The Occurrence of Psilocybin in *Gymnopilus* Species

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ABSTRACT.—An accidental case of mushroom poisoning led to the detection of psilocybin in *Gymnopilus validipes* (Cortinariaceae). This compound was subsequently isolated (0.12% yield) by anion exchange and cellulose chromatography. Eighteen additional species of *Gymnopilus* were screened by a method capable of detecting 0.0004% psilocybin in dried carpophores. Psilocybin was detected in *G. aeruginosus, G. luteus, G. viridans* and *G. spectabilis*. The latter species has been previously reported to be hallucinogenic. This is the first report of psilocybin from this genus.

This investigation was initiated following a case of accidental mushroom poisoning which occurred in Dearborn Heights, Michigan, October, 1976. Two individuals ingested an undetermined quantity of *Gymnopilus validipes*, which they mistook for the edible mushroom *Armellaria mellea*. The initial symptoms of the intoxication developed rapidly after ingestion and consisted of dysphoria, dizziness, and abnormally colored vision. Within an hour the individuals experienced difficulty in expressing their thoughts, anxiety, time distortion, and vivid visual hallucinations. Both received an emetic and supportive therapy at a local hospital and recovered completely within 18 hours.

The symptoms displayed in this case are very similar to those produced by psilocybin [4-phosphoryloxy-3-(2-dimethylaminoethyl)indole], an hallucinogenic tryptamine derivative previously isolated from several genera of mushrooms (1, 2). Because of this similarity, a methanol extract of the *G. validipes* carpophores was chromatographically examined for the presence of psilocybin. A constituent was detected which gave a psilocybin-like R_F value and chromophore with Ehrlich's reagent (3). However, the extract also contained pigments which obscured the chromatogram and prevented unequivocal chromatographic identification of the detected compound. For this reason, the constituent suspected to be psilocybin was isolated from *G. validipes* by ion exchange and cellulose chromatography. The freeze-dried carpophores yielded 0.12% of a compound which was identical chromatographically (3 tlc systems) and spectrally (ms and uv) to standard psilocybin. The isolated material also yielded psilocin, the dephosphorylated derivative of psilocybin, when subjected to hydrolysis (4). The level of psilocybin present in *G. validipes* carpophores was not determined. However, the amount isolated indicates that the mushroom could cause psilocybin intoxication if enough (less than 100 g fresh weight) was ingested (1).

This is the first report of psilocybin from the genus *Gymnopilus* and from the family Cortinariaceae. Psilocybin has been previously isolated or detected in species of *Psilocybe, Stropharia* (Strophariaceae), *Panaeolus, Panaeolina, Copelandia* (Coprinaceae), *Conocybe* and *Pholiotina* (Bolbitiaceae) (5–9).

A closely related species of *Gymnopilus, G. spectabilis,* has also been reported to be hallucinogenic. In 1965, Walters (10) described a case in which visual and auditory hallucinations were experienced by an individual who had eaten a "few nibbles" of a mushroom identified by Walters as *Pholiota* (*Gymnopilus*) *spectabilis*. This identification cannot be considered certain, however, since only a small portion of the pileus was available for identification. It is

possible that the mushroom involved was one of several species now recognized which are closely related to *G. spectabilis* (11).

Buck (12) reported another case involving three individuals in 1967. One developed symptoms suggestive of psilocybin intoxication whereas two others, who had eaten less of the mushroom, experienced only alcohol-like effects. Buck stated that the mushroom involved was *Gymnopilus spectabilis* and that no psilocybin was found in a portion which had been chromatographically (tlc) examined. This report should not be accepted as unequivocal evidence that an hallucinogenic collection of *G. spectabilis* was devoid of psilocybin since the assay procedure utilized may have precluded the detection of this compound. The carpophores were dried at 80° and extracted with methanol in a Soxhlet apparatus. Both of these procedures could have led to thermal decomposition of any psilocybin present (3). Also, as mentioned previously, psilocybin is difficult to detect in methanol extracts of these mushrooms if it is tested for only by tlc.

Reports concerning the hallucinogenic effects of *G. spectabilis* have also come from Japan. Romagnesi (13), in a paper dealing with poisonous mushrooms of Japan, mentioned that *Gymnopilus spectabilis* is considered hallucinogenic in that country and that chemical analysis of the "active compound" indicated it was entirely different from psilocybin. Although this report appeared in 1964, no additional information has been published regarding the nature of this or other constituents of *G. spectabilis* of Japanese origin. Sanford (14) has published an ethnobotanical investigation of *ō-waritake,* the so called "big laughing mushroom" of Japanese folklore, and has proposed that this hallucinogenic fungus is *G. spectabilis*.

