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Developing a Presumptive Test for Select Synthetic Cannabinoids

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by

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Abstract

Synthetic cannabinoids (SC's) began to gain popularity around the world in 2009. Since then, many of the compounds have been outlawed and methods developed to detect them and their metabolites using mass spectrometry. Our work investigated the possibility of developing a colorimetric presumptive test. The SC JWH-019 was synthesized and its ketone targeted as a possible reaction site. Many SC's contain ketones and thus a reaction at this site would be applicable to many of the compounds. Since JWH-019 is costly and time consuming to synthesize, much of the experimental work was done using benzophenone (BP). BP contains a diaryl ketone making it comparable to JWH-019. Our initial work studied existing presumptive tests, one for SC's and one for cannabis. Both gave negative results for JWH-019. From there, we looked at synthesizing imines that might be colored. We studied reactions using dinitrophenylhydrazone, hydrazine, aniline and neutral red. Through these reactions it became apparent that the ketones on BP and JWH-019 were reluctant to react. Finally, we studied forming imines of BP with either ethylenediamine (en) or semicarbazide. The resulting product was then used to produce a metal complex. A complex formed between the en-BP product and Cu^{2+} provided a change in color, but the en-BP imine proved difficult to obtain and the results were not consistent.

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1. Introduction

1.1 Synthetic Drugs

In recent years there has been an influx of "synthetic drugs." Labeled as plant food, incense, bath salts or various other innocuous commodities these substances were easily obtained in "head shops," gas stations or through the internet. [1, 2] Internet drug forums touted their affects and celebrated the fact that these products were legal alternatives to other drugs[3]. However, emergency room visits and calls to poison control centers soon revealed that these "legal" alternatives were not safe alternatives.[1] Authorities and researchers began to investigate the products and found two major kinds. One was a substitute for amphetamines. Generally sold as bath salts, the product is a white crystalline powder. It was first identified in 2007 as mephedrone (4-methylmethcathinone).[4-6] Subsequently, 3.4-methylenedioxypyroverlone (MDPV) and methylone have also been identified in the products and on September 7, 2011 the Drug Enforcement Administration (DEA) temporarily designated all three compounds a Schedule I substance, thus making it illegal to possess or sell any of the compounds[7]. The second synthetic drug mimicked the effects of marijuana and is generally marketed as "herbal incense." The first psychoactive component in the incense was identified in December 2009 as JWH-018 (1-pentyl-3-(1-naphthoyl)indole).[8] Soon after several other compounds were identified and in March 2011 the DEA designated JWH-018, JWH–073 (1-butyl-3-(1-naphthoyl)indole), JWH–200 (1-[2-(4-morpholinyl)ethyl]-3-(1-naphthoyl)indole), CP–47,497 (5-(1,1-dimethylheptyl)-2-[(1R,3S)-3-hydroxycyclohexyl]-phenol), and CP–47,497 C8 homologue (5-(1,1-dimethyloctyl)-2-[(1R,3S)-3-hydroxycyclohexyl]-phenol) as Schedule1 substances.[1]

However, the story does not end there. The producers of these drugs anticipated that these compounds would become illegal and were prepared to begin production of new psychoactive

compounds to replace the illegal ones. Reports have shown new, legal products to appear on the market just four weeks after compounds in the old products have been illegalized.[3, 8] New products even claim on their labels that they do not contain any of the illegal compounds[5]. This is where the primary problem lies. Forensic labs have to constantly analyze an ever changing product and law makers have to continually push legislation to make the new compounds illegal. Even as the compounds are made illegal, many drug screening tests cannot yet detect them.[6, 9, 10] Such limitations make the use of these synthetic drugs appealing to many people, especially those with jobs subject to random drug tests.[4, 5] The need for fast and reliable ways to identify these compounds is imperative.

Our work dealt with synthetic marijuana. The psychoactive compounds found in the herbal mixtures are referred to as synthetic cannabinoids (SC's). The DEA defines SC's as: "a large family of chemically unrelated structures functionally (biologically) similar to THC[1]." While a considerable amount of work has been done to identify SC's and their metabolites using gas chromatography mass spectrometry (GC-MS) or liquid chromatography mass spectrometry (LC-MS), there is little progress in areas that would enable these compounds to be identified outside of a laboratory, or, at least, quickly and inexpensively in the laboratory. Our goal was to begin to look at ways to make identification in the field possible.

1.2 Dangers of Synthetic Cannabis

As mentioned before SC's are generally sold as "herbal incense." The "incense" consists of SC's that have been dissolved in a solvent and sprayed onto different types of plants.[1] Only the plant matter is listed on the packaging as ingredients. Some plants that are commonly listed are Indian warrior, Lion's Tail, Bay Bean, Blue Lotus, vanilla, honey, beach bean, marshmallow, red clover and rose.[3, 4] Several of the ingredients such as Bay Bean and Lion's Tail are rumored

to be mildly psychoactive, but alone are not capable of causing the psychoactive effects associated with smoking the product[3]. The most common brand names for the herbal incense are Spice and K2, but it is sold under 100s of different names.[2] The packaging always contains the warning "not for human consumption" making it easy for producers to claim no responsibility for its adverse effects.

The danger of SC's became apparent as calls to poison control centers and emergency room visits increased. In 2010, approximately 2800 calls to poison control centers involving SCs were reported. In 2011 that number more than doubled to 6348 and in 2012 the number was still high at 5205.[11, 12] The majority of case reports involve young adults and teenagers.[13] The most common complaints associated with use of the drug are extreme anxiety, paranoia and tachycardia, but the drug occasionally has more extreme side effects including hallucinations, nausea, psychosis, unconsciousness and seizures.[6, 14] The most devastating result associated with the use of SC's is death. The first reported suicide of an individual under the influence of a SC was Daniel Rozga in 2010.[15] In 2011, Brandon Murphee took his life a few months before he was to start college.[16] Lamar Jack collapsed during basketball practice after smoking a SC. A few days later his coroner's report said he died from "drug toxicity and organ failure."[17] Max Dobner, 19, crashed his car into a house a few minutes after telling his brother he had just smoked "legal marijuana."[18] This list only describes a few of the deaths associated with SC's. The dangers of the SC's are significant and as use of the compounds continues more dangers are being discovered.

There is also an inherent danger associated with using SC's that goes beyond what the compounds do in the body. The compounds can have much stronger effects than marijuana. This is because many SC's demonstrate a higher affinity for cannabinoid receptor 1 (CB_1) and/or

cannabinoid receptor 2 (CB_2) then marijuana exhibits. Additionally, several of the SC's act as full agonists for cannabinoid receptors, marijuana is only a partial agonist and thus its effect on the cannabinoid system is not as great.[6, 19] Such differences make SC's dangerous for users who are familiar with marijuana and assume that the SC's will be similar. An additional danger is the amount of SC's in an herbal incense package can vary. Thus, if a user switches the brand of product he is using or even buys a new package of the same brand there is a chance smoking the same amount could lead to an overdose.[20] N. Uchiyama et al. analyzed several different brands of the incense and found the amount JWH-018 in one gram of incense to vary from 2.03 mg to 35.9 mg; this danger is compounded when many packages contain 2 or 3 different kinds of SC's.[21] Then, of course, the SC's present in the products are always changing and each SC has a different potency.[11] In 1999 Aung et al. reprted that the naphthylindoles show the highest binding affinity to CB_1 and CB_2 when there is a 3 to 6 alkyl chain from the nitrogen on the indole, with 5 being the highest. JWH-018, interestingly, has a 5 carbon chain and was the first compound used by the producers of Spice.[22] Even the metabolites of SC retain varying amounts of activity towards the CB_1 and $CB_{2;}$ with continually changing compounds comes the risk for continually changing dangers.[2, 22] It is very difficult for health care providers to establish what damage SC's can do to a person and what the best courses for treatment are when every SC and incense product are different.[2, 11]

1.3 Synthetic Cannabis Compounds

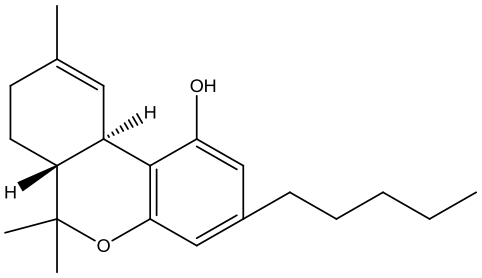
THC Pharma, a German pharmaceutical company, was the first to identify one of the psychoactive ingredients in the herbal incense as JWH-018.[8] The compound received its name from its creator, John W. Huffman. Ever since the elucidation of the psychoactive ingredient in marijuana, Δ^9-THC, a great deal of research has gone into determining how Δ^9-THC reacts in the

body and into creating other compounds that will react similarly. Huffman, along with others, have been involved in this research and it has led to the creation of a large library of SC's. Many of these SC's have been studied at length and their affinity for cannabinoid receptors analyzed and discussed in scientific literature. Thus, the producers of herbal incense had a plethora of information from which to begin their work. The structures of the many SC's derived from this research and subsequently found in the designer drug market can be seen on the next page, as well as the structure for Δ^9-THC. [20, 23] As can be seen, most of the compounds do not resemble Δ^9-THC at all. Many of the compounds are simply analogues of each other. Almost all of the compounds had been studied by scientific researchers before they appeared in the incense products. The exceptions to this are UR-144, XLR-11 and AKB48; before their appearance in incense products there was no information about them in scientific literature.[23]

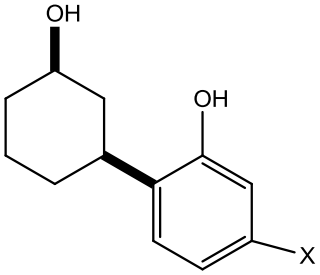
Lawmakers have now outlawed many of the SCs. The temporary ban of the 5 SC's in 2011 lasted one year; the ban was extended for 6 months in 2012. Finally, in July 2012 President Obama signed into law section 1152 of Food and Drug Administration Safety and Innovation Act which contains the Synthetic Drug Abuse Prevention Act of 2012[24]. The act makes most of the SC's found in incense products Schedule 1 substances. Despite the many compounds this act outlawed, in May 2013 the DEA had to temporarily make UR-144, XLR-11 and AKB48, the three new SC's incense producers found, Schedule 1 substances. This temporary placement will be in effect for two years.[23]

The producers of the herbal incense have clearly demonstrated their ability to create a product that could be appealing to drug users for years to come. They have done their research to determine which SC's will provide the most potent high and they have successfully stayed one step ahead of US federal law to keep their product legal. Even as different SC's are made illegal,

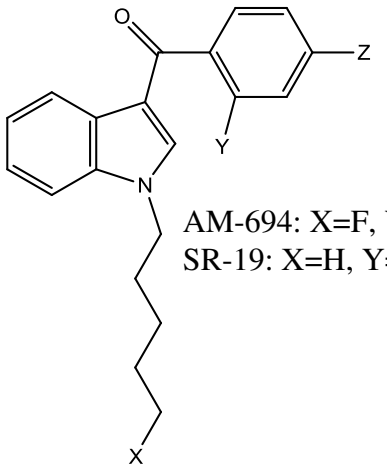
users can still view them as “safer” because they may not be detected on many drug screening tests. Laws can do no good if they cannot be enforced and the laws for SC’s cannot be enforced without ways to quickly detect the compounds in SC products. With the large number of SC’s in existence, forensic scientists and researchers have a huge task to develop methods that will identify which one is in a particular sample.



Δ^{9}-THC

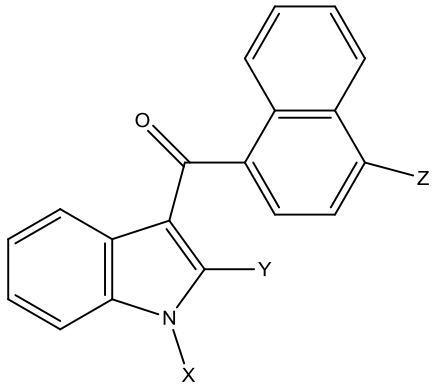


CP 47, 497 X= 1, 1-dimethylheptyl
CP 47, 407 (C8) X= 1,1 dimethylnonyl

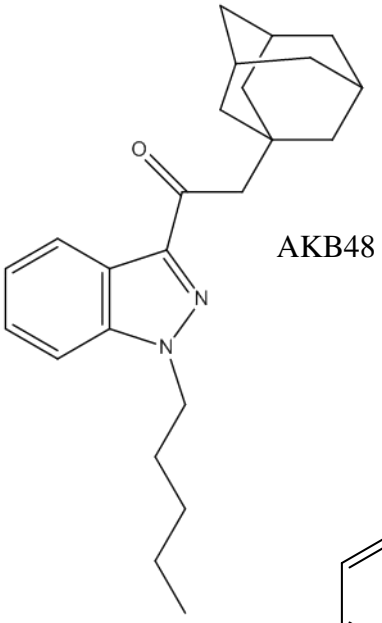


AM-694: X=F, Y=I, Z=H
SR-19: X=H, Y=H, Z=methoxy

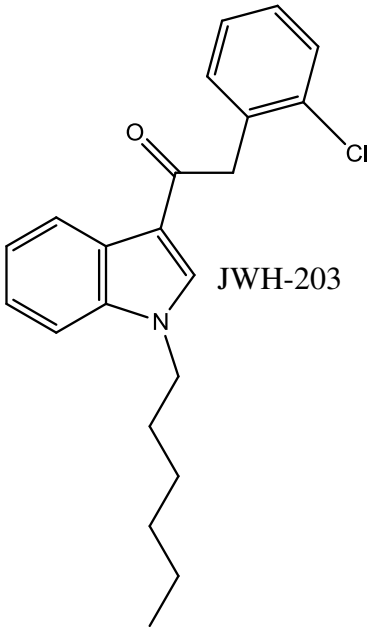
Figure 1: (cont. on next page) Structures of THC and many SC’s. The SC’s shown are the compounds specifically named in Synthetic Drug Abuse Prevention Act of 2012 and the 3 new SC’s banned in May 2013.



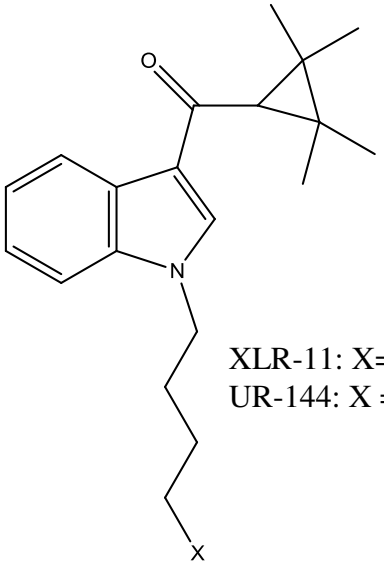
JWH-018: X= pentyl, Y=H, Z=H
AM-2201 X=fluorpentyl, Y=H, Z=H
JWH-200 X=(4-morpholinyl)ethyl, Y=H, Z=H
JWH-398: X= pentyl, Y=H, Z=Cl
JWH-019: X=hexyl, Y=H, Z=H
JWH-122: X=pentyl, Y=H, Z=methyl
JWH-073: X=butyl, Y=H, Z=H
JWH-081: X=pentyl, Y=H, Z=methoxy



AKB48



JWH-203



XLR-11: X=CH_2-F
UR-144: X = CH_3

Figure 1: (cont. from previous page) Structures of THC and many SC's. The SC's shown are the compounds specifically named in Synthetic Drug Abuse Prevention Act of 2012 and the 3 new SC's banned in May 2013.

1.4 Presumptive Tests

Our goal was to develop a presumptive test for as large a subset of these compounds as possible. Presumptive tests are frequently used by law enforcement personnel for rapid screening at a crime scene. A presumptive test can indicate that a sample *does not* contain the compound of interest, or that the compound *might* be present. A presumptive test is not conclusive; further testing must be done to confirm a positive result. Presumptive tests come in several different forms.[25] The most common for drug analysis is a spot test. In a spot test, a small amount of sample is added to the test reagents and the appearance of a particular color indicates a positive result. Microscopy can be used to identify trichomes on plant matter; this is commonly used for cannabis. Microcrystalline tests can identify certain drug types by their crystal structures.

The concept of a presumptive test is quite simple and may even seem archaic when the technology for portable Fourier Transform Infrared (FT-IR) spectrometers and mass spectrometers is rapidly growing. These instruments are capable of identifying an unknown compound in only a few minutes and are very easy to use. However, these instruments are also very expensive. A police department might be able to afford one, but certainly not one for every police officer.

Presumptive tests also have some inherent pitfalls. They are susceptible to false positives and even a positive result will always require further testing. Spot tests are somewhat subjective since they rely on color identification which can vary depending on impurities present and the testing conditions. However, a good presumptive test offers several benefits. The most apparent are they are inexpensive and do not require much time. Forensic labs are subject to a large work load and the faster they can analyze a sample the better. A presumptive test can decrease the number of samples that need to be submitted for more time consuming and more expensive

confirmatory test. If the presumptive test can be accomplished in the field, the number of samples a forensic lab even receives can be lowered.

There are many spot tests in existence that are relatively specific for particular drugs.[26] The Scott's test for cocaine uses cobalt (II) thiocyanate to form a blue metal complex with cocaine.[27] Eherlic's reagent can test for indoles that are not substituted at the 2 position; it forms blue to violet product with hallucinogens such as LSD and ergotamine. And Simon's reagent can be used to form a blue Simon-Awe complex with secondary amines such as methamphetamine.

Our investigation has focused primarily on developing a spot test and understanding the limitations of the chemistry on which the test is built. Given the vast number of SC's and their varying structures, a spot test probably could never be selective enough and still apply to all of them; ultimately a series of tests will be needed. Given 1) the many well-known reactions that involve ketones and 2) the fact that a large portion of the compounds of interest contain a ketone flanked by aromatic groups, we chose to focus our efforts on this functional group as a basis for a spot test. Obviously a spot test that relies only on a reaction with a ketone will not be specific; steps will have to be taken to increase specificity. However, our goal was to first find a viable reaction. Specificity can be increased later using techniques such as solubility, reactivity and chromotography.[26, 27]

1.5 Requirements for Spot Tests

There are many spot tests commercially available that are used in field by police officers. They generally consist of an ampoule or package of ampoules containing the testing reagent(s) with the "positive" color printed on the side. The tester simply places a small amount of the substance in question into the container, mixes the reagents and compares the resulting color to

the color printed on the package. If the colors match, the substance can be sent for further testing to confirm its identity.

Such tests must meet several requirements outlined by the National Institute of Justice (NIJ). [28] The test must be specific for the compound being tested; if the test produces a red color with the drug for which it is designed, it must not cause a red color when reacted with other substances. The protocol also provides a list of common compounds for which the presumptive test should not yield a positive result (Table 1). In addition to specificity, all of the reagents used in a presumptive test must be safe and stable. The test must also be robust and withstand varying testing conditions such as heat and humidity. Finally, the limits of detection and the time required for the test to be completed must be well documented. While these requirements make sense, they add significantly to the challenge of designing a spot test. Our strategy in this

Acetominophen	Mace
Alprazolam	Meperidine HCl
Aspirin	Methaqualone
Baking Soda	Methylphenidate HCl
Brompheniramine Maleate	Nutmeg
Chlordiazepoxide HCl	Phencyclidine HCl
Chlorpromazine HCl	Propoxyphene HCl
Contac	Pseudoephedrine HCl
Diazepam	Quinine HCl
Doxepin HCl	Salt
Dristan	Sugar
Ephedrine HCl	Tea
Exedrine	Tobacco
Hydrocodone tartrate	

Table 1: A list of compounds provided by the NIJ that must test negative when tested with a spot test.[28]

investigation was to determine what types of reactions would be capable of causing a change in color for these SC's. If a reaction was found, ideally it could be made applicable to a field test or at least be used in the laboratory to quickly determine if a SC is in a sample.

