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An Investigation into the Effects of Structural Isomers of Salicylic Acid on  
*Propionibacterium Acnes*

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Southern Scholars Project 2004

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## Abstract

Acne is caused when the sebaceous glands in the skin are unable to secrete an oily substance, called sebum, onto the surface of the skin. This is because the opening of the follicle is clogged from a mixture of skin cells and sebum. The opening of the follicle becomes plugged when the cells from the lining of the follicle are shed too quickly and clump together. Because *Propionibacterium acnes* (*P. acnes*) bacteria feed off sebum, they are then able to grow in the plugged follicle. Acne is caused by the inflammation that results from the chemicals and enzymes produced from the bacteria.

Salicylic acid, or ortho-hydroxybenzoic acid, is often used to treat acne by encouraging the sloughing of dead skin cells and, therefore, reestablishing a normal skin-cell replacement cycle. Whether or not salicylic acid directly kills *P. acnes* is currently debated. The first goal of this research was to determine if salicylic acid kills the bacteria. The second purpose of the research was to determine how the related meta and para isomers of salicylic acid affect *P. acnes*.

The method which produced the most useful data was the measurement of the bacterial growth by examining the change in turbidity of the samples. The results indicate that meta and para do inhibit growth and salicylic acid (ortho) is the most effective at killing *P. acnes*.

## Introduction

Acne is the most common skin disorder in the United States. Almost 17 million people suffer from this disease, with approximately 85% being between the ages of 12 to 25. At least 350,000 of those affected have had acne severe enough to seek treatment from a professional.<sup>1</sup> Although the presence of acne is not life-threatening, it can cause permanent scarring, disfigurement, and an intense psychological effect on the sufferer.<sup>2</sup> Those inflicted with this disease actively search for a cure, as is demonstrated in the amount of money spent on acne treatments each year. The sales of over the counter acne treatments reach more than 100 million dollars annually and patients spend even more money on prescription medications.<sup>1</sup>

Acne is a disorder of the sebaceous, or oil, glands in the skin. Generally, acne is found only in the areas of the body that have the largest and most abundant sebaceous glands -- the face, neck, chest, upper back, and upper arms. The role of the sebaceous glands is to make an oily substance, called sebum, to lubricate the skin and hair. Normally, the sebum moves through the follicles and is excreted onto the surface of the skin.<sup>3</sup> A diagram of a normal sebaceous (oil) gland can be found in figure 1.

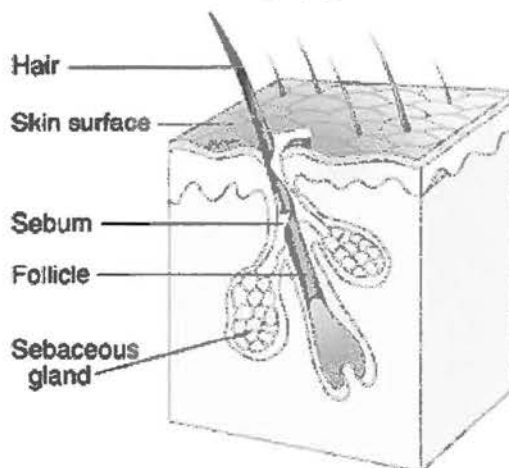


Figure 1

This diagram shows the structure of a normal oil gland. The gland produces the sebum which then moves through the follicle to the surface of the skin.<sup>4</sup>

Acne develops when there is a change in the inner lining of the follicle that prevents the sebum from passing through. In these defective follicles, the cells from the lining of the follicles are shed too quickly and clump together. The clumps plug up the opening of the follicle and the sebum is unable to reach the surface of the skin. In most cases, the sebum carries the cells shed by the glands to the skin's surface, but because the opening of the gland is blocked there is a buildup of both cells and sebum. This mixture of skin cells and oil cause *Propionibacterium acnes* (*P. acnes*), which normally live on the surface of the skin, to grow in the plugged follicles.<sup>3</sup> The *P. acnes* digest the sebum that is excreted onto the skin's surface as well as the sebum that is found in the clogged sebaceous glands. When a gland becomes plugged, the *P. acnes* quickly multiply and break down the sebum; at the same time the bacteria produce chemicals and enzymes that cause inflammation.<sup>5</sup> A drawing of a plugged follicle can be found in figure 2.

