LIBERTY UNIVERSITY Column Chromatography of an Oxepine Synthesized from Meldrum's Acid

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Abstract and/or Background

Column chromatography is a purification technique used to separate organic compounds from a heterogenous mixtures based on their unique polarities that induce different adsorption rates into the adsorbent. Chromatography is generally done with two phases: a mobile phase (eluent) and a stationary phase (absorbent). Silica gel often serves as the adsorbent, because of its ubiquitous ability to be used for the separation of compounds having a variable range of polarities. Typically, the mobile phase (called the eluent) is used to carry the dissolved compound through the stationary phase to allow the adsorption to occur. The mobile phase functions as an eluting agent allowing different components of the compounds to elute from the column. Because the stationary phase is polar, the components of the compound that elute from the column faster are the most nonpolar. The different components of the compound are separated out into the column because of the polarity differences affecting the absorbance of the compounds thus giving separate fractions of the compound to be collected. Synthetic oxepine compounds have the potential for use in various biological activities, such as anti-breast cancer, anti-implantation agents, and anti-HIV. Synthesis of 1,3,7,7-tetramethyl-4H,10H-6,8,9-trioxa-2-thiabenz[f]azulen-5-one using 2,2-dimethyl-1,3-dioxane-4,6-dione, Meldrum's' acid, triethylamine, 3,4-bis(chloromethyl)-2,5-dimethylthiophene, and DMSO was attempted, and it was discovered that the starting material (Meldrum's' acid) was contaminated. Purification using column chromatography was performed with a fifty percent solution of dichloromethane in pentane. MP and IR analysis were performed to verify purity.

Introduction and/or Research Question

For the organic chemist, purification processes are a very important element in synthesis and daily lab operations. An understanding of these processes is pertinent when working in an organic lab as it is common to use separation techniques such as column chromatography to isolate a compound from a heterogenous mixture. Sulfur compounds contain a polar functional group which allows their different properties to be used for selective sorption. Column chromatography works on the basis of these properties such as polarity. The purpose of this research was to practice a column chromatography and develop the skills needed to seamlessly execute this form of purification. Additionally, the uses and applications of column chromatography were investigated through the purification process of 3,4-bis(chloromethyl)-2,5-dimethylthiophene. This research adds to the established organic chemistry community by providing insight on the benefit to this technique and highlighting its usefulness in proper isolation of a compound. Additionally, this research gave way to the possibility of completion of the Meldrum's acid reaction to synthesize oxepines. Oxepines are naturally occurring compounds that have proven to be valuable in the pharmaceutical industry as novel bioactive molecules and drugs. However, synthetic oxepine compounds showcase potential for anti-cancer and anti-implantation application. Therefore, this process of column chromatography adds to the general library of knowledge in both the organic chemistry field, but also has applications in medicinal chemistry and pharmacology through the opportunity that it presents for oxepine synthesis.

Methods

Eluent was prepared using a 50% mixture of dichloromethane in hexanes or pentanes and was used as the mobile phase for both column and thing-layer chromatography. The column was packed with a thin layer of sand and a few milliliters of the mobile phase as added. The column was tapped gently with a cork ring to allow even settling of the sand. Extra care was taken to avoid bumping or disruption of the sand to avoid any uneven surfaces. Silica slurry was made and gently poured into the column for packing. Bubbles were quickly tapped away with a cork ring before packing was completed to avoid cracking. Another thing layer of sand was gently deposited on top of the packed silica gel along with some solution to keep the sand and column wet. 400 mg of product was mixed with sufficient solution to dissolve. The mixture was added to the column gently with a polystyrene disposable pipette and washed down the column with solution. As the product ran down the column a consistent flow of solution was added to avoid the column drying out. Ten fractions were collected in test tubes in two ml intervals. Each fraction was analyzed using thin-layer chromatography to test for purity. Each fraction was left in its respective test tube overnight for crystallization. Infrared spectroscopy was used to identify the functional groups of the obtained compound and further analyze its presence or absence in each fraction. The spectra obtained was compared to the starting product to conclude whether the expected product was isolated and purified. Additionally, melting point data was obtained.

