

SYNTHESIS AND CHARACTERIZATION OF PUTRESCINE-SULFUR ANALOGUE

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ABSTRACT

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Cancer is a disease which has high motility rate worldwide. The current treatment has a high potential to kill cancer cells, however it is lacking of specificity and therefore cause the side effects in normal cells. The alternate pathway has been explored including targeting to cancer cells via polyamine transport system (PTS). The PTS activity is highly upregulated in cancer cells compared to normal cells. In this study, one of the polyamines called putrescine was being exploited to be a vector for sulphur. This putrescine-sulphur analogues were synthesized using two different methods. The synthesized product was later characterized using various physicochemical techniques such as gas-chromatography and Fourier transformed-infrared spectroscopy for structural determination. Several putrescine-sulphur analogue was successfully synthesis with the expected structures

Keywords: Polyamine moiety; Putrescine-sulfur compounds; Polyamine-sulfur derivatives; Sulfur containing compounds; Structural determination.

1. INTRODUCTION

Cancer treatment has been improved significantly throughout the years. New drugs and treatments have been found and discovered. However, the selective delivery of drugs to cancer cells is remaining as a major challenge in oncology (Palmer and Wallace, 2010). By enhancing specific targeting to cancer cells by selective drug delivery, the non-specific toxicities can be minimized by reducing the uptake by healthy cells (Abdul Ghani *et al.*, 2015). One of the promising strategies is the drug delivery via polyamine transport system. As cancer cells have been reported to have increased polyamine uptake, it increases the pharmacological activity of drugs and reducing their side effects by enhancing delivery to the target site (Igarashi *et al.*, 1975).

Mammalian polyamine such as putrescine is small naturally occurring aliphatic cations that are required in many physiological functions including cell proliferation and apoptosis (Marton and Pegg, 1995; Seiler and Raul, 2005; Rato *et al.*, 2011; Tsujinaka *et al.*, 2011; Cerrada-Gimenez *et al.*, 2011). Since polyamines are crucial for cancer cells, one method in reducing the effect of cancer cells is by adding elements that have anti-cancer properties in the polyamine structure. In this study, sulphur atoms are known for its anti-cancer a property is inserted into polyamine structure (Omar and Wabel, 2010). Thiourea, which is a compound containing two sulphur atom, have been opted because of their diverse anticancer activity against various leukemias and solid tumors attributed to the presence of amine and sulfur

atoms (Fuks *et al.*, 2010). An experiment on rats induced with mammary tumor had been done to determine anticarcinogenic activity in sulfur containing compounds (Ip *et al.*, 1992).

Therefore, this study aimed to synthesize and characterize a series of putrescine-sulphur analogues using two different methods. It is hypothesized that the newly synthesized compounds to contain both anti-cancer properties and the ability to target the cancer cells effectively.

2. EXPERIMENTAL

All chemical compounds were used as received, without any purification. All synthesis and analyses were done in Kulliyah of Science, IIUM Kuantan Campus. The putrescine-sulfur analogues were synthesized using cold temperature mediated synthesis and room temperature mediated synthesis.

2.1 Cold temperature mediated synthesis (CTS)

Putrescine dihydrochloride (2.66 g, 0.016 mol) dissolved in 90% ethanol (10.0 ml) was added with potassium hydroxide (1.85 g, 0.033 mol) in 90% ethanol (30.0 ml). Precipitation formed (potassium chloride) was filtered and the mass was recorded. Additional potassium hydroxide (0.18 g, 0.016 mol) dissolved in 90% ethanol was later added to the filtrate. Carbon disulfide (1.0 ml, 0.033 mol) was added dropwise to the filtrate with continuous stirring and maintained in an ice-salt bath. Upon completion, the solution was added with 10.0 ml of 40% ethanol and benzyl chloride (2.0 ml, 0.033 mol) was added dropwise with vigorous stirring. The solution was left to stir for about 3 hours after the addition of benzyl chloride. The solution was left standing overnight to give two layers of solutions. The yellow organic layer was extracted using a separating funnel and labeled as CTS.

2.2 Room temperature mediated synthesis (RTS)

Ethanol solution of potassium hydroxide (2.77 g, 0.049 mol) and putrescine dihydrochloride (2.66 g, 0.016 mol) were added together to give precipitation (potassium chloride), which was filtered and the mass recorded. Carbon disulphide (1.0 ml, 0.033 mol) in was added dropwise into the filtrate with continuous stirring in room temperature. Upon completion, the solution was stirred continuously for 6 hours. Benzyl chloride (2.0 ml, 0.033 mol) was added dropwise with continuously stirring for 3 hours. The solution was left standing overnight to allow precipitation. The precipitate formed was filtered, dried and labeled as RTS.

