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# Synthesis and Characterization of an Anacardic Acid Derivative of Thiazolidinedione

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**Abstract:** An anacardic acid derivative of Thiazolidinedione was synthesized. The preparation of the target compound which may have potential activity against Mycobacterium tuberculosis and cancer cells involved the extraction of anacardic acid from cashew nutshell by base precipitation. The olefinic part of the isolated anacardic acid was cleaved and converted to an aldehyde precursor via performic acid epoxidation. The resulting 2-Hydroxy-6-(8-oxooctyl)benzoic acid was coupled with thiazolidine-2,4-dione via Knoevenagel Condensation. The characterization of the precursor and target compounds were carried out using the ESI-MS and FTIR. Reactions were monitored via thin layer chromatography. The obtained results showed that the compound was successfully synthesized. However, the accomplished characterization was incomplete. Further characterization via $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR) and mass spectrometry (MS) and the testing for activity against cancer cells and Mycobacterium tuberculosis are recommended.

**Key Words:** anacardic acid; thiazolidinedione; organic synthesis; cancer; tuberculosis

## 1. INTRODUCTION

Cancer and Tuberculosis greatly affect a large number of population worldwide. Data from the Health Organization accounts that 8.2 million people across the globe suffer from cancer. It is expected that disease incidence will rise by 70% for the next two decades. Tuberculosis on the other hand killed 1.5 million people in 2013. (World Health Organization, 2015)

Treatment of cancer and tuberculosis require the use of natural medicines or synthetic drugs (World Health Organization, 2015.) Altering the structure of a compound may result to the production of new compounds with increased bioactivity and minimized side effects.

One natural product exhibiting bioactivity is Anacardic acid (AA). Most of its derivatives have shown activity against several types of bacteria. Some of these compounds have known pharmacological uses against cancer cells. Anacardic acids are found in *Anacardium occidentale*, most commonly known as Cashew nuts. Many parts of the said fruit are used as herbal medication (M. Balali-Mood & K. Balali-Mood, 1996.) Moreover, Thiazolidinedione (TZD) is a drug that is used for the treatment of type 2 diabetes mellitus (T2DM), a disease that causes insulin resistance (Diabetes.org, 2015.) This group of drugs is

known to improve the sensitivity of insulin to regulate the right amount of glucose in the body (D. Zhao, Z. Shi, A.H. Warriner, P. Qia, H. Hong, et al. ,2014) The aim of the research was to synthesize and characterize an Anacardic acid derivative of TZD. The synthesis involved the preparation of 2-Hydroxy-6-(8-Oxooctyl)Benzoic Acid from base-precipitated Anacardic acid obtained from cashew nut shells *(Anacardium occidentale)*. Subsequent coupling with TZD produced the desired compound.

## 2. METHODOLOGY

The methods used were adapted from R. Paramashivappa et.al.

Cashew nutshell liquid (3.1633 g) was obtained from Soxhlet extraction of osterized cashew nutshells (6.6765g) using ethanol (200 ml) as the solvent. It was done for 3 hours at 78$^0$C with constant stirring and the solvent was removed under reduced pressure. The crude extract was a viscous oil with dark brown color. The extraction of CNSL was done four times with a resulting mass of 10.8975 g.

CNSL (10.8975 g) was dissolved in methanol (60 ml) and calcium hydroxide (5.0009 g) was slowly added to the mixture. It was done for 3 hours at 50$^o$C. The resulting salt (8.16071 g) was vacuum filtered and the AA mixture were isolated via liquid-liquid extraction using ethyl acetate-hydrochloric acid (1.5:1) and ethyl acetate-water (1:1) mixtures.

Formic acid (0.10 ml) was added to AA followed by drop wise addition of hydrogen peroxide (1 ml) for 30 minutes at 40-50°C. The reaction was done for 5 days with constant stirring. In addition, performic acid (2.0 ml) was added on the third day. After which, liquid-liquid extraction was done with dichloromethane (DCM)-water mixture (1:1). Sodium metaperiodate (0.0510 g) and triethylamine hydrochloride (TEA HCl)(2.053 g) were added to the organic layer obtained from the extraction. Solution was stirred for 30 minutes at room temperature.

Thiazolidinedione (0.0213 g) was added to the precursor compound synthesized (0.040 g) with toluene (5 ml) solvent. Piperidine (0.15 ml) was added as a phase transfer catalyst. Reflux was done for 1 hour and 30 minutes at 127 $^o$C with constant stirring.

## 3. RESULTS AND DISCUSSION

CNSL was obtained from cashew nutshell (*Anacardium occidentale)* with a yield of 47.38%. CNSL is a mixture containing cardols, cardanols and anacardic acids. Base precipitation was done to form anacardate salt. Moreover, calcium anacardate was reacted with hydrochloric acid to form AA.

