NEW HAVEN SCIENCE FAIR

BY

GERALD PUTTTERMAN, COUNCILOR

The New Haven Public Schools Science Fair Program has several components all aimed at improving the quality of science and math education in the New Haven public schools. The students conduct projects during the year and the culmination of the program is the actual science fair where the students preK-grade 12, present posters describing their projects including their results and conclusions. The program is administered by the Greater New Haven Chamber of Commerce. In previous years the Fair was held at Yale Commons. However, this year the Commons has been undergoing renovations so the Fair was held at the Floyd Little Athletic Center located at Hillhouse High School.

In addition to the numerous judging categories and criteria assigned by the Fair administrators there are also Special Awards given by volunteer sponsors such as ACS, the CT Agricultural Experiment Station, the American Society of Metals and many others. For these Special Awards the sponsors judge based on their own criteria. For the first time representatives of the sponsors were invited to attend a reception for the science mentors which was held at the Leitner Family Observatory and Planetarium on Prospect Street in New Haven. I attended the reception, explored the facility and heard an interesting presentation about the fair.

The Fair was run from Monday May 14 to Wednesday May 16. All the judges reviewed the posters on Monday from 4-8 pm in the absence of students and on Tuesday from 9 AM-1 PM in the presence of students. At that time the judges discussed the projects with the students attempting to learn how much of the projects came from input from the students and how much was understood by the students. When the students left, the judges met and determined the prize winners.

The Chamber of Commerce does an excellent job of making the judges feel welcome, even in a new facility, Not only was a buffet dinner provided at the athletic center on Monday evening but a buffet breakfast and buffet lunch were also available there. Furthermore free parking was available on both days and parking passes were not required.

After notifying the Program Consultant for the Fair that ACS would again be a sponsor, my next task was to obtain ACS members who were ready and willing to serve as judges. The response to my call was excellent and the following members served with me as judges: Chris Zuzi, Bob Davis and Raj Bandaranayake. After the winners were selected I purchased the prizes (Visa gift cards) and gave them in marked, sealed envelopes to the Program Consultant for presentation at the Science Fair Awards Ceremony held this year at Hillhouse High School on May 16.

The New Haven Section awards two prizes of $100 each: one for the best project from grades preK-8 and one for the best project from grades 9-12. Unfortunately, the science projects are listed by the Fair organizers in just two major categories: physical science and biological science. Thus we had to first identify and list the projects containing chemistry and then decide on the best projects on our own list.

For grades preK-8 the winning poster was prepared by 7th graders Eris Daraouk and Destiny Torres from Bishop Woods School who studied the formation of Bismuth Crystals. As summarized by Chris Zusi: "The purpose of the experiment was to examine the effect of varying the cooling rate of molten bismuth on the crystal structure (notable since the crystals are known to grow faster at the outside edges rather than the inside edges of nascent crystals). A constant amount of elemental bismuth was melted on a stove, transferred to a tin container, covered, and incubated at either room temperature or refrigerator temperature. The samples (run in triplicate) were retrieved after a set duration and the remaining molten bismuth was poured off. The residual crystals were weighed. The slower-cooling, room temperature samples produced 2-3 times

the mass of crystals compared to the refrigerated samples." A photo of the poster is shown below.

For grades 9-12 the winning poster was prepared by Prastik Mohanraj, a senior from the Engineering and Science University Magnet School. As summarized by Bob Davis: "Chemically altered usnic acid derivatives were prepared in an effort to enhance tumoricidal properties. Cytochrome P450, an active enzyme of heptacellular carcinoma, was targeted by synthesis of analogs of disrupted aromaticity or enhanced electron deficiency in the aromatic ring of usnic acid." A photo of the poster is shown below.

The New Haven ACS Section's Dinner is usually held around the time of the Science Fair. This year the dinner was after the Fair and the 7th graders were able to attend with their teacher. The students had a great time participating in the hands-on part of the speaker's presentation.


BISMUTH
CRYSTALS
Observations
Crystallization
Data Table
Trials by Temp.
Trial 1
Trial 2
Trial 3
Room Temp.
17 grams
3 grams
9 grams
Fridge Temp.
5 grams
4 grams
2 grams
Bismuth
COLD
HOT
TRIAL #1
TRIAL #2
TRIAL #3
Research Paper
Bismuth Crystals
by
Eric Carsecuk & Destiny Torres
169

Novel Organic Synthesis of Usnic Acid Derivatives with Tumoricidal Properties

Background

Cytochrome P450 can cause cancer in hepatic cells and in other cell types through over-expression or inducement. In oxidative stress, this enzyme causes the production of increased reactive oxygen species, which are carcinogenic through chemically modifying the DNA content of a cell.

Inhibitory chemicals that can target such cancerous activity and discriminate such cells from healthy ones remain unknown.

Usnic acid is a complex antibacterial agent with the potential to inhibit oxidative phosphorylation in cells, through attacking mitochondrial or microsomal enzymes. There exist parallels between this feature of usnic acid and the targeted over-expression of microsomal Cytochrome P450.

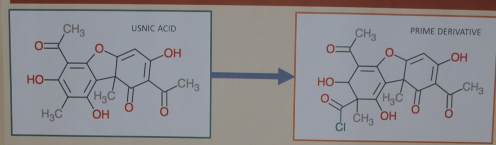
Hypothesis

By chemically altering usnic acid to become more selective towards active Cytochrome P450, it was hypothesized that cancerous activity would be inhibited or reduced. This could possibly be achieved by making the aromatic ring less electron-rich, and thus exposure of induced Cytochrome P450 to the derivative analog would inhibit Cytochrome P450, blocking the persistence of cancer in the cell. The hypothesized route of best efficiency was by adding an acyl chloride group to the aromatic ring, effectively removing resonant *pi* bonds.

