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December 1, 2017

Organic Chemistry II

Addition of Benzylic Bromines to Ethyl Paraben

Abstract

Parabens are chemical compounds that have antibacterial properties and are found in many products such as antiperspirants. However, these current parabens in antiperspirants have been found in studies to bind to the estrogen receptors. Because of this binding site, many researchers have determined a correlation to the formation of breast cancer. In this experiment, a paraben was synthesized from p-cresol and had bromines added beside the OH on the benzylic ring to prevent it from binding to the estrogen receptor, with hopes that it could maintain its antibacterial properties. Next, it's MIC (minimum inhibitory concentration) was tested through a serial dilution assay. This was to compare the new design of paraben (ethyl paraben with bromines) to a known antibiotic, ethyl paraben without bromines (positive), and a row with no compound (negative). It was then discovered that the ethyl paraben with bromines successfully killed bacteria with a MIC of 256 μg/mL and the octyl paraben killed bacteria the best with an MIC of 2 μg/mL. The ethyl paraben without bromines however had a MIC of >256 μg/mL. Since the bacteria was killed at a lower concentration, it's believed that adding bromines to the paraben is a safer alternative to regular parabens. These bromines not only prohibited the binding of estrogen receptors, but the paraben was still able to continue killing bacteria at similar

antibiotic levels. This alternative can be used in antiperspirants to help prevent breast cancer formation, but still prevent bacterial growth.

Introduction

Parabens are antibacterial compounds that are commonly found in products such as cosmetics, medications, and food preservatives (Crinnion, 2010). They are used worldwide because of their low costs and their assumed "low toxicity" rates to humans and to the environment (Crinnion, 2010). They are also commonly used because of their ability to easily dissolve in water as well as have a wide stability range of pH (Terasaki et al, 2009). However, in recent studies, parabens have been found in blood streams, urine, and skin tissues (Crinnion, 2010). They are added to cosmetics at concentrations of about 0.8% (Darbre, 2006) and are then applied superficially to the skin (Lange et al, 2014).

The daily use of products containing parabens such as deodorants have been raising concern because parabens have been detected in breast cancer tumor tissue with an average concentration of 20 ng/g tissue (Darbre, 2006) in the same regions where deodorants are usually applied (Crinnion, 2010). With increased cosmetic usage, permeation of parabens through human skin can increase with repeated doses (Darbre, 2008) and failure to remove previous products containing parabens.

It has been discovered that parabens are endocrine disrupting compounds (EDCs) that tend to bind to the estrogen receptor (Lange et al, 2014). Binding to the estrogen receptor prevents estrogen from binding and therefore creating an increase in estrogen levels (Crinnion, 2010). They also indirectly affect the estrogen levels by blocking the sulfotransferase causing the levels to remain higher than normal and increasing the risk of breast cancer (Crinnion, 2010).

Due to the lack of evidence for the direct development of breast cancer in relation to parabens, further research is needed (Darbre, 2006). Since the majority of the population is exposed to parabens on a daily basis, it's very important to determine if the estrogenic activity in parabens can be detected in estrogen-sensitive human cells (Byford et al, 2002).

In 2014, a study was conducted to determine the estrogenic activities of different kinds of commercially available deodorants. Three different types of deodorants were used, sprays (S), roll-ons (R), and sticks (ST). The E-Screen assay used showed the increased amount of proliferation of breast cancer cells in the presence of estrogen active substances. The samples were then dissolved into *n*-hexanes and then dried over anhydrous sodium sulfate and eventually incubated on an E-Screen assay for 5 days. It was determined that 7 out of the 10 sprays showed estrogenic effects ranging from 0.12 ng g^{-1} to 8.98 ng g^{-1}. However, only one stick and one roll-on showed estrogenic effects of 1.66 ng g^{-1} and 1.35 ng g^{-1}. It is concluded that all three types of deodorants increase the proliferation and estrogenic activities, but the spray deodorant, depending on manufacturer, increases the risk more than stick or roll-on (Lange et al. 2014).