According to Heim (15), the European *G. spectabilis* is not known to be hallucinogenic although its morphologic characters resemble those of the Japanese species on all points. Recently, Gerault (16) reported that a collection of *G. spectabilis* from France was devoid of psilocybin.

Very little is known about the phytochemistry of *G. spectabilis* and other members of this genus. Hatfield and Brady (17, 18) reported the isolation of *bis*-noryangonin [4-hydroxy-6-(4-hydroxystyryl)-2-pyrone] and hispidin [4-hydroxy-6-(3,4-dihydroxystyryl)-2-pyrone] from several *Gymnopilus* species including *G. spectabilis*. These styrylpyrones are structurally related to the α-pyrones found in kava, an intoxicating beverage prepared from *Piper methysticum* (19). However, it is doubtful that the fungal pyrones contribute to the purported hallucinogenic activity of *G. spectabilis*. *Bis*-noryangonin was recently tested for hallucinogenic activity and was found to be inactive up to a dose of 50 mg/kg in rats.[1] It is interesting to note that both *bis*-noryangonin and hispidin give an indole-like chromophore with Ehrlich's reagent (tlc). This fact may have lead to the reports that indole derivatives other than psilocybin were present in *G. spectabilis* (12, 13).

Following the discovery of psilocybin in *G. validipes,* a number of collections of *G. spectabilis* were screened for the presence of this compound. The screening procedure involved methanol extraction of dried carpophores at room temperature followed by ion exchange chromatography and tlc analysis for psilocybin. The minimum detectable concentration of psilocybin in dried mushrooms was found to be 0.0004%. Below this level the presence of psilocybin would be toxicologically insignificant. A total of 13 collections were screened and, of these, four were found to contain psilocybin. Thus, it appears that this compound is responsible for the hallucinogenic effects reported for this species.

The inconsistent occurrence of psilocybin in the *G. spectabilis* samples assayed may in part be due to the age of some of the collections (up to 21 years).

[1]E. F. Domino, Professor of Pharmacology, University of Michigan. Personal communication, 1977.

However, this is probably not the only factor involved since psilocybin has been found to be quite stable in dried herbarium samples. Leung (3) found psilocybin in a *Psilocybe* collection over 25 years old, and in the present study a *Gymnopilus* sample collected in 1950 contained psilocybin.

Another explanation of these results is that two or more subspecies of *G. spectabilis* may exist which vary in their ability to produce psilocybin. Sanford (14) has proposed this to reconcile the purported difference between Japanese (hallucinogenic) and European (nonhallucinogenic) *G. spectabilis*. If this is the case for this species in the United States, the psilocybin containing strain(s) may be more prevalent in the East. Four of eight collections from Ohio, Michigan, Massachusetts, and Ontario were found to contain psilocybin, whereas none of those from the West were positive. This distribution coincides with the reports of *Gymnopilus* intoxication which have occurred in Massachusetts, Michigan and Ohio (10, 12). Inconsistent occurrence of psilocybin within a species has also been noted in the genus *Panaeolus* (8). Further chemotaxonomic studies will be necessary to clarify fully this situation.

In addition to *G. spectabilis,* a number of other *Gymnopilus* species were screened for psilocybin. In the most recent taxonomic treatment of the North American species of *Gymnopilus,* Hesler (11) divided the genus into two subgenera (*Annulati* and *Gymnopilus*) based on the presence or absence of a persistent annulus. The type species of the subgenus *Annulati* is *G. spectabilis*. Of the 16 species in this group, five were screened for psilocybin (table 1). The only additional species found to contain this compound was *G. luteus*. As was the case with *G. spectabilis,* not all collections contained psilocybin.

