TECHNICAL NOTE

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A Technique for the Rapid Isolation and Identification of Psilocin from Psilocin/Psilocybin-Containing Mushrooms

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ABSTRACT: A method has been developed for the rapid isolation and identification of psilocin from psilocin/psilocybin-containing mushrooms. Based on the difference in the solubility properties in butyl chloride of psilocin and other constituents present in psilocin/psilocybin-containing mushrooms, psilocin was easily separated in pure form.

KEYWORDS: toxicology, psilocin, psilocybin, solubility

The analysis of psilocin/psilocybin-containing mushrooms has been done by thin-layer chromatography (TLC) [*1*], ultraviolet (UV) spectrophotometry [*2*], infrared (IR) spectroscopy [*3*], and, more recently, high performance liquid chromatography (HPLC) [*4*]. In each case, lengthy procedures are needed to prepare the mushrooms for analysis. When ultraviolet spectrophotometry or infrared spectroscopy are used, thin-layer chromatography is the method relied upon to isolate and obtain the desired component.

The technique developed here involves a method which takes advantage of the solubility property difference between psilocin and the other constituents present in psilocin/psilocybin mushrooms in the solvent butyl chloride. Psiolocin and psilocybin in psilocin/psilocybin mushrooms are soluble in methanol and in dilute sulfuric acid and sodium hydroxide solutions. However, only psilocin is soluble in butyl chloride. Because of this psilocin can be extracted from mushrooms, purified by solvent/solvent extraction methods using butyl chloride, and identified using ultraviolet spectrophotometry or infrared spectroscopy or both in less than 1 h. The ease of extraction and purification using this method eliminates the need for any special time-consuming preparation of the mushrooms and allows analysis to proceed rapidly.

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Experimental Procedures

Ultraviolet and Infrared Spectroscopy

The UV and IR spectra of the samples were recorded using a Beckman model DB-G grating spectrophotometer and Perkin-Elmer model 1430 ratio recording infrared spectrophotometer with a model 3600 data station.

Reagents

Samples of pure psilocybin, psilocin, and *N,N*-diethyltryptamine fumarate were obtained from Applied Science Laboratories, State College, PA. Pure bufotenine monooxalate was obtained from Sigma Chemical Co. Methanol and butyl chloride were high purity grade solvents. All chemicals were used as received.

Mushroom Extraction

A weighed amount of mushroom material, about 2 g, consisting of stool caps and stems were placed into a closed vial with 15 mL of methanol. The material was allowed to soak for ½ h, after which, the methanol was removed and evaporated to dryness. The residue was taken up in 25 mL of 0.1*N* sodium hydroxide (NaOH) and extracted with 25 mL of butyl chloride. The butyl chloride was then extracted with 15 mL of 0.1*N* sulfuric acid (H_2SO_4) and the UV spectrum of the resulting aqueous fraction was recorded at both acid and basic pH. After recording the UV spectrum at basic pH, this solution was re-extracted with 25 mL of butyl chloride. This was followed by the separation of the phases and evaporation of the butyl chloride layer to dryness in a mortar for the preparation of a KBr pellet. An IR spectrum was then recorded from the KBr pellet.

Results

Figure 1 shows the UV spectrum obtained after the solvent/solvent purification of the mushroom extract which was obtained after only ½ h of contact with the mushrooms. The UV spectra were recorded at both acid and basic pH. For comparison purposes, Fig. 2 shows the UV spectra from a standard of pure psilocin which was also recorded at acid and basic pH. After recording the UV spectra at basic pH from both the mushroom sample and the standard psilocin solution, each material was re-extracted into butyl chloride and prepared for an IR analysis. Figure 3, spectra (*a*) and (*b*), shows the spectrum of the purified material from the mushroom and the spectrum of the pure psilocin standard, respectively.