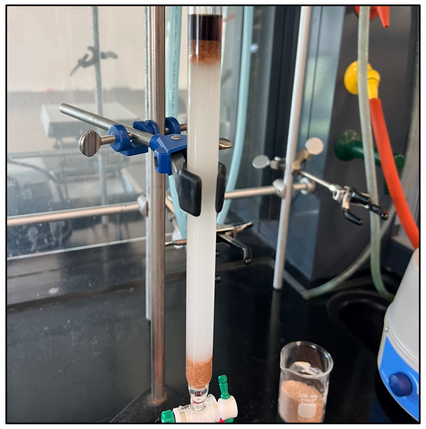


Figure 1. Photo By: Destiny Braynen. Packed column upon addition of product

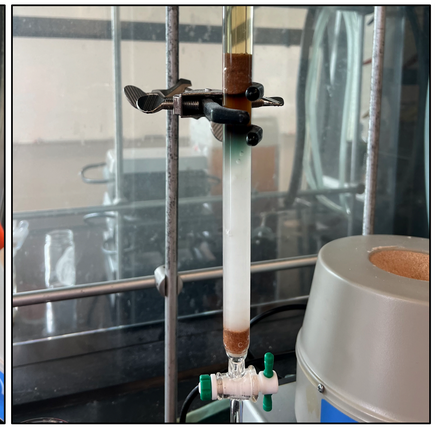


Figure 2. Photo By: Destiny Braynen. Initial phase of product running through the column

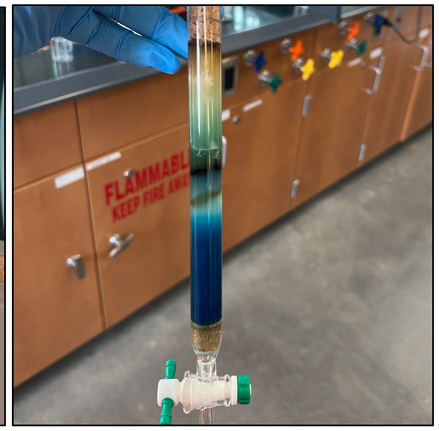


Figure 3. Photo By: Destiny Braynen. Column after complete fraction collection

Figure 4. IR Spectroscopy was run on each fraction collected through the column chromatography and compared to literature values. IR spectroscopy of the purified 3,4-bis(chloromethyl)-2,5-dimethylthiophene. Peaks from 588 cm-1 to 813 cm-1 corresponded to alkyl halide of the form C—X. The peak at 1176 cm-1 indicated the expected bond between H—C—H and Cl. IR spectroscopy of the final purified 3,4-bis(chloromethyl)-2,5-dimethylthiophene. The strong peak present at 2918 cm-1 is an indicator of the sp^3 hybridized C—H. All fractions displayed consistent IR peaks which presents possibility of the isolation of the same compound.

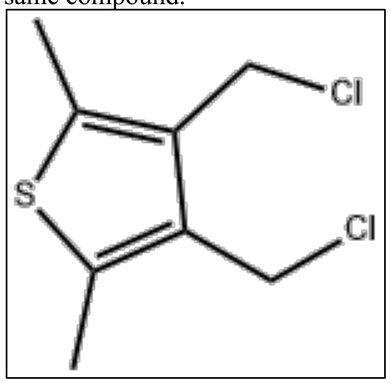


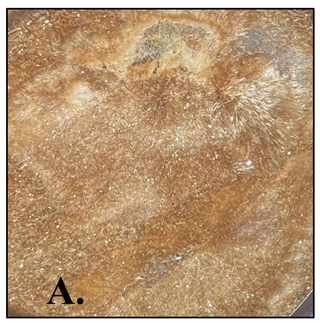
Figure 5. Structure of 3,4-bis(chloromethyl)-2,5-dimethylthiophene. From Chemical Book. Retrieved from https://www.chemicalbook.com/ChemicalProductProperty_EN_CB6145200.htm