2. Background

2.1 JWH-019

As discussed above we wanted to investigate the SC's that contain ketones. Given the reactivity of ketones and the conjugation of the compounds, we hoped a colored product was feasible.

Before a presumptive test could be developed though, at least one of the SC's was needed as a test standard. We chose to make one of the naphthoyl indole compounds. Synthesis of the compounds is well documented and can be done given a few days. Step 1 adds the desired carbon chain to the indole through nucleophilic substitution.[29] The second step of synthesis adds the naphthoyl group to the indole through a Friedel-Crafts acylation(Fig. 2). [30] JWH-019 (Y and Z are hydrogens, and X a hexane chain) was chosen for synthesis because at the time it was not federally banned.

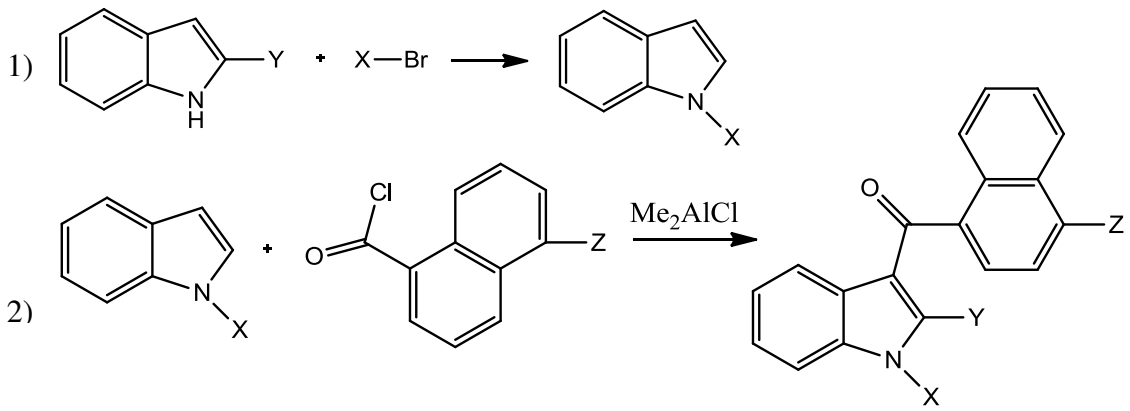


Figure 2: Synthesis of naphthoyl-indole SC's 1) the desired carbon chain is added to the indole through nueclophillic substitution. 2) the naphthoyl group is added to the indole through a Friedel-Crafts acylation.

2.2 Current Test for Synthetic Cannabis

A few months after we began our work, a spot test for herbal incense appeared on the market. The test is sold by M.M.C International B.V. and claims to be able to for identify JWH-018, JWH-073, JWH-250, CP-47, 497, HU-210 and AM-2201. The test kit comes with enough supplies to test 10 samples for eighteen dollars. The direction say when a small amount of sample that contains any of the compounds listed above is added to the testing reagent, the reagent will go from clear to a yellow/brown color. It appeared that our work might already be done. The product was purchased and tested and it quickly became apparent that the test was not capable of what it claimed.

2.3 Duquenois-Levine Reagent

The Duquenois-Levine reagent is a common presumptive test for marijuana. When a small amount of marijuana is added and the reagents of the test mixed in the appropriate order 2 layers form. The bottom layer is a transparent light purple to pink color while the top is very dark purple. There is a considerable amount of controversy surrounding the test due to the several compounds that result in similar colors, such as mace and nutmeg.[31] Since SC's are not molecularly similar to Δ^9-THC, ideally the test should not be positive for SC's. However, since both marijuana and SC products are generally found as plant material it is possible that the herbal incense could be mistaken as marijuana and tested with the Duquenious-Levine reagent. Given that the test is known to become colored with several other substances SC's may yield a positive result or an entirely different color. Documenting the results will provide useful information.

2.4 Benzophenone

While having one of the SC's on hand is possible, there are many benefits to doing much of the experimental work with a more readily available compound. Synthesis of SC's is time

consuming and can be problematic. Buying JWH-019 is not a realistic option when a mere 5 mg costs $73.23 from Cayman Chemicals.[32] Also, having a large amount of a known recreational drug in the lab is undesirable. Thus, a compound was needed that is easily obtained, inexpensive, and has structural similarities with the JWH analogues. Benzophenone (BP) was chosen to fill this role. Although it is certainly different than the compounds found in SC products, it contains some key similarities and offers numerous benefits. As can be seen in Figure 3, BP contains a ketone centered between aromatic groups; this is structurally similar the SC's that will be targeted in this study. BP is inexpensive, does not have to be synthesized and is easily available in large quantities. Finally, many studies have already been done regarding BP's reactivity. Thus, all of our experimental work began with BP. Once an experiment was successful using BP, the experiment was done using JWH-019.

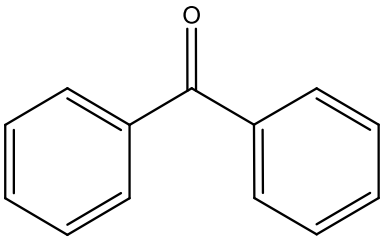


Figure 3: Benzophenone

2.4.1 Sodium-Benzophenone Ketyl

The first compound we looked at was the sodium-BP ketyl (Fig 4). This ketyl is often used to dry organic solvents because it reacts with O_2 and water to form non-volatile species. Thus, when the solvent containing the ketyl is distilled, it is very pure. What makes this reaction significant to us is the Na-BP ketyl is dark blue.[33] Sodium, obviously, is not the ideal reactant for a spot test, but the reaction is known to work and is colored. By comparing the Na-BP ketyl with the Na-JWH-019 ketyl we could gain valuable information: would the JWH-019 ketyl form, how quickly would it form and what color would it be?

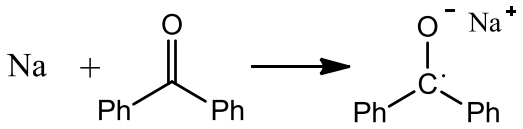


Figure 4: Synthesis of the sodium BP ketyl.

2.4.2 Benzopinacol

A well-known reaction using BP uses UV light to produce benzopinacol. The mechanism for this reaction can be seen in Figure 5. When a solution of BP in isopropanol is irradiated hydrogen from the isopropanol moves to the oxygen on BP leaving the diphenyl ketyl and the dimethyl ketyl. The radical from the dimethyl ketyl then transfers to BP resulting in acetone and another diphenyl ketyl. The dimerization of 2 diphenyl ketyls results in benzopinacol.[34, 35] It is reasonable that the same reaction could occur with JWH-019.

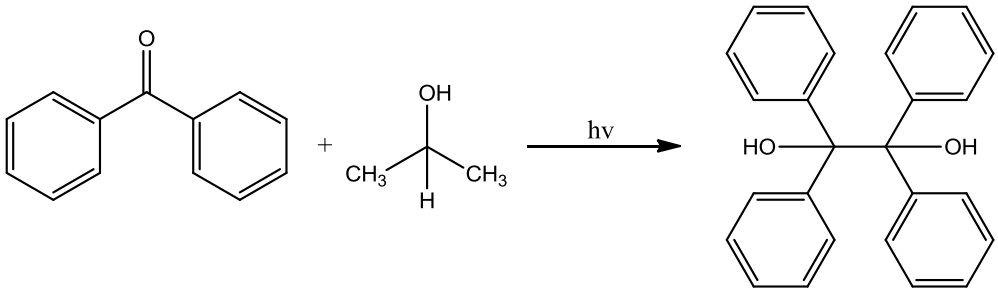


Figure 5: Exposing a solution of BP in isopropyl alcohol to UV radiation results in the formation of benzopinacol.

2.5 Producing Colored Compounds

Since our goal was to produce a colored compound, we first had to consider what makes a compound colored. Colored compounds absorb wavelengths in the visible region (380-780 nm). JWH-019 (and BP) by itself is white and absorbs in the near ultra-violet (UV) region (200-380 nm). There are some generalizations about what will cause a compound to absorb in the

visible region. It needs to be capable of π-π* electronic transitions, and thus have double bonds. The electron transitions σ-σ* and n-σ* both require too much energy and will absorb wavelengths that are too short to provide color. Molar absorptivity and the wavelength absorbed tend to increase when a compound becomes more conjugated. JWH-019 already contains several conjugated double bonds, increasing the conjugation slightly may cause the absorption maximum to shift to a longer wavelength and make the compound colored.[36, 37]

2.6 Ketone Chemistry

We needed a bathochromic shift to occur as a result of some reaction at the ketone. Ketones can form imines, also known as Schiff bases, when reacted with a primary amine. The ketone first forms a hemiaminal with the amine. The hemiaminal then loses water and a double bond between the carbon and nitrogen forms. The resulting product is an imine. There are several amine derivatives such as hydrazine, H_2NNH_2, and semicarbazide, $H_2NNHC(=O)NH_2$, that undergo the same condensation reaction to form imines called hydrazones and semicarbazones respectively. These imine products are often highly crystalline and colored.[38] Given the already conjugated structure of JWH-019 we wanted to see if there was an amine that would react with it and produce a colored imine. We investigated several different amines: 2,4-dinitrophenylhydrazine (DNHP), hydrazine, neutral red and aniline. DNHP is already known to react with most ketones and hydrazine already has a well-established procedure to make a colored compound with BP. While the reagents and protocol required for these reactions cannot be used in a field test, we wanted to see if they would happen with JWH-019 and establish a baseline for producing a colored compound from the SC.

2.6.1 Brady's Reagent

A common test for ketones and aldehydes is Brady's reagent. Brady's reagent is a solution of DNHP in methanol and sulfuric acid. When a ketone or aldehyde is added to the reagent a yellow to red crystalline dinitrophenylhydrazone (DNPH) is formed (Fig. 6).[39] The reagent does not make an ideal spot test. The first problem is DNHP is explosive and thus cannot be used in the field. Second, DNHP reacts with any aldehyde or ketone and while the resulting precipitates vary slightly in color depending on the conjugation of the compound, the colors are not different enough to exclusively identify a JWH analogue.

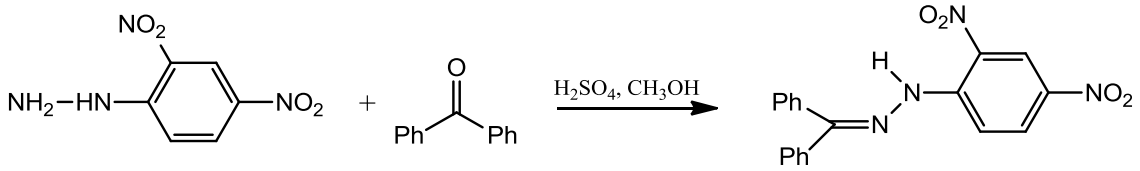


Figure 6: Reaction of DNHP with BP. Resulting DNPH is an orange crystal.

2.6.2 Diphenyldiazomethane

BP hydrazone was of interest because when oxidized to diphenyldiazomethane the solution changes from clear to purple. BP hydrazone can be produced by refluxing BP with hydrazine hydrate (Fig. 7).[40] Mercuric (II) oxide is often used to oxidize BP hydrazone.[41] For the oxidation to go to completion is reported to take 6 hours, however, we do not need the reaction to go to completion, we just need enough to be produced that a change in color can be detected. Other oxidants such as $NaNO_3$, K_2CrO_4, $FeCl_3$ and Ag_2O can also be used.[42] The color change is excellent for a spot test, but the synthesis of benzophenone hydrazone is time consuming (about 10 hours) and requires heat.

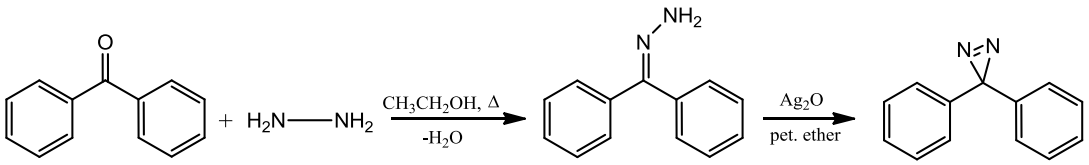


Figure 7: BP reacts with hydrazine to make BP hydrazone. When BP hydrazone is oxidized purple diphenyldiazomethane forms.

2.6.3 Aniline

Aniline is another primary amine that was tested. Given the benzene ring on aniline, it was feasible that the resulting immine of reacting aniline with BP might be colored (Fig 8). Enchev et. al. reported the formation of a yellow product when 2-acetyl-indane-1,3-dione was reacted with aniline.[43] We hoped that the reaction of BP and aniline might occur similarly.

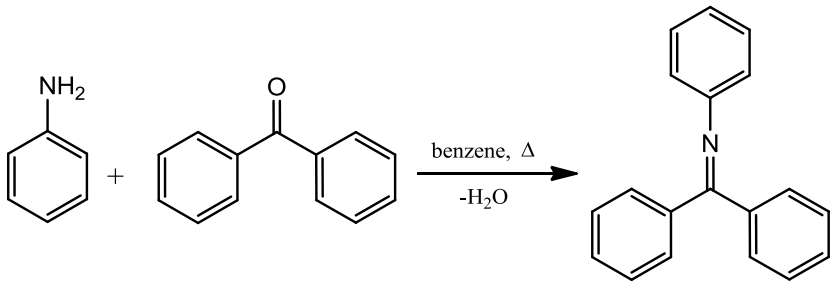


Figure 8: Reaction of aniline with BP.

2.6.4 Neutral Red

Another possible Schiff base could come from neutral red (Fig. 9). A significant benefit of neutral red is it is already colored and reacting it with JWH-019 could significantly change its absorption and thus its color.

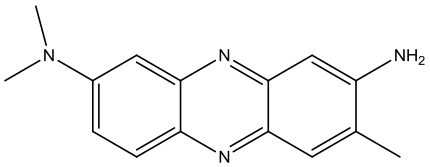


Figure 9: Neutral red contains an amine that could form a possible Schiff base with JWH-019.

2.7 Metal Complexes

Another possible way to achieve color is with transition metals.[36] Transition metals readily form metal complexes in an array of colors when they bind with molecules or ions called ligands. The ligands donate electrons to the metal to form a covalent bond. Each metal is capable of binding with a particular number of ligands; this number is called the *coordination number.* The array of resulting colors can be explained by crystal field theory. Crystal field theory assumes that the d orbitals in the metal lose their degeneracy when the metal complex is formed. The d orbitals located between the ligand-metal bond axis decrease in energy and the d orbitals along the ligand-metal bond gain energy. When there are empty d orbitals an electron can be excited from the lower energy d orbital to the higher. The energy required for this transition is in the UV-Vis region and thus the complexes are often colored. The spot test for cocaine actually uses this chemistry. Cobalt (II) thiocyanate is used to form a bright blue complex with cocaine.[26, 27]

Another possibility when working with metal complexes is the occurrence of a charge transfer complex. When an electron from the ligand moves to the metal a charge transfer occurs and is often accompanied by a change in color. These transfers often have very high molar absorptivities which could be very beneficial for a presumptive test in which there would only be a small amount of sample.[36, 44]

Applying this chemistry to JWH-019 (or BP) will take a few steps. The ketone on the compounds needs to be made into a good ligand. Again, we looked at a condensation reaction to form an imine.

2.7.1 Semicarbazide

Semicarbizide is capable of both being a ligand and reacting with the ketone (Fig. 10). Vijayan et al. reports producing crystals of the semicarbozone of BP.[45] The crystals were colorless, but showed strong absorption at 280 nm. Semicarbazones forming metal complexes is well established and it is possible that the resulting semicarbazone products of BP and JWH-019 may be colored when they form a complex with a metal.[46]

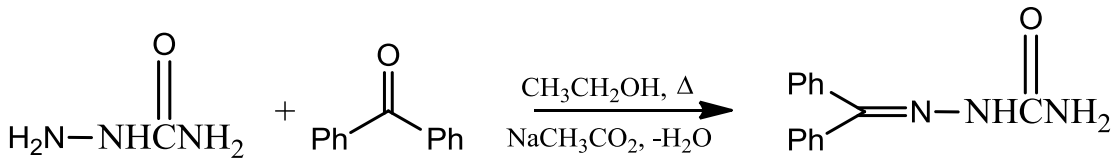


Figure 10: Reaction of semicarbazide with BP. The product may make a colored metal complex.

2.7.2 Ethylenediamine

Ethylenediamine (en) is a common ligand and amine that could make an imine with BP as well. By itself en is known chelate Cu^{2+} ions in an aqueous solution. When 1 en forms a complex with Cu^{2+} the solution changes from the bright blue to dark blue. When 2 ens form a complex with $Cu^{2+}$the color changes to dark violet.[47] It is possible that the imine formed with BP and en will still form a complex with Cu^{2+} and if the resulting color is different from the $[Cu(en)2]^{2+}$ complex. There are two possible products that could come from reacting en with BP, in one BP reacts with the en, in the other both ends of the en react with a BP (Fig. 11).

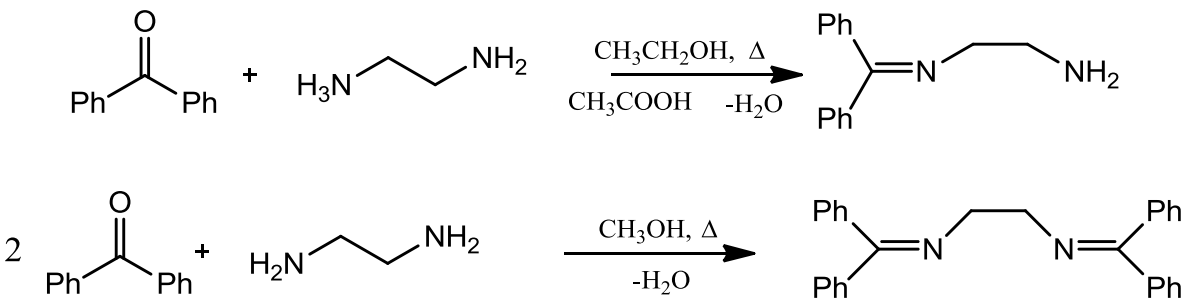


Figure 11: When en is refluxed with BP, it could react with 1 or 2 BP's

2.8 Microcrystalline Identification

Microcrystalline identification of illicit substances is another form of presumptive testing. A small amount of the sample in question is dissolved in a few drops of a specific solvent and the resulting solution it spotted onto a microscope slide.[48] If the resulting crystals have the characteristic structure of the drug they are being tested for, the sample is positive and is sent for confirmatory testing. The tests are relatively easy to perform and require a very small amount of sample and reagents. The sample can even be recovered from the slide and used in the confirmatory test, which is very beneficial if the original sample is very small.[49] Of course such a test cannot be used by police officers in the field, but it can save time and money in forensic laboratories.[50] There are several common microcrystalline tests. The "date rape drug" γ-hydroxybutyrate (GHB) can be identified be its crystals in a solution of silver nitrate and lanthanum nitrate.[49, 51] Methadone produces unique crystals in mercuric chloride. Recently, the designer drugs mephadrone, benzylpiperzine and 5,6-methylenedioxy-2-aminoindane have also been shown to yield unique crystals in mercuric chloride.[52]

Many of the SC's are structurally similar enough that their crystals should have the same shape and a microcrystalline test could be designed for them. A significant problem in using a microcrystalline test to identify SC's is that SC's are usually found sprayed on plant material,

thus the drugs would have to first be extracted and then crystallized. This could create two problems. First, a large amount of the incense might be needed to extract enough of the SC to yield crystals. Second, impurities in a sample can cause interferences in crystallization, so the extract would have to be relatively clean.