Discussion

In the past, attempts to separate psilocin from psilocybin by solvent systems were generally unsatisfactory [*4*]. The similar structure of psilocin and psilocybin (Fig. 4) suggests that the two materials would be similar in their solubility properties in given solvents. For example, both materials are soluble in methanol, and also in dilute acids and in dilute bases, a result that is reasonable considering the fact that both the structures exhibit similar polarity characteristics and have present in their structures electronegative atoms of oxygen and nitrogen. Also, both substances have replaceable hydrogen atoms in their molecular structures as well as having basic amine groups which can probably be attributed to their solubility in dilute acids and in dilute bases.

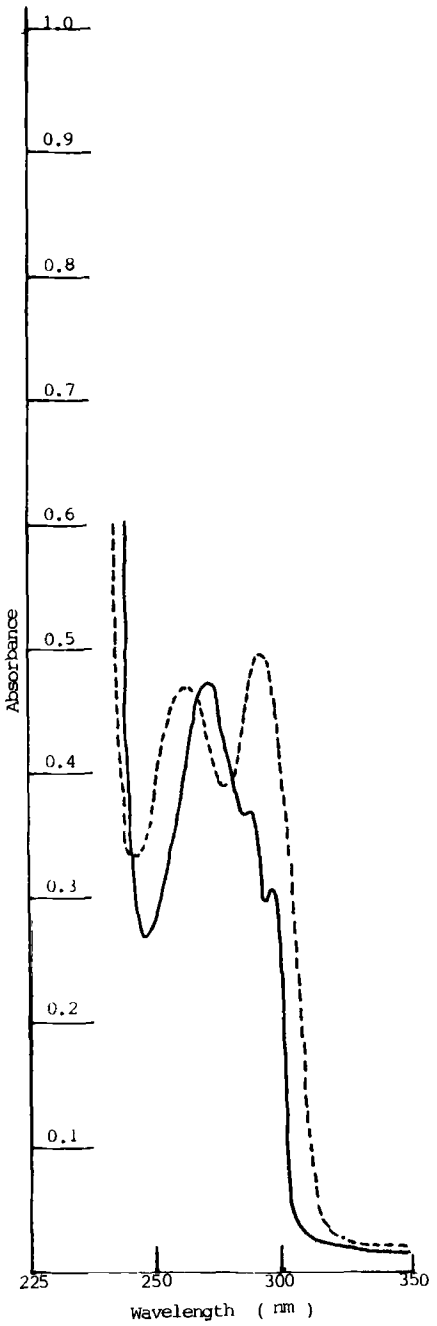

FIG. 1—*Solvent/solvent purification of mushroom extract where solid line is 0.1* N H_2SO_4 *and broken line is NaOH.*