2.3 Physical measurements

All analyses were done in the Instrumentation room in Kulliyah of Science at IIUM Kuantan Campus. Melting points were determined using an Electrothermal digital m.p. apparatus (IA9000 Series). The IR spectra were recorded using Perkin Elmer Frontier FT-IR Spectrometer in the range of 400 – 4000 cm^{-1} using KBr pellets. The TLC was done using silica gel as the stationary phase and dichloromethane as the mobile phase. The GC-MS were done using Perkin Elmer Clarus 680 with 600S MS Gas Chromatography Mass Spectrometer.

3. RESULTS AND DISCUSSION

Figure 1 showed that there are two possible structures upon completion of the reaction. The RTS was crystalline white solids while CTS was yellow liquid. It was found that RTS decomposed at 160 °C. CTS was subjected to TLC and showed that there was an obvious of four spots with the retention values of 0.88, 0.50, 0.33 and 0.09.

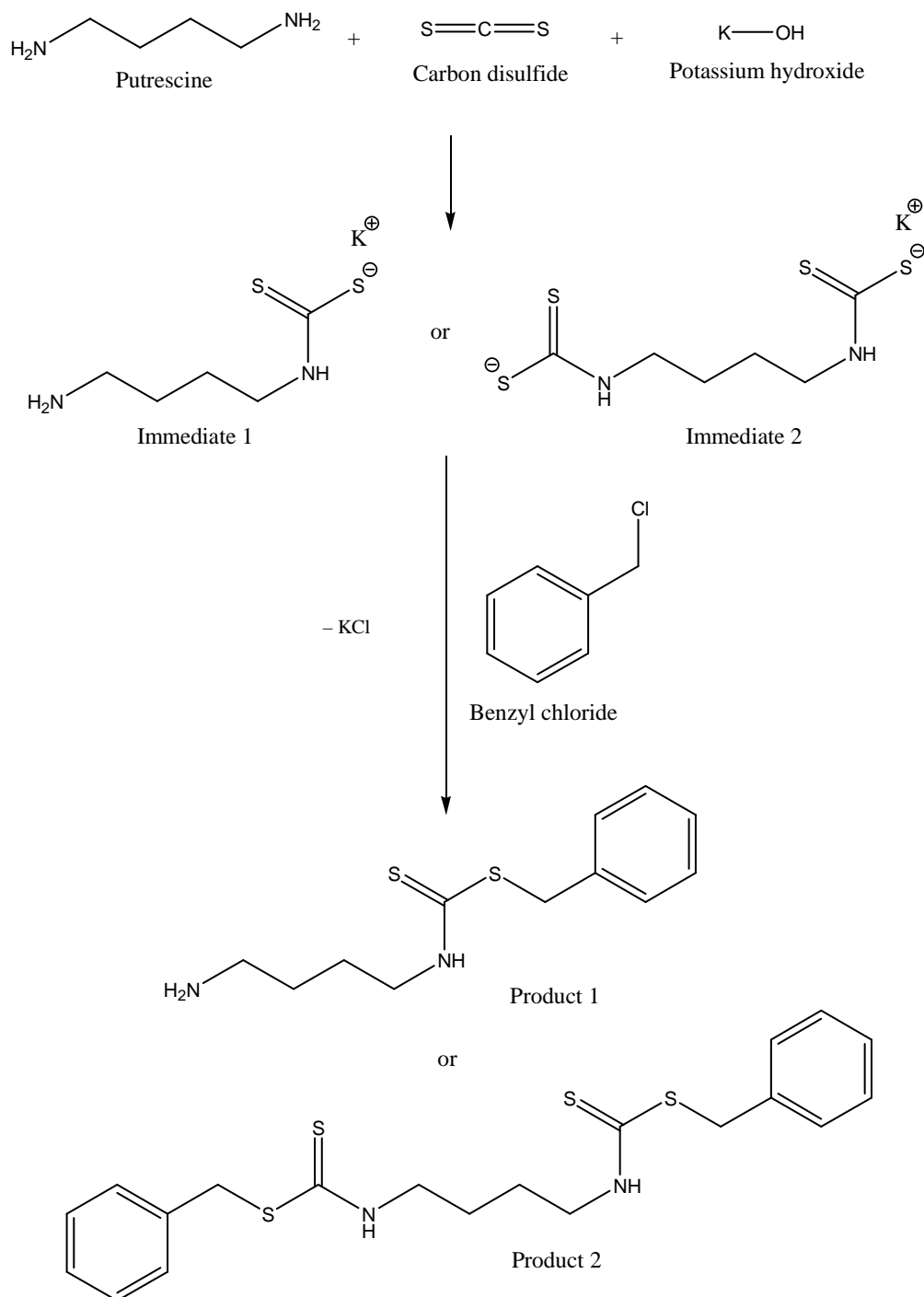


Figure 1: The reaction pathway of the synthesis

3.1 Fourier Transformed Infrared Spectroscopic Analysis

Selected FTIR bands were tabulated in Table 1. The IR spectra of putrescine dihydrochloride, CTS and RTS exhibited sharp peaks in the region of 3215-3250 cm^{-1} attributed to $\nu(\text{NH}_2)$ as shown in Table 1. These IR peaks are important to distinguish whether the structure of CTS and RTS has an unoccupied free amine group. This is because putrescine dihydrochloride possess two amine group at both end of the alkyl chain are able to react with CS_2 , as postulated as Product 1 and Product 2 shown in Figure 1. Both CTS and RTS exhibited