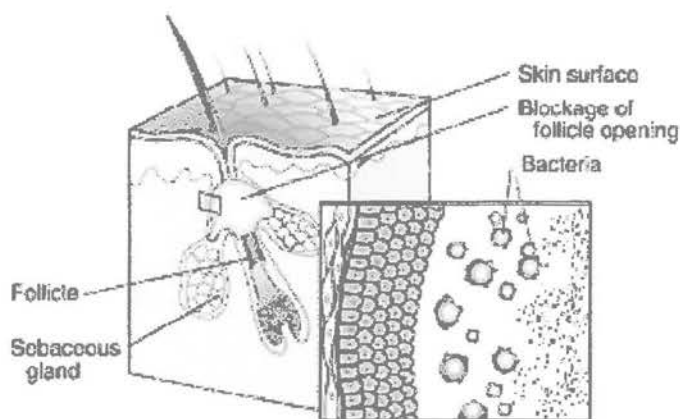


Figure 2

Acne develops when the opening of a follicle becomes blocked and the sebum is unable to pass through the pore to the skin's surface.<sup>4</sup>

*Propionibacteria* are found on the skin's surface and in the nasal pharynx, oral cavity, and gastrointestinal and genitourinary tracts. *P. acnes* are the most common non-

spore forming species found in clinical specimens. These bacteria are gram-positive, anaerobic, rod-shaped, and ferment glucose to produce propionic acid.<sup>6</sup>

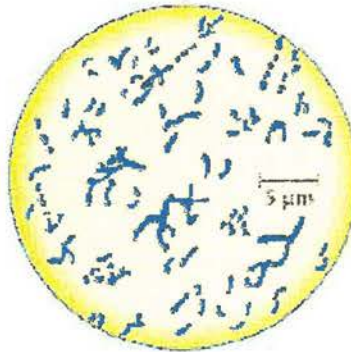


Figure 3  
This is a plate of *Propionibacterium acnes*.<sup>7</sup>

Acne develops in relation to where *P. acnes* colonize. As stated earlier, the bacteria live on the surface of the skin and in the sebaceous glands, where it feeds on skin oil. As the bacteria digest the oil, it produces fatty acids that cause an inflammatory response and burst the hair follicle. The lesion that forms on the surface of the skin is referred to as acne.<sup>6</sup>



Figure 4  
An example of a patient who has acne.<sup>6</sup>

Most acne treatments focus on either reducing the production of sebum, killing *P. acnes*, or normalizing the shedding of skin cells.<sup>8</sup> These treatments include non-prescription benzoyl peroxide and prescription drugs such as Accutane, tetracycline,

erythromycin, and Retin-A.<sup>7</sup> Another common active ingredient in acne medications is salicylic acid.

Salicylic acid, or 2-hydroxybenzoic acid, is a beta hydroxy acid that is obtained from the bark of the willow tree (see figure 5 for its structure).<sup>9</sup> Beta hydroxy acids are better than alpha hydroxy acids at penetrating and exfoliating within the pore. This is because the beta hydroxy acids are lipid soluble and, thus, are able to enter into the oil-containing pores and exfoliate the dead skin cells that have built up inside.<sup>10</sup> An effective concentration of salicylic acid for acne treatment is between 1-2%. A 1% concentration is used on sensitive skin types and 2% on persistent acne.<sup>9</sup> Salicylic acid is found in many over-the-counter medications such as Clearasil Clearstick Maximum Strength, Stridex, and Oxy Night Watch.<sup>3</sup>

Salicylic acid works as a keratolytic acid; it encourages the sloughing of dead skin cells. The plugged follicles are reopened and a normal skin-cell replacement cycle is reestablished. The issue of whether salicylic acid directly kills *P. acnes* is still being debated. Some sources state that salicylic acid does not kill *P. acnes*<sup>11</sup> while others state the contrary, that salicylic acid does kill the bacteria.<sup>12</sup> Thus, one of the purposes of this research is to determine whether or not salicylic acid directly kills *Propionibacterium*. The second purpose of this research is to determine the effect that different structural isomers of salicylic acid have on *P. acnes*. Only ortho-hydroxybenzoic acid, commonly known as salicylic acid, is used in the treatment of acne. This research investigates the effect of all three structural isomers (ortho, meta and para) of this molecule on the growth of *P. acnes*.



