

SYNTHESIS OF 1,4-BENZODIAZEPINE THROUGH PHOTOCHEMICAL REACTION OF 3-PHENYL-3,6-DIHYDRO-[1,2]DIOXINE WITH ETHYLENDIAMINE

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ABSTRACT

Synthesis of 1,4-Benzodiazepine via reaction of ethylenediamine with 3-phenyl-1,2-dioxine has been carried out. The first step of this experiment was conversion of triphenyl phosphine to triphenyl methyl phosphine iodide which was then used to form phenyl butadiene from cinnamaldehyde. The synthesis of phenyl dioxine was done via photochemical reaction of phenyl butadiene with oxygen gas in the presence of tetraphenyl phosphine. The coupling reaction between phenyl phosphine and ethylenediamine was carried out under atmospheric pressure at room temperature. All steps of these reactions were confirmed using TLC with appropriate eluents and the resulting compounds were purified using column chromatography. In addition, all products structure including phenyl dioxine and 1,4-benzodiazepine were determined using HNMR.

Keywords: Benzodiazepine, photochemical reaction, dioxine

INTRODUCTION

Historically, benzodiazepines started to be used in pharmaceutical by 1960 when chlordiazepoxide was produced and introduced commercially as a medicine under the trade name of Librium®. Chlordiazepoxide which initially called *methaminodiazepoxide* was the first benzodiazepine that has been synthesized in 1950s. This compound was accidentally produced when the chemical work of dye was carried out, later when the test was made the scientist found that this compound had anxiolytic, hypnotic and muscle relaxant effects. Soon after this first invention, many benzodiazepine derivatives were discovered, produced and introduced to the market.

Due to clinical and biological activities as well as commercial success of 1,4-benzodiazepine family of CNS active agents, the interest in extensive development of this compound derivatives has risen among medicinal chemistry community as privileged structures.

Through modification of origin structures many compounds which were proven to give the biological activities have been extensively derived, some of

important compounds including diazepam which was first marketed as Valium®, alprazolam was designed by introducing the heterocyclic ring fused to different faces of the benzodiazepine ring nucleus. Moreover, the development of this drug can also carried out by replacing benzene ring with other ring constituents like naphthalene, imidazole, furan, pyridine and pyrimidine. The following diagram shows some important benzodiazepines.

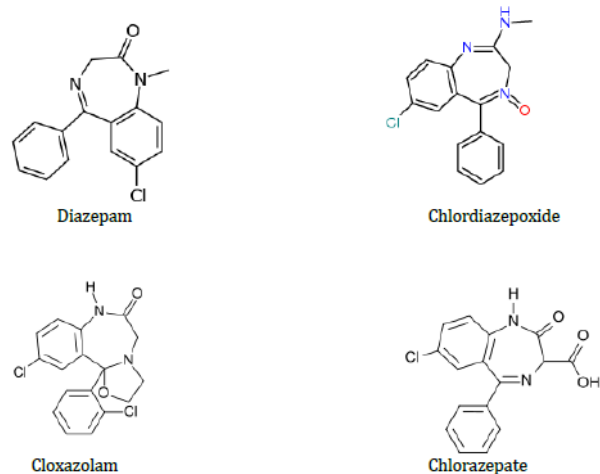


Figure 1. Some important benzodiazepines

Synthesis of diazepam

Even though some diazepam have been found naturally from several sources like Liposidomycins which was found in *Streptomyces griseosporous* (Knapp *et al*, 1992), Asmarines A and B (Bridson and Weirich, 1988; Colombo *et al*, 1989) were isolated from sponge *Raspallia* sp. and benzomalvin A was extracted from the culture of *Penicillium* sp., the development of this family compounds remains interesting to chemists until present days.

Two main approaches including chiral synthesis and combinatorial synthesis can be employed to obtain the family compound of benzodiazepines. Gilman *et al*, 1990 pioneered the synthesis of first optically active benzodiazepine compound and Herrero *et al*, 2003 have also successfully synthesized some important chiral 1,4-benzodiazepine derivatives. However, a versatile approach which has been used by many chemists is combinatorial synthesis. This method allows researchers to develop various derivatives of benzodiazepines with varied biological activities. By changing the substituents or rings of reactants involved in synthesis of benzodiazepines, many chemists have successfully synthesized bunch of diazepam derivatives which some of them will be discussed in this section. For example, Bunin *et al*, 1994 reported the successful combinatorial synthesis of 1,4-benzodiazepine library and had also evaluated the biological activities of synthesized compounds.