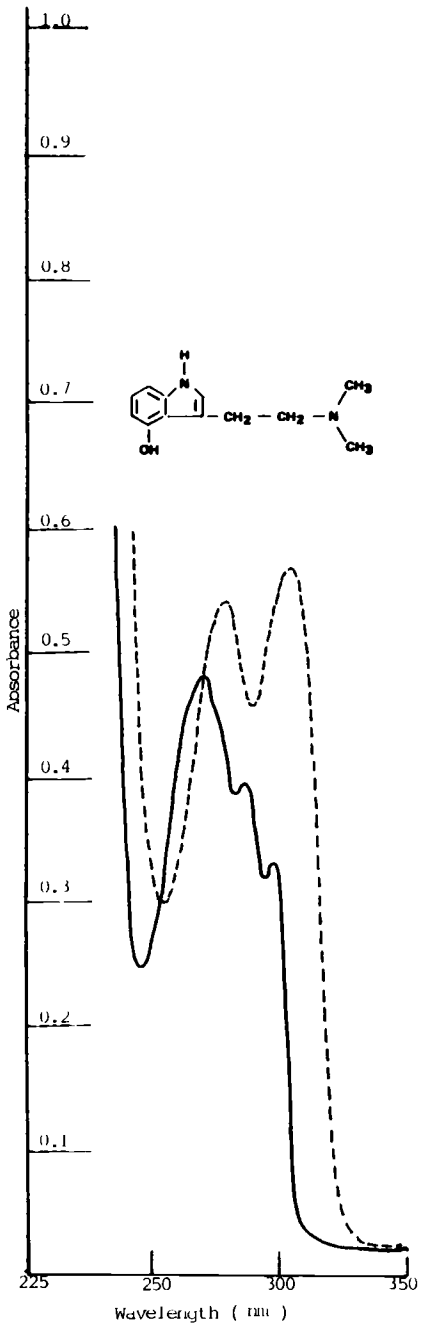

FIG. 2—*Pure psilocin 35 μg/mL where solid line is 0.1* N H_2SO_4 *and broken line is NaOH.*

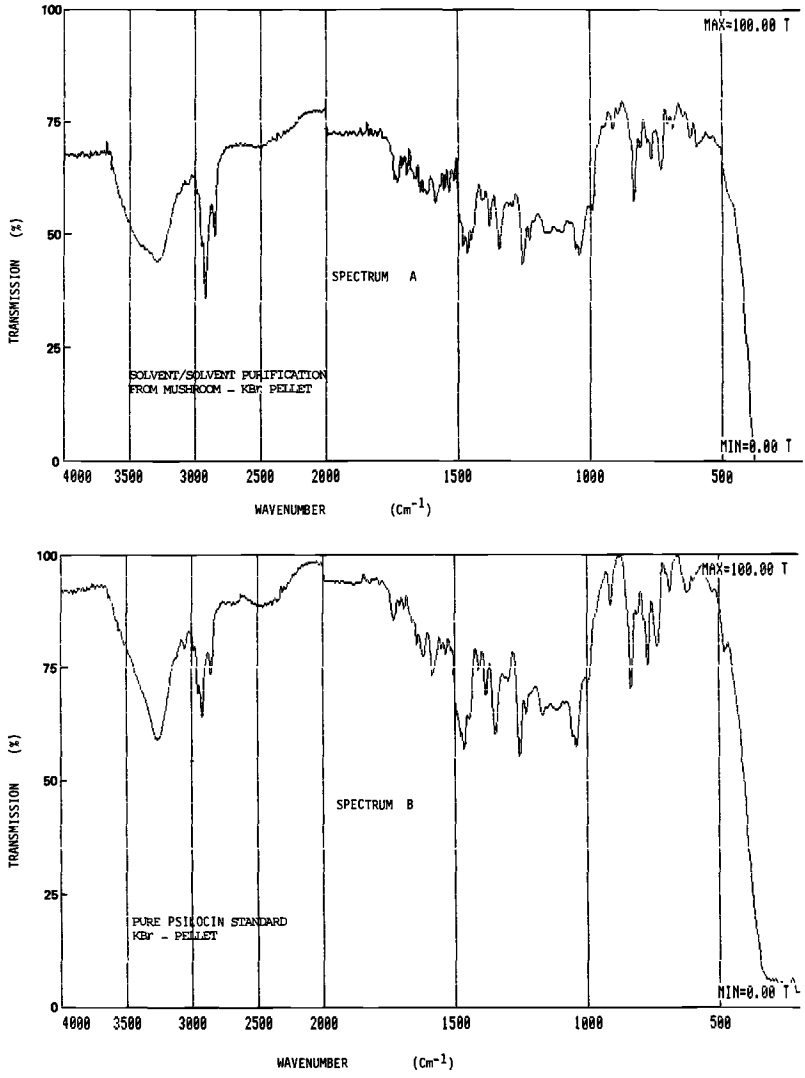

FIG. 3—(a) *Solvent/solvent purification from mushroom-KBr pellet and* (b) *pure psilocin standard—KBr pellet.*