Hesler (11) subdivided the subgenus *Gymnopilus* into two sections (*Microspori* and *Gymnopilus*) based on spore size. Four of the 22 species found in section *Microspori* were screened and none contained psilocybin, as shown in table 1. *Gymnopilus* sect. *Gymnopilus* contains 33 species, of which 10 were screened (table 1). Both *G. aeruginosus* and *G viridans* were found to contain psilocybin. Singer (20) stated that *G. aeruginosus* contains "an alkaloid which is said to have psychotropic effects," but no reference is given to the

TABLE 1. *Species of Gymnopilus examined for psilocybin.*

Subgenus	Species	Psilocybin[a]
Annulati	*G. luteus* (Pk.) Hesler	+
	G. spectabilis (Fr.) A. H. Smith	+
	G. subspectabilis Hesler	−
	G. validipes (Pk.) Hesler	+
	G. ventricosus (Earle) Hesler	−
Gymnopilus sect. *Microspori*	*G. punctifolius* (Pk.) Sing.	−
	G. sordidostipes Hesler	−
	G. subtropicus Hesler	−
	G. terrestris Hesler	−
Gymnopilus sect. *Gymnopilus*	*G. aeruginosus* (Pk.) Sing.	+
	G. aurantiophyllus Hesler	−
	G. flavidellus Murr.	−
	G. liquiritiae (Pers. ex Fr.) Karst.	−
	G. luteofolius (Pk.) Hesler	−
	G. mitis Hesler	−
	G. penetrans (Fr. ex Fr.) Murr.	−
	G. picreus (Fr.) Karst.	−
	G. sapineus (Fr.) R. Marie	−
	G. viridans Murr.	+

[a]A species is considered positive if one or more collections were found to contain psilocybin.

original work, and no other publication concerning the hallucinogenic effects or psilocybin content of *G. aeruginosus* could be found in a review of the literature.

EXPERIMENTAL

PLANT MATERIAL.—The date and site of collection of each mushroom sample are given below. Samples obtained from G. M. Hatfield were freeze-dried whereas others were air dried (<50°). The identification of all collections was determined according to Hesler's monograph of the genus (11). Samples of all collections utilized have been deposited in The University of Michigan Herbarium.

THIN-LAYER CHROMATOGRAPHY.—The following tlc systems employing silca gel 60 F–254 (E.M. Reagents) were utilized in this study. Tlc system A: *n*-butanol-acetic acid-water, 2:1:1. Tlc system B: *n*-butanol-pyridine-water-acetic acid, 15:10:10:3. Tlc system C: *n*-propanol-5% NH_4OH, 5:2. The R_F values of psilocybin were A 0.25, B 0.10, and C 0.12. A 2% solution of *p*-dimethylaminobenzaldehyde (PDAB) in conc. HCl-ethanol (1:1) was used as a detection reagent.

ISOLATION OF PSILOCYBIN FROM *G. validipes*.—Five grams of freeze-dried *G. validipes* carpophores were ground to a 20-mesh powder and extracted with 500 ml of methanol by stirring at room temperature for 22 h. The marc was removed by filtration, and the extract was reduced to dryness *in vacuo*. Ten ml of distilled water was added to the extract residue (2.01 g), and the pH was adjusted to 9.5 using 58% NH_4OH. Undissolved material was removed by centrifugation (1300xG), and the solution was applied to a 9.0 ml column (0.9 x 15 cm) of AGlX–4 anion exchange resin (Bio-Rad, bicarbonate, 100–200 mesh) which was equilibrated with 0.01 M NH_4HCO_3, pH 9.0 buffer. After the sample volume passed into the resin, the column was washed with 70 ml of 0.01 buffer (flow rate <0.5 ml/min). This eluate, which was free of psilocybin, was discarded. The resin was then eluted with 75 ml 0.1 M buffer. This eluate was freeze-dried to remove the volatile buffer. Tlc analysis of the residue (48 mg) indicated the presence of psilocybin. The residue also contained several other constituents that could be detected by tlc system A.

The freeze dried residue from the 0.1 M buffer eluate was dissolved in 1 ml distilled water and mixed with 2.0 g of Avicel (Brinkmann, 38 μ). This mixture was allowed to dry overnight. The absorbed sample was applied to a 1.5 x 38 cm column of Avicel which was poured as an acetone slurry and equilibrated with a *n*-butanol saturated with water. The column was then eluted with this solvent at a rate of 0.18 ml/min. Fractions (4 ml) were collected, reduced to dryness *in vacuo*, and evaluated (tlc system A) for the presence of psilocybin. Fractions 42–45 contained psilocybin and were free of other constituents (tlc systems A, B, C). The uv spectrum of the combined fraction was identical to standard psilocybin [λmax(MeOH) 220, 267, 288 nm]. Quantitative analysis based on the absorbence at 267 nm showed that 5.93 mg of psilocybin had been isolated. The EI mass spectrum of the isolated psilocybin was identical to that obtained for standard psilocybin and contained major ions at m/e 204, 160, 159, 146, 130, 117, and 58 (100%).

