

CASE REPORT

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Psychotropic Drugs in Developmental Mushrooms: A Case Study Review

ABSTRACT: Psilocyn and psilocybin can be identified in different stages of developing *psilocybe* mushrooms. Knowing the various stages of the mushroom development can be useful when receiving evidence from illicit mushroom growing operations. Exhibits from three separate cases were submitted to the drug analysis section of the Minnesota Bureau of Criminal Apprehension Forensic Science Laboratory. Each case contained different stages of developing mushrooms. This report describes the evidence in each case, the sample preparation, the sample analysis and the final report that was written.

KEY WORDS: forensic science, psilocyn, psilocybin, psychotropic mushrooms

Psilocybe mushrooms can easily be grown with the correct materials in a controlled setting. Analysis of psychoactive drugs in the developmental stages of the mushroom has been previously reported (1). In the present study, three separate cases of developing mushrooms were submitted to the Bureau of Criminal Apprehension (BCA) Forensic Science Laboratory in St. Paul, Minnesota for analysis. This report gives a brief background on psilocyn and psilocybin that are found in psilocybe mushrooms and describes the three cases received by the BCA.

Psilocyn (also spelled psilocin) and psilocybin (Fig. 1) are regulated under both Minnesota State and U.S. Federal laws as a Schedule I substance. Schedule I substances have a high potential for abuse, have no current accepted medical use in treatment in the U.S., and have a lack of accepted safety for use (2). In Minnesota, psilocyn and psilocybin are controlled by Minnesota Statute 152.02 Subdivision 2 and also under Minnesota Rule 6800.4210 Section C. Federal regulations classify psilocyn and psilocybin as Schedule I substances under Section 812 of the CSA (21 U.S.C. § 812).

Hallucinogens are drugs that affect the central nervous system and produce sometimes profound and/or bizarre changes in perception, thought and mood (2). Effects can include sensory and perceptual distortions (2). Terms used to describe these substances include psychedelic, psychosomatic, psychotropic and psychoactive.

Psilocyn and psilocybin are naturally occurring substances in certain families of mushrooms. The four families of mushrooms that contain these psychoactive drugs are Strophariaceae, Bolbitiaceae, Coprinaceae, and Cortinariaceae (3–8). The four stages making up the life cycle of a mushroom have been previously de-

scribed (1), and consist of spores, mycelium, primordia, and the mature fruit. Illicit growing operations often use spores, mycelium or primordia to start their crops. This study examined the detection of psilocyn and psilocybin in the developmental stages of mushrooms using thin layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS). Additional studies have examined the analysis of psilocybe mushrooms by capillary electrophoresis (9), by ion mobility spectrometry and GC/MS (10), by morphological, microscopic, microchemical, and high performance liquid chromatography (11) and by TLC, GC/MS and LC/MS (12).

Case History

Three separate cases of developmental mushrooms were submitted to the BCA drug analysis section for analysis. Case A contained syringes which contained an aqueous solution containing spores; Case B contained jars with growing media containing mycelium growth; and Case C contained jars containing primordia. Each case is described herein with the physical characterization of the evidence, sample preparation, sample analysis and the report that was written stating the conclusions of the testing.

Case A

Evidence Description

Three 10 cc syringes were submitted to the laboratory for analysis (Fig. 2). One syringe, sealed with masking tape, contained approximately 10 mL of a clear aqueous solution. The other two syringes (unsealed) each contained approximately 8 mL of a clear aqueous solution. All three solutions contained spores dark purplish brown in color and elliptical to oblong elliptical in shape. A $\times 400$ microphotograph of the spores suspended in the aqueous solutions is shown in Fig. 3.

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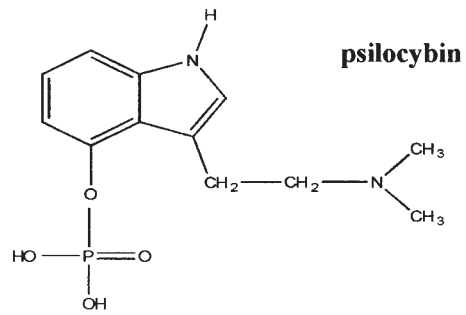
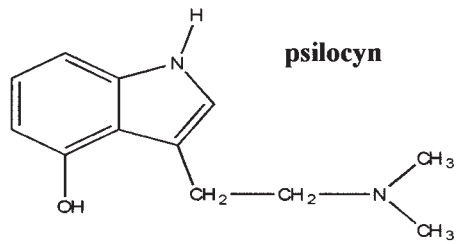


FIG. 1—Chemical structures of the psychoactive drugs psilocyn and psilocybin.