3. Experimental

3.1 Reagents

Indole, potassium hydroxide (KOH), 1-bromohexane, magnesium sulfate anhydrous ($MgSO_4$), benzophenone (BP), vanillin, aniline, semicarbazide HCl, copper (II) nitrate hemi(pentahydrate) ($Cu(NO_3)_2 \cdot 2.5H_2O$) and silica gel were purchased from Alfa Aeser. Dimethylaluminum chloride (Me_2AlCl), 2-naphthoyl chloride, acetaldehyde, sodium metal, ethylenediamine (en), and neutral red were purchase from Sigma Aldrich. Sodium bicarbonate ($NaHCO_3$), hydrochloric acid (HCl), isopropyl alcohol and hexanes were purchased from EMD. Ethanol was purchased from Kotec. Chloroform was purchased from BDH. 2,4-Dinitrophenylhydrazine (DNPH) was purchased from DCI America. Mercuric (II) oxide (red) and silver nitrate ($AgNO_3$) were purchased from Fisher Scientific Company Sodium hydroxide (NaOH) and acetic acid were purchased from VWR. dimethyl-sulfoxide (DMSO) was purchased from ACPOS Organics and the deuterated NMR solvent acetonitrile (CD_3CN) was purchased from Cambridge Isotope Laboratories.

3.2 Instrumentation

All proton NMR (^1H NMR) experiments were performed on a Bruker 300 MHz spectrometer, equipped with a broad-band probe. Spin works 2.5 was used to process the data.

A Hewlett Packard 8452A Diode Array spectrophotometer was used for all of the ultraviolet-visible spectrophotometry (UV-Vis) experiments. The data obtained was transferred to Microsoft Excel for further processing.

Fourier- Transform Infrared spectroscopy (FT-IR) was performed on a Perkin Elmer spectrum 100 with a tri-glycine sulfate (TGS) detector and a potassium bromide beam splitter

with a wavelength range of 7800-370 cm^{-1}. All data was transferred to Microsoft Excel for further analysis.

GC-MS analyses were performed on a Varian 450-GC coupled to a 320-MS triple quad mass spectrometer (Bruker Daltonics, Billerica MA). The column used was a Phenomenex Zebron ZB-5HT Inferno column (30 M x 0.25 ID). The injector was set to 310^{o}C at a split ratio of 10:1 and a 1 μL injection volume. The initial column temperature was 70 ^{o}C and was increased at a rate of 15^{o}C/min until 310^{o}C was reached and finally held for 5 min.

Electrospray ionization mass spectrometry (ESI-MS) was performed on a Bruker Esquire-LC ion Trap LC/MS using standard conditions.

Crystals were analyzed using an Olympus BH2 microscope equipped with a crossed polarizer.

3.3 Synthesis of JWH-019

JWH-019 was synthesized in two steps. First, 1-hexylindole was synthesized. Indole (5.86 g, 50.0 mmol), KOH (2.81 g, 50.0 mmol) and 21.05 mL of bromohexane were added to 100mL of DMF. This solution was stirred in a round bottom flask overnight. Then, the solution was washed with water and extracted with ether (3 x 100 mL) and the ether layer was dried with $MgSO_4$. The product was recovered by rotary evaporation. The product was purified using chromatography on silica gel and eluted with petroleum ether.[29] Solvent was removed by rotary evaporation resulting in a light brown oil. The product was analyzed using GC-MS.

1-Hexalindole (5.5 g, 27.3 mmol) was stirred in CH_2Cl_2 under N_2 at 0^{o}C and Me_2AlCl (1 M in hexanes) was added drop wise. The solution was stirred for 30 min., then naphthoyl chloride (6 mL, 24.9 mmol) in 50 mL of CH_2Cl_2 was added and the solution stirred for another hour. The resulting solution was dark pink. The solution was added to 200 mL of cold, 1 M HCl

and then extracted with three portions of dichloromethane. The combined extracts were washed with saturated $NaHCO_3$ and dried with $MgSO_4$. Solvent was removed by rotary evaporation. The product was purified by chromatography on silica gel using 9:1 ether/ethyl acetate to elute the product.[30] Poor separation was achieved as revealed by TLC and GC-MS. The product was further purified by recrystallized in 1:1 hexanes: ethyl acetate. The resulting crystals were white and powdery. The product was confirmed with 1H NMR and GC-MS.

3.4 Current Test for Synthetic Cannabis

The presumptive test sold by M.M.C International B.V. was bought and tested on several different compounds according to package directions. The unopened ampoule was knocked several times against a hard surface to ensure all the test reagents were at the bottom of the ampoule. The top of the ampoule was snapped off and the spatula used to place a small amount of the sample in question into the ampoule. The solution was mixed and then the resulting color compared to the "positive" color printed on the ampoule. The test was performed on an SC product called "space" that is known to contain JWH-018. Another test was performed on the pure JWH-019, pure JWH-018 and Lipton tea.

3.5 Duquenois-Levine Reagent

The Duquenois-Levine test consists of three reagents. Reagent 1 was made by adding acetaldehyde (0.8 mL) and vanillin (0.64 g) to 32 mL of ethanol. Reagent 2 was concentrated HCl and reagent 3 was chloroform. The test was performed by adding a small amount of sample to a test tube along with 200 µL of reagent 1. The test tube was shaken for 1 min. and 200 µL of HCl were added. The solution was shaken for a few seconds then chloroform was added and mixed.[31] Upon sitting the solution formed 2 layers. The color of each layer was observed. Using the above procedure nutmeg was tested and used as a positive control. Then, pure JWH-019 and

the K2 product "Astral Blast Berry Blend" were tested as well as a negative control in which nothing was tested.

3.6 Initial Comparisons of Benzophenone and JWH-019

3.6.1 UV-Vis Comparison of Benzophenone and JWH-019

JWH-019 and BP were compared using UV-Vis spectrophotometry. A 93.8 μM solution of BP in methanol was prepared, and its absorbance spectra taken. A 46.9 μM solution of JWH-019 was prepared and its absorbance spectra taken.

3.6.2 Sodium-Benzophenone Ketyl

Air was purged from a round bottom flask with N_2. BP was dissolved in dry THF and added to the flask. Then, Na wire was added. The solution immediately turned dark blue. The same procedure was done using JWH-019 instead of BP. The solution took approximately one hour to turn dark yellow.

3.6.3 Benzopinacol

BP (0.084 g) was dissolved in 1 mL of isopropyl alcohol in a quartz cuvette containing a stir bar. After a few minutes of stirring all of the BP dissolved and the cuvette was irradiated with a 1000 watt UV lamp. The cuvette was held in a temperature controlled brass block. After 30 min a precipitate could be seen in the solution indicating benzopinacol had formed.[34, 35]

The same was done using JWH-019 instead of BP. JWH-019 (0.084 g) was dissolved in 1 mL of isopropyl alcohol in a quartz cuvette containing a stir bar. However, after 30 min of stirring the JWH-019 had still not dissolved. The cuvette was heated to about 50 □ C and the all the solid dissolved. However, upon cooling the solid precipitated. An additional 1 mL of isopropyl alcohol was added and the solution heated to 50 □ C. The JWH-019 dissolved and remained in the solution. The brass block was heated to 45 □ C to prevent the unreacted JWH-

019 from precipitating. The cuvette was irradiated for 1 hr and 20 min, no precipitate formed. Upon cooling, small crystals began to slowly fall out of the solution. Crystals were allowed to form overnight and the following day a ^{1}H NMR of the crystals was taken.

3.7 Ketone Chemistry

3.7.1 Brady's Reagent

To make Brady's reagent 1 mL of concentrated H_2SO_4 and 0.25 g of DNHP were added to 50mL of methanol[39]. The resulting solution was bright yellow. Approximately 1 mL aliquots of the reagent were added to two different vials. To one vial a small amount of BP was added and the other a small amount of JWH-019 was added. The vials were observed for several hours and the formation of colored precipitates noted.

3.7.2 Synthesis of Diphenyldiazomethane

BP hydrazone was prepared by adding hydrazine monohydrate (0.8 mL, 16.49 mmol) and BP (2.0 g, 10.98 mmol) to 3.2 mL of ethanol. The solution was refluxed for 9 hours. The product was recrystallized in ethanol. The resulting crystals were thin, long and white with a melting point of 98^{o}C[40]. The crystals were analyzed using FT-IR.

BP hydrazone (0.1 g, 0.51 mmol) and mercuric (II) oxide, red (0.15 g, 0.69 mmol) were added to 0.6 mL of petroleum ether in a small vial. The solution was clear and the red HgO did not dissolve. The vial was shaken for approximately 6 hours and the formation of diphenyldiazomethane observed as the solution turned dark purple.[41]

The same was done using $NaNO_3$, K_2CrO_4, $FeCl_3$, and $(NH_4)_2Ce(NO_3)_6$ instead of HgO, except each vial was only shaken each for 5 minutes. The change in color was observed.

Ag_2O was freshly made using NaOH and $AgNO_3$. 10 mL of 0.5 M NaOH was mixed with 10 mL of 0.1 M $AgNO_3$. Ag_2O precipitated from the solution as a brown powder. Ag_2O was

filtered off. A small amount of the Ag_2O was placed into a well of a spot plate with BP hydrazone and several drops of petroleum ether. The formation of diphenyl-diazomethane was observed as indicated by the solution turning purple.[42]

3.7.3 Reaction of JWH-019 with Hydrazine

JWH-019 (50 mg, 0.141mmol) and hydrazine monohydrate (20 μL, 0.412 mmol) were added to approximately 2 mL of ethanol. The reaction mixture was refluxed at 40°C for 9 hours. The reaction flask was placed in the refrigerator and small light yellow crystals formed in the solution. The powder was analyzed using FT-IR.

The reaction was done again increasing the hydrazine monohydrate to 50 μL and refluxing the solution for approximately 16 hrs. Product was recrystallized in ethanol resulting in small light yellow crystals. Crystals were analyzed using FT-IR.

3.7.4 Reaction of Benzophenone with Aniline

BP (1 g, 5.5 mmol) and aniline (0.5 mL, 5.5mmol) were added to 10 mL of dry benzene. Reaction mixture was refluxed for 5 hours then the solvent was removed by rotary evaporation resulting in a yellow oil. Methanol was then added to crystallize the product. Several attempts of this reaction were made. Crystals would not form. A probable cause for this was the presence of water in the reaction mixture, thus drying the mixture with $MgSO_4$ after 5 hours of refluxing was tried. Still, crystals did not form. Excess solvent was evaporated and an NMR of the product in CD_3CN was taken.

3.7.5 Reaction of JWH-019 with Neutral Red

A stock solution of neutral red was prepared by dissolving neutral red (5 mg, 0.017 mmol) in 25 mL of ethanol. A negative control was prepared by diluting 150 μL of the stock solution with 4 mL of ethanol. A solution of JWH-019 was made by dissolving a few JWH-019

crystals in 3 mL of ethanol. The reaction solution was made by diluting 150 µL of the neutral red stock solution with 3 mL of ethanol and 1 mL of the JWH-019 solution. A UV-Vis spectrum was taken immediately after each solution was made. Both solutions were then gently heated for 1.5 hours and diluted back to 4 mL with ethanol. No significant difference in the color of the solutions could be seen. A UV-Vis spectrum of both solutions was taken. The solutions were allowed to sit overnight. The next day no significant difference in the color of the solutions could be seen and again and UV-Vis spectrum was taken.

3.8 Metal Complexes

3.8.1 Synthesis of Benzophenone Semicarbazone

Semicarbazide HCl (1g, 9.0 mmol) and crystallized sodium acetate (1.5 g, 18.2 mmol) were dissolved 10 mL of water. BP (0.5 g, 2.7 mmol) was then added to the solution. BP is not soluble in water so 50 mL of ethanol was slowly added and the solution shaken until all the BP had dissolved. The solution was allowed to sit for several days.[53]

BP (0.5 g, 2.7 mmol) was dissolved in ethanol. Semicarbazide HCl (0.438 g, 3.9 mmol) and sodium acetate (0.974 g, 11.9 mmol) were dissolved in water. The two solutions were mixed; the resulting solution was cloudy. Ethanol was added and the solution gently heated. Two distinct layers could then be seen in the reaction mixture so more ethanol was added and solution mixed and heated again until a clear solution was obtained. After 3 days there was no crystallization, excess ethanol was evaporated and small white crystals formed[45]. An NMR of crystals in DMSO was taken.

3.8.2 Benzophenone Semicarbazone Metal Complex

0.25 g of the semicarbazide-BP product were dissolved in 2 mL of ethanol to make a 0.520 M solution. 0.1 M solutions of $Cu(NO_3)_2 \cdot 2.5H_2O$, $FeSO_4$ and $NiCl_2$ were made in an

acetic acid buffer with a pH of 5. In different wells of a porcelain spot plate 10 drops of the metal solutions were mixed with 2 drops of the SC-BP solution. The colors of the metal solutions and the metal SC-BP solutions were compared.

3.8.3 Benzophenone Reaction with Ethylenediamine

BP (0.5 g, 2.7 mmol) was refluxed at 60 ^{o}C for 1.5 hours with en (183 μL, 2.7 mmol) in approximately 10 mL ethanol. The reaction was allowed to sit overnight to crystalize; no crystals formed. The reaction was attempted several times; increasing the reaction time to 2 hours and drying the product with $MgSO_4$ both failed to yield crystals. The solutions were analyzed using 1H NMR. Finally, the reaction was done in 5 mL of isopropyl alcohol. Excess solvent was removed by rotary evaporation and ethyl ether added. The reaction sat overnight and crystals formed. The crystals were analyzed using FT-IR and had a m.p. of 50 ^{o}C.

Acetic acid was added to the procedure to act as a Lewis acid catalyst.[54] In a small flask, BP (0.5 g, 2.7 mmol) and en (94 μL, 1.4 mmol), 2 drops of acetic acid and 10 mL of ethanol were added. The mixture was heated for 20 min and yielded a thick yellow solution. The next day small yellowish brown crystals were in the solution. They were recovered by filtration and allowed to dry in a desiccator for several days. The dried crystals were analyzed using ESI-MS.

3.8.4 Benzophenone Ethylenediamine Metal Complex

BP-en was tested to see if it would form a complex with any metals. 0.1M solutions of $Cu(NO_3)_2{\cdot}5H_2O$, $FeSO_4$ and $NiCl_2$ were made in an acetic acid buffer with a pH of 5. The BP-en solution was approximately 0.11 M in ethanol. In separate wells of a spot plate a few drops BP-en solution were mixed with a few drops of each of the metal solutions. The solution containing iron resulted in no change in color. The solution containing copper changed from blue to purple. The solution containing nickel went from a light green to a slightly darker green.

UV-Vis absorption of the BP-en Cu complex was obtained and compared to the absorption of $CuNO_3$ alone, and the en-Cu complex. A UV-Vis spectrum of the following solutions was taken: 0.5 mL of the 0.01M $Cu(NO_3)_2$ diluted with 0.5 mL of ethanol, 0.5 mL of the BP-en solution mixed with 0.5mL of the $CuNO_3$ and 0.5mL of 0.1 M en in ethanol and 0.5mL of the $CuNO_3$ solution. The reaction of en-BP and Cu^{2+} was monitored for 15 minutes.

A comparison of the two complexes was also done in which the en-BP product was not crystalized. A 10 mL stock solution of 0.07 M en was made and 5 drops of acetic acid added. The en-BP product was made by taking 2 mL of the en solution and adding BP (0.0255 g, 0.014 mmol). The molar ratio of en to BP was one to one. The solution was gently heated (~ 50^{o}C) for 30 min and then diluted with 2 mL of ethanol. The resulting solution was yellow. A 0.02 M solution of $CuNO_3$ was prepared in an acetic acid buffer (pH=5) and 2 mL added to the en-BP product. The absorption of the solution was monitored for 20 min. The same procedure as above was done to form the en-Cu complex except BP was not added to the en solution.

3.8.5 Benzophenone Reaction with Two Ethylenediamines

The en was dried by adding 7 g of 4A molecular sieves to 50 mL en. The solution was shaken for 19 hours then the en decanted off. 0.75 g KOH and 2.5 g CaO were added to the en that was removed and shaken for another 19 hrs. The en was then distilled.

2 g (11 mmol) of BP were added to 15 mL of anhydrous methanol in a round bottom flask and stirred until BP was dissolved. 370 µL of dried en (5.5 mmol) were added to the solution and the solution was refluxed at 70^{o}C for 6 hrs. The methanol was then evaporated off under low pressure. A thin yellow oil resulted. Once the product was slightly cooled approximately 5 mL of hexanes were added. The oil and hexanes separated in two distinct layers. The solution was placed in the refrigerator and allowed to sit overnight. The next morning white

powdery crystals had formed. The crystals were filtered off and allowed to dry in a desiccator. The product was analyzed using FT-IR.

The same reaction was tried again except on a larger scale. BP (10.87 g, 60 mmol) and en (2 mL, 30 mmol) were added to 50 mL of anhydrous methanol. The solution was refluxed for 6 hrs, the methanol removed using rotary evaporation and 15mL of hexanes added.[55] The resulting crystals were analyzed on FT-IR.

3.9 Microcrystalline Identification

A small amount of JWH-019 (~10 mg) was dissolved in ethyl acetate (1 mL) and spotted onto a microscope slide. Photomicrographs of the resulting crystals were taken. Upon drying, a drop of ethanol was placed on top of the resulting crystals and allowed to dry. Photomicrographs of the resulting crystals were taken.

The same procedure was done using JWH-018 except only ~5 mg in 1 mL of ethyl acetate was used. Analysis under the microscope revealed he JWH-018 oiled out and no crystals were obtained. The slide was gently heated on a hot plate and placed in a desiccator to dry in hopes of initiating crystallization, but the JWH-018 remained in oil form.

5 mL of ethyl acetate were added to 0.8 g of "Astral Blast: fragrant blend" herbal incense to extract any SC's. The incense was known to contain JWH-018. The solution was stirred for 1 min and the solvent filtered off. The resulting solution was dark green. Excess solvent was then removed by gently heating the solution. Drops of the resulting solution were placed on a microscope slide and when dry a drop of ethanol was added. Crystal began to form, but then turned to oil. Again, the slide was gently heated on a hot plate and placed in a desiccator to initiate crystallization, but no crystals formed.

4. Results and Discussion

4.1 Synthesis of JWH-019

Synthesis of JWH-019 consisted of 2 steps. In the first, 1-hexylindole was synthesized by adding the hexane chain to indole through nueclophillic substitution (Fig. 2).[29] The resulting product was a light brown oil. The product was confirmed to be 1-hexylindole using GC-MS as shown in Figure 12. The GC chromatogram reveals 2 major peaks at 4.3 min and 9.2 min representing unreacted indole and 1-hexylindole respectively. The mass spectrum shows the [M^+] peak at m/z 201.1 and the fragment ion peak resulting from the loss of the pentyl group at m/z 130.