To confirm the identification of the isolated material, Troxler's *et al.* (4) procedure was used to hydrolyze a sample (approximately 1 mg) to psilocin. Psilocin was detected in the hydrolysis reaction mixture by tlc analysis (system A R_F 0.52, B 0.55, C 0.76) with PDAB as a detection reagent.

SCREENING PROCEDURE FOR PSILOCYBIN.—The dried mushroom sample was ground to a 20-mesh powder and 0.5 g was extracted with 75 ml of methanol by stirring at room temperature for 12 h. After removal of the marc by filtration, the extract was reduced to dryness *in vacuo*, and 2 ml of 0.01 M NH_4HCO_3 was added to the residue. The pH of the resulting solution was adjusted to 9.0 with 5% NH_4OH, and any undissolved material was removed by centrifugation.

The sample solution was applied to a column (50 ml buret) containing 2–3 ml of AGlX–4 anion exchange resin and eluted with 5 times the column volume of 0.01 M NH_4HCO_3, pH 9 buffer, followed by an equal volume of 0.1 M buffer. The 0.01 M eluate was discarded, and the 0.1 M eluate was freeze-dried for tlc analysis.

One ml of methanol was added to the freeze-dried residue, and the mixture was heated on a steam bath for 1 min to aid dissolution. Any undissolved material was allowed to settle, and 50 μl of the solution was applied to the tlc plate. After the plate was allowed to develop at least 10 cm (tlc system A), the chromatogram was sprayed with PDAB reagent as previously described. The plate was heated in a forced air oven at 90° for 2 min. Psilocybin formed a purple-blue chromaphore which allowed detection of 0.1 μg per sample. If detected, the presence of psilocybin was confirmed by additional tlc analysis (systems B and C).

The limit of detection (0.1 μg) was determined by the analysis of mushroom extracts to which known quantities of psilocybin were added. With this level of sensitivity, 0.0004% psilocybin in dried samples could be detected.

MUSHROOM COLLECTION ASSAYED FOR PSILOCYBIN.—Each collection listed below is designated by the collector's initials and collection number followed by the location and year of collection. An explanation of the sample abbreviations utilized is given below. Samples found to contain

psilocybin are indicated with an asterisk. *G. aeruginosus:* AHS 35829–MI50*, WBC 33124–OH61, GMH 111–WA68*, GMH 4–WA69*, AHS 62277–MI77*. *G. aurantiophyllus:* GMH 18–WA69. *G. flavidellus:* HDT 18035–CA66, AHS 76104–ID68. *G. liquiritiae:* WK 5230–AK71. *G. luteofolius:* ICB 2427–MI65, GMH 13–WA69. *G. luteus:* JFA 2582–MI68*, JFA 4030–MI69, FVH 1721–MI70. *G. mitis:* HDT 12014–CA64. *G. penetrans:* SJM 6251–WS70. *G. picreus:* AHS 83374–WA72. *G. punctifolius:* GMH 28–WA66. *G. sapineus:* HDT 21715–CA67, GMH 3–WA69. *G. sardidostipes:* GMH 7–WA66. *G. spectabilis:* HDT 4048–MI56, AHS 58269–MI57, RLS 3581–MA61, CB 1340–NM62, AHS 72862–MI65, AHS 73836–ID66, HDT 21307–CA67, AHS 60600–ID68, AHS 87286–MI71*, AHS 80521–EG71, JFA 7281–ON75*, AHS 87301–MI77*, WS 62977–OH77*. *G. subspectabilis:* HDT 21275–CA67. *G. subtropicus:* HDT 7913–LA60. *G. terrestris:* AHS 82794–MI72. *G. validipes:* AHS 87285–MI76*. *G. ventricocus:* GMH 15–WA67, GMH 135–WA68. *G. viridans:* JFA 7751–ON76*.

The abbreviations used above are as follows. Collectors: JFA—Ammirati, CB—Barrows, ICB—Bartelli, WBC—Cooke, GMH—Hatfield, FVH—Hoseney, SJM—Mazzer, RLS—Schaffer, AHS—Smith, WS—Sturgeon, HDT—Thiers, and WK—Wells, Kempton. Collection sites: AK—Alaska, CA—California, EG—England, ID—Idaho, LA—Louisiana, MA—Massachusetts, MI—Michigan, NM—New Mexico, OH—Ohio, ON—Ontario, WA—Washington, and WS—Wisconsin.

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