FIG. 2—Three syringes submitted in Case A. Each syringe contained an aqueous solution.

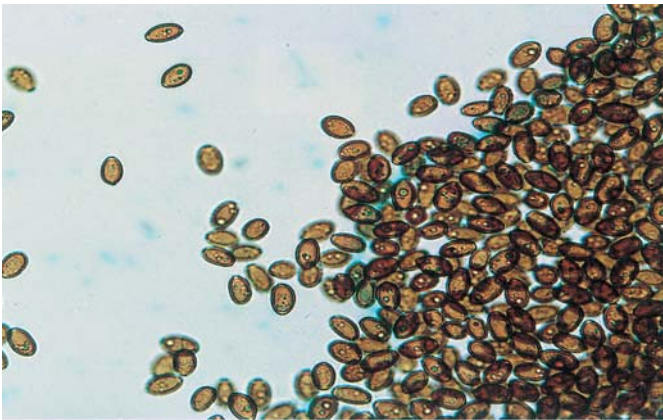


FIG. 3—Photomicrograph ($\times 400$) of spores found in the aqueous solution submitted in Case A.

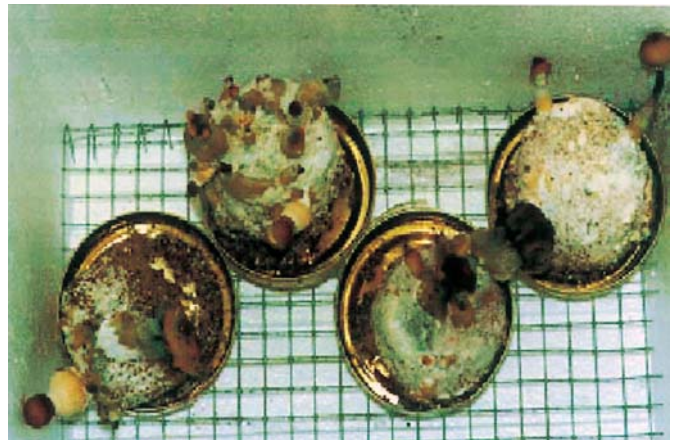


FIG. 4—Primordia and mature mushrooms in terrarium that were grown from the spores submitted in the aqueous solution in Case A.



FIG. 5—Mature mushrooms grown from the spores submitted in the aqueous solution in Case A.

Sample Preparation

The growing media mixture and sample preparation were according to the method previously described (1). Three jars were each inoculated with approximately 1/3 of the solution from one syringe. This was repeated for the other two solutions in additional jars for a total of nine jars inoculated. The jars were then allowed to grow under indirect light. Within five days, two of the jars inoculated with the 10 mL solution had white mycelium

growth in them. Primordia appeared in these two jars 26 days after inoculation. A sample of the primordia from each jar was taken for analysis. The samples were allowed to continue to grow and were transferred to a terrarium when the primordia was too large for the jars. Samples continued to grow and were removed from the terrarium when the mushrooms were mature. Figure 4 illustrates the primordia and mature mushrooms that were grown in the terrarium. Figure 5 is a picture of a mature mushroom.

Sample Analysis

Samples from the primordia were screened using a color test with paradimethylaminobenzaldehyde (ρ -DMAB), also known as the Van Urks reagent. The reagent consists of 2 g ρ -DMAB dissolved in 50 mL of ethanol. One drop of the reagent followed by 1 drop of concentrated hydrochloric acid turns a purple color in the presence of indoles (13). The primordia samples were prepared and analyzed by TLC and GC/MS according to the methods previously described (1). Both of the samples contained psilocyn and psilocybin. The liquid from the syringes (i.e., the spores) was extracted with chloroform and analyzed by GC/MS. No controlled sub-

stances were detected. Figures 6 and 7 show the total ion chromatogram (TIC) and the mass spectrum of the psilocyn standard that was analyzed, respectively. The samples that were inoculated from the two unsealed syringes never grew.