Materials and Methods

Research was divided into two stages: the production and characterization of derivatives, and the analysis of targeting Cytochrome P450 in cancer cells and healthy cells. Organic reactions would be performed to react usnic acid with standard organic reagents using various reaction schema. Reactions would occur in flash reactor conditions using an Anton Paar microwave flash reactor, at generally 200° C and 20 bars of pressure. Thin-layer chromatography would be performed throughout the reactions to track kinetics data. Products would be distilled after a 24 hour reaction period, and separated from waste byproducts using column chromatography, a rotary evaporator, and vacuum distillation. Compounds would be characterized using NMR, mass spectrometry, and chromatographic methods. Candidates for tumoricidal activity would be tested against PLHC-1 hepatocellular carcinoma cells, compared with healthy hepatic cells, both rich in Cytochrome P450 enzymes. Cells would be cultured using bovine fetal serum and essential amino acids and nutrients. P450 activity, through tagging the enzyme using the activity-dependent 2EN-ABP proteomic molecule, would be compared before and after exposure to analog compounds for a constant period. Cell death would be measured through flow cytometry through measurement of propidium iodide uptake. Biomarking and tagging essential components of the sampled cells would reveal the mechanism of action in such tumoricidal properties, which would validate hypotheses.

Results

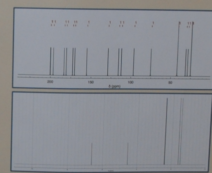


Multiple reaction types were examined at constant flash reactor settings for a 24 hour reaction period. The top reaction, in terms of yield, was the acylation reaction. The yield was approximately 45% with a margin of error of +/- 2.67%, the maximum yield seen in the observed reactions. Moreover, this reaction appealed strongest to the hypotheses.

Reactions occurred at 200° C and at 20 bars of pressure. Hydrazine and potassium hydroxide were used as catalytic agents in solution within the flash reactor setting, at equal molarity to the usnic acid, added as solid heterogeneous to the reaction mixture.

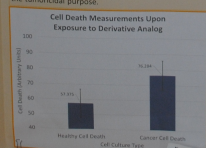
The acylation reaction occurred, producing an acyl chloride group on the aromatic ring, and removing the aromaticity to remove the electron-rich *pi* bonds as well as the accompanying resonance. Stereochemistry was kept constant, as both the starting compound and the product analog compound were dextrorotatory.

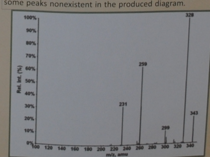
Reaction kinetics were measured, and the reaction was determined to proceed in a first-order mechanism.



Above are the NMR results. NMR was performed against Hydrogen-1 (bottom) and Carbon-13 (top), and the NMR diagrams produced were similar to the predicted simulated models of the analog, within a maximum of +/- 5% variation. Below are the mass spectrometry results. Variation came within +/- 7.5% variation, with some peaks nonexistent in the produced diagram.

A trial run of cell death measurements was performed. Propidium iodide uptake and flow cytometry were used to identify cell death with exposure to the derivative compound. Statistically significant results ($P < 0.05$) were obtained, and cell death was near 33% greater in the hepatocellular carcinoma cells than in the healthy hepatic cells. This demonstrates effective behavior of the analog compound in the tumoricidal purpose.





Tagging Cytochrome P450 using the fluorescent 2EN-ABP activity-dependent marker yielded a statistically significant change ($P < 0.01$) in Cytochrome activity and induction. This occurred in the hypothesized direction of lower activity in hepatocellular carcinoma cells following exposure to the analog compound, where activity was lowered by 24% between the groups.

Discussion

The results show that the acylation reaction was primarily successful, and the high yield of the reaction over a constant time period provides support for its dominant property. Still, other reactions may have more effective products in terms of tumoricidal properties, but this reaction produced a compound that was sufficient, as given by the data on the reaction kinetics.

The acylation removed a double bond and thus removed the aromatic resonance of *pi* bonds, which decreased the electron-richness of the aromatic ring. The acyl chloride had NMR and mass spectroscopy measurements at a manageable deviation from the predicted results, which could be accounted for due to simulation inconsistency and data analysis faults.

Doing a trial run on hepatocellular carcinoma cells does not prove as sufficient evidence to conclude tumoricidal properties, but the data collected give a reasonable premise as to the existence of such properties. Tagging the Cytochrome P450 molecules showed that intensity of the enzyme decreased upon exposure to the acylated derivative, and comparing cell death using flow cytometry between healthy and tumor cells showed a substantially higher death rate for tumor cells.

Further research must be performed to validate the claims from this second stage of the research, but there lies promise in tumoricidal properties in this derivative. Further characterization of this compound must be performed as well.

More precise equipment and methodologies, as well as more intense laboratory work, would validate all claims set forth here. A repetition of this research would be sufficient to conclude causal relationships are present.

Conclusions

It can be concluded that the acylation reaction was a success, and that novel derivatives of the usnic acid compound have been identified to have tumoricidal properties. The reaction has a high yield, indicating high efficacy in producing the analog from usnic acid starting compound. Further research would explore different flash reactor settings and various catalytic agents. Further repetition of the cancer cell tests would provide support for the tumoricidal properties demonstrated here. The acylated analog of usnic acid could become a widely used anti-cancer chemical agent, if further research goes into demonstrating the various chemical and biological properties it has.

Acknowledgments

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New Haven Science Fair Program

226