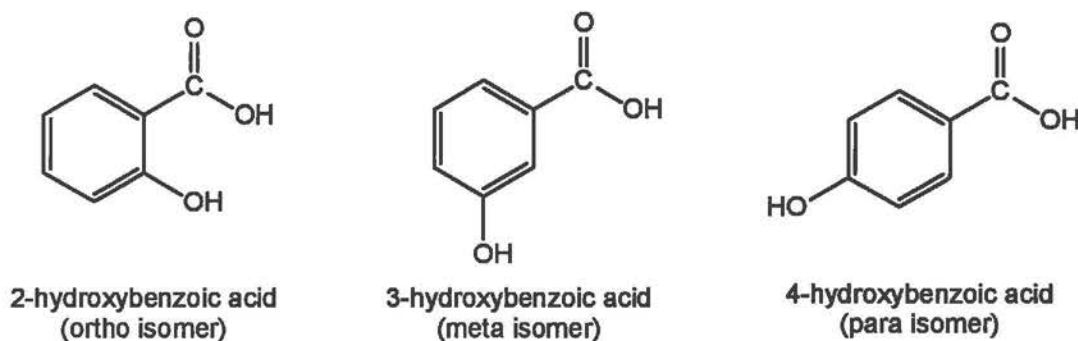


Figure 5

These are the structures of ortho-, meta-, and para-hydroxybenzoic acid.

To summarize, the two goals of this research are 1) to determine if salicylic acid directly affects the growth of *P. acnes* and 2) to determine how the other two structural isomers of salicylic acid affect *P. acnes*.

## Materials and Methods

The original bacteria, *Propionibacterium acnes* (purchased from American Type Culture Collection), used in this experiment were grown in approximately 5 mL of reinforced clostridial medium over a period of 72 hours. The bacteria were mixed with the medium under aerobic conditions, but allowed to grow under anaerobic conditions at 37°C.

The first procedure used in this experiment involved the plating of the bacteria onto agar plates.<sup>13</sup> Two 2-fold dilutions of the bacteria were performed and 50 µL from each solution were plated onto freshly prepared agar (Trypticase™ soy agar) plates. Thus, the first plate received 50 µL of the bacteria, the second 25 µL of bacteria, and third plate 12.5 µL of bacteria. The plates were incubated at 37°C for 48 hours in a BBL Gas Pak Jar. The procedure was repeated because of experimental problems in the first

run. Next, the same protocol was tried using blood agar plates (Trypticase<sup>TM</sup> soy agar with 5% sheep blood).<sup>13</sup>

Ortho-, meta-, and para-hydroxybenzoic acid were made in 5% solutions and it was found that a complex solvent mixture (by volume: 86.00% ethanol, 4.25 % water, 4.75 % methanol, and 5.00% isopropyl alcohol) had to be used because none of the three solids were very soluble in water.

Next, 100  $\mu$ L of the bacteria were plated onto a blood agar plate. A small amount of each of the 5% solutions was diluted 3 times by a 2-fold dilution to produce 5%, 2.5%, 1.25%, and .625% solutions of each structural isomer of salicylic acid. Each plate was then divided into four quadrants and a small piece of round filter paper soaked in the original 5% concentration was placed in the middle of one of the quadrants. Three more pieces of filter paper were soaked in each of the other lower concentrations and placed in the other three quadrants. Separate plates were prepared for each of the structural forms of salicylic acid. Plates were incubated at 37°C for 48 hours. The diameter of the zone of inhibition around each disk was measured. This procedure was repeated and altered to include only 5% and 2.5% concentrations. At this point in the research project, the experimental procedure was changed dramatically.

The third procedure involved measuring the change in turbidity (the measurement of light absorption) of the sample to determine the amount of bacterial growth in broth cultures.<sup>14</sup> First, the bacteria were regrown to ensure a fresh batch for the next part of the experiment. Two-fold dilutions of the salicylic acid were performed (with reinforced clostridial medium as the solvent) to obtain 5 mL of 2.5%, 1.25%, .625%, and .3125% of salicylic acid. Bacteria (100  $\mu$ L) were pipetted into each of four test tubes. The

absorbance of each of the test tubes at 650 nm was then recorded using a spectrophotometer (Spectronic® 20 Genesys™) which was set at 650 nm.<sup>14</sup> The control contained 5 mL of reinforced clostridial medium and 100 µL of bacteria. The machine was blanked with the reinforced clostridial medium. All the samples were grown anaerobically at 37°C for 48 hours after which the absorbance of each sample was again recorded. This procedure was run twice. The procedure was then modified by including controls that lacked bacteria. This modification allowed the final absorbances to take into account other factors, besides the growth of the bacteria, which would affect the change in turbidity of the sample. (These other factors will be discussed in a later section.)