strong bands indicating the presence of $\text{C}=\text{S}$ at 1086 cm^{-1} and 1091 cm^{-1} , respectively (Ali and Livingstone, 1974). The is further supported by the appearance of $\nu(\text{CSS})$ in the spectra of CTS and RTS. The presence of $\nu(\text{ArCH})$ bands showed the presence of the benzyl ring in both CTS and RTS.

Table 1: Selected FTIR bands

Compounds	$\nu(\text{NH}_2)$	$\nu(\text{C}=\text{S})$	$\nu(\text{CSS})$	$\nu(\text{ArCH})$
Putrescine.2HCl	3250	-	-	-
CTS	3246	1086	1009	739, 699
RTS	3215	1091	1018	734, 699

3.2 Gas chromatography mass spectroscopic (GCMS) analysis

The chromatograms of CTS (in Figure 2) showed that there are four compounds eluted as expected according to the TLC result with the retention time 23.48 mins, 25.77 mins, 28.76 mins and 31.03 mins. The chromatograms of RTS (in Figure 2) showed that there are five compounds eluted when dissolved in dichloromethane. The retention time for them was 21.81 mins, 23.49 mins, 25.77 mins, 28.78 mins and 31.03 mins. The major component was obtained from the retention time at 25.77 and 21.81 mins, respectively. However, the compound of interest was found to occur at 28.76 and 28.78 mins for both CTS and RTS. The mass spectrum of CTS at retention time 28.76 mins was shown in Figure 2. The fragmentation pattern deduced based on the mass spectrum was shown in Figure 3. CTS gave a base peak at $m/z=71.9$. Traces of putrescine ($m/z = 89$) was found to be present in the mass spectrum. Fragment ion at $m/z=91$ is corresponded to tropylium ion, which was evident that benzyl group is present in the structure.

