

## TECHNICAL NOTE

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### An Aqueous-Organic Extraction Method for the Isolation and Identification of Psilocin from Hallucinogenic Mushrooms

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**REFERENCE:** Casale, J. F., "An Aqueous-Organic Extraction Method for the Isolation and Identification of Psilocin from Hallucinogenic Mushrooms," *Journal of Forensic Sciences*, JFSCA, Vol. 30, No. 1, Jan. 1985, pp. 247-250.

**ABSTRACT:** A simple aqueous extraction method for the isolation and identification of psilocin from *Psilocybe cubensis* mushrooms is reported. This method employs a dephosphorylation of the phosphate ester to psilocin, which facilitates a greater product yield and simplifies identification. Psilocin extracted by this method is sufficiently concentrated and free of cocontaminants to allow identification by infrared spectroscopy and gas chromatography/mass spectrometry.

**KEYWORDS:** toxicology, psilocin, extraction

The tryptamines are one of four categories of hallucinogenic indoles in more than 20 classes of indole compounds comprising approximately 600 alkaloids [1]. Considerable research has been conducted with psilocin and psilocybin since their isolation by Hofmann et al [2]. Several extraction techniques [1,3-6] have been used to isolate psilocin and psilocybin from more than two dozen species of mushrooms in four genera (*Conocybe*, *Panaeolus*, *Psilocybe*, *Stropharia*). The techniques that use methanol coextract other compounds such as urea, ergosterol, ergosterol peroxide,  $\alpha,\alpha$ -trehalose, baecocystin, and norbaecocystin [3,4,7]. At present, a useful aqueous extraction procedure has not been reported for psilocin and psilocybin.

The dephosphorylation of psilocybin to psilocin in vivo has been well documented [1,8,9] and is thought to account for most or all of its central nervous system activity [8]. Conversion of psilocybin to psilocin is also necessary for aqueous extraction with organic solvents because of the very low lipid solubility of psilocybin. Extraction of only one compound also permits infrared analysis of the extract.

Concentration and detectability of psilocin and psilocybin are dependent on several variables, including:

1. The absence of glucose, which will prevent the production of psilocybin [10].
2. Low levels of ammonium succinate, which will give poor yields of psilocybin [10].
3. The growing medium, which requires a pH of less than 7 [10].
4. Timing: maximum protection of psilocybin occurs on the seventh day after germination, while maximum production of the mycelium is reached by the ninth day [10].

Received for publication 24 March 1984; accepted for publication 21 May 1984.

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5. Temperature: complete loss of psilocin and psilocybin will occur in harvested mushrooms left at room temperature for an extended period of time [3].

6. Oxidation: psilocin will oxidize to a blue product (possibly accounting for the bluing color in the four genera containing psilocin and psilocybin) [9].

Because of the increasing popularity of these mushrooms and kits available from drug-oriented publications for growing mushrooms containing psilocin and psilocybin in cow manure a simple aqueous extraction procedure has been developed that extracts reasonably pure psilocin from mature mushrooms. This extraction method greatly simplifies the identification of psilocin from those mushrooms by infrared spectroscopy and gas chromatography/mass spectrometry (GS/MS).

### Experimental Procedure

A representative sample of 2 to 10g of dried mushrooms is ground to a fine powder by mortar and pestle. The powder is mixed with 100 mL of dilute acetic acid in a 250-mL beaker. The pH is readjusted to pH 4 with glacial acetic acid. After standing 1 h, the beaker is placed in a boiling water bath for 8 to 10 min or until the internal temperature of the acid mixture reaches 70°C. The beaker is removed and cooled to room temperature under running water. The acid mixture is separated from the mushroom powder by suction filtration using glass wool. The filtrate is brought to pH 8 with concentrated ammonium hydroxide and quickly extracted with two 50-mL portions of diethyl ether. Gentle mixing instead of shaking should be used to prevent an emulsion. The ether is dried over sodium sulfate, filtered, and evaporated under nitrogen with no applied heat.

Crude psilocin will appear as a greenish residue. Recrystallization from chloroform/*n*-heptane (1:3) yields white crystals. The resulting powder can then be submitted to infrared and mass spectral analyses.

Infrared spectra were obtained from potassium bromide disks on a Beckman Microlab 600 spectrophotometer. A Finnigan Model 3200 GC/MS with Model 6000 data system was used for producing the mass spectra.