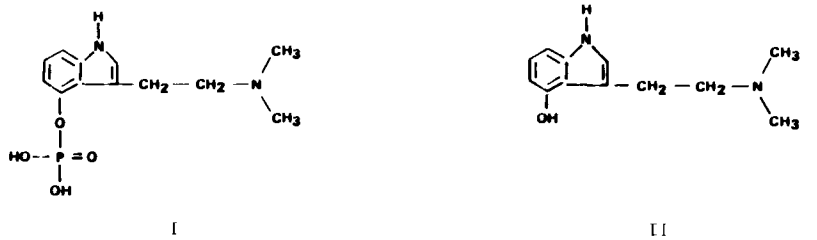

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FIG. 4—*Structure I is psilocybin and Structure II is psilocin.*

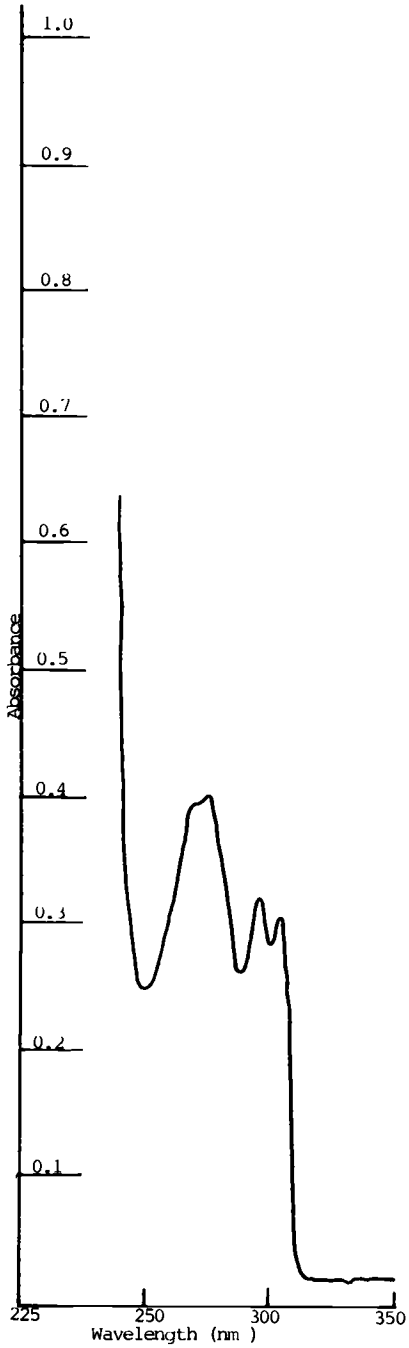

FIG. 5—*Pure psilocin 31 μg/mL in butyl chloride.*

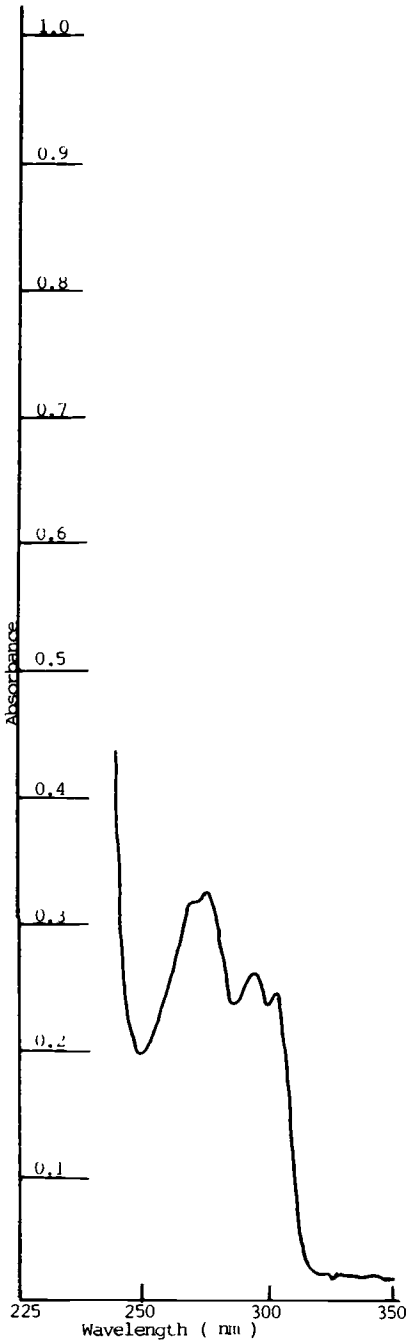

FIG. 6—*Solvent/solvent purification of mushroom extract—the butyl chloride phase.*