The method was then altered slightly to include only 2.5% and 1.25% concentrations of the structural isomers. Each of 6 test tubes (3 test tubes containing the 2.5% concentration of each isomer and 3 test tubes containing the 1.25% concentration of the isomer) received 100 µL of the bacteria. Six more test tubes were prepared with the same contents, excluding bacteria. A control was also prepared as before. The absorbances were recorded at 650 nm and the bacteria were allowed to grow for 48 hours at 37°C under anaerobic conditions. The absorbances were again recorded at the same wavelength. This procedure was repeated 2 more times.

## Data and Results

Table 1: Run #1

Form and Conc.	Original Absorbance (650 nm)	Abs after 48 hrs. (650 nm)	$\Delta$ in Absorbance
Ortho 2.5%	0.146	0.121	-0.025
Ortho 1.25%	0.333	0.399	0.066
Ortho .625%	0.1	0.04	-0.06
Ortho .3125%	0.02	-0.004	-0.024
Meta 2.5%	0.151	0.013	-0.138
Meta 1.25%	0.052	0.013	-0.039
Meta .625%	0.035	0	-0.035
Meta .3125%	0.009	-0.016	-0.025
Para 2.5%	0.093	-0.048	-0.141
Para 1.25%	0.028	-0.021	-0.049
Para .625%	0.026	-0.024	-0.05
Para .3125%	0.022	-0.04	-0.062
Control	0.01	0.739	0.729

Table 1 displays the data collected from the first run of the turbidity procedure.

The numbers in the first column represent the absorbance of the samples before any bacterial growth. The second column represents the absorbance of the samples 48 hours later. The third column is the change in absorbance that occurred during the 48 hours. A control was included to ensure that bacteria growth was present. Because the control does not contain salicylic acid it should contain the greatest amount of bacterial growth.

Table 2: Run #2

Form and Conc.	Original Absorbance (650 nm)	Abs after 48 hrs. (650 nm)	$\Delta$ in Abs
Ortho 2.5%	0.141	0.142	0.001
Ortho 1.25%	0.498	0.74	0.242
Ortho .625%	0.16	0.059	-0.101
Ortho .3125%	0.047	0.007	-0.04
Meta 2.5%	0.146	0.032	-0.114
Meta 1.25%	0.099	0.094	-0.005
Meta .625%	0.072	0.022	-0.05
Meta .3125%	0.036	-0.001	-0.037
Para 2.5%	0.104	0.004	-0.1
Para 1.25%	0.05	0.089	0.039
Para .625%	0.04	0.009	-0.031
Para .3125%	0.02	-0.006	-0.026
Control	0.005	0.819	0.814

The data in Table 2 follows the same format as Table 1. The data will be analyzed in the discussion section.

Table 3: Run #1 of Revised Procedure

Form and Conc.	Original Absorbance (650 nm)	Abs after 48 hrs. (650 nm)	$\Delta$ in Abs
Ortho 2.5% w/bact.	0.153	0.117	-0.036
Ortho 1.25% w/bact.	0.531	0.277	-0.254
Ortho 2.5% no bact.	0.136	0.08	-0.056
Ortho 1.25% no bact.	0.511	0.265	-0.246
Meta 2.5% w/bact.	0.153	0.024	-0.129
Meta 1.25% w/bact.	0.114	0.024	-0.09
Meta 2.5% no bact.	0.14	0.049	-0.091
Meta 1.25% no bact.	0.085	0.061	-0.024
Para 2.5% w/bact.	0.182	0.035	-0.147
Para 1.25% w/bact.	0.079	0.04	-0.039
Para 2.5% no bact.	0.185	0.016	-0.169
Para 1.25% no bact.	0.055	0.001	-0.054
Control	0.083	0.692	0.609

In Run #3 the procedure was revised to account for the absorbance change that occurred due to the interaction between the medium and the different isomers of salicylic

acid. This was done by including the controls for each isomer and concentration (samples with no bacteria). Thus, the change in absorbance in a sample containing no bacteria provided information to help obtain a more accurate value of the growth that occurred in the same sample to which bacteria was added. The change in absorbance of the controls represented the change in turbidity that occurred which is not related to the bacterial growth.