Report

The report for Case A read as follows:

Item 13A-1 contained approximately 10 milliliters of an aqueous solution. Spores were observed in this solution. Germination of these spores was observed after exposing

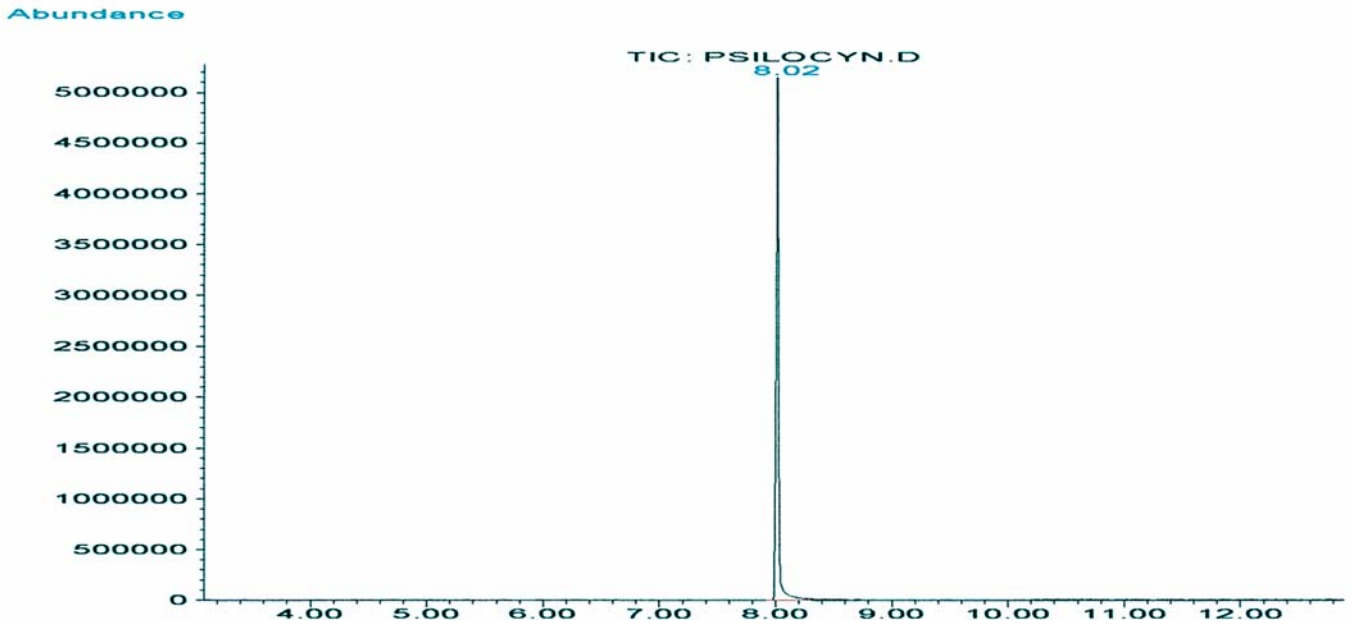


FIG. 6—Total ion chromatogram (TIC) of psilocyn standard analyzed on the GCMS.