Table 1. Accepted frequency values from IR Spectroscopy bonds and the corresponding experimental values from each spectrum. Table By: Destiny Braynen

Bond	Pure 3,4-bis(chloromethyl)-2,5-dimethylthiophene (cm^{-1})	Purified 3,4-bis(chloromethyl)-2,5-dimethylthiophene (cm^{-1})
C—Cl	550-850	588-813
CH_2—Cl	1196	1176
C—H sp^3	2915	~2918

Table 2. Melting point ranges for 10 collected fractions. Table By: Destiny Braynen

Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8	Fraction 9	Fraction 10
66.0-69.0	66.6-69.3	65.3-68.6	65.5-67.2	63.6-68.9	66.4-70.6	64.8-67.8	65.8-68.0	62.8-64.9	66.1-67.4



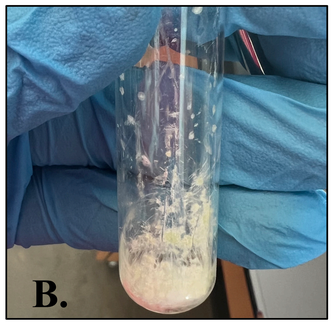
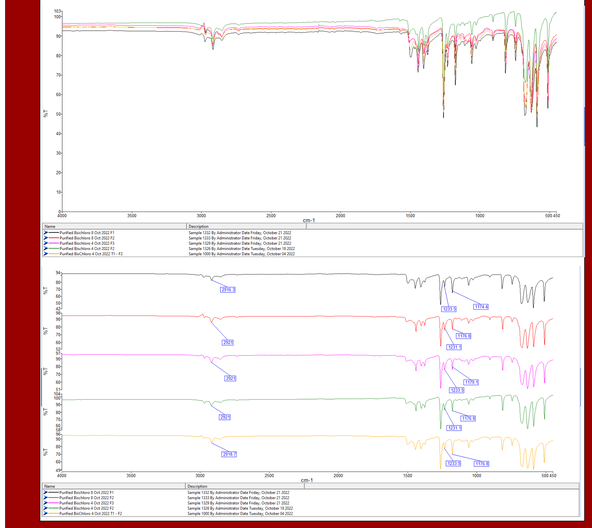


Figure 6. Photos By: Destiny Braynen. State of 3,4-bis(chloromethyl)-2,5-dimethylthiophene before **(A.)** and after **(B.)** purification

Results and/or Conclusion

After analysis through infrared spectroscopy and melting point data, it can be assumed that the sample of purified 3,4-bis(chloromethyl)-2,5-dimethylthiophene was "impure" due to an incomplete reaction or possible contaminants that caused a single chloro attachment as opposed to two. The column chromatography worked well in purifying the substance of the contaminants and the results are consistent throughout the collected fractions, however further investigation is required to determine whether the product of the chromatography is in proper condition to be used in future Meldrum's acid reactions. Although it is unclear, the result shows promising signs of being suitable for future work. Characteristic peaks at 1176 cm-1 and 2918 cm-1 suggest proper isolation of the expected compound. The expected melting point range is 70-71 degrees Celsius. Minimal deviations by 3-4 degrees suggest a relatively purified compound. The work done gave rise to valuable exposure to column chromatography skills and techniques along with troubleshooting when attempting to purify a substance.



Future Work

1. Completion of the Meldrum's Acid reaction with purified compound must be done.
2. Further analysis, characterization and identification of the collected compound must be performed to confirm its purification and proper isolation from the impure starting material
3. Purification using other processes as a point of comparison
4. Analysis of the application and usefulness of this purification method for other organic compounds

References and/or Acknowledgments

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