Table 4: Amount of Bacterial Growth from Run #1 of Revised Procedure

Salicylic Acid Isomer and Concentration	<i>fI</i> in Absorbance (at 650 nm)
Ortho 2.5%	0.02
Ortho 1.25%	-0.008
Meta 2.5%	-0.038
Meta 1.25%	-0.066
Para 2.5%	0.022
Para 1.25%	-0.015

The bacterial growth from each of the samples is shown as an absorbance. These values were obtained by subtracting the change in absorbance in the sample with no bacteria from the change in absorbance in the sample with bacteria (refer to column 3 in Table 3). By including all of these values in the final calculation it is possible to compensate for the absorbance change that is produced from the interaction between the form of salicylic acid and the medium. The more positive the absorbance in this table the more bacterial growth occurred. The results show that the least amount of growth occurred in the presence of 1.25% 3-hydroxybenzoic acid (meta isomer).

Table 5: Run #2 of Revised Procedure

Form and Conc.	Original Absorbance (650 nm)	Abs after 48 hrs. (650 nm)	$\Delta$ in Abs
Ortho 2.5% w/bact.	0.169	0.129	-0.04
Ortho 1.25% w/bact.	0.397	0.227	-0.17
Ortho 2.5% no bact.	0.2	0.156	-0.044
Ortho 1.25% no bact.	0.384	0.205	-0.179
Meta 2.5% w/bact.	0.152	0.068	-0.084
Meta 1.25% w/bact.	0.075	0.045	-0.03
Meta 2.5% no bact.	0.164	0.028	-0.136
Meta 1.25% no bact.	0.042	0.006	-0.036
Para 2.5% w/bact.	0.12	0.024	-0.096
Para 1.25% w/bact.	0.06	0.032	-0.028
Para 2.5% no bact.	0.136	0.013	-0.123
Para 1.25% no bact.	0.033	-0.002	-0.035
Control	0.112	1.026	0.914

Table 5 displays the data obtained from the second run of the revised procedure.

Because the data from this run was somewhat different from the data from the first run, it was necessary to perform this procedure a third time.

Table 6: Amount of Bacterial Growth from Run #2 of Revised Procedure

Salicylic Acid Isomer and Concentration	$\Delta$ in Absorbance (at 650 nm)
Ortho 2.5%	0.004
Ortho 1.25%	0.009
Meta 2.5%	0.052
Meta 1.25%	0.006
Para 2.5%	0.027
Para 1.25%	0.007

Table 6 shows that the results from the second run are not the same as those from the first run. Unlike the first run (revised procedure), the data from the second run shows that the least amount of bacterial growth occurred in the samples containing 2-hydroxybenzoic acid (ortho isomer).

Table 7: Run #3 of Revised Procedure

Form and Conc.	Original Absorbance (650 nm)	Abs after 48 hrs. (650 nm)	$\Delta$ in Abs
Ortho 2.5% w/bact.	0.137	0.111	-0.026
Ortho 1.25% w/bact.	0.473	0.421	-0.052
Ortho 2.5% no bact.	0.119	0.101	-0.018
Ortho 1.25% no bact.	0.506	0.472	-0.034
Meta 2.5% w/bact.	0.154	0.081	-0.073
Meta 1.25% w/bact.	0.082	0.068	-0.014
Meta 2.5% no bact.	0.158	0.054	-0.104
Meta 1.25% no bact.	0.068	0.014	-0.054
Para 2.5% w/bact.	0.161	0.063	-0.098
Para 1.25% w/bact.	0.093	0.046	-0.047
Para 2.5% no bact.	0.158	0.04	-0.118
Para 1.25% no bact.	0.038	0.007	-0.031
Control	0.023	0.879	0.856

The results from run #3, shown in Table 7, are similar to the results from the previous run, displayed in Table 6.

Table 8: Revised Procedure, Run #3

Salicylic Acid Isomer and Concentration	$\Delta$ in Absorbance (at 650 nm)
Ortho 2.5%	-0.008
Ortho 1.25%	-0.018
Meta 2.5%	0.031
Meta 1.25%	0.04
Para 2.5%	0.02
Para 1.25%	-0.016

The calculations in this table show that ortho-hydroxybenzoic acid is the most effective at preventing the growth of *P. acnes* and that the meta form is the least effective. These results are similar to those found in the second run.



