Catalyzing Brominated Parabens and Their Antimicrobial Abilities

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Abstract:

Parabens are compounds used as a preservative in makeup and food products. The purpose of parabens is to help combat bacterial growth in these products. However, recent studies have suggested that parabens may be linked to the development of certain types of breast cancer due to their ability to mimic estrogen. The goal of the experiment is to create a paraben that will still maintain its anti-microbial properties by varying the carbon chain. These parabens can bind to estrogen receptors and cause them to activate, creating more estrogen. The overall experiment was done through; aromatic substitution, benzylic bromination and substitution, filtration through a column, jones oxidation, fischer esterification, and a microdilution assay to guarantee the paraben is both successful in killing bacteria and not existent in breast tumors. The product was purified and tested for efficacy on S*. salivarius.* An MIC of 1 was found for the brominated paraben with an octyl chain. With these results, we have found the favorable paraben carbon chain that can be potentially used as a safer alternative in these products.

Introduction

Throughout the years, parabens had become a common aspect in food and makeup products with their ability to resist bacterial growth. These are products used on a daily basis, but somehow manage to avoid bacterial growth. The differentiation between parabens in the length of the alkyl chain and solubility. The chemical preservative that makes this feasible is the alkyl esters 4-hydroxybenzoic acid. In recent years, incorporating parabens in these daily products has diminished. It has been suggested that parabens might be linked to the increased amount of breast cancer in women. In 2004, parabens were detected in human breast tumor tissue (Darbre et al., 2004), and it is thought that their ability to develop these breast cancer cells is through their estrogenic properties. Further studies proved that the parabens stimulate oestrogen receptors but they not only can bind the oestrogen receptors, they can activate it.

Initially, the breast tumor tissue discovered was found to have five commonly used paraben esters. In the circumstance of their distinguished oestrogenic activity in breast cancer cells, this has elevated concerns for a possible connection in the development of breast cancer (Byford et al., 2002; Darbre et al., 2002). When used through dermal application, parabens can find their way into the blood in less than an hour (Janjua et al, 2007) and the concentration in the blood is positively correlated to the amount of product used (Sandanger, et al, 2011). They can then easily find their way into human tissues and have been found in breast cancer tissues (Darbre et al, 2004). In study conducted in 2017, the exposure of MCF-7 breast cancer cells and a patient derived xenograft to physiological levels of methyl paraben, which is commonly used in personal care products. About 4.4g of methyl paraben pellets per day led to increased tumor size of MCF-7 xenografts and ER+ PDX tumors (Lillo, et al, 2017), proving methyl parabens increase breast cancer tumor proliferation.

The addition of Bromine to a paraben reduces their ability to bind to estrogen. They are safer to use because they then do not mimic estrogen as well. However, it is unknown how effective these parabens are in killing bacteria. Improved understanding of molecular interactions is important to differentiating binding mode in estrogen receptors and non-specific binding mode bacteria. Researchers have successfully attempted to deactivate the oestrogenic receptor binding ability from parabens, therefore; discovering a safer product that is still successfully antimicrobial.

The purpose of this study was to test these new brominated parabens’ antimicrobial abilities. This will be done through testing the effects of varied carbon chains in multiple parabens against bacteria. We will then measure the effectiveness of the parabens by recording the percentage of bacteria killed.

Results:

 

**Figure 1:**A; Aromatic Substitution, B; Benzylic Bromination, C; Benzylic Substitution, D; Oxidation, E; Fischer Esterification

The figure shown was a series of reactions where the brominated esters were synthesized. After the aromatic substitution was finalized, the 2,6-dibromo-4-methylphenol product had a percent yield of 77%. The benzylic bromination and benzylic substitution had a percent yield of 80.4%. The overall percent yield of the entire synthesis was 71% and was confirmed using 1H NMR.