In the second step of synthesis naphthoyl was added to the 1-hexylindole through a Friedel-Crafts acylation (Fig. 2).[30]The resulting crystals were white and powdery. GC-MS data of the crystals dissolved in methanol revealed the presence of JWH-019 (Fig. 13). The GC chromatogram revealed 1 major peak at 16.25 min. The mass spectrum shows the M^+ at m/z 355.2, cleavage at the secondary carbon of the hexyl chain resulted in the peak at m/z 284.1, cleavage of the naphthalene resulted in the fragment at 228.3 m/z and the naphthalene ion peak at m/z 127.0, and cleavage of the 1-hexylindole resulted in the peak at m/z 155. The fragmentation matched literature data for JWH-019.[56]

The final product was further confirmed by ^{1}H-NMR. The solvent used was DMSO (δ 2.5). The NMR and signal assignments can be seen in Figure 14 and is consistent with literature data.[8]

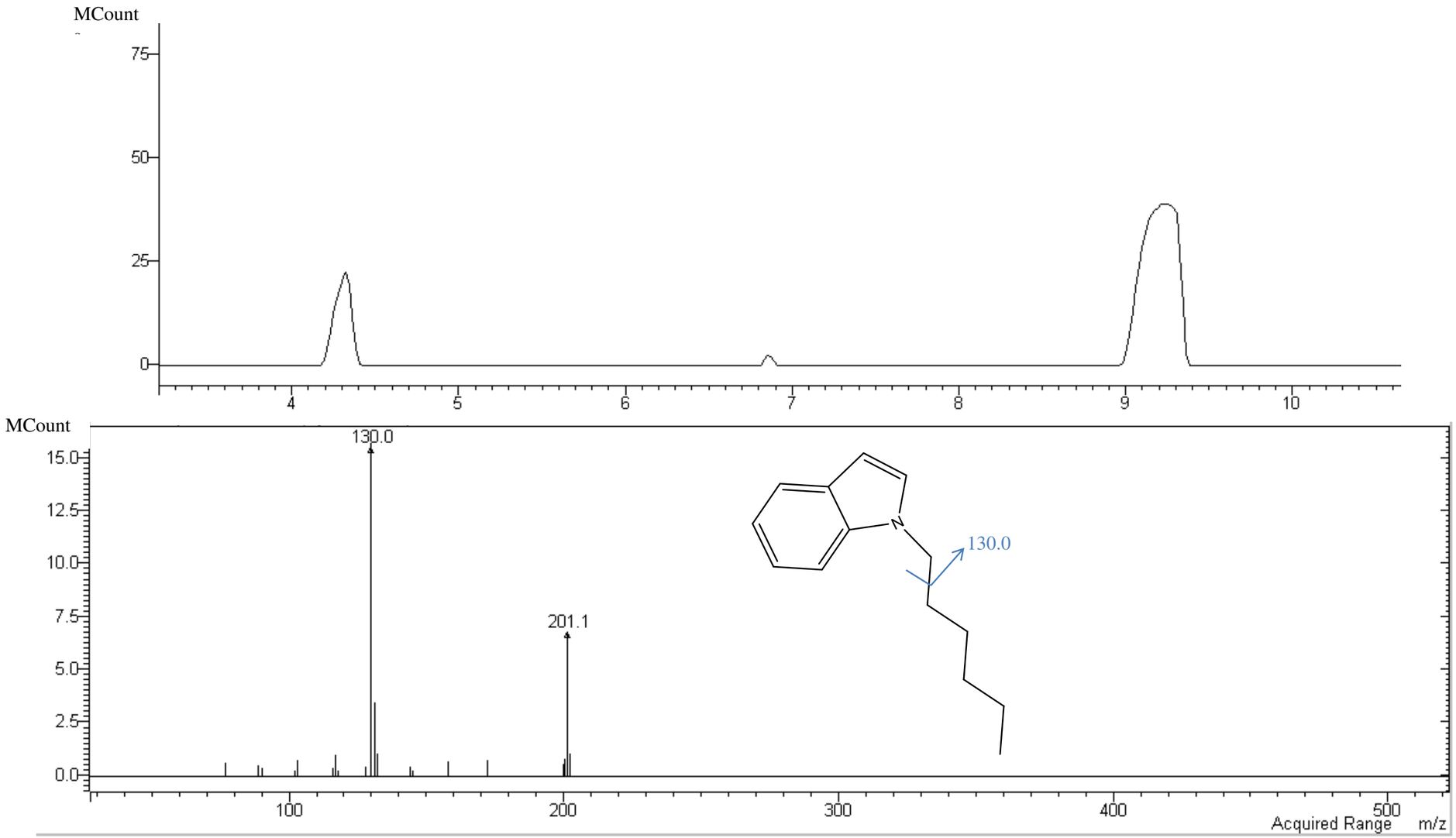


Figure 12: GC-MS data for the first step of the JWH-019 synthesis. The GC chromatogram (top) reveals 2 major peaks at 4.3 min and 9.2 min representing unreacted indole and 1-hexylindole respectively. The mass spectrum shows the $[M]^+$ peak at m/z 201.1 and the fragment ion peak at m/z 130 resulting from the loss of the pentyl group.

m/z	Fragment
355.2	M^+
338.2	$[M\text{-}CH_3]^+$
284.1	$[M\text{-}CH_3(CH_2)_4]^+$
228.3	$[M\text{-}C(=O)C_{10}H_7]^+$
155.0	$[M\text{-}C_{14}H_{18}N]^+$
127.0	$[M\text{-}C_{15}H_{18}NO]^+$

Table 2: Shows fragmentation of JWH-019 as revealed by the mass spectrum below.

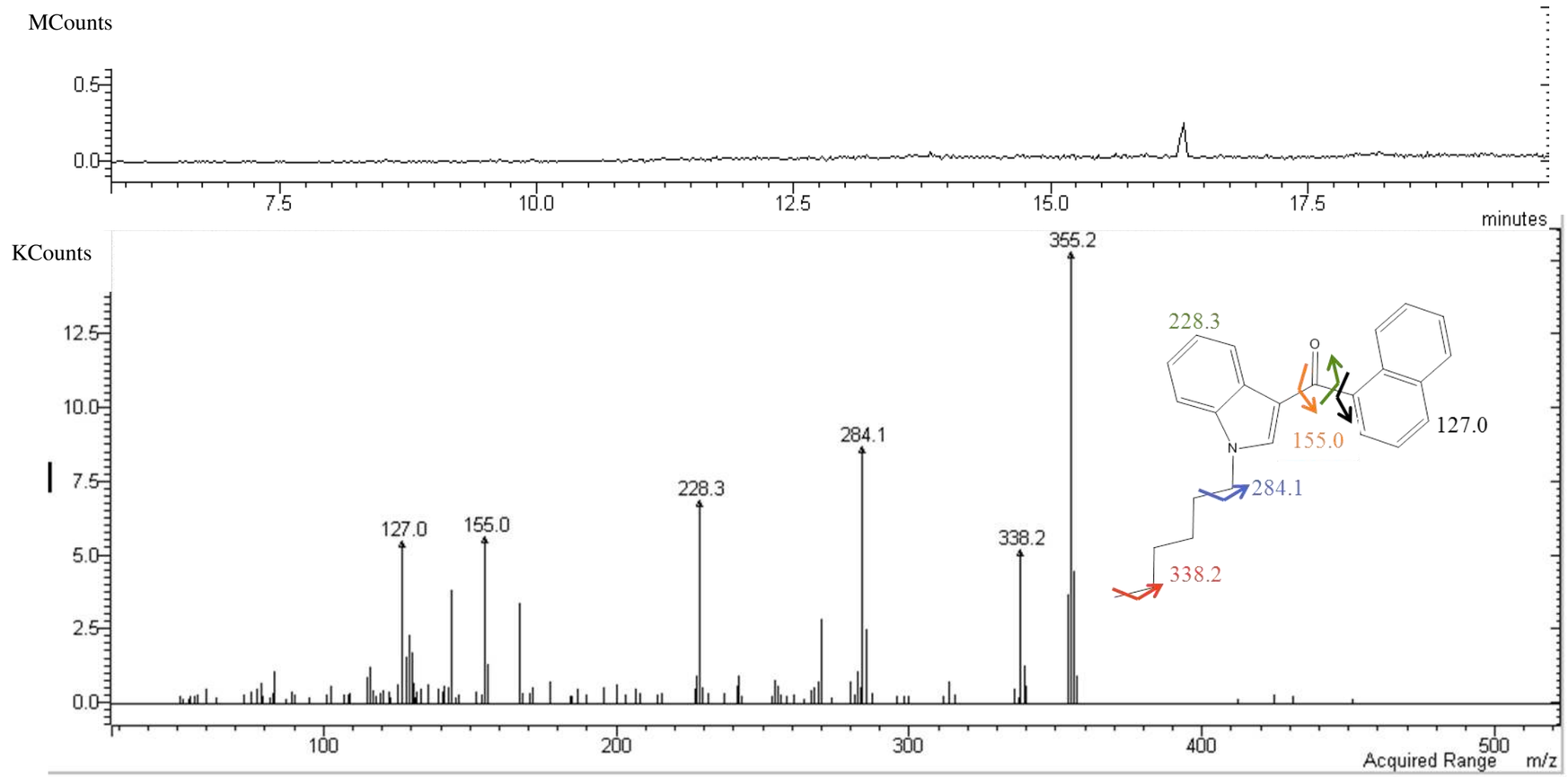
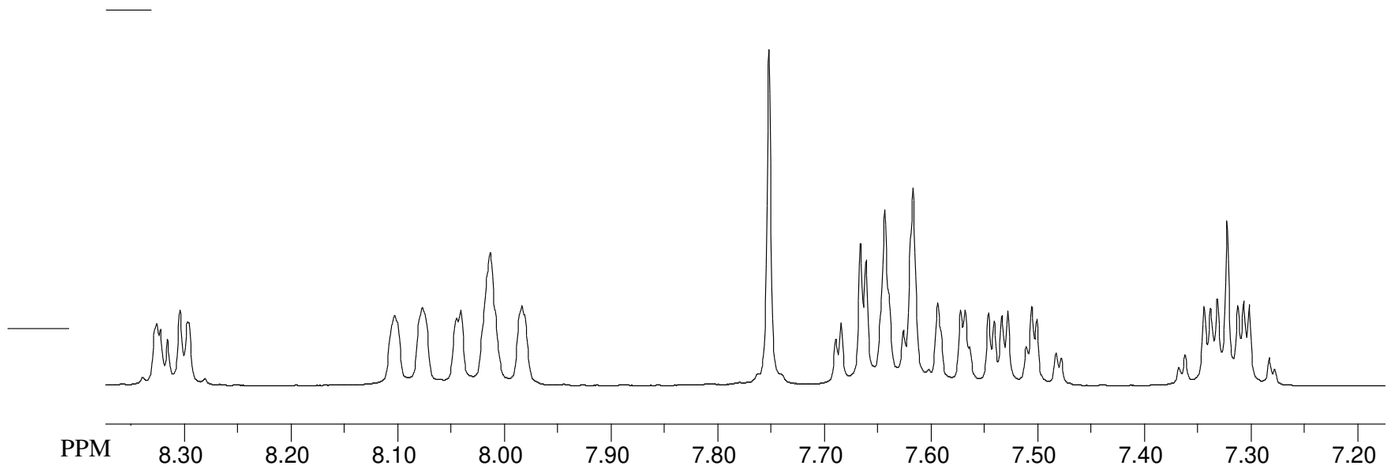
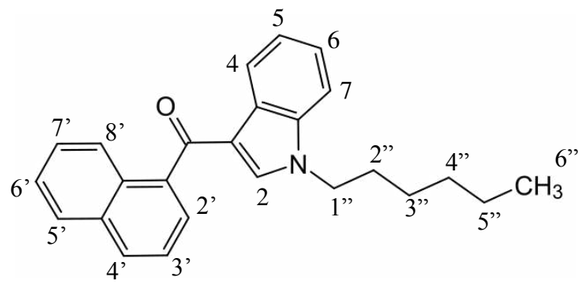


Figure 13: GC-MS of JWH-019. The GC chromatogram reveals 1 major peak at 16.25 min. The mass spectrum shows the fragmentation as pictured.





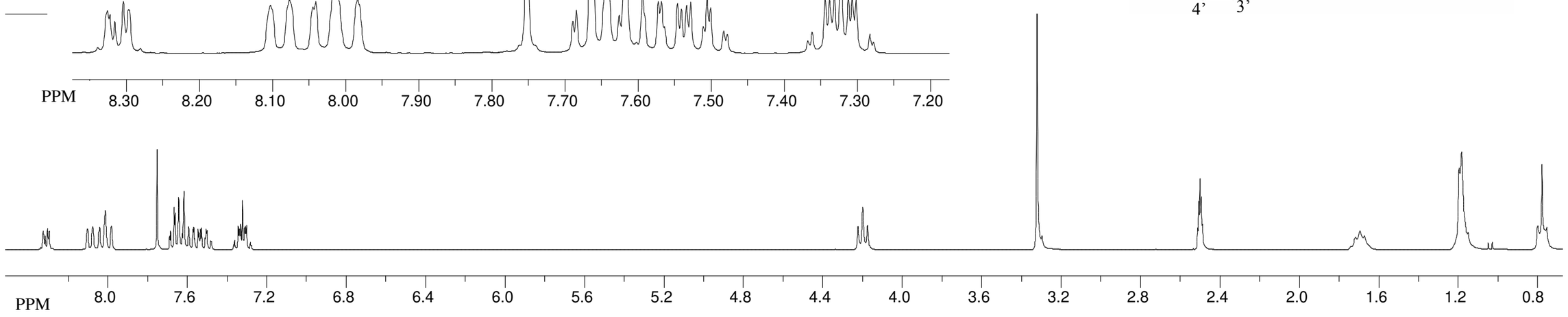


Figure14: The ^{1}H NMR for JWH-019. The peak assignments are as follows: H1”: 4.2 Hz, H2”: 1.7Hz; H3”, H4”, H5”: 1.18 Hz; H6”: 7.78 Hz; H-2: 7.75 Hz; H4: 8.3, 8.32 Hz; H5, 6, 7, 2’, 7’ 7.47-7.70 Hz; H3’, 6’: 7.27-7.4 Hz; H4’, 5’: 7.98, 8.01, 8.04 Hz.

4.2 Current Test for Synthetic Cannabinoids

The commercial presumptive test for SC's sold by M.M.C International B.V. was purchased and tested to determine how effective it was and possibly to use as a benchmark for developing our own test. Pure JWH-019 and JWH-018, a SC product called "Space" and tea, a recommended control for colorimetric tests, were tested.[28] The samples were tested using the procedure provided in the box of tests. Within 5 min. of adding the "Space" incense to the test ampoule the color of the solution began to change from clear to a "rust" yellow-brown color that matched the "positive" color printed on the ampoule. However, when pure JWH-019 was added to a new test, there was no change in color. The addition of pure JWH-018 also caused no change in color. However, when Lipton Tea was added and the color quickly changed to a brown color that was slightly darker than the "positive" color. These results indicate that the test does yield positive results for incense containing SC's, however, it is not the SC's that cause the positive result. Given that tea turned the solution a similar color to the "positive" a reasonable assumption is that the plant material in the incense causes the rust color to appear. Such results indicate that the test cannot be used to identify SC and likely to have an abundance of false positives. A more reliable test is still needed for SC.

4.3 Duquenois-Levine Reagent

A commonly used spot test for cannabis is the Duquenois-Levine test. The test uses 3 reagents the first consists of acetaldehyde and vanillin added to ethanol, the second is concentrated HCl and the third is chloroform. The solutions are added to the sample one by one in that respective order.[31] When reacted with cannabis, the lower layer of the solution is purple and the top layer dark purple. This test was performed on JWH-019 and a K2 product known to contain JWH-018. We were interested in attaining these results since SC's are often found on plant

material that could be mistaken for marijuana and because the test is known to have several interferences. One of these interferences is nutmeg and since nutmeg does not require special permits to possess, as marijuana does, it was used as a positive control. When nutmeg was tested the top layer turned a pale gray purple as described in the literature.[31] When pure JWH-019 was tested the solution turned bright yellow upon the addition of HCl, when chloroform was added the top layer was a bright light yellow and the bottom layer pale yellow. With the SC product, "Astral Blast Berry Blend," the solution was a gold/yellow when HCl was added, when chloroform was added the top layer was gold/yellow and the bottom layer was clear. When no sample was added, the solution turned light yellow after the addition of HCl and when chloroform was added the top layer was green/yellow and the bottom layer clear. In summary, all of the samples (except nutmeg) turned some shade of yellow. The bright yellow of the pure JWH-019 was distinct from the yellow of the incense. The difference between the two is likely due to varying concentration of SC's in each and the interfering plant material from the incense.

Such results are not significantly useful in the context of a presumptive test. Shades of yellow cannot be defined well enough to allow for a positive or negative identification with the naked eye; especially when the intensity of the color will vary depending on concentration. Yellow is also not an ideal "positive" color since various plant materials will likely be yellow in the solution. Tea has already been shown to yield a yellow color.[31] However, the results do reveal that SC's (at least JWH-019 and JWH-018) do not yield a false positive for when tested with the Duquenois-Levine reagent.

4.4 Initial Comparisons of Benzophenone and JWH-019

4.4.1 UV-Vis Comparison of Benzophenone and JWH-019

UV-Vis spectra of JWH-019 and BP **c**an be seen in Figure 15. BP has two major peaks at 210 nm and 254 nm with molar absorptivities of 9660 $M^{-1}cm^{-1}$ and 12300 $M^{-1}cm^{-1}$ respectively. JWH-019 has three major peaks at 224 nm, 246 nm and 316 nm with molar absorptivities of 35100 $M^{-1}cm^{-1}$, 12200 $M^{-1}cm^{-1}$ and 14100 $M^{-1}cm^{-1}$ respectively. The increased conjugation of JWH-019 causes it to absorb at a higher wavelength.

