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Lab Paper

Abstract

Parabens are commonly used antimicrobial agents in many personal care products including makeup and hygiene products. Parabens can help prevent the growth of bacteria and mold, but they can also have adverse effects on humans. Recent studies in 2011 have exhibited bonding of parabens to the estrogen receptor. Binding causes accelerated proliferation of cells, allowing for an increase in cancer development chances. This study analyzes the synthesis and antimicrobial prowess of brominated paraben derivatives; specifically aiming to reduce the rate of binding to the estrogen receptor by modifying the phenol. Paraben derivatives were synthesized using a wide range of chemical synthesis processes involving reactions on the benzene rings. After paraben derivatives were constructed, the antimicrobial properties of the paraben derivatives were tested against *Streptococcus salivarius* using a dilution assay in order to analyze and compare the antimicrobial properties of the paraben derivatives. The purpose of this project is to create a modified paraben and examine its antimicrobial properties. This study was completed using both biological methods to create brominated parabens. Benzylic bromination and substitution were used to modify the starting material. Jones Oxidation, Fischer esterification, and thin layer & column chromatography methods were used to purify the final product and prepare it for examination of antimicrobial properties.

Parabens are materials found in many personal care products including makeup and lotions. Parabens form microscopic crystals on the surface of these products to protect the growth of bacteria, and this activity cannot be observed with the naked eye. A large portion of the parabens discovered within the human body were found to have been sourced from personal care products containing parabens[1]. In some instances, parabens have been linked to cases of breast cancer developing after years of using certain products[2]. This was investigated partially due to the discovery of parabens within breast tumor tissue in 2004[4]. With a study of the parabens in question it was discovered that parabens not only shared a similar structure to estrogen due to their phenolic group[5], the parabens could then bind to the estrogen receptor, causing the propagation of cells, and therefore increasing the chances of cancer within the body[5]. From this research, the industry shifted to investigating modes of decreasing or eliminating the binding of parabens to the estrogen receptor, opening the door to new parabens as alternatives to traditional, cell-propagating ones. Recent discoveries have shown that the double halogenation of traditional parabens results in the decrease or weakening of the phenolic binding[6]. Although these halogenated parabens reduce binding to the estrogen receptor, it is unsure yet as to whether or not these parabens have the antibacterial prowess to rival that of traditional parabens as well as other microbial agents.

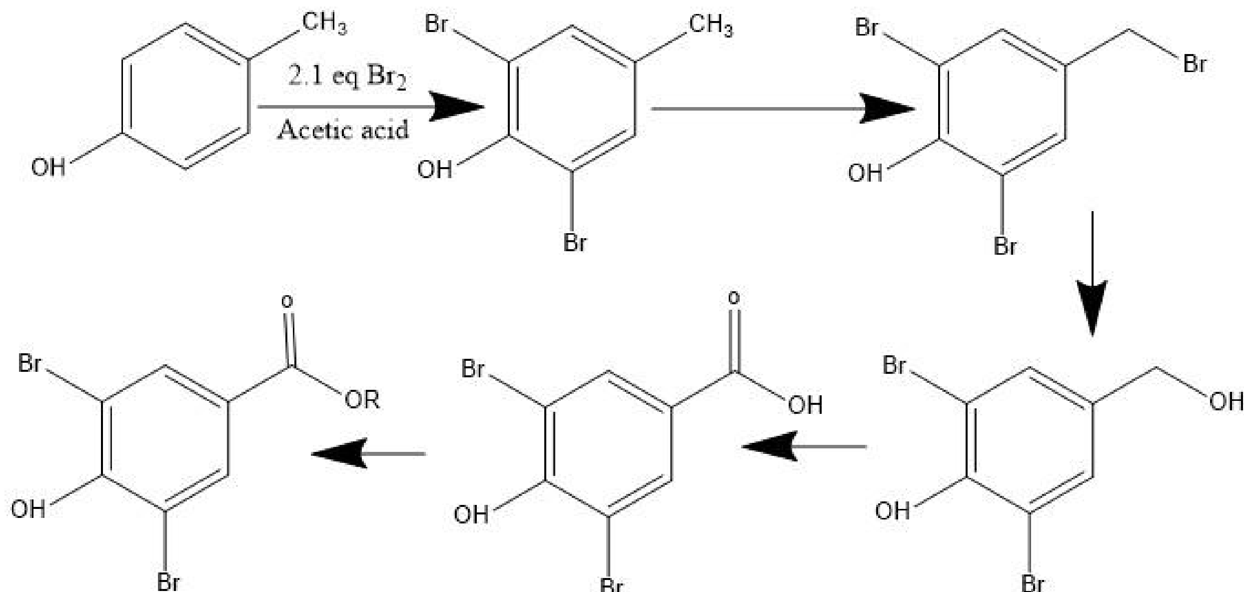
The topic being investigated in this research project is focused on modifying the antimicrobial abilities of brominated parabens. The study focuses on looking into ways to make them safer for human usage while still keeping the antimicrobial properties in the products. Starting with P-cresol, several steps were taken to modify the source structure into a brominated paraben. Bromination uses electrophilic aromatic substitution to add bromine to an aromatic system[3]. After benzylic bromination and substitution, the remaining product was