## Discussion

The first procedure that was used in this experiment was the plating of the bacteria onto TSA plates. After the bacteria failed to grow it was discovered that this type of bacteria had to be grown on blood agar plates and the procedure was modified.

Thus, the second method was the plating of the bacteria onto blood agar plates. Despite successive dilutions of the bacteria it was not possible to grow colonies, only lawns developed. This was because not enough dilutions were done. The original procedure<sup>13</sup> called for the growth of the colonies in order to quantify and to later rate the ability of each form of salicylic acid to kill the *P. acnes*. The results showed that the dilution factors that were used were not sufficient to yield colonies, but instead produced lawns. For this reason a different procedure was pursued.

It was then decided to continue with the plating of the bacteria, but instead to change the procedure to utilize inhibition zones on bacterial lawns. The salicylic acid on filter paper disks did prevent the growth of the bacteria, but only in the places where the solution was in direct contact with the agar. Initial results were inconclusive and it was soon realized that the method being used was ineffective. Next, the filter papers were first placed on the plates and then 5  $\mu\text{L}$  of the solution was pipetted onto the paper. The results from this procedure were also inconclusive and it was discovered that the problem lay in the lack of diffusion of the solution throughout the blood agar plate. The solutions were not diffusing throughout the plate and it was only possible for the inhibition of bacterial growth to occur in the places where the solution was in direct contact with the bacteria. Because each piece of filter paper adhered to the plate in a different way it was not possible to further modify this procedure and it was abandoned.

The third method used was the quantitative measure of the bacterial growth by studying the change in turbidity of broth cultures. The first two runs of this procedure examined the change in turbidity in mixtures that contained the same amount of *P. acnes* (100  $\mu$ L) but different concentrations of the salicylic acid isomers. The absorbance of each sample (at 650 nm) was recorded on the day of its preparation and again 48 hours later. It was hypothesized that the absorbance level would increase during the 48 hours due to the growth of the bacteria but this did not happen. In most cases the absorbance decreased. The blank that was used for the spectrophotometer was freshly prepared reinforced clostridial medium. The absorbance of the blank was recorded on the day of the samples' original readings and the same absorbance was used to blank the machine 48 hours later. This was done by making up some of the reinforced clostridial medium on the second day and then allowing the solution to settle and clarify. Once the mixture's absorbance decreased to match the blank's absorbance on the first day, the machine was blanked with this value. A control was also included during each run that did not contain any bacteria. The change in absorbance of the control indicates the amount of bacterial growth that would normally occur. This value was positive for each control. Thus, the assumption was made that the negative absorbance change of each sample was likely due to a combination of little bacterial growth, the settling of the medium in the solution, and the color change that was produced from the interaction between the salicylic acid and the medium. The procedure was expanded to account for these circumstances.

The last three runs of the procedure involved only 2.5% and 1.25% concentrations of the salicylic acid. It was decided not to include the two lower concentrations because most acne medications contain between 1 and 2% salicylic acid.<sup>9</sup> Controls for each

sample were made that did not contain bacteria. The change in absorbance of the controls are representative of the amount of settling of the medium (which causes the solution to clarify and thus have a lower absorbance) and the clarity change (or turbidity change) that is produced from the interaction between the salicylic acid and the medium. The absorbances of the controls were included in the calculations of the total amount of bacterial growth (final values found in Tables 4, 6, and 8).

In the first two runs of the turbidity experiment, the data indicate that ortho-hydroxybenzoic acid is the least effective at inhibiting the growth of *P. acnes* while meta- and para-hydroxybenzoic acids have a comparable effect on their growth. This conclusion was made by examining the last column of Tables 1 and 2. These values represent the absorbance of the bacteria that grew during a 48 hour time period. The level of absorbance is directly related to the amount of bacterial growth.

Of all the information collected during this experiment the data from the last three runs should lead to a more accurate calculation of the amount of *P. acnes* growth. This is because of the measures taken to remove outside influences, leaving only the effects of the salicylic isomers to be observed. The values produced from the first run indicated that the meta form of salicylic acid was the most effective at killing *P. acnes* and the ortho and para forms were the least effective. However, it was realized that the glassware had not been properly cleaned from the previous run and thus residual bacteria had likely been present at the beginning of the growth process. Each sample would not have had the same amount of bacteria and the data collected would not be accurate. New glassware was obtained and the run was repeated.