Another study was conducted to investigate the paraben's estrogenic activity, specifically inside the MCF7 human breast cancer cells which require estrogen to grow and develop. Multiple types of parabens were diluted into a growth media where the breast cancer cells were then incubated as a monolayer culture for a minimum of 3 days. They were then tested inside of a competitive binding assay in cytosol at 4°C using the method of dextran-coated charcoal. RNA analysis by northern blotting and cell proliferation tests were performed at the conclusion of the incubation. It was then concluded that there was estrogenic activity of parabens in the human breast cancer cells in the cultures. They also found that there was an increase in the expression of genes regulated by estrogen and the proliferation of those cells (Byford et al. 2002).

The purpose of this study is to determine how a paraben can be altered to reduce the estrogenic activity levels while still maintaining its antibacterial properties. It is hypothesized that adding bromines beside the OH on the benzylic ring of the paraben, which will prevent the binding of estrogen receptors, will still maintain its antibacterial properties.

Results

Chemical

Starting with 1, a successful aromatic bromination occurred resulting in a percent yield of 68.9% and a mass spec was taken. Using 2, a benzylic bromination was conducted to produce product 3 which then went through a benzylic substitution which gave product 4 with a percent yield of 80.6% and a NMR was performed. 4 then went through a jones oxidation where the percent yield was 71.58% and a boiling point was taken at 265℃due to some chromium still left in product. 5 then went through a fischer esterification where a NMR was performed and the percent yield was more than 100% due to ethyl acetate still in the product from the vacuums not working properly and the scales not being accurate enough to weigh out a 500 mL round bottom flask.