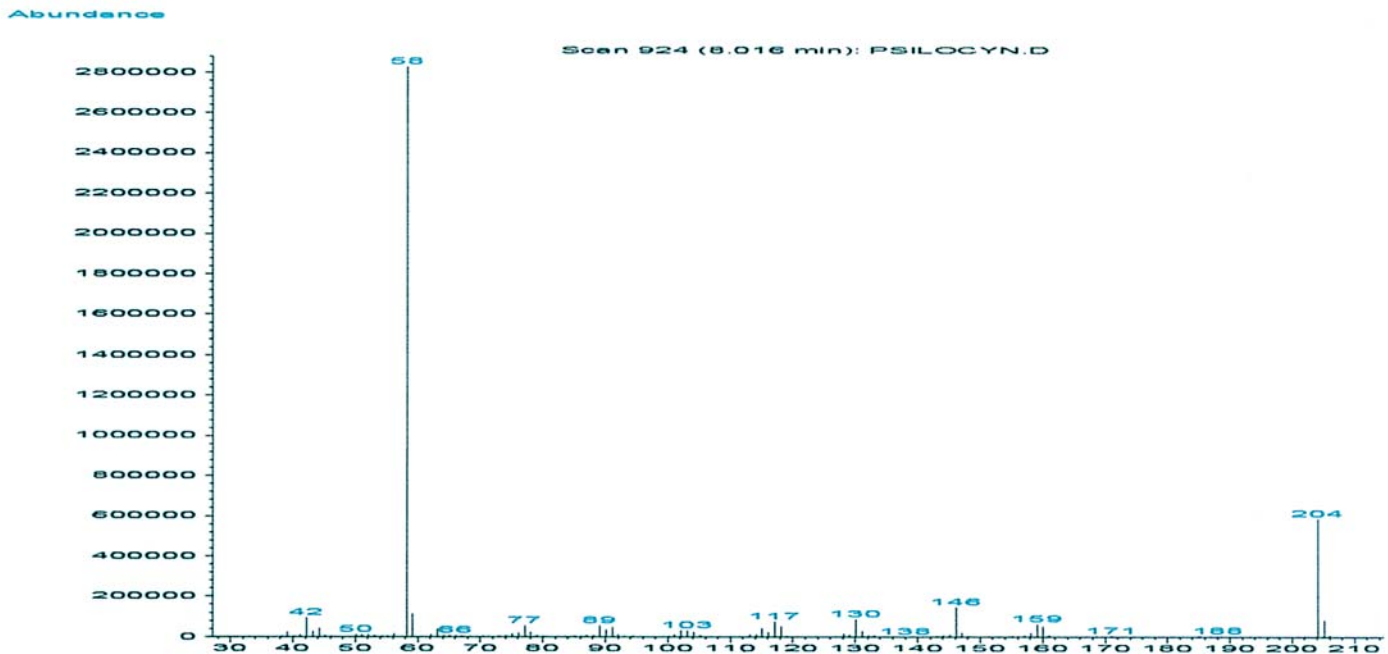


FIG. 7—Mass spectrum of psilocyn standard analyzed on the GCMS.

them to the appropriate growing medium and environmental conditions. Mushrooms containing psilocyn and psilocybin were grown from these spores. Item 13A-2 and 13A-3 each contained approximately 8 milliliters of an aqueous solution. Spores were observed in these solutions also. No germination of these was obtained.

The spores of the mushrooms containing the psychoactive drugs psilocyn and psilocybin are not controlled by the state of Minnesota. The subjects that possessed the spores in Case A also possessed all of the other necessary materials needed to propagate the spores. However, the suspects were not charged, even though there was enough evidence according to Minnesota Statute §2D1.1 for the suspects to be charged with conspiracy to manufacture a controlled substance.

Case B

Evidence Description

Two boxes, each containing 12 half-print jars, were received (see Fig. 8). Each jar contained growing media with white mycelium growth (Fig. 9). The mycelium appeared to have been growing 10 to 13 days based on comparisons with previously grown mushrooms (1).



FIG. 8—Twelve of the jars containing mycelium growth submitted as evidence in Case B.



FIG. 9—Photograph of the mycelium growth submitted as evidence in Case B. Growth period is approximately 10 to 13 days.



FIG. 10—Primordia and mature mushrooms in terrarium grown from the mycelium that was submitted in Case B.

Sample Preparation

The mycelium was allowed to grow under indirect light. The samples were monitored and photographed during the growing process. Samples were transferred to terrariums when the pinheads were plump. The humidity was kept high inside the terrariums by spraying the plexiglass shield with water approximately three times a day. The terrariums were also aired out approximately three times a day to ensure high oxygen levels and rid carbon dioxide. The mushrooms were picked when mature, as previously described (1). Three of the jars grew pinheads, but those pinheads did not get large enough to transplant. The remainder of the jars all grew pinheads that eventually grew into mushrooms after transplantation into the terrarium (Fig. 10). All of the mushrooms and pinheads were weighed after drying.

Sample Analysis

A sample from each jar was screened using the Van Urks reagent. Two mushroom samples from each box were prepared and analyzed by TLC and GC/MS in accordance with the previous literature (1). All of the samples analyzed contained psilocyn.

Report

The report for this case read as follows:

Item 12 contained twelve jars containing a growing media that contained mycelium—the vegetative portion of the mushroom. The mycelium was allowed to continue to grow under appropriate environmental conditions. 28.8 grams of apparent mushrooms containing psilocyn were grown from the mycelium submitted in Item 12.