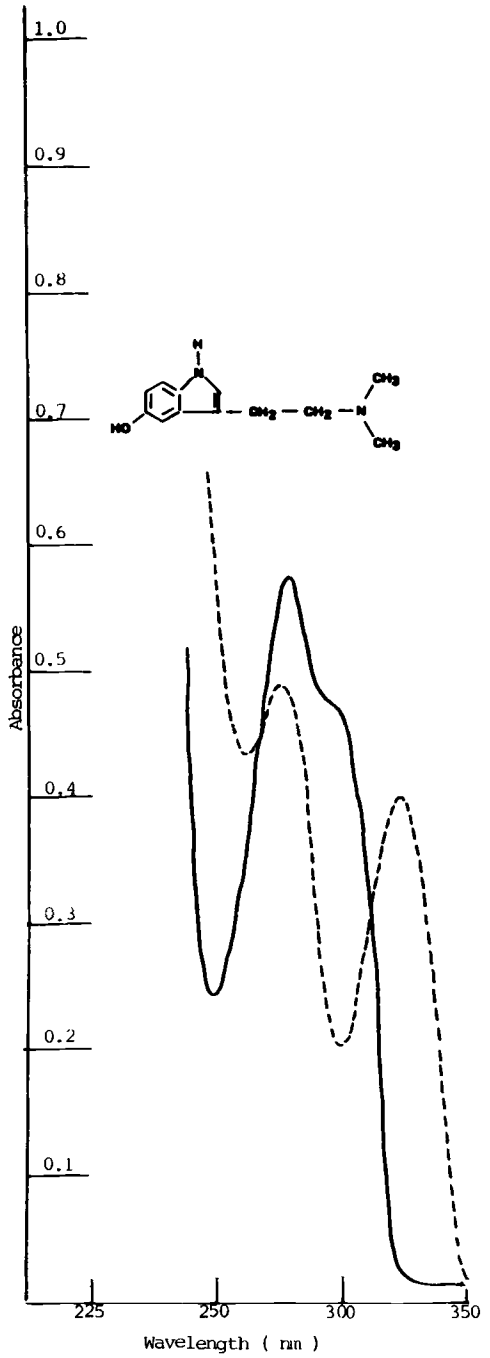

FIG. 7—*Pure bufotenine 30 μg/mL where solid line is 0.1* N H_2SO_4 *and broken line is NaOH.*