In 1993, Akssira *et al* had successfully synthesized the compound of 1,4 benzodiazepine 2,5-diones through combination of anthranilic acid derivative and amino esters by using HCl gas as a catalyst. Furthermore, diazepam can also be made through the reaction of 1, 3-diketoester compounds with ethylenediamine by heating at 60°C as suggested by Kumar and Joshi, 2009. The following scheme illustrates the synthesis of benzodiazepines.

Some Important clinical activities of 1,4-benzodiazepines

Anti-malarial agents

Falcipain-2 (FP-2) inhibitors was designed by Micale *et al*. which is based on a 1,4- benzodiazepine scaffold. This compound is introduced internally to a peptide sequence, which mimics the fragment D-Ser-Gly, and on a C-terminal aspartyl aldehyde building block, which inhibits the enzyme by forming a reversible covalent bond at the active site. The following diagram shows the structure of Falcipain-2.

Anti Bacterial Agents

Tibenzonium iodide with systematic name of Diethylmethyl (2- (4-(p (phenylthio) phenyl) – 3 H – 1 , 5 - benzodiazepin-2-yl) thio) ethyl) ammonium iodide is one of benzodiazepine derivatives which has anti-infective activity and anti-bacterial activity and has been reported to have antibacterial activity against *Streptococcus pyogenes*. The following image illustrates the structure of tibenzonium iodide.

Anti-viral agents

Drug from the family of Non-nucleoside reverse transcriptase inhibitors or usually abbreviated as (NNRTIs) were reported to give the therapeutic effect against human immunodeficiency virus-1 (HIV-1) which are targeted at a specific site of reverse transcriptase.

Anti-hypertensive agents

Some of family compound of diazepam were found to possess anti-hypertensive activity. When guanidine group combined with tetrahydro-1,4-benzodiazepine the resulted compound give the anti-hypertensive action. In addition, the fusion of benzodiazepine to indole also showed the anti-hypertensive activity. The following figure illustrates the chemical structures of drugs synthesized by Kim, 1995 which were proven to give the therapeutic effect against blood hypertension.

RESEARCH METHOD

Chemicals and Instruments

The chemicals involved in this experiment including dichloromethane, ethylacetate, triphenyl phosphine, tetraphenylphosphine, potassium

butoxide, cinnamaldehyde, methanol, diethyl ether, methyl iodide, saturated sodium carbonate, oxygen gas, ethylenediamine petroleum spirit and silica gel. In purification using Thin Layer Chromatography the aluminum base silica plate was used while in column chromatography silica gel was utilized. For structural analysis, HNMR was used.

Preparation of triphenylmethyl phosphine salt

46 g of methyl iodate was dissolved in 150 mL dried diethyl ether and stirred. In to this stirred solution 92.8 g triphenyl phosphine was gently added resulted in precipitation of product in the bottom of the flask, subsequently the resulting product was then filtered under reduced pressure and washed several times using diethyl ether.

Synthesis of 1-phenyl-1,2-butadiene

Dried diethyl ether was prepared by distillation. 50 mL of distilled diethyl ether was taken using a syringe and placed in to 250 mL round bottle flask. 16.2 g of triphenyl phosphonium iodide was then added and dissolved by stirring for 30 minutes followed by addition of potassium butoxide while the stirring was continued, After 30 minutes, 5 g of cinnamaldehyde was added to the flask resulted in turn of mixture colour to pale orange solid. In order to ensure that all cinnamaldehyde completely react, couple grams of potassium butoxide was added to the mixture. Upon the completion of reaction the brown oily product was obtained and TLC of product along with starting material was carried out using the eluent of 2.5% ethyl acetate in petroleum ether. The product obtained then was purified by column chromatography using silica as stationary phase and the same mobile phase as in TLC. The fraction of 3, 4 and 5 were combined together followed by evaporation of the eluent under reduced pressure. 2.35 g product which was obtained then was stored in the refrigerator for further reaction.