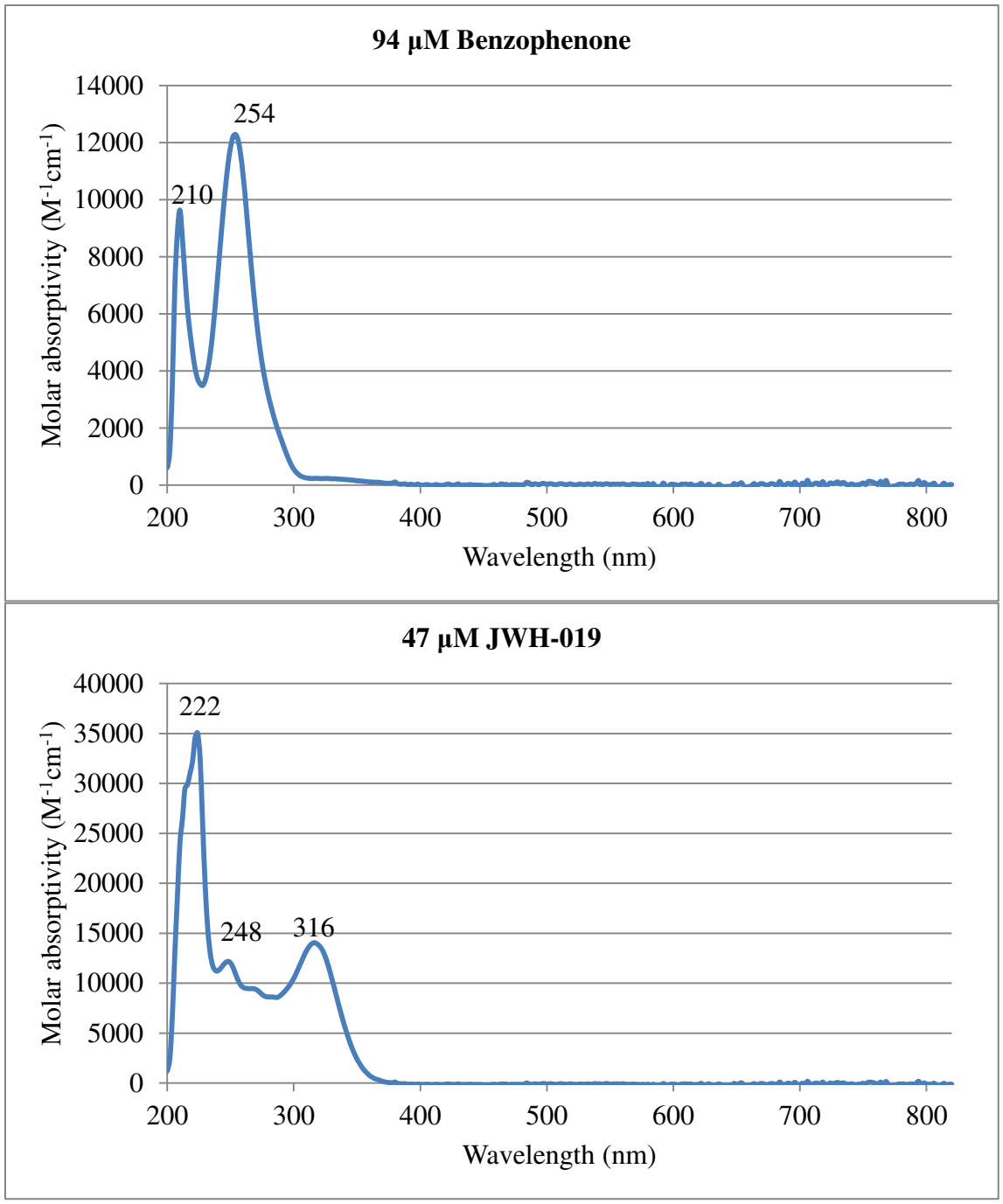


Figure 15: UV-Vis spectra of BP and JWH-019. BP has a λ_{max} 210 (ε = 9660) and 254 (ε = 12300). JWH-019 has a λ_{max} 224 (ε = 35100), 246 (ε = 12200) and 316 (ε = 14100).

4.1.2 Sodium-Benzophenone Ketyl

The Na-BP ketyl formed in dry THF almost immediately after Na was added to a solution of THF containing BP. However, when the same was done using JWH-019 instead of BP the solution took an hour to turn dark yellow. A UV-Vis spectrum was desired to compare the shifts in absorption of BP and JWH-019 and their respective ketyls. This quickly proved difficult for the BP ketyl. When the ketyl was exposed to air it immediately began to turn colorless as it reacted with oxygen. However, when the reaction was carried out in the cuvette and its exposure to air limited the solution was too dark to obtain a good UV-Vis spectrum. The JWH-019 ketyl behaved differently. When exposed to air it remained its original yellow color. Since exposure to oxygen causes the reaction to reverse, it was assumed that the JWH-019 ketyl never formed and something else was causing the yellow color. Since Na is difficult to handle and would not be appropriate for a presumptive test this experiment was abandoned for a less sensitive reaction. However, the results indicate that JWH-019 is much less reactive than BP and it does not form a stable ketyl radical.

4.1.3 Benzopinacol

Benzopinacol formed after 30 min of radiating a solution of isopropyl alcohol and BP with a 1000 watt UV lamp. The formation of benzopinacol was indicated by the formation of a white precipitate in the solution. JWH-019 proved difficult to dissolve in isopropyl alcohol. The solution had to be heated to 45^{o}C for the JWH-019 to remain in solution. The solution was exposed to the UV light was for 1 hr and 20 min; no precipitate formed. After the solution cooled, crystals slowly formed in the cuvette. The resulting crystals were analyzed in DMSO using ^{1}H-NMR to determine if the pinacol of JWH-019 had formed. The resulting NMR was identical to JWH-019 indicating that no reaction had taken place. ^{1}H NMR chemical shifts for JWH-019 pinacol reaction were as

follows: H1”, t, 4.2 Hz, H2”, qi, 1.7Hz; H3”, H4”, H5”, m, 1.18 Hz; H6”, t, 7.78 Hz; H-2, s, 7.75 Hz; H4, dm, 8.3, 8.32 Hz; H5, 6, 7, 2’, 7’, m, 7.47-7.70 Hz; H3’, 6’ m, 7.27-7.4 Hz; H4’, 5’, tm, 7.98, 8.01, 8.04 Hz (see Fig. 14 for δ assignments).

4.5 Ketone Chemistry

4.5.1 Brady’s Reagent

The reaction of BP and JWH-019 with DNHP can be seen in Figure 16.[57] BP began to form a precipitate relatively quickly. Within 10 minutes a bright orange precipitate was beginning to collect at the bottom of the vial. Precipitate continued to form for approximately 2 hours. JWH-019 took much more time to react. At 10 min there was no change in the reaction vial and by 30 min the solution was just beginning to darken. After 1 hr and 45 min a dark rust orange precipitate was just starting to form. At 4.5 hrs precipitate was still forming and collecting on the bottom of the vial.

This reaction was successful; the fact that JWH-019 took so long to react was disappointing, but provided valuable information. The formation of hydrazones from ketones has been shown to be effected by the steric and electronic hindrances from the ketone; ketones with a large amount of steric hindrance often take longer to react and/or require a catalyst.[58] The difference in color between the two hydrazones was expected; as the conjugation of the ketone increases the color of the DNPH crystal shifts from orange to red.[39]

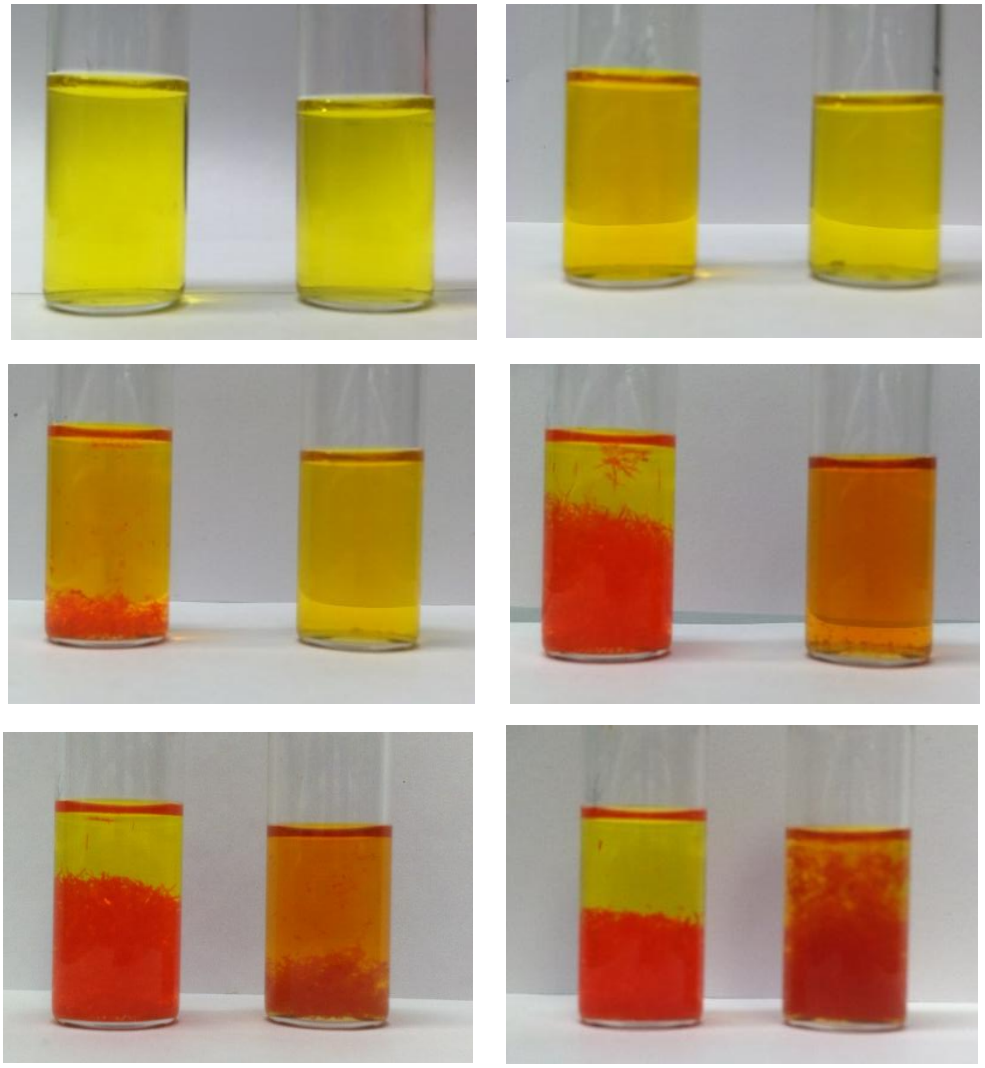


Figure 16: Reaction of DNHP with BP (left vial) and JWH-019 (right vial). The time intervals seen starting from the top left are 0 min, 10 min, 30 min, 1 hr and 45 min, 4 hrs and 30 min and 24 hrs.[57]

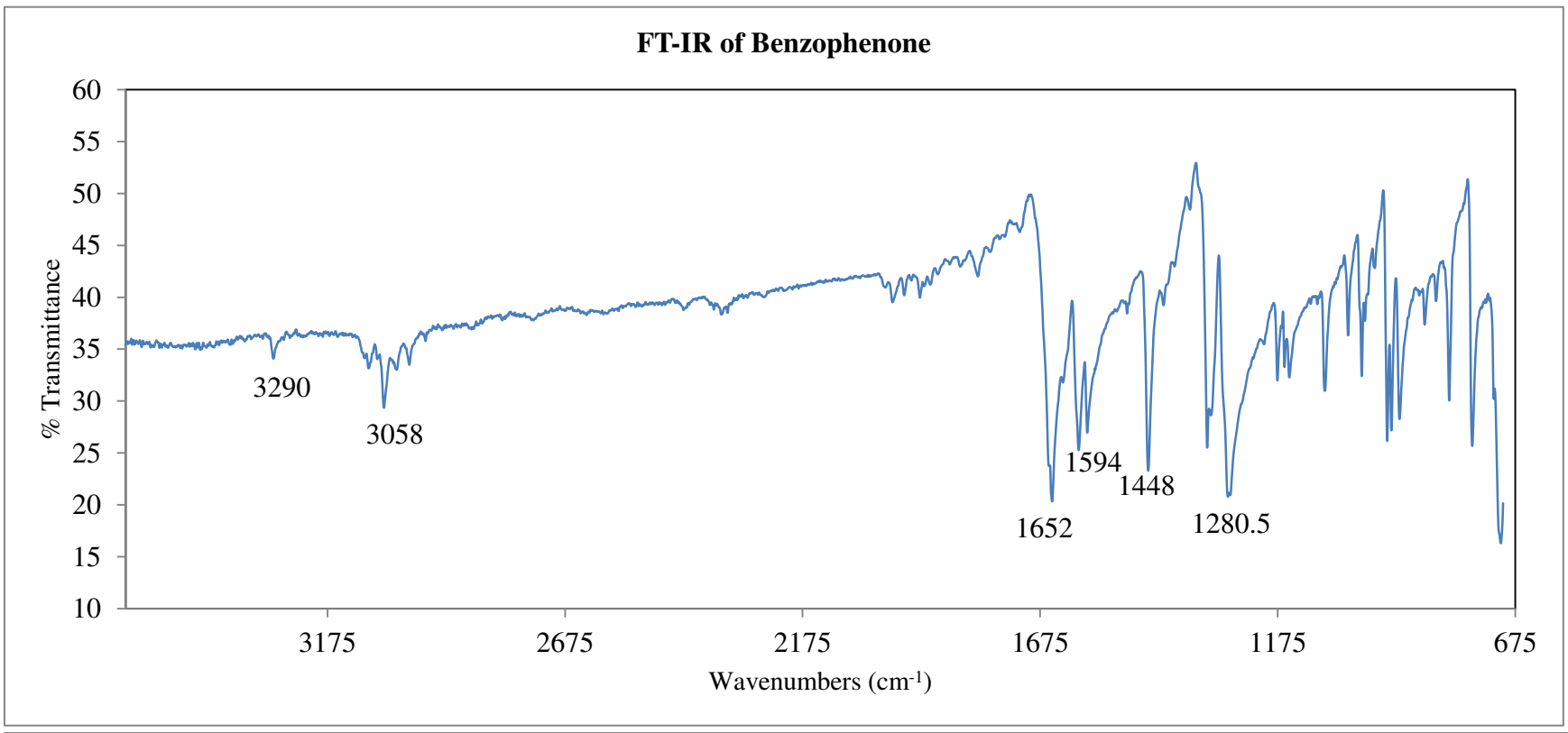
4.5.2 Synthesis of Diphenyldiazomethane

Synthesis of BP hydrazone was achieved by refluxing BP and hydrazine monohydrate in methanol for 10 hrs as described in the literature. The resulting crystals were thin, white sticks. The crystals had a melting point of 98^{o}C, matching literature values.[40] The BP hydrazone product was pressed into a KBr pellet and the identity confirmed using FT-IR. The spectra of BP and BP hydrazone can be seen in Figure 17. The BP spectrum shows a strong peak at 1652 cm^{-1} indicative of a ketone, as well as peaks in the 1500 cm^{-1} region from the carbon bonds from the benzene ring. The ketone peak is absent in the spectrum of BP hydrazone, instead there is a strong peak at 3422 cm^{-1} which is indicative of an amine.

Success was further indicated when the hydrazone was oxidized to diphenyldiazomethane and thus turned purple. A common route to oxidation is to shake the hydrazone with mercuric (II) oxide for 6 hours.[41] This was done and the solution began to turn purple within 45 min. The solution was allowed to shake for 5 more hours and gradually became darker. At the end of 6 hrs the solution was dark purple. Other oxidants were tried to determine if a color change could be detected in less than 45 min. $NaNO_3$, K_2CrO_4, $FeCl_3$, and $(NH_4)_2Ce(NO_3)_6$ were all tried and each proved to take longer than 5 min. Finally Ag_2O was tried. When mixed with a few granules of BP hydrazone dissolved in petroleum ether on a porcelain well plate, the solution began to turn purple in less than a minute.

FT-IR Data for Benzophenone		**FT-IR Data for Benzophenone Hydrazone**	
Wavenumber (cm^{-1})	**Functional Group**	**Wavenumber (cm^{-1})**	**Functional Group**
3290	C=O overtone	3422, 3273	Primary amine N-H vibrations
1652	C=O stretching	1581, 1443, 1492	Carbon bonds in phenyl groups
1448, 1594, 1576	Carbon bonds in phenyl groups	1336	C=N stretch
1280.5	C-C(=O)-C stretching and bending	767.5, 700	Bending of ring C-H bonds
765.5, 705	Bending of ring C-H bonds		

Table 3: FT-IR data for BP and BP hydrazone



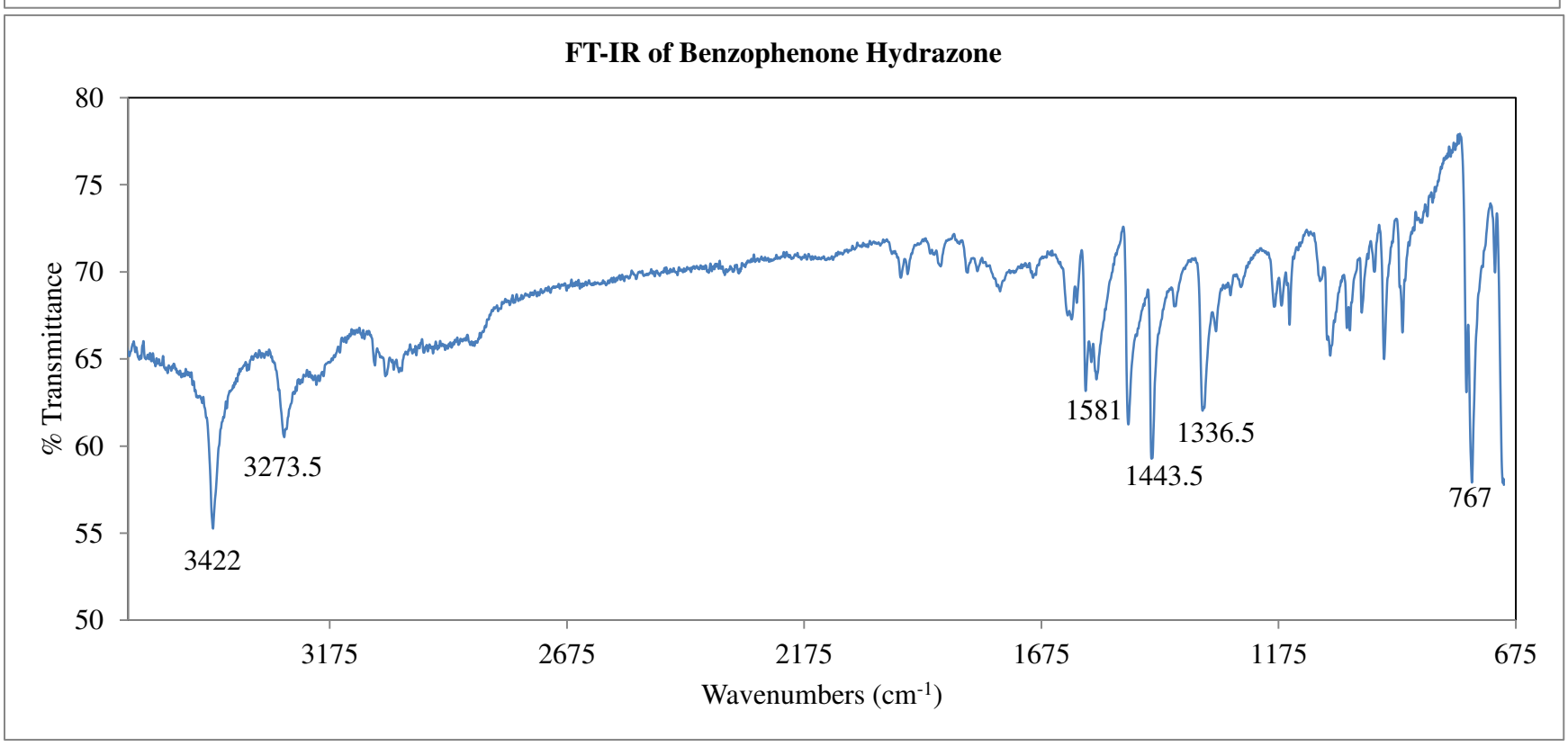


Figure 17: FT-IR spectra of BP (top) and BP hydrazone bottom. The IR for BP shows a strong absorption peak at 1652 for the ketone, this peak is absent in the BP hydrazone, instead there is a strong peak at 3422 which is indicative of a primary amine.

