Synthesis of 4,5-Benzoxepin for use in Enzymatic Studies

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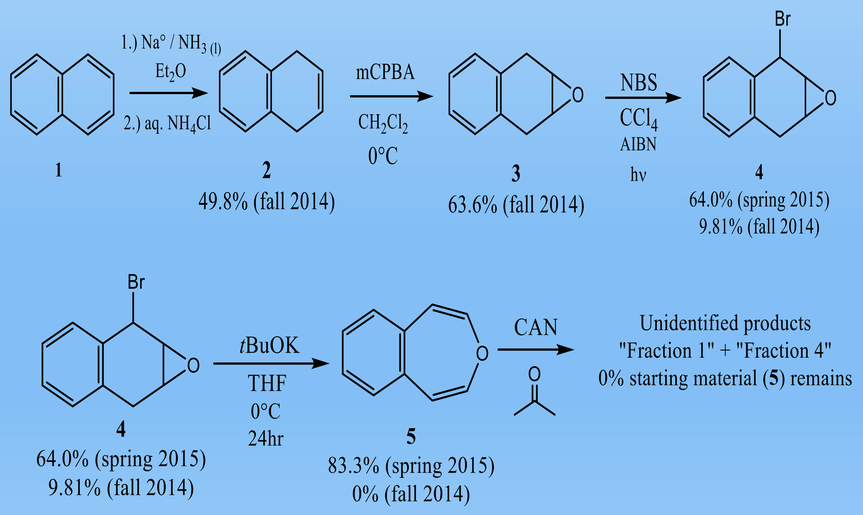
Introduction:

Benzene is a known carcinogen and dangerous chemical used in industry. Knowing the mechanistic pathways which potential toxins break down in the body is useful to prevent illnesses and help those ailing presently. Oxepin derivatives are useful models in investigating the ring opening mechanism of oxepin as seen in benzene metabolism. 4,5-benzoxepin is a good substrate for use in enzymatic oxidation studies with cytochrome P-450. This project focuses on improving the synthesis of 4,5-benzoxepin for use in enzymatic reactions by the Greenberg research group at UNH to establish the current metabolic pathway of benzene.[3]

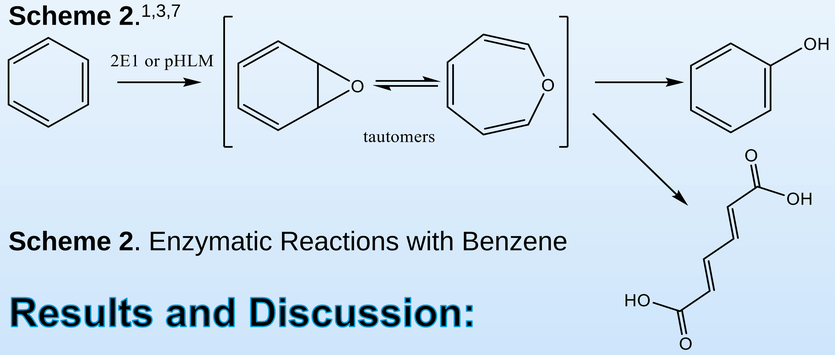
Experimental Work:

A multistep synthesis was performed starting with naphthalene **1** to yield purified 4,5-benzoxepin **5** for a further reaction to yield unknown compounds. The synthesis is shown in **Scheme 1**. A free radical bromination was performed on **3** using NBS to yield brominated epoxide **4**. The brominated epoxide **4** was subjected to dehydrohalogenation using potassium tert-butoxide to yield 4,5-benzoxepin **5**. **5** was reacted with CAN to yield to unknown structures. Each step of the synthesis was analyzed with proton NMR to verify purity and if unreacted starting material still remained.[1,3,7]



Scheme 1. Synthetic Pathway to 4,5-Benzoxepin

An attempt at a control enzymatic reaction was performed on benzene with the enzymes 2E1 and pHLM. This is shown by **Scheme 2**.[1,3,7]



Scheme 2. Enzymatic Reactions with Benzene

Results and Discussion:

The free radical bromination with NBS and AIBN radical initiator was improved by using light as a radical initiator instead of heat. Also, the dehydrohalogenation of the brominated epoxide **4** was improved by using THF as a solvent instead of diethylether. Reactions with CAN were also performed on 4,5-benzoxepin **5** yielding unknown products with no **5** remaining.