Photolytic synthesis of 3-phenyl-1, 2-dioxine

1-phenyl-1,3-butadiene which was obtained previously was diluted in dichloro methane and 20 mg of tetra phenyl phosphine was added and the resulting solution was violet, the solution was put in a reactor and was bubbled by O₂ and emitted by light source for 7 hours. The product then was purified by TLC followed by column chromatography involving 10% ethyl acetate in petroleum ether as an eluent. Upon completion of chromatographic purification, all fractions were tested by TLC then the fraction of 11 to 15 were collected and combined and the solvent was evaporated under reduced pressure rotary evaporator. 410 mg product was obtained and stored for further reaction with ethylenediamine to produce the final product.

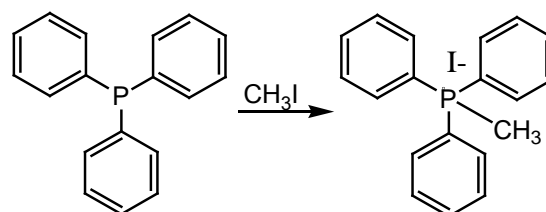
Reaction of 3-phenyl-1,2-dioxine with ethylenediamine

Synthesis of 1,4-benzodiazepine was carried out at room temperature and pressure. 200 mg of product from previous reaction 3-phenyl-1,2-dioxine was mixed and reacted with 1.5 molar ratio of ethylenediamine in dichloromethane and stirred for 3 days until the reaction complete.

RESULTS AND DISCUSSIONS

Preparation of triphenylmethyl phosphine salt

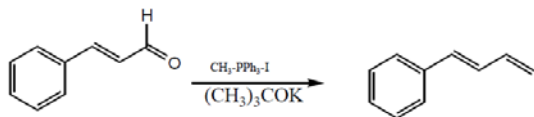
The first step of this research was the preparation of triphenylmethyl phosphine salt by reacting the methyl iodide and triphenyl phosphene in dried diethyl ether which produced the white powder. The reaction is depicted below.



Synthesis of 1-phenyl-1,2-butadiene

The reaction was carried out under the fumehoodand. Triphenyl phosphonium iodide was reacted with potassium butoxide. Upon the addition of potassium

butoxide the mixture turned yellow. The reaction then proceeded by adding cinnamaldehyde which resulted pale brown solid. The reaction is illustrated by the figure 3



The TLC of 1-phenyl-1,2-butadiene compared to the starting material can be seen in figure 1.

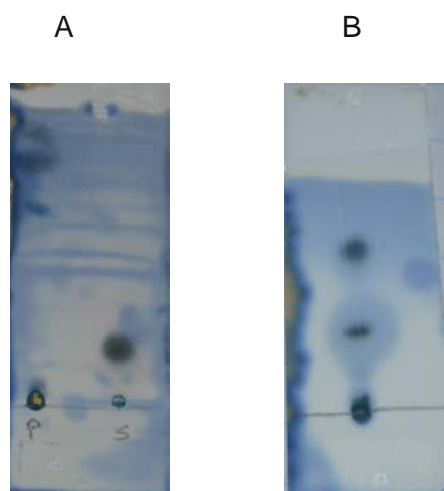


Figure 1 . TLC of 1-phenyl-1,3-butadiene vs cinamaldehyde (A) and Dioxine (B)

The product with $R_f = 0.375$ was obtained, the TLC spot of product was close to the spot of dye (tetraphenyl phosphine). TLC of dioxine (B) after purification using column chromatography with eluent of 10% ethyl acetate in petroleum spirit. The following figure depicted the TLC of final product (1,4-benzodiazepine) fragments collected after purification using column chromatography involving silica gel as adsorbant and the eluent of 10% ethyl acetate in petroleum spirit.

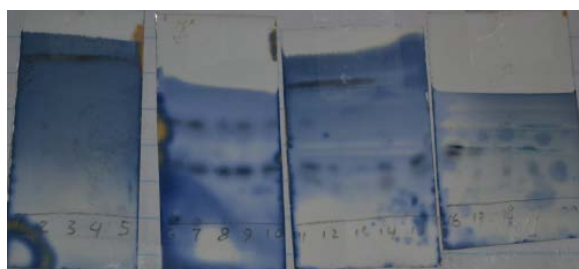
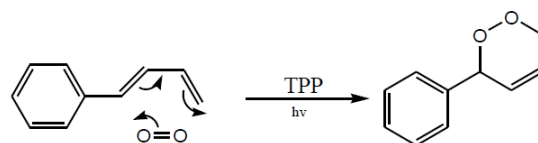


Figure 2. TLC spectra of 1,4 Benzodiazepines

Fractions of 11, 12, 13, 14, 15 and 16 were collected and combined followed by evaporation under reduced pressure. Mass of product obtained was 0.41 g.