Item 13 contained twelve jars containing a growing media that contained mycelium—the vegetative portion of the mushroom. The mycelium was allowed to continue to grow under appropriate environmental conditions. 33.9 grams of apparent mushrooms containing psilocyn were grown from the mycelium submitted in Item 13.

The lower limit of detection for both psilocyn and psilocybin was determined to be 0.03 mg/mL by the TLC method and 0.1 mg/mL by the GC/MS method. It has been previously determined (1) that drugs are not found above these levels in mycelium using

these methods; therefore, the mycelium in Case B was not analyzed until mature mushrooms were developed. The subjects in this case were charged with conspiracy and aiding and abetting a control substance crime. They pled guilty to a lesser charge.

Case C

Evidence Description

Two half-pint jars that contained growing media with apparent primordia were submitted to the laboratory for analysis. Figures 11 and 12 illustrate two views of one jar that contained the primordia.

Sample Preparation

No additional sample preparation was required.

Sample Analysis

The primordia were removed from the growing media and weighed. A sample from each jar was screened using Van Urks reagent. Primordia samples from each jar were also prepared and

analyzed by TLC and GC/MS in accordance with the previously described methods (1). One sample contained psilocyn and the other contained psilocyn and psilocybin.

Report

The report for this case read as follows:

Item 11 contained 7.8 grams of apparent mushrooms containing psilocyn and psilocybin.

Item 12 contained 4.3 grams of apparent mushrooms containing psilocyn.

The subjects in this case were charged with possession of a controlled substance with intent to sell, and pled guilty.

Discussion

It is not the responsibility of the forensic drug chemist to determine the charges that are issued against a suspect. It is the chemist's responsibility to determine what the evidence possesses and to educate law enforcement personnel. Knowing the developmental stages of the mushroom and the illicit growing procedure can assist a drug chemist in his or her duties.

Photographs of the various stages of the mushroom along with examples of mushroom growing operations have been dispersed to law enforcement personnel in Minnesota. This has resulted in numerous arrests and convictions for growing and possessing the controlled substances of psilocyn and psilocybin.

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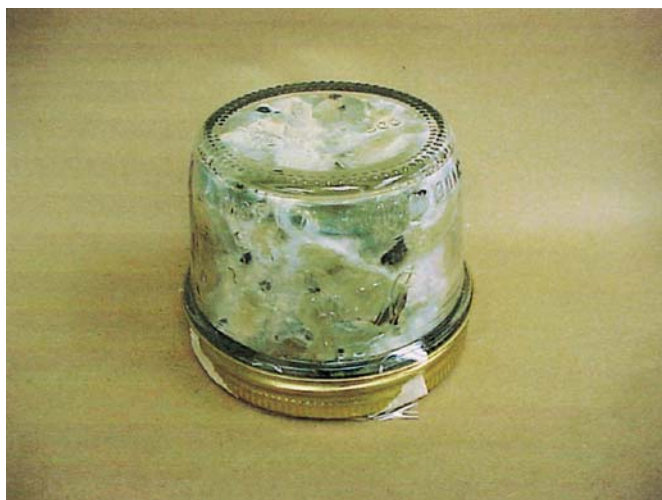


FIG. 11—Mason jar containing primordia submitted as evidence in Case C.

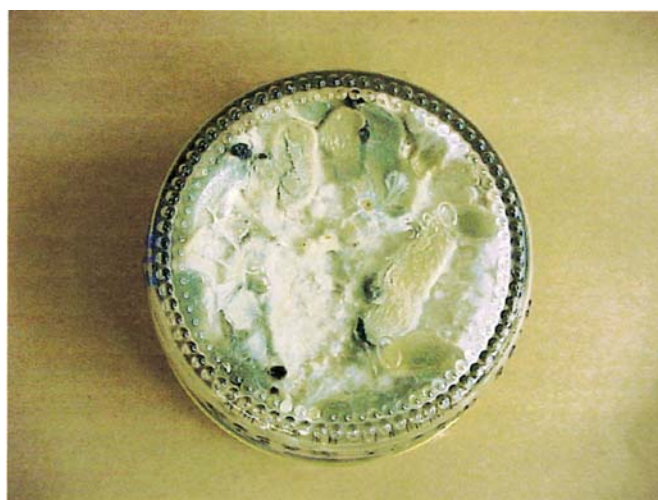


FIG. 12—Bottom view of a mason jar containing primordia submitted as evidence in Case C.