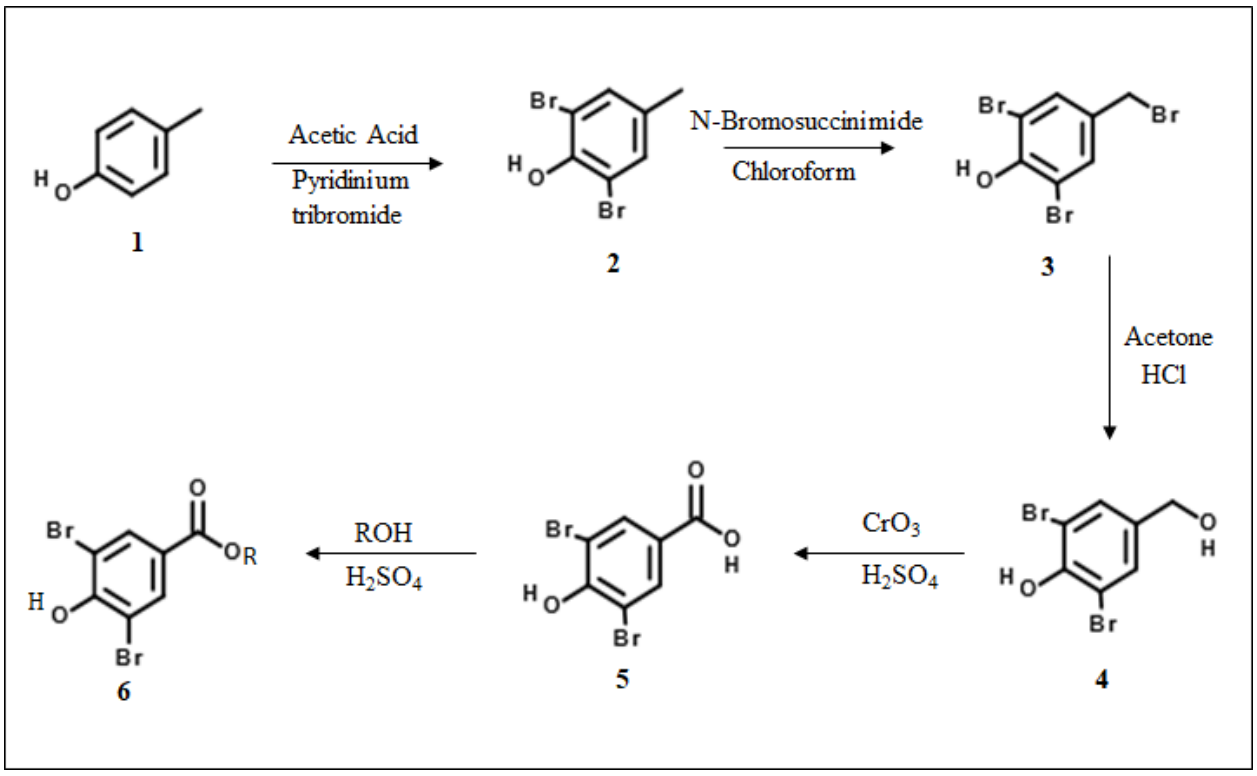

Figure 1. Chemical Procedure. (1) p-Cresol. (2) 2-6-dibromo-4-methylphenol. (3) 2,6-dibromo-4-(bromomethyl)phenol (4) 2,6-dibromo-4-(hydroxymethyl)phenol. (5) 2,6-dibromo-4-hydroxybenzoic acid. (6) Ethyl 3,5-dibromo-4-hydroxybenzoate.

Biological

12 ester groups were tested in their effectiveness in killing *S. salivarius.* The ester carbon chains between 6a-6e all had MIC's of >256, 256, 256, 128, and 64 respectively. It wasn't until the esters carbon chains of 6f-6h had MIC's of 4, 4, and 2 respectively that the MIC's decreased by a larger margin. Ester groups with branch carbons (6i-6l), had MIC's of 128, N/A, 64, N/A respectively.

Table 1. Brominated Paraben MIC's compared to Commercial Paraben MIC's. R being the produced ester chains. Compound being the commercial parabens.

R	Compound	MIC
Methyl	6 a	>256
Ethyl	6 b	256
Propyl	6 c	256
Butyl	6 d	128
Pentyl	6 e	64
Hexyl	6 f	4
Heptyl	6 g	4
Octyl	6 h	2
Isobutyl	6 i	128
Secbutyl	6 j	N/A
Isopentyl	6 k	64
Capryl	6 l	N/A
	Me Paraben	>256
	Et Paraben	>256
	Pr Paraben	256
	Bu Paraben	128
	Erythromycin	0.007813
	Penicillin	0.5

Conclusion

Based on these results, it can be concluded that the addition of bromines to parabens does not affect its antibacterial capabilities which supports the hypothesis of this study. The parabens with bromines kill bacteria at similar MIC's to those parabens without bromines. This is important because these new parabens would be safer to use in products such as antiperspirants because the bromines prevent the binding of the estrogen receptor which can lead to breast cancer (Darbre, 2006). Based off of the biological results, a trend can be noted showing that the longer the esters carbon chain, the lower the MIC. Another trend that can be noted is that the farther the branch carbons are from the oxygen, the lower the MIC is. These new parabens with bromines could also be further tested to see if there are any other kinds of modifications that could be made to lower their MIC's to make them even more effective at killing bacteria.

Experimental

Chemical Procedures

2-6-dibromo-4-methylphenol (2)

p-Cresol(1) (3.000 g) and 2.1 mol equivalent of pyridinium tribromide in 15 mL of acetic acid were all added into a round bottom flask. Flask was heated inside heating mantel with stir bar and set to reflux for 30 minutes. Flask was allowed to cool before reaction was added dropwise into ~50 mL of 0.5 M HCl in an ice bath. Once white precipitate formed, rest of reaction was poured in and then vacuum filtrated. Percent yield: 68.9%. Color: White. Mass: 5.088 g. Next, 2-8 mg of *(2)* was used to make a solution of 1 mg/ mL in dichloromethane and was diluted by 1000 for GC-MS. GC-MS m/z 266 [$(M\cdot)^+$; calculated mass for $BrC_6H_2(CH_3)OH^+$: 265.9 amu].