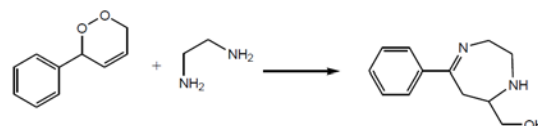
synthesis of 3-phenyl-1, 2-dioxine

The photochemical reaction of 3-phenyl-1, 2-dioxine was carried out in the reactor equipped by the O_2 filled balloon and light source to initiate the following reaction:



In the reaction depicted, the diatomic molecule of O_2 started to open the double bond initiated by the photon and formed the ring with two carbons exist in the molecule of 1-phenyl-1,2-butadiene.

After the reaction of 3-phenyl-1, 2-dioxine was completed about 7 hours. The synthesis of 1,4-benzodiazepine was carried out by mixing the 3-phenyl-1, 2-dioxine with ethylenediamine as shown by the following figure.



The final product produced was then analysed by TLC and H-NMR. The following TLC spectrum shown the spot of product.

The TLC of final product (1,4-benzodiazepines); eluent= 10% methanol in DCM



Figure 3. TLC spectrum of 1,4-benzodiazepines

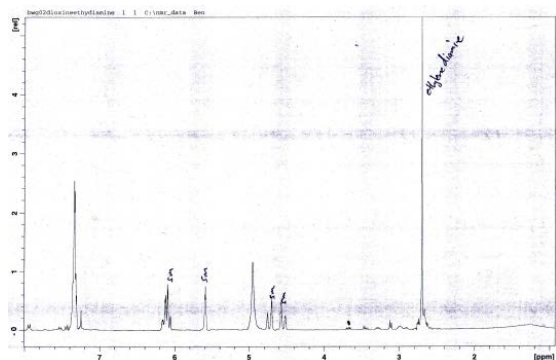


Figure 4. HNMR at time of reaction of t=0

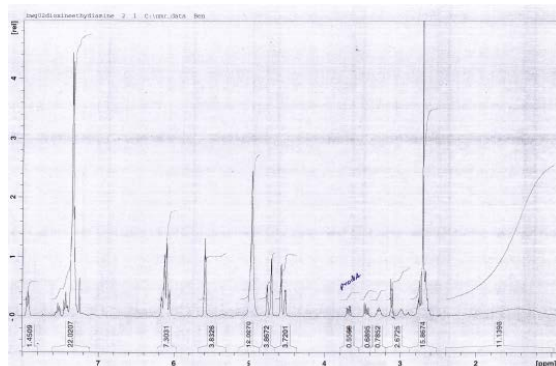


Figure 5. HNMR at time of 1 hour

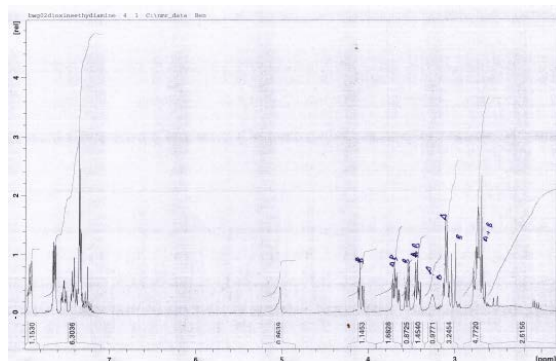


Figure 6. HNMR Spectra at t=16 hours

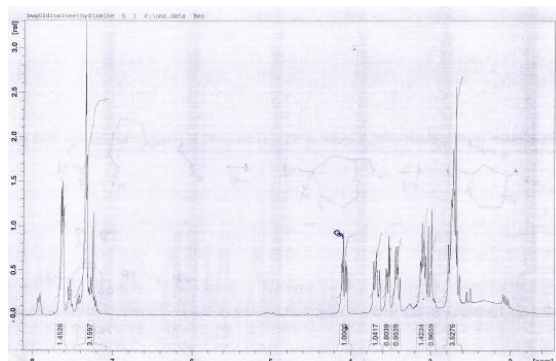


Figure 7. HNMR Spectra at t=24 hours

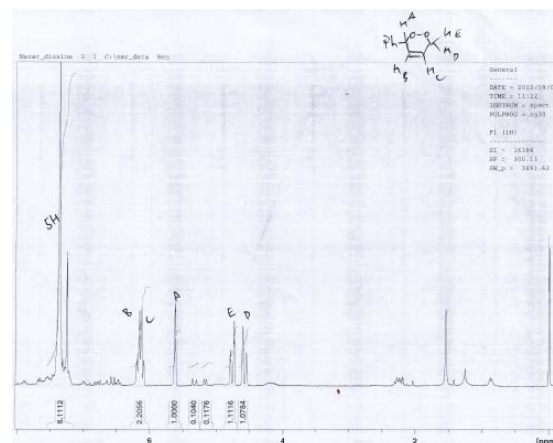
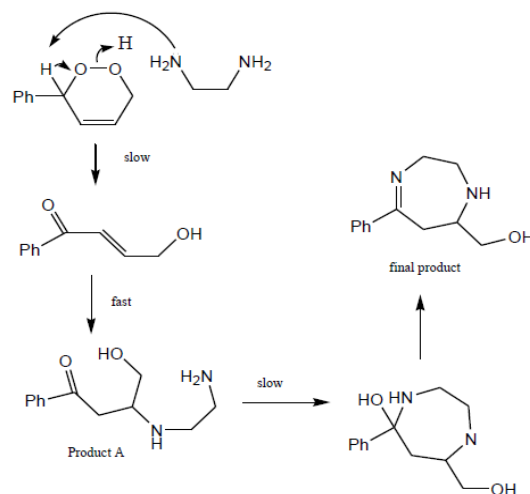


Figure 8. HNMR Spectra of dioxine

The overall reaction can be written as the following figure.



The above diagram depicted the overall reaction of dioxine with ethylenediamine resulted in the final product of 1,4-benzodiazepine. As can be seen in the scheme that the reaction proceeded through nucleophilic attack by ethylenediamine and the reaction went to some slow process and fast process, during the reaction there is an intermediate formation of product A as marked in the reaction scheme above thus product A can be observed in the spectra at t=1 and t= 16 hours.

However, as the reaction complete i.e at t=24 hours, the signal for product A no longer appear in the spectra. If we compare the spectra of reaction at t=16 against t=24, it is clearly observed that the signal at 5.2 ppm which appears in t=16 but not at t=24. In addition, the overlapping signal between 2.5 ppm and 4.2 ppm at t=16 indicates the mixture of