2,6-dibromo-4-bromomethylphenol. This product was then purified using flash column chromatography and a TLC was run to check for the correct product. This step was done by adding the product to a column with sand and silica gel. Then, ethyl acetate with hexanes is added in different concentrations until the product comes out of the column. After purification, a Jones oxidation was performed. A Jones oxidation is used to convert alcohols to carboxylic acids. Finally, we will move on to a fischer esterification and final purification to isolate the final paraben product.

The final step of this research is to test the antimicrobial ability of the new, substituted paraben. To do this, our team is going to create an assay with 96 wells diluted to different concentrations. We will add a liquid culture of bacteria into each well and allow it to incubate for 16 hours. After 16 hours, we will observe the wells and see which concentrations of the parabens were successful in preventing bacteria growth. We are hoping to find that the modifications of the paraben will make it safer for human usage in different products like makeup and lotion.

To begin the synthesis we started with p-cresol and went through multiple reactions to create the final product. The best anti microbial reaction occurred with our brominated paraben. The MIC of our paraben (hexyl) is 8. In the positive control of the microdilution assay, the MIC

was higher, meaning that it was less effective in killing the bacteria.



Synthesis of 2,6-dibromo-4-methylphenol

P-cresol (3 g) and acetic acid (5 mL) were mixed in a 25 mL round bottom flask. This mixture was stirred on an ice bath, then BrP-cresol (3 g) and acetic acid (5 mL) were mixed in a 25 mL round bottom flask. This mixture was stirred on an ice bath, then Br_2 (2.9 mL) in acetic acid was added. In a 250 mL Erlenmeyer flask, HCl (50 mL) was stirred in an ice bath. The reaction solution was added dropwise until a precipitate was formed. Then, the remaining solution was added, and the product was rinsed with cold water and collected using vacuum filtration to provide a white solid (2.547 g, 7.9 mmol, 84.9% yield). GC-MS (EI+) m/z 266

Synthesis of 2,6-dibromo-4-(hydroxymethyl)phenol

2,6-dibromo-4-methylphenol (3.5 g, 11 mmol) and N-bromosuccinimide (2.1 g, 11.6 mmol) were added to a round bottom flask (100 mL) and dissolved in chloroform (50 mL). This was put under a UV light for a week. Then, the solution was poured into separatory funnel and washed with hydrochloric acid three times. Then, the organic layer was put into an Erlenmeyer flask and dried with magnesium sulfate, after dried it was filtered into a round bottom flask and