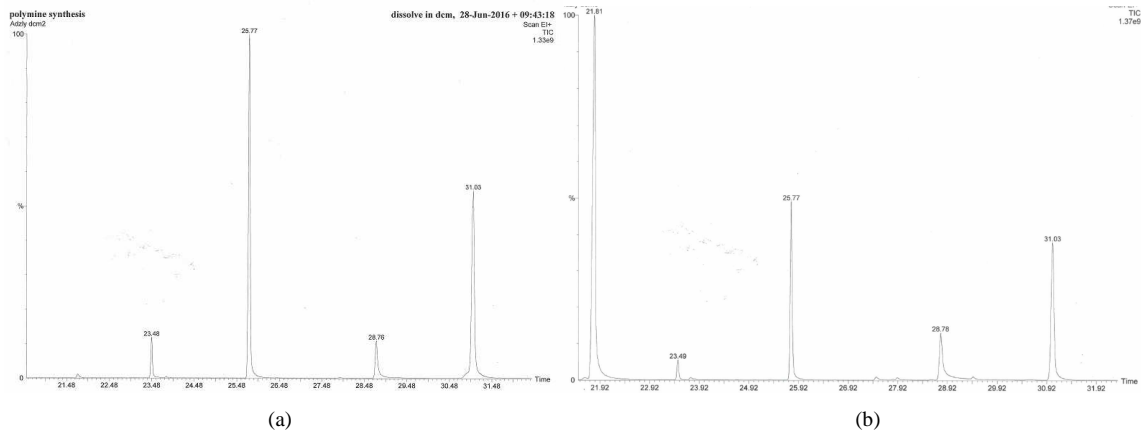


Figure 2a Gas chromatogram of RTS

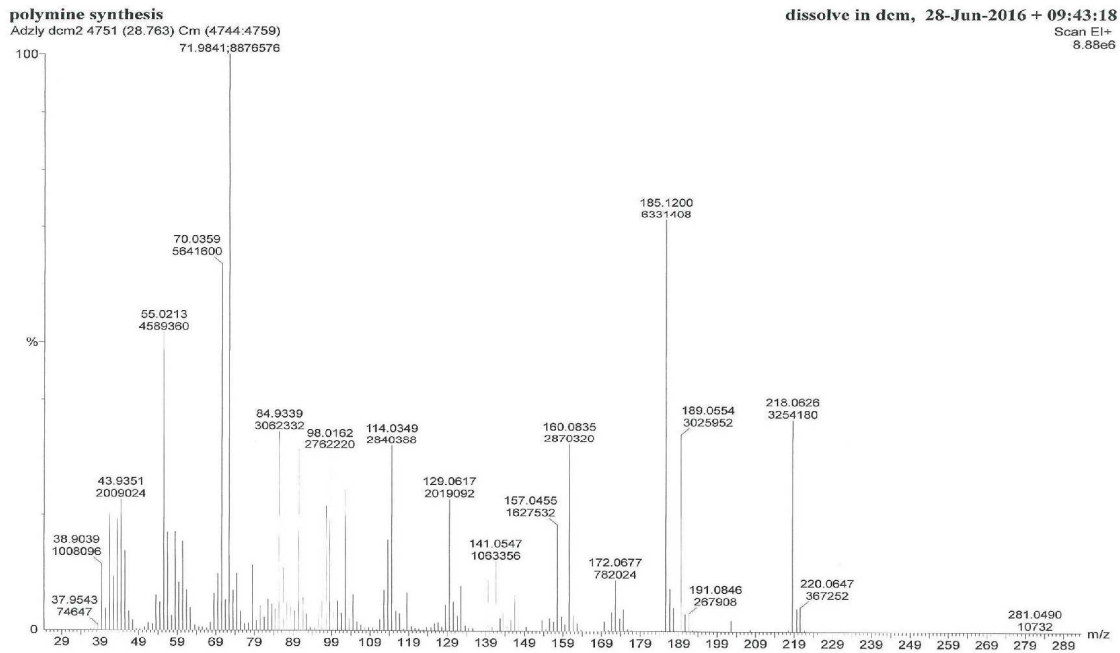


Figure 2b: Mass spectrum of CTS

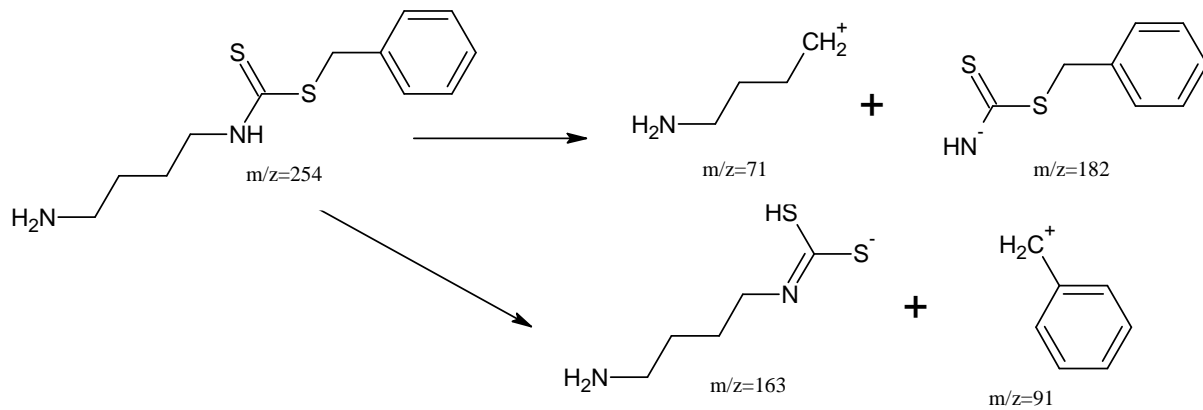


Figure 3: Selected fragmentation patterns

4. CONCLUSION

In conclusion, the synthesis of the putrescine containing sulfur was a success. Based on the results obtained, it is certain that the sulfur and the benzyl ring has attached to putrescine.

5. ACKNOWLEDGEMENT

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