product A and final product is no longer be seen at $t=24$ which is the indication of complete reaction.

The HNMR spectra for the final product at time zero of reaction to $t=24$ hours are given in the appendixes 3, 4, 5 and 6. As can be seen that at $t=0$ the signal of ethylenediamine and starting material of dioxine are observed. When we compare the spectra for dioxine (Figure 8) to the spectra of reactant mixture at $t=0$ (Figure 4) the similar spectra can be seen except the presence of ethylenediamine in the mixture which cannot be seen in the dioxine spectra.

The compound resulted in this experiment was determined by HNMR but the spectra produced was not sufficiently good enough. In addition, unfortunately the purification of the resulted compound also was not very successful since at the end of purification process, the desired spectra which were expected to appear did not show up.

TLC was run to investigate the completion of coupling reaction between ethylenediamine and dioxine but it seems that the eluent used (10% methanol in dichloromethane) for TLC and column chromatography was not suitable enough. Therefore we would suggest the comprehensive investigation in order to find an appropriate eluent that can be used to purify the compound that produced in this experiment.

CONCLUSION

In conclusion, the synthesis of 1,4-benzodiazepine through coupling reaction between ethylene diamine and 1-phenyl-1,3-dioxine was handy to perform since the reaction can be carried out at room temperature and atmospheric pressure.

However, this reaction is time consuming process and dioxine as an important reactant was prepared through photochemical reaction involving oxygen gas. This experiment has only covered the synthesis of 1,4-benzodiazepine compound which has the chemical structure as depicted in the last reaction. However, the biological or clinical activities of this compound is necessary to be investigated and by obtaining the pharmaceutical activities we can then

know whether this compound can be used as a medicine with specific therapeutic effect in order to cure particular disease.

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