^{1}H NMR was used to characterize crude and pure products.

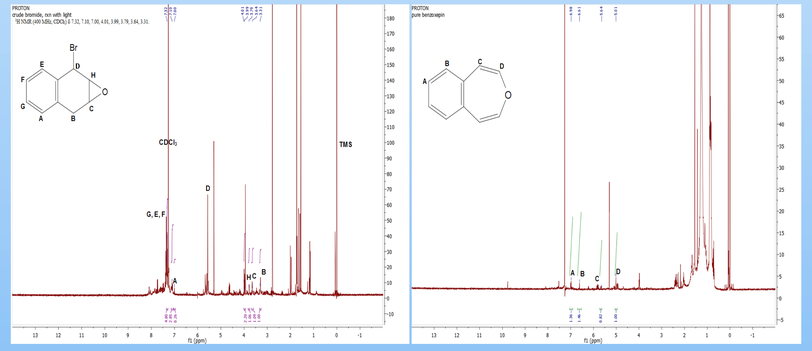


Figure 1. ^{1}H NMR of crude **4** and purified **5.** (From left to right)

Enzymatic reactions with benzene as a substrate were explored also.

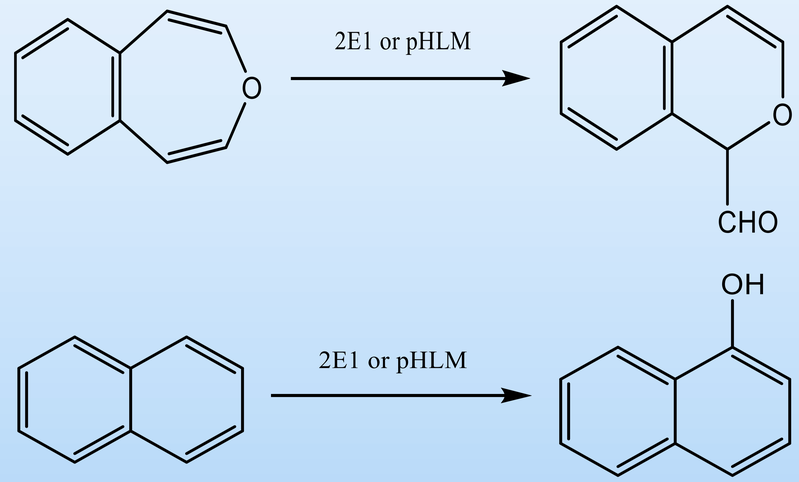
Table 1. Concentrations of Enzymes used with Benzene substrate.

Trial	Substrate	Enzyme	[Enzyme]
2E1 (1)	Benzene	2E1	5.0pmol/mL
2E1 (2)	Benzene	2E1	50pmol/mL
pHLM (1)	Benzene	pHLM	0.05mg/mL
pHLM (2)	Benzene	pHLM	0.50mg/mL

The amount of enzymes were improperly added to the first trial of both 2E1 and pHLM. The second trials were corrected to the desired concentration.

Future Work:

Explore enzyme reactions with naphthalene and 4,5-benzoxepin as well as correcting the benzene enzyme reactions.



Conclusions:

The organic synthesis of 4,5-benzoxepin **5** was completed and improved. Enzymatic studies using benzene were started but require further improvement.

Each synthetic step now has satisfactory yields and can be run from the beginning for even better results. “Fraction 1” and “Fraction 4” remain unidentified and will require further analysis.

Acknowledgements:

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References:

(1) Vogel, E., Gunther, H., *Angew. Chem. Int. Ed*. **1967**, *6*, 385-476

(2) Davies, S. G., Whitham, G. H., *J. Chem. Soc. Perkin Trans. I*, **1977**, 1346-1347

(3) Morgan, J., Greenberg, A., *J. Org. Chem*. **2010**, *75*, 4761-4768

(4) Greenberg, A., et. al., *Structural Chemistry*, **1998**, *9(3)*, 223-236

(5) Nauduri, D., Greenberg, A., *Tetrahedron Letters*, **2004**, *45*, 4789-4793

(6) Paquette, L. A., Barrett, J. H., *Org. Synth*. **1969**, *49*, 62.

(7) Rabideau, Peter W., Burkholder, E., *J. Org Chem*. **1978**, *43*, 4283-4288