4.5.3 Reaction of JWH-019 with Hydrazine

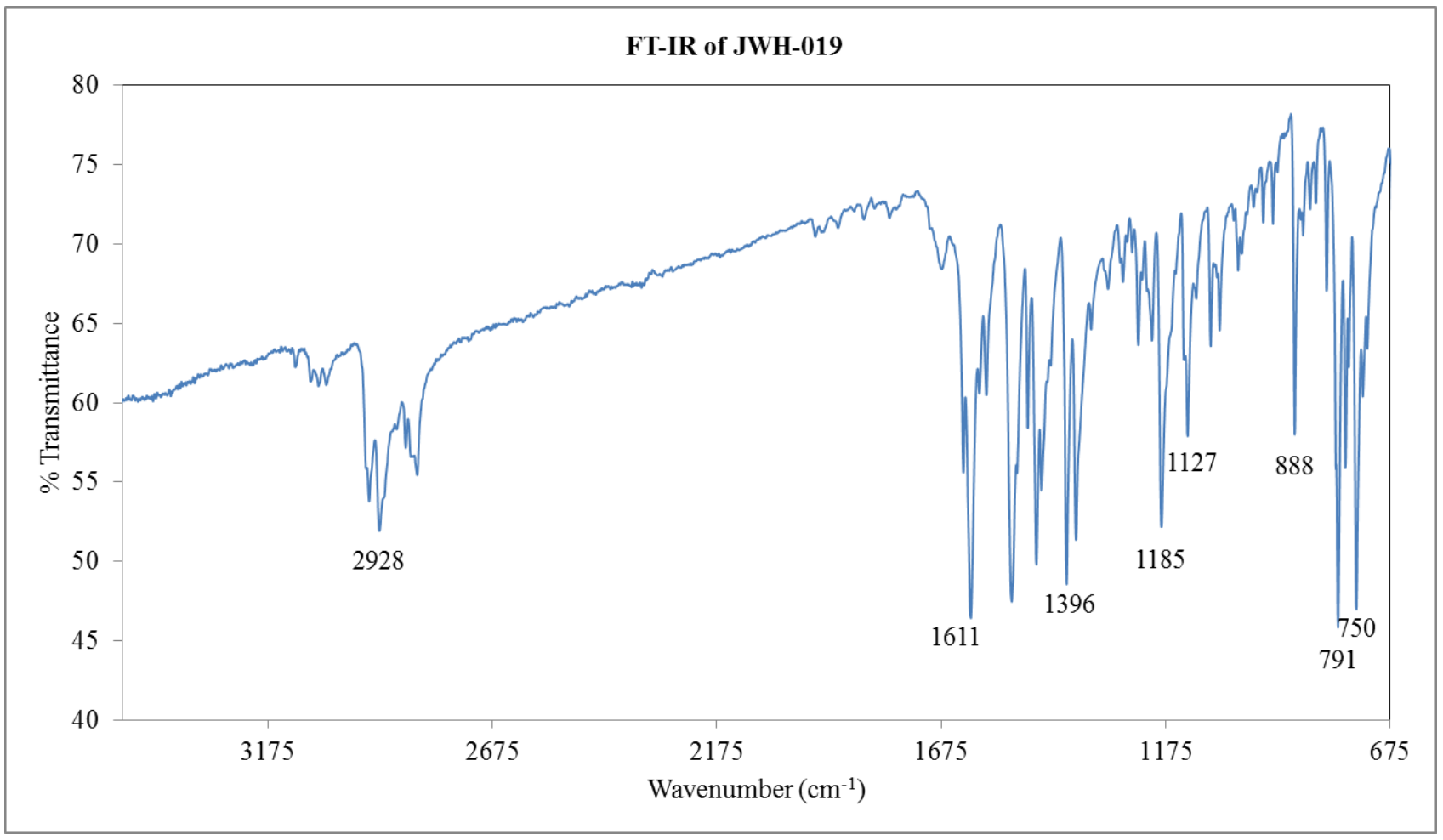
The synthesis of JWH-019 was unsuccessful. Reaction conditions were kept nearly the same as they were the synthesis of the BP hydrazone, but the amounts of reactants used were smaller because the amount of JWH-019 available was limited. The crystals that resulted from the reaction were analyzed in a KBr pellet using FT-IR. The resulting spectrum revealed JWH-019 was crystallizing from the solution unreacted. As can be seen in Figure 18, the FT-IR of JWH-019 and that of the reacted JWH-019 are identical with a peak at 1611 cm^{-1} indicative of the carbonyl group, peaks in the 1500 cm^{-1} region indicative of the benzene ring and a strong peak at 1396 indicative of a tertiary amine.

Since it was possible that the JWH-019 might be more reluctant to react than BP, the reaction was tried again and allowed to reflux for 16 hrs instead of the original 9 hrs. This also proved ineffective and again JWH-019 crystallized unreacted.

FT-IR Data for JWH-019 and JWH-019 Hydrazone

Wavenumber (cm^{-1})	**Functional Group**
3115	C=O overtone
2950, 2928, 2845	alkane C-H bonds
1611	C=O
1463	CH_2 bending
1520	carbon bonds in phenyl groups
1396,1375	C-N stretch from naphthoyl
1185, 1123	C-C(=O)-C stretching and bending
791, 750	bending of ring C-H bonds

Table 4: FT-IR data for JWH-019 which was identical to the data obtained after reacting JWH-019 with hydrazine.



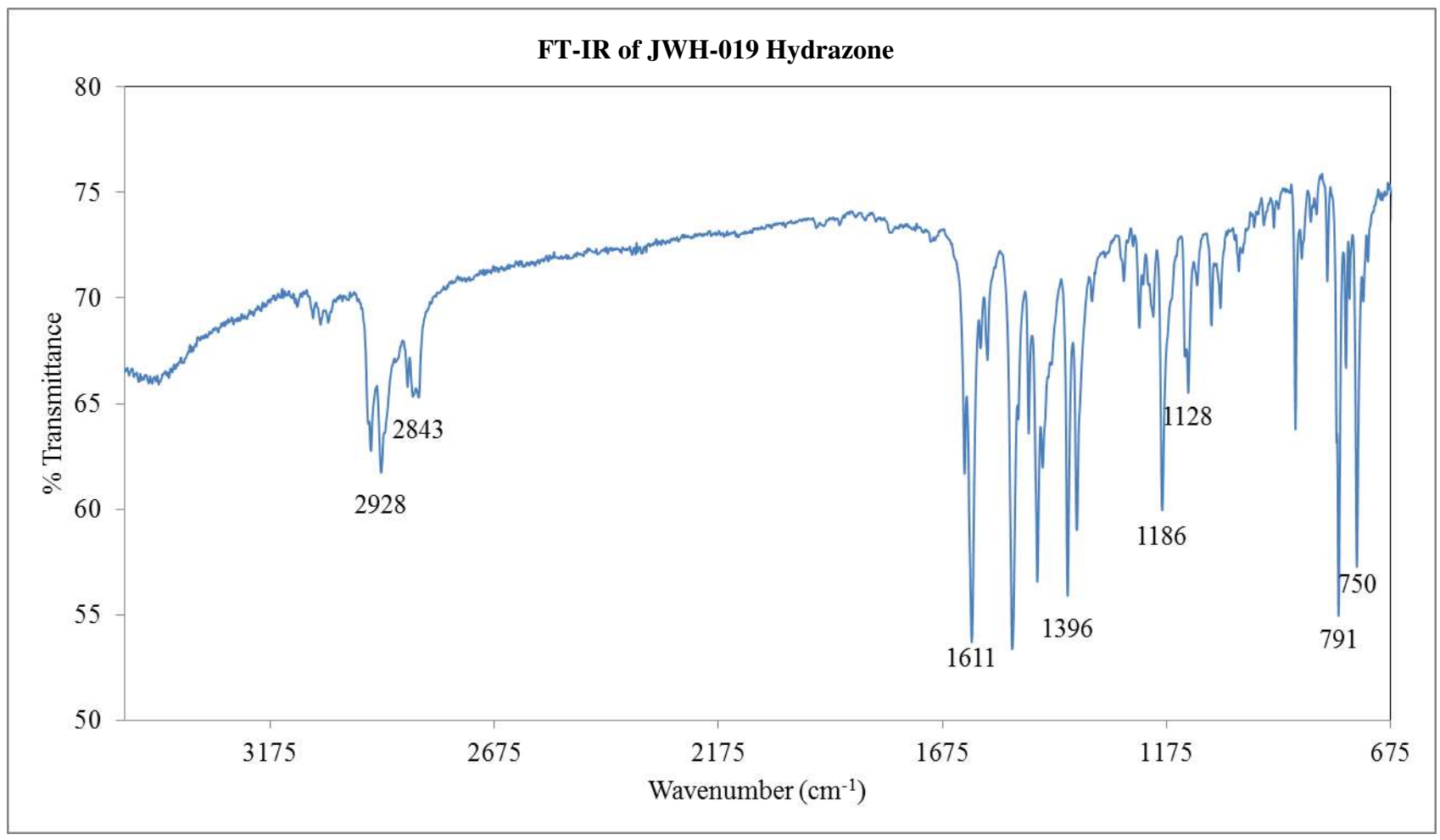
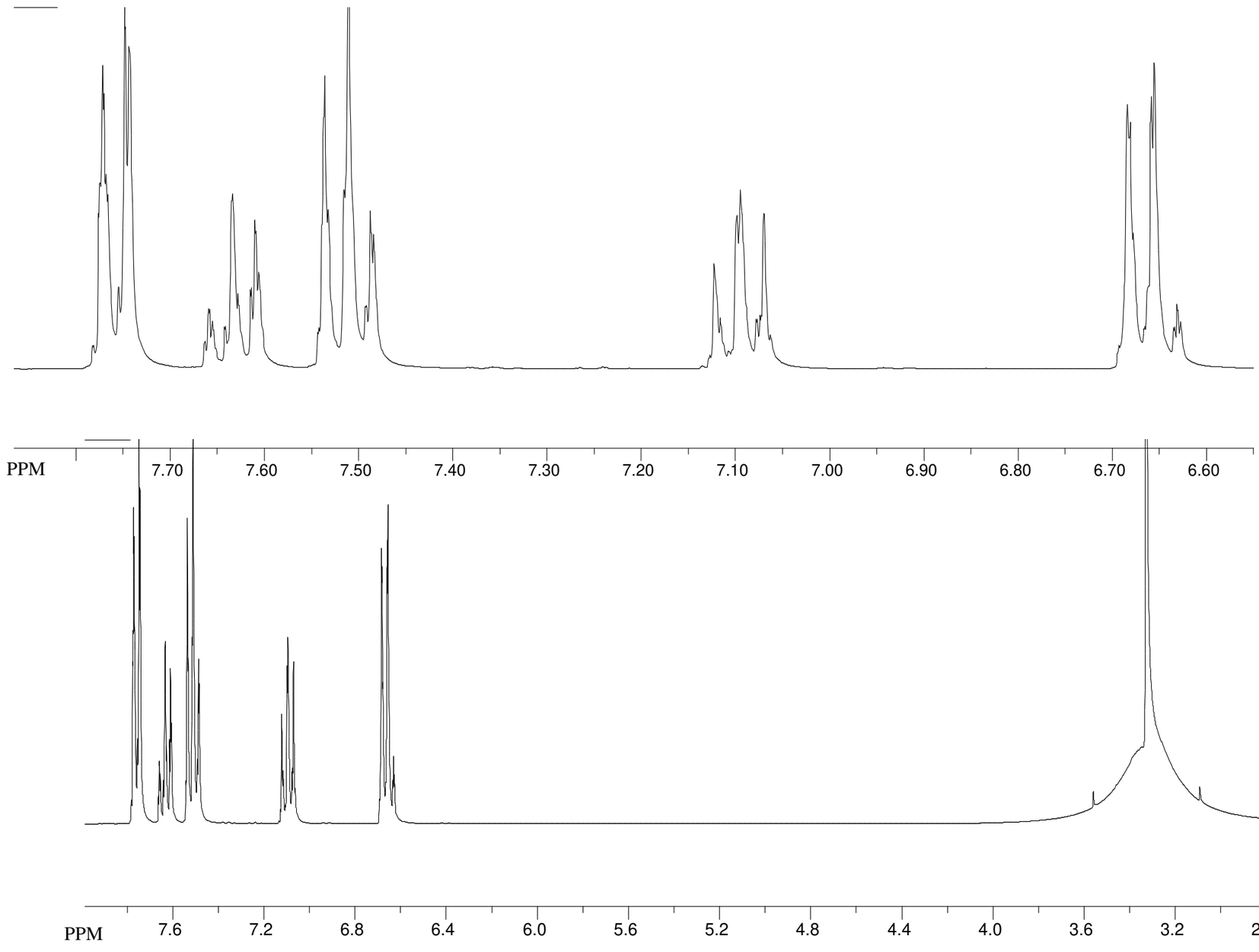
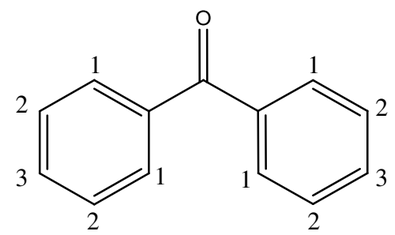


Figure 18: FT-IR spectra for JWH-019 (top) and JWH-019 hydrazone (bottom). The spectra have identical peaks indicating that the reaction was not successful.

4.5.4 Reaction of Benzophenone with Aniline

The reaction of aniline and BP was tried several times. The procedure provided by Enchev et al. in which 2-acetyl-indane-1,3-dione and aniline were refluxed for 5 hrs. in benzene to yield a Schiff base refused to produce crystals.[43] The reason for this was presumably the water that was being produced by the reaction, so $MgSO_4$ was added after refluxing and the product filtered off. Still no crystals resulted from the dried solution. A ^{1}H-NMR in CD_3CN was taken of the resulting yellow oil. The spectrum showed no shifts from unreacted BP and aniline, and thus it was assumed the reaction had not occurred. The ^{1}H-NMR and peak assignments can be seen in Figure 19. Aniline was thus proving to more reluctant to react than 2-acetyl-indane-1,3-dione. This was reasonable given the additional steric hindrance on BP and aniline was abandoned for an amine that might more readily react.





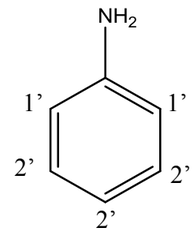


Figure 19: ^{1}H NMR of BP after being reacted with aniline. The peak assignments are: 1: 7.76 Hz, 2: 7.51 Hz, 3: 7.63 Hz, 1': 7.1 Hz, 2': 6.65 Hz, excess methanol and the amine: 3.33 Hz, water: 2.1, CD_3CN: 1.90 Hz.

4.5.5 Reaction of JWH-019 with Neutral Red

Neutral red was the final Schiff base reaction that was attempted. Since neutral red is already brightly colored, if a Schiff base were to readily form it was possible that the product would result in a change of color. Two solutions of neutral red were prepared and to one JWH-019 was added. The absorption of the solutions were monitored immediately after they were made, after heating for 1.5 hrs, and after allowing them to sit overnight. The UV-Vis spectra can be seen in Figure 20. As can be seen, the λ_{max} remained at 536 nm for both solutions throughout the entire experiment. The shape of the curve did change slightly in the region from 400 to 480 nm, the change is more significant for the sample containing JWH-019. This is likely due to the solutions becoming more basic; the color of neutral red is red at a pH below 6.8 and yellow at a pH above 8. The change in JWH-019 is more significant because the amine on the indole ring is being protonated. The results reveal that neutral red does will not work as a presumptive test for JWH-019.

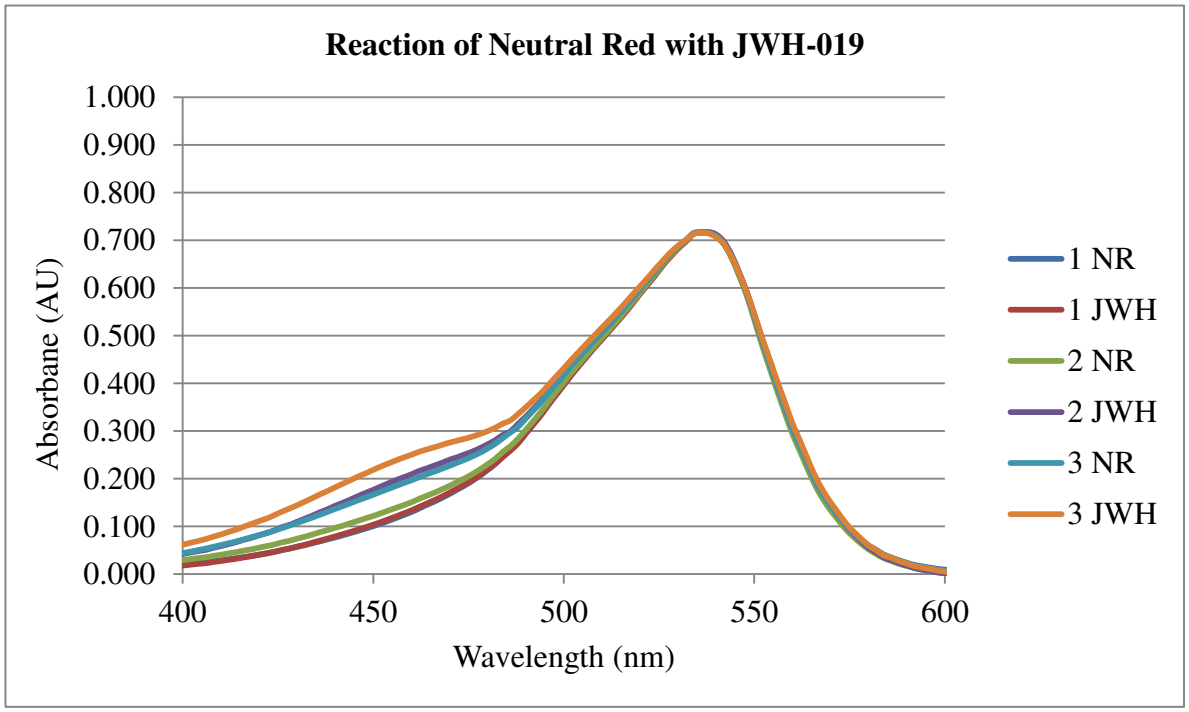


Figure 20: Shows the UV-Vis spectra for the reaction of JWH-019 with neutral red. On the legend NR is for neutral red alone and JWH is the reaction. 1 is the initial absorption before heating, 2 is after heating and 3 is after sitting overnight. As can be seen the λ_{max} for both solutions remained at 536 nm. (The absorbance of the solutions has been corrected as concentration changed during heating; this allows curve shape to be better compared.)

4.6 Metal Complexes

4.6.1 Synthesis of Benzophenone Semicarbazone

Vogel's Textbook of Practical Organic Chemistry 5th ed. provides a generic procedure for reacting semicarbazide with a ketone or aldehyde.[53] According to Vogel's procedure the product should begin to precipitate out of the solution in a few minutes to several hours depending on the ketone used. The reaction was done with BP, and after several days no crystals had formed.[45] Some of the excess solvent was removed by heating and upon cooling there were a few, very small crystals, but nothing significant enough to recover.

Vijayan et al. reports forming the semicarbozone of BP crystals by allowing the solution to sit in warm conditions for several days.[45] This was tried and a ^{1}H-NMR of the resulting crystals in DMSO was taken. The ^{1}H NMR showed chemical shifts characteristic of BP; ^{1}H NMR δ 7.73 Hz (dm, 4H), 7.65-7.7 Hz (tt, 2H), 7.55 Hz (tm, 4H). However, there was also a series of peaks further up-field between 7.5 and 7.2 Hz (these were not well resolved). Due to the small amount of product obtained no further analysis was done, instead we investigated whether or not the compound would form a colored metal complex.

4.6.2 Benzophenone Semicarbazone Metal Complex

The BP semicarbazone crystals were dissolved in ethanol and small aliquots of the resulting solution mixed with solutions of $Cu(NO_3)_2{\cdot}5H_2O$, $FeSO_4$ and $NiCl_2$. We hoped that metal complexes would form and result in a color change, unfortunately no change in color occurred.