Isolated AA is a thick, brown oil with a percent recovery of 24.76%. Infrared (IR) spectrum showed phenol alcohol (O-H) stretch at 3420.15 cm$^{-1}$ and carboxylic acid carbonyl stretch at 1643.49 cm$^{-1}$. Mass spectrum in negative mode showed peaks at 341.2422, 343.25803 and 345.2576 all of which correspond to AA mixture with molecular weight assignments of 342, 343 and 346 amu.

The precursor compound synthesis has three steps: performic acid epoxidation, its diol conversion and diol cleavage to form the aldehyde functionality needed for the subsequent coupling with TZD via Knoevenagel condensation. Furthermore, there were three trials done for this step.

For the first trial, performic acid (8 ml) was added to AA (0.1383 g) for 30 minutes at 40-50°C. Tetrahydrofuran(THF)-water was used to extract the organic layer. This compound was a thick, brown oil by appearance with a percent recovery of 28.13%. Mass spectrum (negative mode) showed peaks at 265.06644, 293.08635 and 282.78554. Molecular weight assignments correspond to 266, 294 and 283. All of which are inconsistent with the target mass of 264 amu. IR results showed no characteristic peaks for a carboxylic acid carbonyl (1710cm$^{-1}$) and carboxylic acid hydroxyl groups (2500-3500 cm$^{-1}$). There were also no characteristic absorptions at 1740-1720 cm$^{-1}$ and 2820-2850 or 2720-2750 cm$^{-1}$ which correspond to the carbonyl and Csp$^2$-H bond of the aldehyde functional group, respectively.

Characteristic peaks appeared at 3501 $cm^{-1}$ consistent with an alcohol hydroxyl and at 1585 $cm^{-1}$ consistent with arene C=C. These information indicated that the target compound was not successfully synthesized

For trial 2, performic acid volume was to 19 ml and the reaction was done at room temperature to prevent the decomposition of performic acid which readily happens at high temperatures. The thin layer chromatography (TLC) of the recovered oil showed no reaction occurred. Mass spectrometry showed peaks that were still consistent with the starting material. These findings were proof that no significant reaction took place.

For trial 3, formic acid (0.10 ml) was added first followed by drop wise addition of hydrogen peroxide (1 ml). The reaction time was also increased to 5 days since the 30 minutes span showed no reaction occurred as evidenced by ESI-MS results that still correspond to the masses of the AA. The stirring was carried out for another three days. Mass spectrometry [M-H] showed a signal at 263 corresponding to 264 amu which indicated that some of the product was formed. However, the intensity was very low compared to that of AA. Performic acid (2.0 ml) was added on the third day. The reaction was monitored by mass spectrometry and TLC. Also, DCM was used in liquid-liquid extraction and TEA HCl was synthesized by reacting hydrochloric acid (12 M) with triethanolamine since it was suspected that the readily availables was not working.

The method for the diol cleavage was also modified [7] The oil recovered was dissolved in DCM and $NaIO_4$ and TEA HCl were added. The mixture was stirred and washed with water. The solvent was removed resulting to thick, brown oil (0.04g).

IR results showed a characteristic peak for an aldehyde functional group at 1699 $cm^{-1}$. Peaks were also found at 3623.80 and 1645.46 $cm^{-1}$ which are the characteristic wavenumbers for the carboxylic acid functional group. Mass spectrometry (positive mode) revealed a significant peak at 263.14070 corresponding to a mass of 262 amu. Instrument was not calibrated before running the sample. This may be the cause of the deviation from the target signal which is 265 as there have been reported incidents where deviant masses were corrected after recalibration of the instrument.

The reaction for the synthesis of the target compound followed a reaction called Knoevenagel condensation. The synthesized compound was a yellowish solution suspended in toluene solvent. Moreover, IR spectroscopy was the only method that was used for the characterization and verification of the compound. The representative fractions from column chromatography were analyzed via IR spectroscopy.

It was inferred that the ninth fraction was the target compound because of the peaks that were observed. The coupling of TZD with AA caused the N-H overlap from TZD to the O-H functionality of the AA. Moreover, the carbonyl stretch that was observed was a sharper peak compared to the first and third fractions which supports the fact that there is an increase in the number of C=O functionality in the compound.

## 4. CONCLUSION

Data obtained indicated the successful synthesis of the target compound. The AA mixture was isolated from the CNSL as evidenced by the MS results. The IR spectrum of the mixture revealed the characteristic feature for O-H group of COOH and for the C=O group of an aromatic-COOH functionality. IR results showed a peak for an aldehyde functional group and the peaks were also found corresponding to wavenumbers for the COOH functional group. Mass spectrometry revealed a significant peak at 263.14070. The deviation from the target signal may have been because of the failure to calibrate the instrument before running the sample. The AA derivative of TZD was also successfully synthesized. The IR spectrum of the ninth fraction of the

purification showed a significant change in the sharpness of the peaks in comparison with the 1st and 3rd fractions. The sharpness of the peak at 1690.38cm-1 was due to the overlap of the C=O functionalities of TZD.

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