### Results and Discussion

This method permits rapid isolation of psilocin from hallucinogenic mushrooms by coextraction of both psilocin and psilocybin. Dilute acetic acid is an excellent solvent for this purpose, because both compounds are very soluble in acetic acid [11] and very little of other interfering substances are extracted. It is most likely some other compounds are coextracted but are removed from psilocin in the ether extraction from the aqueous base. Psilocybin is completely dephosphorylated to psilocin by heating the acid extract. After addition of the base, extraction into ether should be performed promptly, because of decomposition of psilocin at a greater than 7 [12]. The extraction and dephosphorylation steps produce reasonably pure psilocin from a small amount of mushroom material. Two grams of mushrooms will often be sufficient to obtain an infrared spectrum of psilocin (Fig. 1). Smaller mushrooms exhibit ample psilocin for mass spectral analysis (Fig. 2).

This method has been used in our laboratory for six months and has given excellent results in separating psilocin from methanol-soluble compounds. Other identification techniques such as gas chromatography and microcrystalline tests are possible on psilocin extracted by this method.

### Acknowledgments

The author would like to thank J. R. Daniel and M. S. Nelson for their help in the preparation of this manuscript.

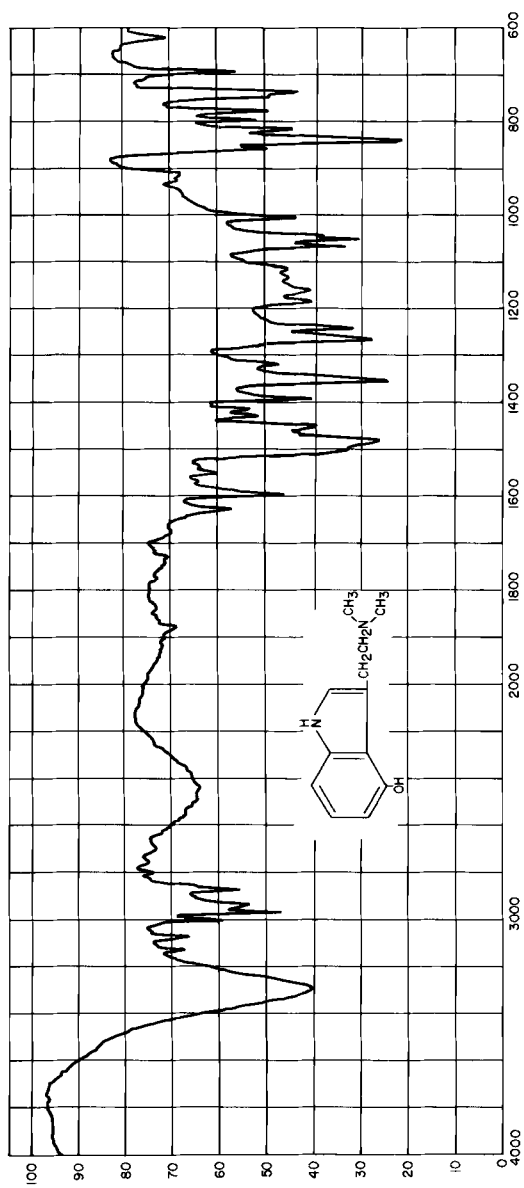


FIG. 1—Infrared spectrum of psilocin.

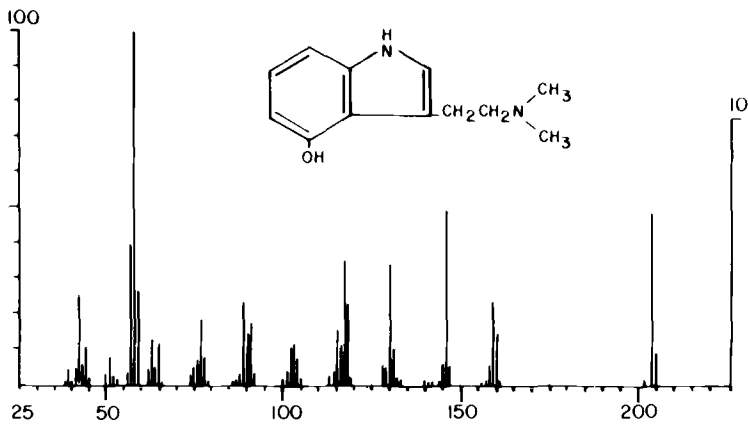


FIG. 2—Electron impact mass spectrum of psilocin.

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