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

(2) (4.001g, 0.015mol) was dissolved in chloroform (50 mL) using a 100 mL round bottom then N-bromosuccinimide (2.108g, 0.025 mol) was added. Round bottom was placed under UV light for 7 days. Next, reaction solution was poured into a separatory funnel and was rinsed with ~5 mL dichloromethane. Organic layer was washed 3 times with 50 mL portions of 1 M hydrochloric acid, dried with magnesium sulfate, and then filtered into round bottom flask. Solvent was then vacuumed using rotary evaporation. This crude material was dissolved in 2:1 of acetone to 1 M HCl to make a 0.25 M solution and allowed to sit for 7 days. Solution was poured into separatory funnel and 75 mL of ethyl acetate was added. Organic layer was washed 3 times with 25 mL portions of deionized water, dried with magnesium sulfate and then filtered

into a round bottom flask. Solvent was then vacuumed with rotary evaporation. Crude sample was analyzed by TLC (25% ethyl acetate/hexanes) then purified by flash chromatography which was loaded using ethyl acetate (3 mL) and eluted (eluent: 200 mL of 20% ethyl acetate/hexanes, 300 mL of 30% ethyl acetate/hexanes, and then 400 mL of 50% ethyl acetate/ hexane) to provide a white solid (1.549 g, 29.8% yield). 20-50 mgs of purified *(B)* was added into a NMR tube, 0.75 to 1 mL of D6-DMSO and was sent to NMR. ^{1}H NMR (D6-DMSO, 60 MHz): δ 10.706 (brS, 1H), 8.441 (S, 2H), 5.372 (S, 2H), 3.160 (brS, 1H).

2,6-dibromo-4-hydroxybenzoic acid (5)

Purified *(3)*(1.549 g) was dissolved in acetone (54 mL) until 0.1 M solution. 1.5 equivalent of 2.0 M Chromium was added dropwise into Erlenmeyer and stirred on stir plate for 1 hour. 100 mL of ethyl acetate was added to the reaction in a 250 mL separatory funnel and organic layer with three 50 mL portions of deionized water, dried using magnesium sulfate and filtered and rotovapped (1.108 g, 71.58% yield). Color: pale yellow Melting Point: 265℃.

Ethyl 3,5-dibromo-4-hydroxybenzoate (6)

300 mg of *(5)*, 1 mL of ethanol, and 5 drops of sulfuric acid were added to a 100 mL round bottom and set to reflux for an hour. In a column, ethanol product was put on top of sand dropwise then 10% ethyl acetate/hexanes solution until product was loaded. Crude sample was purified by flash chromatography and eluted (eluent: 400 mL of 10% ethyl acetate/hexanes and then 400 mL of 15% ethyl acetate/hexanes). Purified samples were analyzed on silica gel plate using 25% ethyl acetate/hexanes. TLC plates were dipped into $KMnO_4$ and allowed to dry.

Purified products was vacuumed using rotary filtration (0.52g, >100%). Color: White. ^{1}H NMR (D6-DMSO, 60 MHz): δ 9.788 (S, 2H), 8.290 (brS, 1H), 6.195 (Q, 2H).

Biological Procedure

Determining Minimum Inhibitory Concentration

For bioassay preparation, 5-10 mg of product was weighed and put into an Eppendorf and dissolved in D6-DMSO with a final concentration of 16 mg/mL. Positive and negative controls were labeled on a 96 well plate, one control with inoculated broth (5 x 10^5 CFUs) without ethanol product and one control with broth and ethyl paraben without bromine. 1 mL of inoculated broth was added to a test tube with sample making a solution with a concentration of 256μg/mL. Test tube was then vortexed and then 200 μL of solution was added into the top well of column. Steps were repeated with ethyl paraben without bromines sample but with a concentration of 256 μg/mL. All three columns were filled with 100μL of inoculated broth in rows 2-12. Row one was mixed 6-8 times and then 100 μL of solution was transferred into well 2, procedure was repeated until last well and was repeated in the penicillin column. Each plate was tightly sealed with GLAD Press n' Seal and placed in damp paper towel inside plastic container. Plates were incubated for a minimum of 16 hours at 37℃. After 16 hours, data was recorded on the MIC for the positive control and the tested paraben.

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