4.6.3 Benzophenone Reaction with Ethylenediamine

Since en was already known to change color when it forms a metal complex, we hoped it would form a complex of a different color when it formed an imine with en. We began by

investigating a procedure described by G.B. Joshi[59, 60]. He reports synthesizing 2-Hydroxy-5-methyl-benzophenone-ethylenediamine-anil and forming a complex with iron (II). The procedure given was vague and indicated that the ligand was formed by refluxing 2 hydroxy–5–methyl–BP (2 moles) and en (1 mole) in alcohol for 1 hr, and then recrystallizing the resulting product in alcohol. This reaction was tried several times, but the reported crystals were never obtained. $MgSO_4$ was added to the resulting product then filtered off to remove the water formed by the reaction, still no crystals formed. Finally, isopropyl alcohol was used as the solvent and the product was recrystallized in diethyl-ether. The result was long, thin, clear crystals. FT-IR was used to analyze the resulting crystals and revealed that unreacted BP had precipitated from the solution. The FT-IR spectrum shows a strong peak at 1652 cm^{-1} indicative of its ketone, as well as peaks at 1595 cm^{-1} from the carbon bonds from the benzene ring.

A few drops of acetic acid were added to the procedure to help catalyze the formation of the imine. Yellow-brown crystals were produced and analyzed using ESI-MS. The spectra showed the $[MH^+]$ at m/z 225 and unreacted BP at m/z 183 (Fig.21).

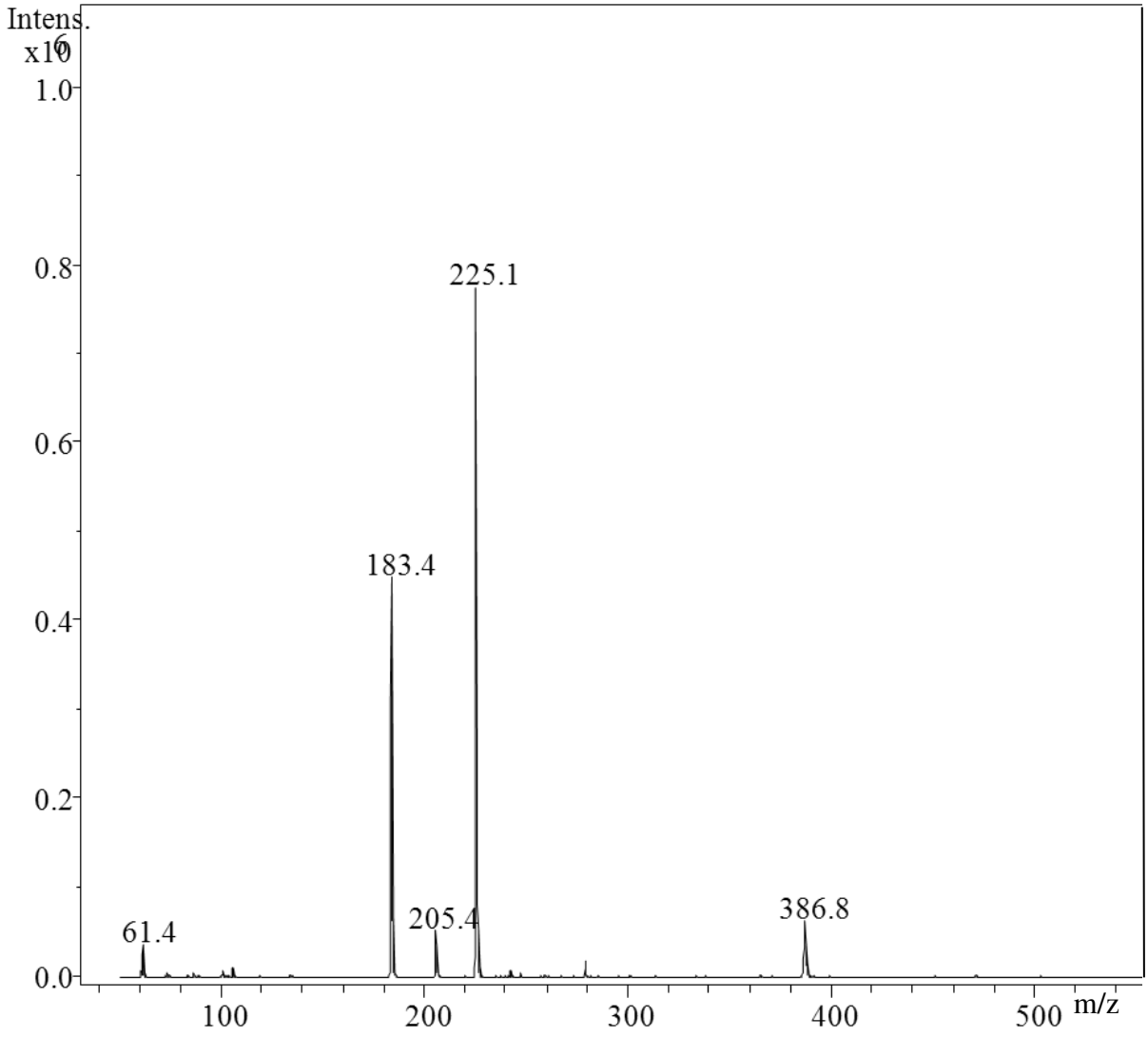


Figure 21: The ESI-MS of BP-en. The spectrum shows the [MH^+] at m/z 225 and unreacted BP at m/z 183.

4.6.4 Benzophenone Ethylenediamine Metal Complex

Preliminary results revealed that the en-BP Schiff base could form a metal complex. When mixed with solutions of $Cu(NO_3)_2$ the solution changed from bright blue to purple. When mixed with Fe^{2+} there was not a significant change in color from the brown of the Fe^{2+} solution. When mixed with the Ni^{2+} the solution became a slightly darker green from its original pale green. Since the Cu^{2+} solution provided the most dramatic color change it was used for the rest of the study.

The complex was analyzed using UV-Vis spectrophotometry. As can be seen in Figure 22, $Cu(NO_3)_2$, the en-BP complex and the en complex each have a different λ_{max}. The λ_{max} of the $Cu(NO_3)_2$ was 734 nm and the solution was bright blue. The en-BP-Cu complex was 610 nm and the λ_{max} of the en-Cu complex was 648 nm. While the difference in colors of the complexes were visible to the naked eye (both were a shade of violet-blue) we had hoped for a more drastic difference. However, there was still the possibility that the JWH-019-en compound would provide the drastic change needed for a colorimetric presumptive test. Before working with it though we wanted to perfect the BP-en synthesis and confirm our initial results.

Unfortunately, reproducing the en-BP proved to be challenging. When we tried to produce the BP-en again, it refused to crystalize. Following the same procedure that worked before, refluxing the reaction longer and varying the amount of acetic acid all proved unsuccessful; either no crystals would form or unreacted BP would precipitate out of solution. Since a spot test would be inefficient if crystallization was required we tried just using the solution. The reactants were heated, the solvent evaporated and the resulting yellow oil mixed with the Cu^{2+} solution. The results appeared comparable to the initial results at first. Figure 23 shows the UV-Vis spectrum for each of the solutions. The maximum absorption of $Cu(NO_3)_2$

remained consistent at 732 nm. The λ_{max} of the en-BP complex was at 636 nm and that of the en complex at 596 nm. These results were reversed in the previous reaction when the en-Cu complex absorbed the longer wavelengths. In addition to this the en-BP complex was slow to equilibrate. Ten minutes after the initial absorption reading, the λ_{max} for the en-BP complex decreased to 626 nm. The en complex remained at 526 nm. Another experiment (using the same conditions) was done to monitor this phenomenon. After adding the Cu^{2+} solution to the reaction mixture, the λ_{max} was monitored for several hours. As can be seen in Figure 24, the λ_{max} continually shifted to shorter wavelengths. This problem was not observed when a solution of en formed a complex with Cu^{2+} or when the original en-BP crystals formed a complex with Cu^{2+} (Fig. 25). Consistent results were not attainable when the en-BP product was left in solution. The concentration of product could not be well controlled which caused shifts in absorption from experiment to experiment as the en-BP product, unreacted en and water competed to form a complex with the copper. The slow equilibration of en-BP solution also made it difficult to determine if the absorption of the two complexes was consistently different. Since this method was proving to be so problematic, we moved on to a slightly different approach.

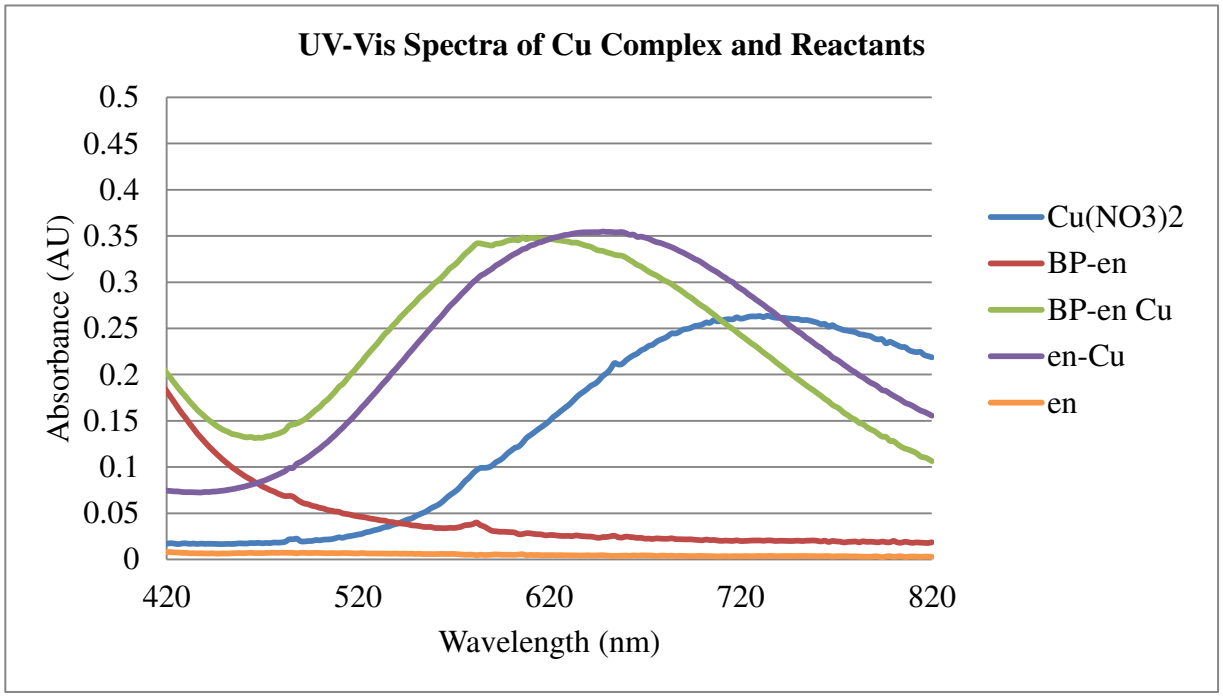


Figure 22: UV-Vis spectra of the BP-en Cu complex compared with the en-Cu complex and the other reactants. The maximum absorption of BP-en Cu is 610 nm, the maximum absorption of en-Cu is 648 nm.

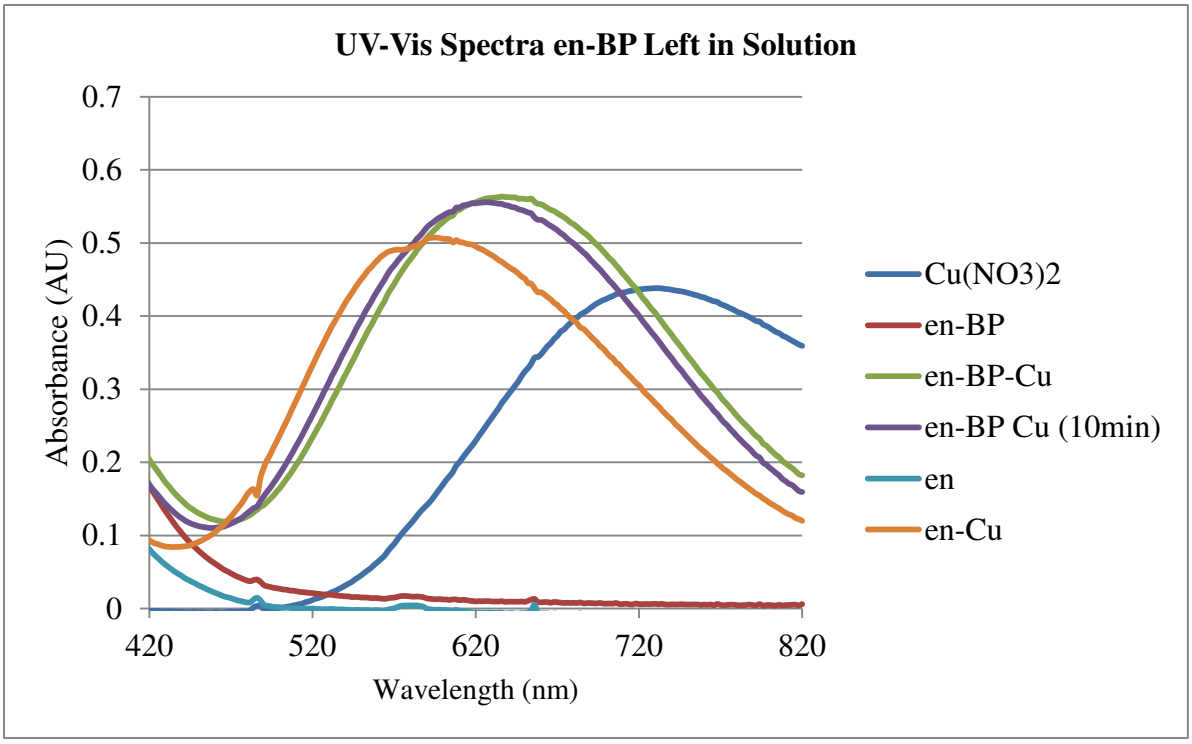


Figure 23: The UV-Vis spectra comparing the en-BP product left in solution forming the complex with Cu^{2+}. The maximum absorption of the en-BP-Cu complex is 636 initially, but decreases to 626 nm after 10 min. The maximum absorption of the en-Cu complex is 596 nm.

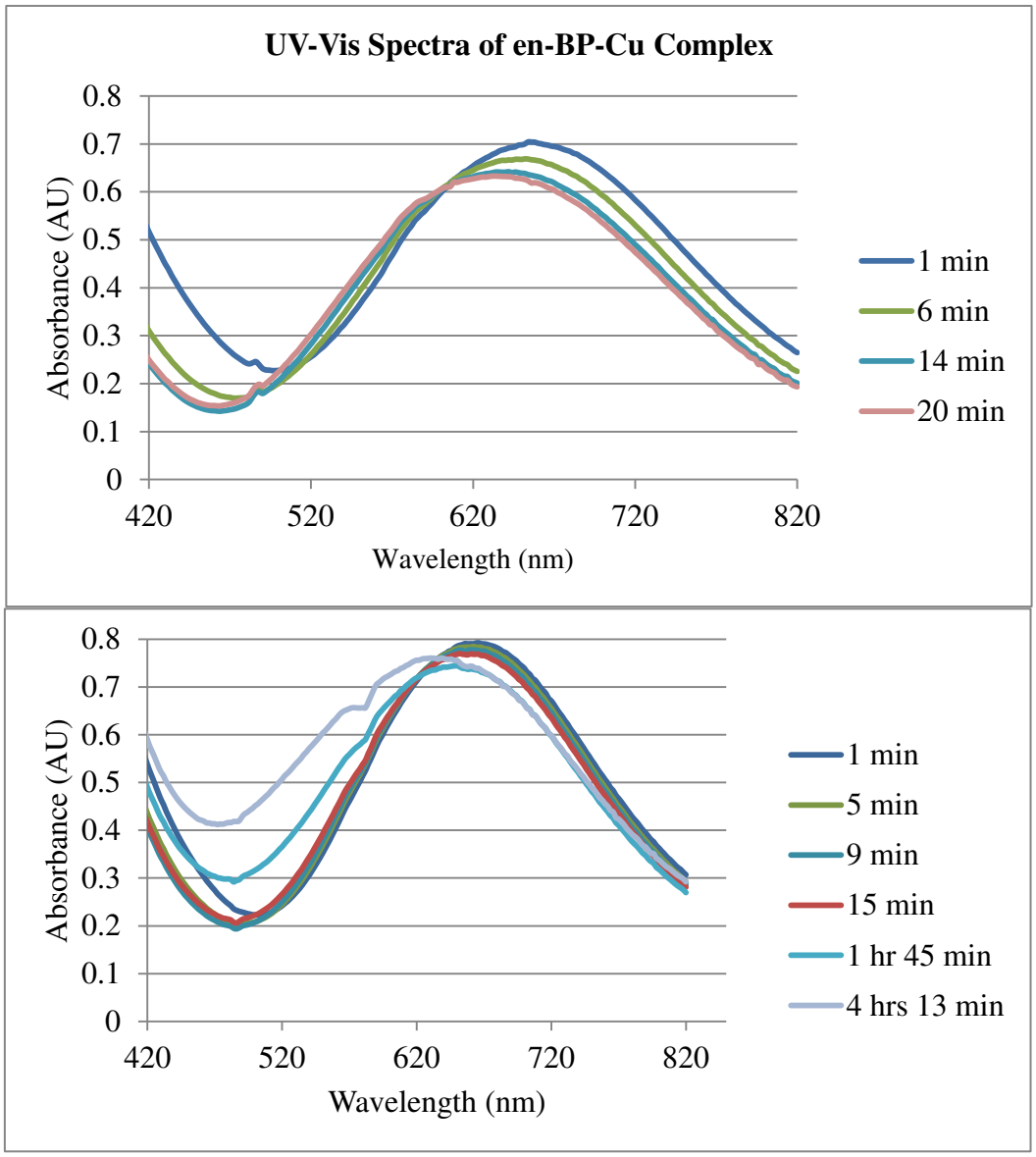


Figure 24: UV-Vis spectra of en-BP Cu complex when en-BP product was left in solution. In both experiments the λmax decreased over time. For the top graph λmax was 656 nm at 1 min, then decreased to 654 nm, then 642 nm and finally 634 nm at 20 min. A 2nd experiment was done and the results were similar. The λmaxes starting at 1 min and going up to 4 hrs are as follows: 666, 664, 660, 652, 650 and 638 nm.