the solvent was removed in the Rotary Evaporator. After the solvent was removed, it was dissolved into a 2:1 ratio of acetone and HCL (xx mL) until it was 0.25 M in concentration. After seven days, the reaction was added to a separatory funnel with ethyl acetate to make an organic layer. Then, the layer was washed three times with deionized water and dried. The solvent was then removed with the Rotary Evaporator. The crude material was then purified by flash chromatography (20%-30%-50% ethyl acetate in hexanes) to provide a white solid (2.82 g, 11 mmol, 91%). ^{1}H NMR (d6-DMSO, 90 MHz) 9.7 (s, 1H), 7.5 (s, 2H), 5.2(s, 2H), 4.4 (s, 2H).

Synthesis of 3,5-dibromo-4-hydroxybenzoic acid

2,6-dibromo-4-(hydroxymethyl)phenol (1.45 g, 5.1 mmol) was dissolved in acetone (51.4 mL) and a Jones reagent (4.11 mL, 2M) and stirred for one hour. Ethyl acetate was added to the reaction and washed with deionized water three times. Then it was dried and put into the Rotary Evaporator to obtain a dried product as a white solid (1.841 g, 6 mmol, quant. yield). Mp = 270-271 (lit. Mp = 270-274 ^{o}C)

Synthesis of hexyl 3,5-dibromo-4-hydroxybenzoate

3,5-dibromo-4-hydroxybenzoic acid (438 g, 1.48 mol), hexanol (1 mL) and H2SO4 (4 drops) were refluxed for one hour in a round bottom flask. The crude material was then purified by flash chromatography (10%-15% ethyl acetate in hexanes) to provide a white solid (0.85g, x mmol, x%). 1H NMR (d6-DMSO, 90MHz) 8.7 (s, 2H), 6.3 (s, 1H), 4.3 (t, 2H). Antimicrobial activity was quantified using a dilution assay that would aid in the finding of the minimum inhibitory concentration (MIC). Brain-heart infusion was inoculated (5×10^5 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 brominated paraben 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 antibiotics 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.

This section of the lab created the brominated paraben Hexyl 3,5-dibromo-4-hydroxybenzoate. After examining the IR, GCMS, and NMR of this final compound created from the experimental section of this section, there were little to no noticeable impurities. As seen in Table 1 and Table 2, the MIC value of the brominated paraben is 8 μg. This is lower than the positive control in this observation, and positive control being Butyl has an MIC value of 32 μg. This means that the brominated paraben has a higher rate of killing *Streptococcus salivarius* than its counterpart. Although it can be said that the brominated paraben and non-brominated paraben used in this section was more successful in killing the bacteria, compared to Erythromycin and Penicillin G both of the brominated and non-brominated parabens were less successful in killing the bacteria. Also looking at lengths of carbon chains, it

can be said that the longer the carbon chain of these parabens, the more successful it will be at killing bacteria.

All together, the brominated parabens did around the same performance as the non-brominated parabens. This in return makes everyday items such as food and cosmetics a viable option for replacement. The best brominated parabens with the highest potential in being a successful antimicrobial agent would be Propyl, Butyl, Pentyl, Hexyl, and Heptyl (Table 1). Although this is a good start, further testing should be performed on paraben derivatives to find more viable options needed for humans, as well as testing the human toxicity of such experimental substances. Ultimately if the side effects of an alternative is even worse, it is not a viable consumer-friendly alternative

Table 1:Brominated Parabens	MIC (µg/mL)
Methyl	>256
Ethyl	>256
Propyl	64
Butyl	32
Pentyl	16
Hexyl	8
Heptyl	4
Octyl	1
Isobutyl	128
Secbutyl	128
Isopentyl	64

Table 2: Positive Controls	MIC (µg/mL)
Methyl	>256
Ethyl	>256
Propyl	256
Butyl	32
Isobutyl	128
Erythromycin	<0.125
Penicillin G	<0.125

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