Antimicrobial properties of S-benzofuran-2-yl ethanol produced by biotransformation

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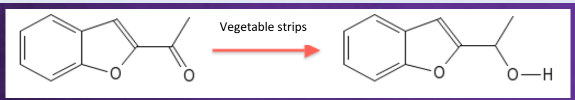
ABSTRACT

All living organisms contain enzymes to carry out biological reactions, which produce substances with a specific three dimensional shape. Enantiomers are two molecules that are mirror images of each other just as hands are mirror images of each other. It is well known in the pharmaceutical world that one of the enantiomers (one of the mirror-image molecules) has a positive biological effect while the other can be harmful or have no effect. Currently, any potential pharmaceutical that could exist as enantiomers must have each enantiomer tested for biological activity prior to FDA approval. In our laboratory, we have focused on one particular reaction in which enzymes in vegetables catalyze a reaction of benzofuranyl methyl ketone (BMK) to benzofuranylethanol (BMA). BMA can have two different three dimensional arrangements, so mirror image molecules are possible. The two enantiomers are designated S-BMA and R-BMA. Several vegetables have been utilized to determine which can catalyze this reaction, as well as to determine whether the various vegetables produce both enantiomers or only a single enantiomer. Several vegetables (carrot, parsnip, and celery) produce only one of the enantiomers, the S-isomer. However, potatoes and radishes produce a mixture of the R- and S-isomer, with the potato producing nearly equal amounts of both. Previous work in the laboratory has shown that the S-BMA produced from carrots has antimicrobial properties, inhibiting the growth of bacteria as well as yeast. Initial studies indicate the mixture of S- and R-BMA from potatoes has less potent antimicrobial activity than the pure S-BMA produced by carrots. Studies are underway to more fully assess the antimicrobial property of the mixture of the R- and S-isomer and compare to the antimicrobial property of the pure S-isomer produced by carrots.

Reaction



benzofuran-2-yl methyl ketone (BMK) → benzofuran-2-yl ethanol (BMA)

Funding and References

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Methods

Determination of BMA-Producing Plants

- The samples were prepared by chopping various plants, adding 1.6 E-4 mole (25 mg) of BMK and 25 mL of dH_2O, and mixing on a rotisserie until BMA was produced

Testing Optical Purity of the Sample

- ~10 mg of purified BMA from each plant were loaded into a polarimetry cell with 10 mL of chloroform and tested for optical rotation using a Jasco P-1010 Polarimeter

Antimicrobial Studies

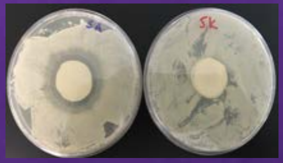
- Baker's Yeast culture was grown overnight in YPD broth. YPD agar plates were inoculated with 20 µL of overnight yeast culture and incubated at 30 °C for 2 hours
- BMA from carrots and BMA from potatoes were each dissolved in ethyl acetate to a concentration of 0.40 mg/mL
- Filter paper discs were impregnated with 10 or 50 mg of BMA from potato and carrot. BMK was used for comparison.
- Control plates used were yeast alone, yeast with plain filter paper, and yeast with filter paper with only ethyl acetate added.
- After two hours, the filter paper discs were placed at the center of each plate, and the plates were incubated at 30 °C for 48 hours
- The zones of inhibition for each sample were measured and recorded

Table 1. Specific Optical Rotation Results

Plant Type	BMA Produced	Conversion Time (hrs)	$[\alpha]^{22}_{589}$
Carrot	Yes	2	-16.621
Potato	Yes	48	-0.741
Radish	Yes	24	-8.953
Parsnip	Yes	4	-16.886
Cucumber	Yes	48	-13.323
Celery	Yes	24	-16.593

Antimicrobial Results

(I)



(II)

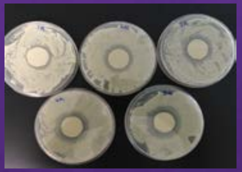


Table 2. Results of Potato and Carrot BMA on Yeast Growth

BMA (mg)	Inhibition zone (cm)	Inhibition zone (cm)
0 (control)	0.0	0.0
	Potato (R-and S-isomers)	**Carrot** (S-isomer only)
10	0.00	0.20
50	0.88	0.98

Discussion

The polarimetry data in Table 1 indicates carrots, parsnips, and celery produced pure S-BMA. Other groups have determined carrots often produce >99% enantiomeric excess of the S-isomer when starting with various prochiral ketones.[1-5] The potato appears to produces a near-racemic mixture, while the radish produces some mixture of the two isomers in which the S-isomer is more prevalent.

The antimicrobial studies show that BMA clearly inhibits yeast growth while BMK did not. In figure (I), the BMA plate 5A (50 mg BMA) had two definite zones of inhibition around the filter paper disc while the BMK plate 5K (50 mg BMK) actually had yeast grow up to, and even on top of, the filter paper disc. Figure (II) demonstrates a significant dose dependence of the BMA's antimicrobial properties. As the BMA concentration increases from 5.0 to 50 mg, the zone of growth inhibition increases.

The preliminary comparison of BMA produced by carrots and potatoes shown in Table 2 suggests the pure S-BMA produced by carrots has slightly greater antimicrobial effects than the mixture of isomers produced by potatoes. However, because only a single trial was performed with two doses used for each sample, no conclusion can be drawn until more trials are performed.

Future plans are to quantify the antimicrobial activity of the S-BMA, further compare the dose-dependence of BMA produced by potato and carrot, and develop a method to determine the percentages of each isomer present of the mixtures produced by potatoes and radishes. Work is currently being done to isolate and characterize the protein from the carrot surface that is responsible for catalyzing this reaction. Furthermore, this experiment will be expanded to other prochiral ketones in hopes of producing other single mirror image molecules with biological activity.