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SYNTHESIS OF SIX PUTRESCINE ANALOGS AS POSSIBLE ANTIOXIDANTS

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Abstract

This investigation will involve the synthesis of several putrescine analogs produced using ethanol as the solvent. These nucleophilic substitution reactions with alkyl hydroxy halides will produce bis (2-hydroxyethyl), bis (3-hydroxypropyl), bis (4-hydroxybutyl), bis (5-hydroxypentyl), bis (6-hydroxyhexyl) and bis (7-hydroxyheptyl) putrescine. In this study, ethanol was used as the reaction solvent which allowed for yields of polyamine analogs to proceed in a greener solvent. It was also found, in this work, that the use of ethanol gave comparable product yields when compared with more toxic alcohol solvents used for previous studies in our laboratory. The putrescine analog products were characterized using FT-IR and FT-NMR, and the antioxidant properties were measured and compared with a standard antioxidant using a Ferric Reduction Antioxidant Potential (FRAP) assay kit.

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Introduction

Polyamines are small organic intracellular cations that are vital for normal cell growth and development in eukaryotes[1]. These molecules, under normal physiological conditions, are produced through an intricate biological transport system through catabolic enzymes and other proteins. Ornithine decarboxylase was identified as a key enzyme in polyamine biosynthesis[2]. The research of polyamines subsequently took off when a triamine named "spermidine" and a diamine, named "putrescine" were isolated from prokaryotic and eukaryotic systems[2]. These experiments showed that polyamines such as spermine, spermidine, and putrescine, promoted growth of fastidious bacteria. Though these molecules are small, they can modulate the functions of RNA, DNA, nucleotide triphosphates, proteins, and other acidic substances[3]. Thus, polyamines play a variety of important roles in the molecular biology realm.

Free radicals are constantly being generated by our bodies due to endogenous systems and exposure to various environmental and chemical factors[3]. These free radicals are extremely reactive, and they function by taking away electrons from different molecules in our body[4]. This removal of electrons causes severe cellular damage to our body's biomolecules, leading to the development of carcinogens, mutagens, inflammation, and cytotoxicity[5]. To counteract these effects humans have been changing their diets to include richer antioxidant foods. Polyamines have been observed to be vital in anti-oxidative and anti-inflammatory responses due to their lysosomal stabilization properties[1].

Previous research has focused on the radical scavenging activity of corn derived polyamine conjugates DCP, CFP, and DFP[5]. These conjugates were proven to be stronger antioxidants than α-tocopherol, a well-known antioxidant, using a DPPH radical scavenging assay.[5]

Earlier studies using polyamines, such as putrescine, spermidine, and spermine, have shown that positive antioxidant results are often correlated with high levels of the three polyamines.[6] However, much of the research that has been conducted with polyamines was completed using spermine, since it is the most readily available and yields the most predictive results.[7-9] Spermine has commonly been seen as an antioxidant specifically with regards to plants and osmotic/salt stress.[10-11] Spermine has also been used *in vivo* to protect antioxidant enzymes in a rat brain and to enhance the antioxidant capacity of rat spleen and liver.[11-13] Little research has been performed using putrescine, or any analogs of putrescine. Thus, putrescine and synthesized analogs will be the focus of this study. The FRAP assay was not used in previous work to identify the antioxidant properties of polyamines.

Putrescine, 1, 4-diaminobutane, is a biological precursor to the polyamines spermine and spermidine.[14] Putrescine, a four-carbon diamine, is produced during tissue decomposition by the decarboxylation of amino acids and acts on growth factors that promote cell division.[14] Previous studies have shown that putrescine concentration was increased in plants that are undergoing ionic and osmotic stress.[15-17] Research has also shown that putrescine increases the synthesis of ATP in plants.[18] Polyamines in general can increase the length of time that fruit and vegetables can stay ripe and fresh.[19] This was seen in studies that treated fruit with putrescine to maintain their firmness and to reduce bruising zones.[19-21]

In this study, putrescine analogs were synthesized, and confirmed using Infrared Spectroscopy, FT-IR and Proton Nuclear Magnetic Resonance Spectroscopy, FT-NMR. Figure 1 represents one of the Sn2 reactions to synthesize one of the polyamine analogs. In this scheme, one equivalent of putrescine was combined with two equivalents of 2-chloro-1-ethanol to produce the analog, N1, N4-bis (2-hydroxyethyl) putrescine dichloride.