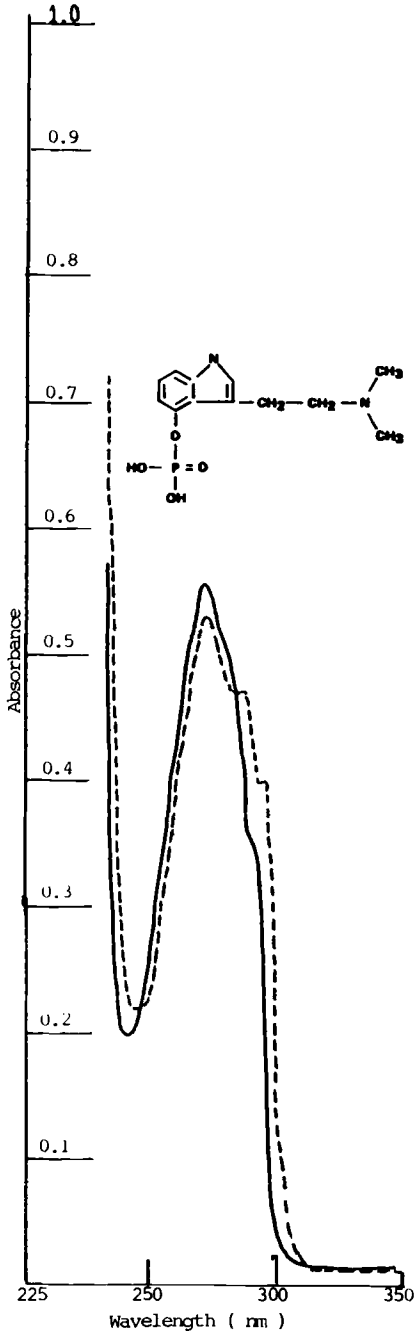

FIG. 8—*Pure psilocybin 30 μg/mL where solid line is 0.1 N H_2SO_4 and broken line is NaOH.*

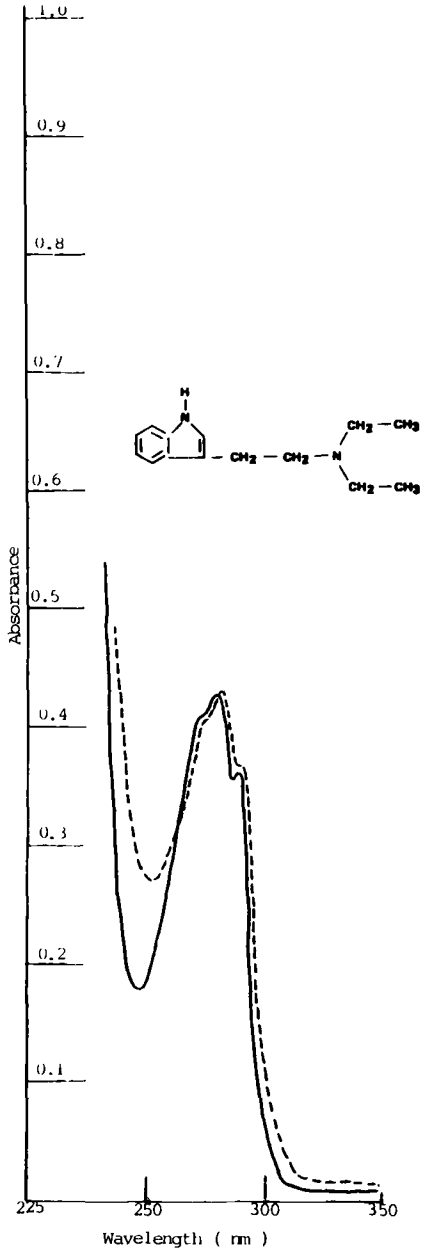

FIG. 9—*Pure* N,N-*diethyltyptamine 31 μg/mL where solid line is 0.1* N H_2SO_4 *and broken line is NaOH.*

On further inspection, it appears that there is nothing in both of the structures that would suggest that only psilocin should be soluble in butyl chloride or other such halogenated hydrocarbon solvents and, indeed, be extracted into a butyl chloride solution from an aqueous basic solution. Normally, it would be expected that psilocin should be extracted, if at all, into butyl chloride from a neutral solution. However, on a closer look at the two structures, it can be seen that the ionic properties of psilocybin is greater than that of psilocin. This is believed to be the reason why psilocin is solubilized in a solvent of low dielectric constant such as butyl chloride or chloroform while psilocybin is not. Figure 5 shows a UV spectrum of the butyl chloride phase after extraction of a basic solution containing mushroom-extracted material. It indicates at this point that there is an apparent absence of the other mushroom constituents and that psilocin is present in relatively pure form. For comparison purposes, Fig. 6 shows a UV spectrum of a pure sample of psilocin in butyl chloride.

Finally, in comparing the UV spectrum of materials having somewhat similar molecular structures to psilocin, such as *N,N*-diethyltryptamine, psilocybin, and, indeed, psilocin's isomer bufotenine, Figs. 7, 8, and 9 show that the UV spectrum of psilocin can be differentiated from the spectra of these other substances.

References

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