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| **R Group** | **Compound** | **MIC against S. *Salivarius* in ug/nL** |
| Methyl |  | >256 |
| Ethyl |  | >256 |
| Propyl |  | >256 |
| Butyl |  | 128 |
| Pentyl |  | 32 |
| Hexyl |  | 8 |
| Heptyl |  | 2 |
| Octyl |  | 1 |
| Isobutyl |  | 128 |
| Secbutyl    |  | 128 |
| Isopentyl   |  | 64 |

**Table 1.** MIC against S. *Salivarius* for the brominated esters (ug/mL).

 The paraben Octyl was the most antimicrobial, it can be identified with a MIC of 1 ug/mL. Methyl, ethyl, and propyl were the least antimicrobial, all three parabens had a MIC value greater than 256 ug/mL. High levels of antimicrobial activity on parabens are generally due to their long carbon chains. The MIC results corresponded to the esters with the same number of carbons, not necessarily the length of the carbon chain.

Discussion

 Before experimentation, it was hypothesized that the longer the carbon chain, the higher the antimicrobial activity. The experiment showed that brominated parabens with longer carbon chains had lower MIC’s, and were more effective against bacterial growth. Brominated parabens with an octyl chain was the most effective overall with an MIC value of 1. In future experimentation brominated parabens with more than eight carbons in the side chain should be tested to narrow down the most effective chain. The developed brominated paraben could be tested for their ability to enter these membranes to further support this experiment.

Experimental

**p-cresol to 2,6-dibromo-4-methylphenol through aromatic substitution**

In glacial acetic acid (5mL), (3g) of p-cresol was dissolved. Adding bromine (2.1 eq) into the round bottom flask (100mL). The reflux apparatus was assembled and then heated for 30 minutes. The reflux was removed off the heat and the reaction was cooled to room temperature. Measured out was 0.5 M HCl (~50mL) into an Erlenmeyer flask and cooled in ice with stirring. Once cooled, add reaction solution dropwise to the cold HCl until a white precipitate persists on the acidic aqueous solution. Vacuum filtrate the precipitate until dry. (percent yield: 77%) GCMS (ESI+) m/*z* 266 [(M)+; calculated mass for C7H6Br2O+: 265.93amu].

**2,6-dibromo-4-methylphenol to 2,6-dibromo-4-bromomethylphenol through benzylic bromination**

In a small flask, (50mg) of 2,6-dibromo-4-methylphenol was set aside to be used later. (3.95g) of the leftover 2,6-dibromo-4-methylphenol was added to a round bottom flask (100mL) and dissolved in (50mL) of chloroform. N-bromosuccinimide (1.05 eq) was slowly added and the round bottom was left under a UV light for a week. A sample of 2,6-dibromo-4-methylphenol (~8mg) was collected in a small Erlenmeyer flask and dissolved with dichloromethane (CH₂Cl₂) to obtain a solution (1mg/mL). The crude material was collected after benzylic bromination and the percent yield was calculated (88.8% Br2C6H2(CH3)OH).

**2,6-dibromo-4-bromomethylphenol to 2,6-dibromo-4-(hydroxymethyl)phenol through benzylic substitution and column filtration**

A (0.25M) solution of acetone and 1M HCl dissolved the 2,6-dibromo-4-bromomethylphenol in the round bottom. The reaction was then left to sit for seven days. After seven days the reacton was poured into a separatory funnel and ethyl acetate was added (75mL). The organic layer was washed three separate times with deionized water (25mL). The 2,6-dibromo-4-(hydroxymethyl)phenol was poured into an Erlenmeyer flask (125mL) and dried with magnesium sulfate(MgSO4), filtered, and concentrated *in vacuo*.. A small portion of the solution was set aside (~1mL) in a vial The 2,6-dibromo-4-(hydroxymethyl)phenol was dissolved in (3mL) of ethyl acetate and added to a column by pipet for filtration. To start off, (200mL) of (20%) ethyl acetate/hexanes solution was added until the solution reached the sand, then (300mL) of (30%) ethyl acetate was added, followed by (200mL) of (50%) ethyl acetate. The solution was drained into test tubes until the top reached the sand and a TLC was run to find the vials that only contained the product. Those vials were then added into a (500mL) round bottom flask.‘H NMR (DMSO, 60 mhz) 9.755 (s, 1H) 7.489 (s, 2H) 4.89 (s, 1H) 4.421 (s, 2H) percent yield was found to be (80.4% C7H6Br2)