Antioxidant effectiveness of putrescine and all six putrescine analogs were tested and compared with an antioxidant standard, ascorbic acid. The antioxidant properties were determined using a FRAP assay.[22]

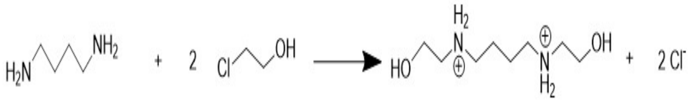

Figure 1. Representative reaction of putrescine with an alkyl hydroxy halide to produce one of the putrescine analogs.

Methods

The synthetic process for each putrescine analog was as follows: 2.0 mL of putrescine is added to a 50 mL, three-neck, round-bottom flask set up for reflux. The putrescine was dissolved in 15 mL of ethanol as the solvent. The addition of 2 molar equivalents of each alkyl hydroxy halide was added to the flask. The mixture was stirred and kept at a constant temperature of 65°C (J-Kem scientific, model 210) for 24 hours to allow reaction completion.

The reaction mixture was vacuum filtered; the solid product was washed with ethanol and allowed to dry in an oven set at 65°C. After the drying process, the product was massed, analyzed by FT-IR, FT-NMR and the percent yield was calculated. The alkyl hydroxy halide reagents used were 2-chloro-1-ethanol, 3-chloro-1-propanol, 4-chloro-1-butanol, 5-chloro-1-pentanol, 6-chloro-1-hexanol and 7-chloro-1-heptanol. The final products synthesized were: N1, N4-bis (2-hydroxyethyl) putrescine (B2HP), N1, N4-bis (3-hydroxypropyl) putrescine (B3HP), N1, N4-bis (4-hydroxybutyl) putrescine (B4HP), N1, N4-bis (5-hydroxypentyl) putrescine (B5HP), N1, N4-bis (6-hydroxyhexyl) putrescine (B6HP) and N1, N4-bis (7-hydroxyheptyl) putrescine (B7HP).

The antioxidant portion of the study included samples of ascorbic acid, putrescine, B2HP, B3HP, B4HP, B5HP, B6HP and B7HP. Each sample was analyzed using a FRAP assay kit (BioAssay Systems, Hayward, CA) with a 96 well plate reader (Fisher Scientific accuSkan FC) set at 590 nm. The lower absorbance value of measured Fe^{3+} concentration would suggest larger antioxidant activity or potential. (All solvents and reagents were from Sigma Aldrich; St. Louis, MO).

Discussion

Previous research in our laboratory developed the synthesis of putrescine analogs involving the di-nucleophilic substitution (Sn2) of alkyl hydroxy halides onto the primary amine sites of putrescine. The addition of these alkyl hydroxy groups created secondary amine salts in methanol, ethanol, 1-propanol and 1-butanol. This method was altered to create derivatives of alkyl hydroxy putrescine in a less toxic solvent, ethanol. Table 1 gives the percentage yields for the first four derivatives using four alcohol solvents. The data shows the yields in ethanol were comparable to the more toxic solvents, 1- propanol and 1-butanol. This method produced clean products in a less toxic solvent that easily precipitated out of the reaction mixture. Our study also aimed to investigate putrescine, and each of the alkyl hydroxy putrescine analogs, to determine their antioxidant potential as compared with other polyamines.[23-25] The putrescine analog products were characterized and confirmed using FT-IR and proton FT-NMR.