The data collected from the second run suggested that the ortho form of salicylic acid was the most effective at killing *P. acnes* and the meta was the least effective. The final run produced the same results. These results make sense because the structural form that is used in acne medications is the ortho-hydroxybenzoic acid. This structural form of salicylic acid is most likely used to kill *P. acnes*, and it was hypothesized its increased activity may be due to its ability to produce intramolecular hydrogen bonds. The hydrogen on the -OH group is able to hydrogen bond with the oxygen in the carboxylic acid group producing an additional six-membered ring in the molecule.

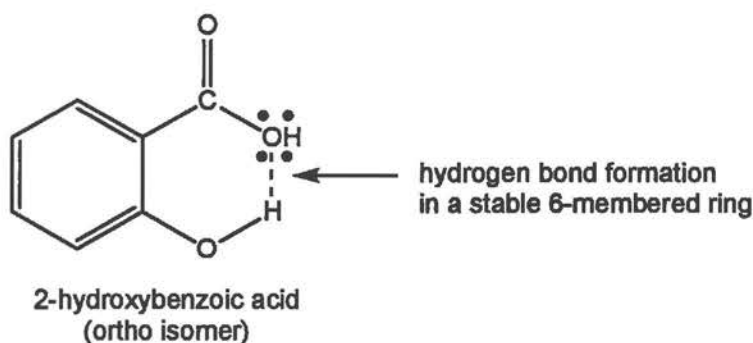


Figure 6  
Hydrogen bond formation in the ortho isomer of salicylic acid.

Because of the increasing distance of the hydroxy and carboxylic acid groups in the meta- and para- structural forms, hydrogen bonding is not as probable.

The results from this research suggest that salicylic acid does indeed directly affect the growth of *P. acnes*. Some sources are adamant that salicylic acid only works by encouraging the sloughing of dead skin cells, but its role should be expanded to include the direct inhibition of the bacteria's growth. The procedure used in this experiment only allows for the salicylic acid to affect the growth of *P. acnes*; the only other ingredient in each mixture was the culture medium which facilitates rather than

inhibits the growth of bacteria. Thus, from the data collected in this research, it has been demonstrated that salicylic acid does kill *P. acnes*.

## Conclusion

Acne affects a large portion of the population and, thus, studies into the treatment of this disease are important. Salicylic acid has been used for years to stop the growth of the bacteria that causes acne, *Propionibacterium acnes*, by acting only as a keratolytic acid.<sup>11</sup> It causes the dead skin cells to slough off the skin which reduces the chance of the follicle to become plugged. It is debated as to whether or not salicylic acid is able to kill *P. acnes*. It is also known that ortho-hydroxybenzoic acid is the only structural form of salicylic acid that is used in acne medications. The goals of the research were to determine if salicylic acid could kill *P. acnes* and to examine the effect of the other two structural isomers of salicylic acid, meta- and para-hydroxybenzoic acid, on *P. acnes*.

The information obtained was able to answer both of these questions. It was concluded that salicylic acid is able to kill *P. acnes* and that although meta- and para-hydroxybenzoic acid are able to inhibit the growth of the bacteria, the ortho isomer, used in acne medication, is the most effective at killing *P. acnes*.

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- <sup>4</sup> [www.acne-care.com](http://www.acne-care.com)
- <sup>5</sup> [www.healthychild.com/database/acne\\_anguish\\_a\\_common\\_teen\\_age\\_problem.htm](http://www.healthychild.com/database/acne_anguish_a_common_teen_age_problem.htm)
- <sup>6</sup> [www.arches.uga.edu/~rch/propionibacterium.htm](http://www.arches.uga.edu/~rch/propionibacterium.htm)
- <sup>7</sup> [www.arches.uga.edu/~bais/prop2.html](http://www.arches.uga.edu/~bais/prop2.html)
- <sup>8</sup> [www.derm-infonet.com/acnetnet/treatment.html](http://www.derm-infonet.com/acnetnet/treatment.html)
- <sup>9</sup> [www.skincarerx.com/salicylic-acid.html](http://www.skincarerx.com/salicylic-acid.html)
- <sup>10</sup> [www.dermatology.about.com/cs.skincareproducts/a/bha.htm](http://www.dermatology.about.com/cs.skincareproducts/a/bha.htm)
- <sup>11</sup> [www.acne.com/t\\_salicylic.php](http://www.acne.com/t_salicylic.php)
- <sup>12</sup> [www.bion-research.com/acne\\_vulgaris.htm](http://www.bion-research.com/acne_vulgaris.htm)
- <sup>13</sup> Winchester, A.M. and P.J. Wejksnora 1996. *Laboratory Manual of Genetics*, 4<sup>th</sup> ed. Wm. C. Brown Publishers, Dubuque, IA, 186-187
- <sup>14</sup> Seely, H.W., P.J. Vandemark, J.J. Lee 1991. *Selected Exercises From Microbes in Action: A Laboratory Manual of Microbiology*. 4<sup>th</sup> ed. W.H. Freeman and Company, 93-95

