: BROWNPRODUCT.D\data.ms

[Graph showing the TIC plot with time on the x-axis and abundance on the y-axis]
File: C:\msdchem\1\DATA\Penaloza\MENADIONECYCLOPENTADIENE.D\Snapshot\BROWNPRODUCT.D
Operator: Instrument: Instrument #1
Sample Name: Misc Info: 3rd trial menadione and cyclopentadiene

Average of 10.600 to 10.640 min.: BROWNPRODUCT.D\data.ms

Abundance

m/z → 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265

172.1
157.0
144.0
129.0
89.0
76.0
51.0
63.0
File: C:\msdchem\1\DATA\Penaloza\MENADIONECYCLOPENTADIENE.D\Snapshots\BROWNPRODUCT.D
Operator: Instrument: Instrument #1
Sample Name: Misc Info: 3rd trial menadione and cyclopentadiene

Average of 18.960 to 18.971 min.: BROWNPRODUCT.D\data.ms

![Mass Spectrogram](image-url)
File: C:\msdchem\1\DATA\Penaloza\MENADIONECYCLOPENTADIENE.D\Snapsh ...

 Operator: 
 Instrument: Instrument #1 
 Sample Name: Mise 
 Misc Info: 3rd trial menadione and cyclopentadiene 

**Figure 8**

Average of 19.560 to 19.572 min.: BROWNPRODUCT.D\data.ms
Data Path: C:\MSDCHEM\1\DATA\PENALOZA\2ND SEMESTER TRIAL\Snapshot\Data File: MENADIONE DERIVATIVE.D
Acq On: 31 Mar 2009 16:01
Operator:
Sample:
Misc: in methanol
ALS Vial: 1 Sample Multiplier: 1
Integration Parameters: events.e
Integrator: ChemStation
Method: C:\msdchem\1\METHODS\highmw.M
Title:
Signal: EIC TIC: MENADIONE DERIVATIVE.D\data.ms
peak R.T. first max last PK peak corr. corr. % of
# min scan scan scan TY height area % max. total
--- -------- -------- -------- -------- -------- -------- -------- -------- -------- --------
1 7.749 807 815 834 BB 1486156 19253683 100.00% 79.676%  
2 11.168 1405 1412 1429 BB 393555 4911223 25.51% 20.324%

Sum of corrected areas: 24164906

highmw.M Tue Mar 31 16:35:04 2009

Figure 9
Library Searched: C:\Database\NIST05a.L
Quality: 94
ID: Benzoic acid, ethyl ester

Figure 10

Average of 7.739 to 7.750 min.: MENADIONE DERIVATIVE.DIdata.ms

#23436: Benzoic acid, ethyl ester
Figure 11

Average of 11.161 to 11.172 min.: MENADIONE DERIVATIVE.D.data.ms