Table 1 Represents triplicate percentage yields of four hydroxyalkyl putrescine derivatives in several alcohol solvents

Putrescine Derivative	Methanol	Ethanol	1-Propanol	1-Butanol
N1, N4-bis(2-hydroxyethyl) putrescine	22.69%	44.19%	43.48%	46.65%
N1, N4-bis(3-hydroxypropyl) putrescine	33.54%	53.71%	56.35%	55.73%
N1, N4-bis(4-hydroxybutyl) putrescine	47.58%	57.35%	42.06%	60.78%
N1, N4-bis(5-hydroxypentyl) putrescine	31.17%	43.54%	41.35%	53.11%

The FT-IR for putrescine shows the primary amine nitrogen-hydrogen stretches at 3400 and 3300 cm^{-1}. By comparison the converted putrescine analog amine salts gave a broad absorbance from 2700-2250 cm^{-1}. The primary hydroxyl groups at the end of the alkyl chains attached to amine groups were confirmed with the broad absorption at 3400 cm.$^{-1}$

As an example, the proton FT-NMR for putrescine and one of the analogs, [N1, N4-bis (2-hydroxyethyl)] putrescine was used to confirm the product formation. Proton NMR for putrescine presents four amine protons at 4.9 ppm (singlet), four methylene protons, attached to the nitrogen at 3.1 ppm (triplet) and four methylene protons in the middle of the molecule at 1.9 ppm (triplet). The proton NMR for N1, N4-bis (2-hydroxyethyl) putrescine shows the two hydroxyl protons at 5.6 ppm (singlet), four methylene protons attached to the oxygens at 4.6 ppm (triplet), four methylene protons attached to the secondary nitrogen at 3.9 ppm (triplet) as well as the proton absorbances for the putrescine backbone.

One of the goals of this study was to determine the effectiveness of our analogs as antioxidants in comparison to other known antioxidants. When using a FRAP assay, the higher the concentration of Fe^{3+} results in less antioxidant activity. The standard antioxidant for this study was 0.5 M ascorbic acid. After running the FRAP assay on iron (III) controls, a standard curve (figure 2) of absorbance vs Fe^{3+} concentration. The standard curve was used to calculate the Fe^{3+} concentrations for each sample. Figure 3 provides a histogram of calculated Fe^{3+} concentrations vs sample type. Figure 3 reports the following antioxidant potentials: ascorbic acid (46 μM), putrescine (30 μM), B2HP (92 μM), B3HP (41 μM),

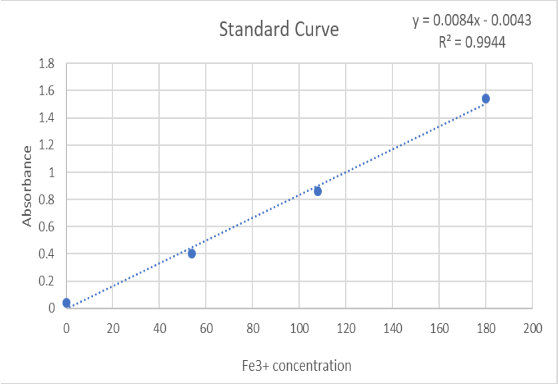

Figure 2. Standard Curve of FRAP Assay

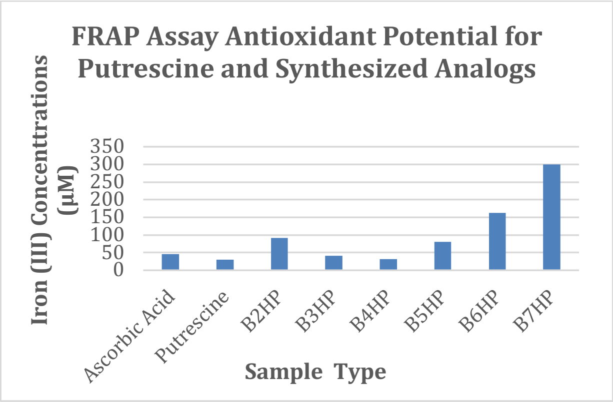

Figure 3. Histogram of Antioxidant Activity or Potential of various synthesized putrescine compounds

B4HP (31 µM), B5HP (81 µM), B6HP (162 µM) and B7HP (300 µM). These results suggest that Putrescine, B3HP and B4HP have comparable antioxidant activity to ascorbic acid, which might suggest these chain lengths were optimal for greater antioxidant activity.

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