## **Acknowledgements**

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SOUTHERN SCHOLARS SENIOR PROJECT

Name: Ireland Burch Date: 4/15/04 Major: Biochemistry

SENIOR PROJECT

A significant scholarly project, involving research, writing, or special performance, appropriate to the major in question, is ordinarily completed the senior year. The project is expected to be of sufficiently high quality to warrant a grade of A and to justify public presentation.

Under the guidance of a faculty advisor, the Senior Project should be an original work, should use primary sources when applicable, should have a table of contents and works cited page, should give convincing evidence to support a strong thesis, and should use the methods and writing style appropriate to the discipline.

The completed project, to be turned in in duplicate, must be approved by the Honors Committee in consultation with the student's supervising professor three weeks prior to graduation. Please include the advisor's name on the title page. The 2-3 hours of credit for this project is done as directed study or in a research class.

Keeping in mind the above senior project description, please describe in as much detail as you can the project you will undertake. You may attach a separate sheet if you wish:

This research will examine the effects of the structural isomers of salicylic acid on Propionibacterium acnes bacteria. It will attempt to determine whether or not these isomers directly kill the bacteria and, if so, which isomer is the best at killing P. acnes.

Signature of faculty advisor [Signature] Expected date of completion 4/16/04

Approval to be signed by faculty advisor when completed:

This project has been completed as planned: ✓

This is an "A" project: ✓

This project is worth 2-3 hours of credit: ✓

Advisor's Final Signature [Signature]

Chair, Honors Committee \_\_\_\_\_ Date Approved: \_\_\_\_\_

Dear Advisor, please write your final evaluation on the project on the reverse side of this page. Comment on the characteristics that make this "A" quality work.



Dear Southern Scholars Committee,

Ireland's work on her research project was exceptional, and I feel that she has demonstrated 'A' quality work for several reasons.

1. Most science-based research projects have components which are not necessarily present in the more traditional, literature-search-based research project. In addition to pursuing the literature search to determine whether or not the study has been performed before and to obtain background information, Ireland also had to obtain laboratory procedures to carry out her work. In Ireland's project, this involved working very closely with the biology department, and with the gracious help of several biology faculty, Ireland was able to modify and combine several methods which enabled her research project to come to a successful conclusion, and will enable future students to use her methods to continue her work.
2. The persistence and independence that Ireland showed, even though many of her initial experiments failed (8+) and many hours were "wasted" (50+), was indicative of a student who understands that results will not come without hard work, and that trial and error is sometimes the only way to determine the truth. Her drive and ability to work, without me looking over her shoulder, during this project was outstanding, I merely was a 'supply store' for chemicals and advice.
3. The science in this paper was a mixture of chemistry and biology. The goal was to prove that different chemical structures should react differently with biological structures. This seems obvious, but no literature information proving our hypothesis could be found. Every once in awhile, projects that everyone assumes are well-researched prove to be merely commonly held beliefs. In Ireland's research project, she successfully 'proved' that the ortho- isomer of hydroxybenzoic acid truly does kill bacteria better than the meta- or para-isomers. While not earth-shattering knowledge, it is important in its own right if seen as shoring up the scientific foundation underlying perceived truths.
4. Finally, skills and techniques not commonly used in the undergraduate setting were required to successfully complete this project. Throughout the course of her work, Ireland learned how to use new instruments and perform new procedures, and by the end of the project, demonstrated proficiency by being able to 'tweak' the methods to fit the required tasks at hand.

Sincerely,

A handwritten signature in black ink, appearing to read "Loren Barnhurst", written in a cursive style.

Loren Barnhurst