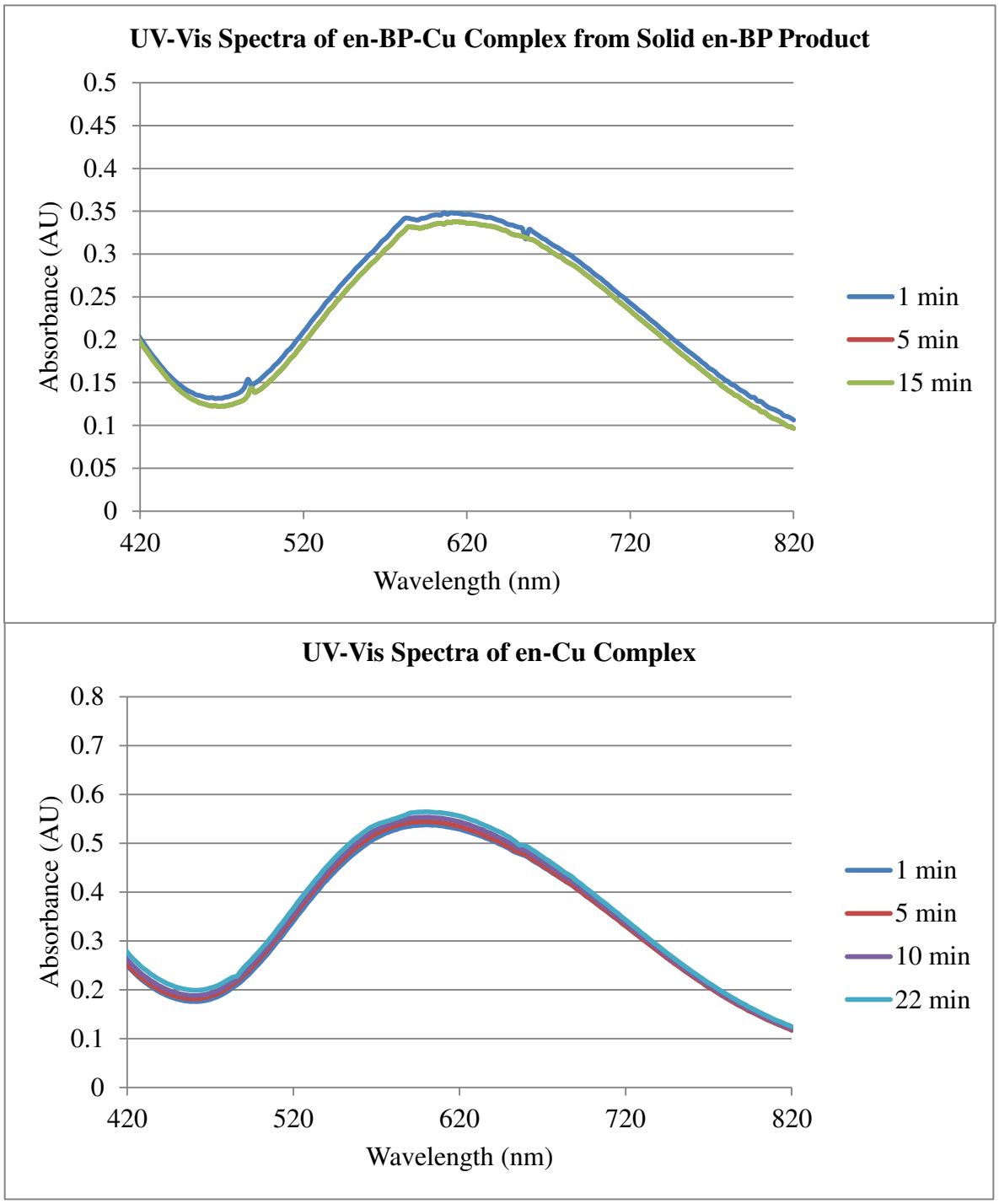


Figure 25: UV-Vis spectra of the en-BP Cu complex from crystallized product and of the en-Cu complex monitored over time. For both the λmax stays constant over time. (λmax top 648 nm and λmax bottom 600 nm).

4.6.5 Benzophenone Reaction with Two Ethylenediamines

In the midst of not being able to reproduce the en-BP product, another promising reaction was found. Chowdhury et al. reported synthesis of photoluminescent Cu^1N_4 chromophores using en and BP[55]. The product they formed had a BP attached to both sides of the en, they then formed a brick red metal complex with $[Cu(CH_3CN)_4]ClO_4$. First, we investigated if JWH-019 was fluorescent on its own. The compound was exposed to a UV light for 1 min and then observed in a dark room, no fluorescence was observed. We then attempted to reproduce Chowdhury's results. The procedure described in the paper is straight forward and involves refluxing the two reactants in methanol then recrystallizing the product in n-hexane. The procedure was followed except the amounts of reactants were lowered. The formation of crystals was more reluctant than expected. Chowdhury reported solid forming upon evaporation of the methanol, we did not see this. Crystals did not form until solution sat overnight in hexanes.

Analyzation of the crystals was also disappointing. Chowdhury reported "white blocks," ours were small white sticks. FT-IR revealed that the resulting crystals were only BP (ketone = 1652 cm^{-1}, 1500 region cm^{-1} = benzene ring). The reaction was attempted several times to ensure no errors were being made, but the result was always the same. A ^1H NMR of some of the resulting crystals was taken in DMSO and can be seen below in Figure 26. The NMR matched all of the chemical shifts for BP; ^1H NMR δ 7.64 (dm, 4H), 7.46 (tt, 2H), 7.58 (tm, 4H). The reactants' amounts were increased to those described by Chowdhury and still only BP crystallized from the solution.

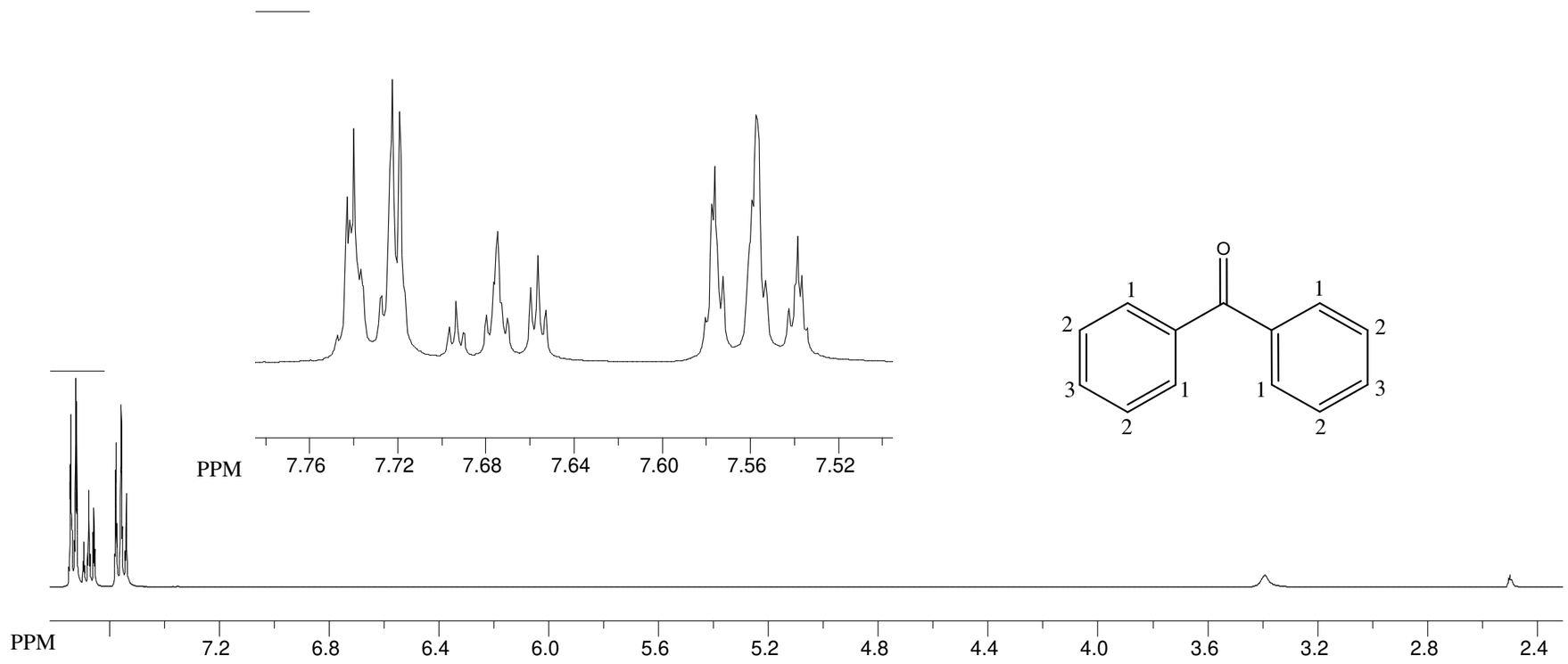


Figure 26: ^{1}H NMR of BP after being reacted with en. There is no change in the chemical shifts from unreacted BP, thus it was concluded that no reaction took place. The peak assignments are: 1: 7.70-7.75, 2: 7.53-7.58, 3: 7.64-7.70 Hz.

4.7 Microcrystalline Identification

Due to the amount of materials we had on hand we could only begin preliminary work on a microcrystalline test for SC's. The photomicrographs of the JWH-019 crystals can be seen in Figure X. The resulting crystals from ethyl acetate (~10 g/L) were short needled rosettes. When the ethanol was added on top of these crystals, the rosettes broke apart resulting in short needles.[61] The JWH-018 refused to yield any crystals from the ethyl acetate (~5g/L) or when ethanol was added. There was only a small amount of JWH-018 available so the problem could not be completely analyzed, but the likely culprits are either concentration or impurities (the JWH-018 had been synthesized over 3 years ago). The herbal incense that was tested also refused to yield good crystals. Crystals could be seen starting to form, but would then oil out and disappear. Again, there was not enough sample to further analyze the problem, but it was thought to be either a concentration or impurity issue. The results from the JWH-019 crystals do show promise. More work would have to be done to investigate the concentration and impurity issues. Then, other SC's would have to be tested to determine if their crystal structures were different. Also, other compounds such as different drugs and incense that does not contain SC's would have to be tested to verify they did not produce crystals similar to those of SC's.

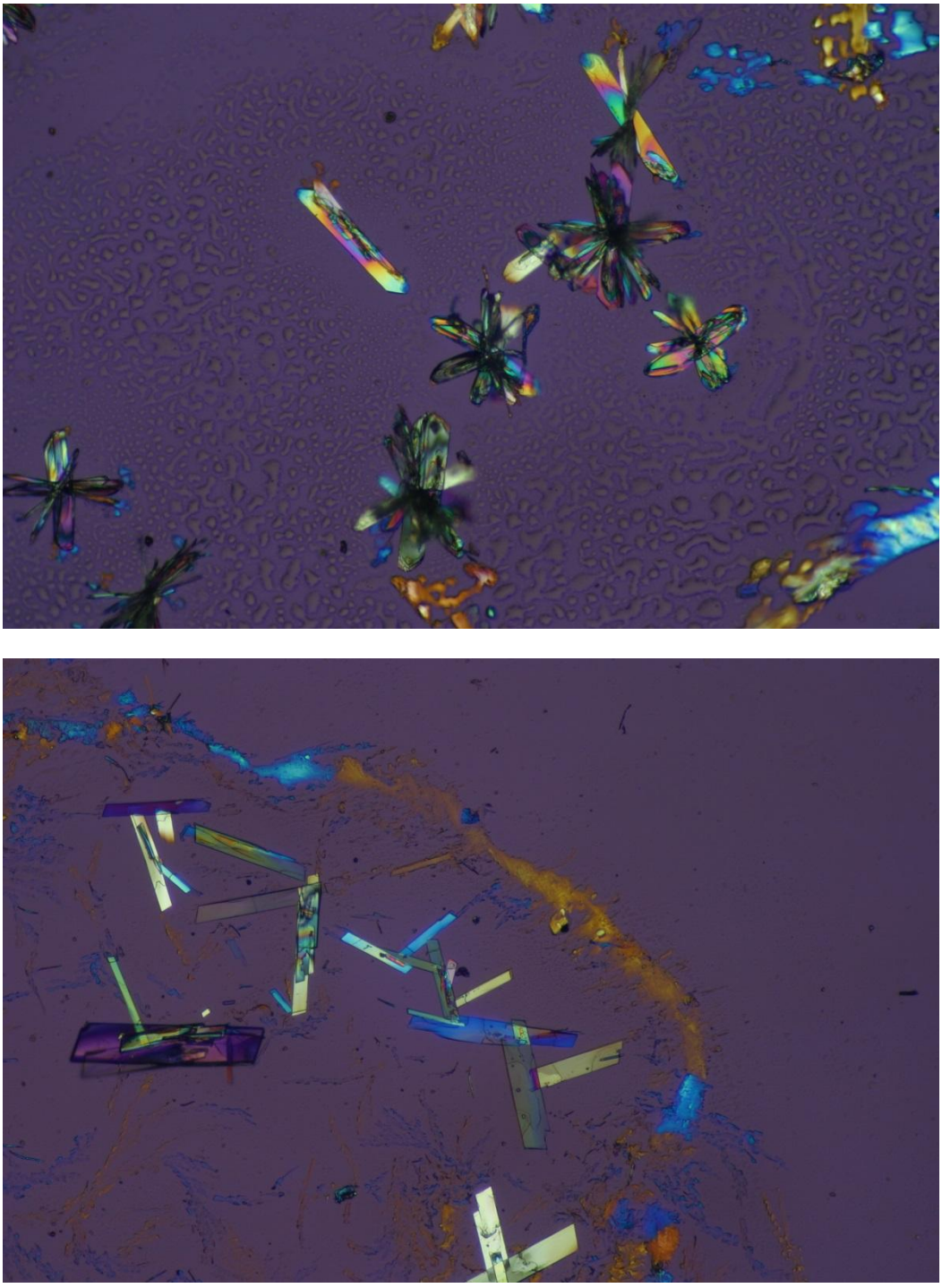


Figure 27: JWH-019 crystals in ethyl acetate (top) and recrystallized in ethanol (bottom).[60]

5. Conclusions

Creating a presumptive spot test for SC's proved to be quite challenging. Our initial tests in which the reactivity of BP and JWH-019 were compared were disappointing. The BP ketyl and benzopinacol were easily made and then the reactions were tried using JWH-019. Both were unsuccessful. This may have been because BP and JWH-019 do not behave similarly, or JWH-019 might be significantly more reluctant to react than BP and the right conditions were not found. The Brady's reagent, which tests for ketones, showed the reluctance of JWH-019 to react. BP took 2 hours to finish reacting with the DNHP while JWH-019 was still reacting at 4.5 hrs. Several more reactions were tried to determine if other Schiff bases could be produced. We continued to use BP in our experiments because even if the two compounds would not react in a similar time frame, BP still has a ketone and thus had the ability to produce a Schiff base. The first Schiff base prepared was BP hydrazone. It was oxidized to diphenyldiazomethane which caused the solution to turn purple. Unfortunately, when this reaction was tried with JWH-019 no hydrazone formed. Ethylenediamine was the only other compound that displayed any success with JWH-019. There were several instances in literature where en was reacted with BP and colored metal complexes produced.[55, 59, 60] We had some success producing a complex using Cu^{2+}, but our results were inconsistent. The en-BP imine often refused to form and resulted in unreacted BP crashing out of the solution.

The difficulty in obtaining crystalized product in any Schiff base reaction is the formation of water. Imines are easily hydrolyzed back to ketone and amine reactants. Using a method in which water is removed or the imine product is immediately reacted further would shift the equilibrium to the right.[38] However, such requirements could complicate the reaction and its compatibility with a field test.

Through this study it became increasingly clear that the ketone on JWH-019 (and BP) is reluctant to react. The Brady's reagent clearly demonstrated this when BP took 2 hrs to finish reacting and JWH-019 over 4.5 hrs (Brady's reagent takes only a few minutes to react with simple ketones). The other Schiff bases reported to form with BP all took at least 6 hours. Looking at both compounds it can be seen that the ketone is not only sterically hindered, but part of a highly conjugated system. The FT-IR data of BP and JWH-019 confirms this observation. The C=O bond in aliphatic aldehydes generally absorb between 1740-1720 cm^{-1} (acetaldehyde: 1727 cm^{-1}).[62] Saturated aliphatic ketones absorb at slightly lower frequencies around 1715 cm^{-1}. Increases in conjugation quickly shift the absorption of the C=O bond to longer wavelengths. The absorption of longer wavelengths is indicative a delocalized π system as the energy difference between filled and unfilled orbitals decreases.[38] This trait is demonstrated by comparing the C=O absorption of several ketones and aldehydes as seen in Table 2.[63] In BP the C=O bond absorbed at 1652 cm^{2}; a significantly smaller frequency than 1715 cm^{-1}. The delocalization of the ketones electrons in BP contributes to its stability and thus its reluctance to react. The ketone in JWH-019 absorbed at an even lower frequency of 1611 cm^{-1}. The increased delocalization seen in JWH-019 is due to the increased conjugation provided from the naphthoyl and indole moieties. Indole alone can greatly shift the absorption of aldehyde as seen in 3-indolecarbaldehyde which absorbs at 1614 cm^{-1}. The non-bonding pair of electrons on indole's nitrogen is likely responsible for this occurrence. A resonance structure can form when the non-bonding electrons on the nitrogen transfer through the conjugated system to the oxygen, thus decreasing the double bond nature of the ketone (Fig. 28). Such delocalization stabilizes the ketone and makes its carbon much less susceptible to nucleophilic attack by the amine. Many

SC's contain the ketone and indole and thus all their C=O absorptions would likely occur at an unusually low frequency. This trait could be used in preliminary identification for many SC's.

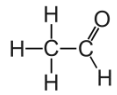
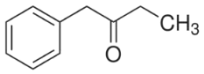
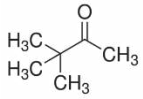
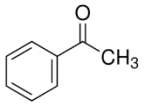
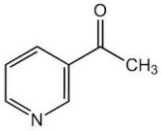
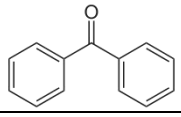
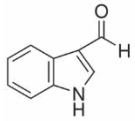
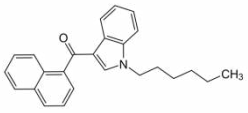
Compound	**Structure**	**Wavenumbers (cm^{-1})**
Acetaldehyde		1727
1-phenyl-2-butanone		1713
3,3-dimethyl-2-butanone		1708
Acetophenone		1691
3-acetylpyridine		1689
Benzophenone		1653
Indole-3-carboxaldehyde		1614
JWH-019		1611

Table 5: Shows the C=O absorptions for various ketones. All data were obtained from the SDBS Spectral Database for organic compounds except BP and JWH-019.[63]

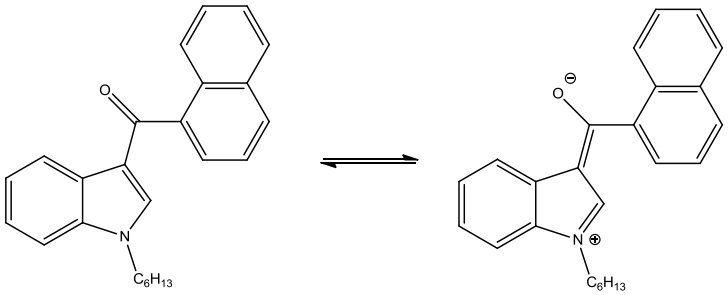


Figure 28: Possible resonance structure of JWH-019. The transfer of nitrogen's electrons to oxygen makes the ketone less susceptible to a nucleophilic attack.

While finding a reaction that could occur at the ketone in a short amount of time would be difficult, its resistance to react could also be useful. A spot test that works with ketones will not be specific for SC's; there would be many interferences with the test. However, if a reaction was found to work with SC's in a reasonable amount of time, impurities containing ketones could be removed by an initial reaction. Presumably, other ketones would react faster, and if they could be reacted and removed from the sample the test could become sufficiently selective for ketone containing SC's. Of course the main challenge in any future work will be finding a reaction that is fast enough. It is possible that an acid catalyst could be used to form a Schiff base in a smaller amount of time.[58] The reaction with BP and hydrazine provided the most consistent results and it is possible that JWH-019 could be reacted with hydrazine if the right conditions were found and the time required decreased with an acid catalyst. However, due to the reagents and time required the reaction would have to be performed in a laboratory.

The microcrystalline test showed promise and further work needs to done to solidify the results. The first step would be obtaining more pure SC's and analyzing their crystal structure. Then, a method could be developed to extract the compounds from the herbal incense. Finally, the crystals would have to be compared to the crystals of other illicit substances.

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