**Extracting 3,5-dibromo-4-hydroxybenzoic acid from 2,6-dibromo-4-(hydroxymethyl)phenol through a Jones Oxidation**

In a (500mL) Erlenmeyer flask, (3.359g) of the product was dissolved in a (1M) solution of acetone.The amount of Jones reagent required to oxidize was added to our sample (10.65mL CrO3). Ethyl acetate (100mL) and deionized water was used to purify the sample through a separatory funnel and dried with magnesium sulfate. An off-white solid was produced after being concentrated *in vacuo*. Set at 270°C, the 3,5-dibromo-4-hydroxybenzoic acid melting point was obtained (225.8°C-259.0°C). Pure 2,6-dibromo-4-(hydroxymethyl)phenol was placed in an NMR tube (~20 mg) and analyzed.

**Adding octanol to 3,5-dibromo-4-hydroxybenzoic acid through fischer esterification**

In a (10mL) round bottom flask, (300mg) of 3,5-dibromo-4-hydroxybenzoic acid was mixed with (1mL) of octanol and five drops of H₂SO₄. The reaction was then refluxed for an hour. A column filtration was run with (400mL) of (10%) ethyl acetate/hexanes and (400mL) of (15%) ethyl acetate/hexanes, followed by a TLC to ensure the product was extracted. The vials containing the product were added to a round bottom flask and concentrated *in vacuo.* Isolation of the purified product was recorded and the bio sample was prepared. Rf=0.25(20% ethyl acetate/ hexanes); IR (CDCl3) 3482, 3447, 3360, 2956, 2930, 2872, 1700, 1684, 1652, 1587, 1558, 1472, 1457. 1H NMR (CDCl3, 60MHz) δ 8.2 (s, 2H), 6.3 (s, 1H), 4.3 (J= 6.5, 3H), 1.4 (m, 15H) GCMS; IR; and NMR.

**Broth microdilution method for antibiotic resensitization.**

Mueller-Hinton broth (MHB) was inoculated (5 x 105 CFU mL-1) with Streptococcus salivarius. The resulting bacterial suspension was aliquoted (5 mL) into culture tubes and test compound (from its DMSO stock) was added to give the final concentration to be tested. Bacteria not treated with the tested 2-AI derivative served as the control. After sitting for 30 min at room temperature, 1 mL of each sample was transferred to a new culture tube and oxacillin was added from 128 mg mL-1 water stock to give a concentration of 128 µg mL-1. Rows 2-12 of a 96-well microtiter plate were filled with 100 µL/well from the remaining 3 mL bacterial subcultures, allowing the concentration of compound to be kept uniform throughout the antibiotic dilution procedure. After standing for 10 min, the samples containing antibiotic were aliquoted (200 µL) into the corresponding first row wells of the microtiter plate. Row 1 wells were mixed 6 to 8 times then 100 µL was transferred to row 2. Row 2 wells were mixed 6 to 8 times, followed by a 100 µL transfer from row 2 to row 3. This procedure was repeated to serially dilute the rest of the rows of the microtiter plate, with the exception of the final row, to which no antibiotic was added. The plate was then sealed with GLAD Press n’ Seal® and incubated under stationary conditions at 37 °C. After 16 h, minimum inhibitory concentration (MIC) values were recorded as the lowest concentration of antibiotic at which no visible growth of bacteria was observed.

Works Cited

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