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Hallucinogenic mushrooms on the German market — simple instructions for examination and identification

F. Musshoff[a,*], B. Madea[a], J. Beike[b]

[a]*Institute of Legal Medicine, Rheinische Friedrich-Wilhelms-University, Stiftsplatz 12, 53111 Bonn, Germany*
[b]*Institute of Legal Medicine, Westfaelische-Wilhelms-University, von-Esmarch Strasse 62, 48149 Muenster, Germany*

Abstract

'Magic mushrooms' is the name most commonly given to psychoactive fungi containing the hallucinogenic components psilocybin and psilocin. Material confiscated by local authorities was examined using morphologic, microscopic, microchemical, and toxicological methods. *Psilocybe cubensis* was the most popular mushroom in the sample collective, followed by *Psilocybe semilanceata*, *Panaeolus cyanescens* and *Psilocybe tampanensis*. The alkaloid content was determined with <0.003–1.15% of psilocybin and 0.01–0.90% psilocin. *Panaeolus cyanescens* was the mushroom with highest levels of psilocybin and psilocin.

Keywords: Hallucinogenic mushrooms; Psilocybe; Psilocybin; Psilocin; Morphology; Analytical toxicology

1. Introduction

'Magic mushrooms' is the name most commonly given to psychoactive fungi [1]. From hallucinogenic mushrooms Hofmann et al. [2] isolated two hallucinogenic components of the tyramine type, psilocybin (4-phosphoryloxy-*N,N*-dimethyltryptamine), the main psychotropic compound, and psilocin (4-hydroxy-*N,N*-dimethyltryptamine). The metabolism and pharmacokinetic properties of psilocybin have been investigated [3]. The rapid and extensive cleavage of the phosphoric ester group of psilocybin by alkaline phosphatase and unspecific esterases indicates that psilocybin acts as a prodrug and that its hydroxy metabolite psilocin represents the true pharmacologically active agent. 4-Hydroxyindole-3-acetic acid and 4-hydroxytryptophole were identified as further metabolites. Today these indole alkaloids have become biochemically important drugs in psychotherapy and psychodiagnostics. Moreover, ancient cults using magic mushrooms are reborn as a ritual in the drug scene [4–6]. Fresh or dried *Psilocybe* mushrooms and also kits consisting of spores, fertiliser and instructions on how to grow cultures are offered worldwide. German consumers get their supplies through the Internet or they buy material in Dutch 'Smart-Shops'. Some of the material has been confiscated by local authorities. With increasing frequency we are asked to analyse these materials. We report about established methods and common findings in morphologic, microscopic, microchemical, and toxicological examination of hallucinogenic mushrooms.

*Corresponding author. Tel.: +49-228-738-316; fax: +49-228-738-368.

E-mail address: f.musshoff@uni-bonn.de (F. Musshoff).

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2. Material and methods

2.1. Chemicals

Psilocin and psilocybin were purchased from Alltech (PA, USA). The other chemicals were obtained from Merck (Darmstadt, Germany). Ehrlich's reagent: 5 g 4-dimethyl-aminobenzaldehyde in 50 ml of 90% ethanol to which 50 ml of 38% hydrochloric acid is added. Melzer's reagent: 1.5 g iodine in 100 ml of an aqueous mixture containing 5 g of potassium iodide and 100 g of chloral hydrate.

2.2. Macroscopic examination

Our material consisted of 37 confiscated samples of dried material. Diagnosis of genus and species of dried hallucinogenic mushrooms is extremely difficult, because the samples often consist either of broken parts of caps and stems or of tissue ground to granules or powders. For the latter, microscopic examination revealed further results. We did not find fresh material, which might have been used for a spore print or the bluing reaction for identification purposes.

2.3. Microscopic examination

A 10% ammoniacal solution (v/v) acts as a mild clearing agent and possesses a contrasting refractive index. It also acts as a swelling agent and allows the dried tissues to return to their previous state, when the agaric was fresh. An extensive microscopic examination is described by Watling [7] who studied three parts of a mushroom, the gill, the scalp of the cap and a longitudinal section through the gill. Spores free of tissue can be obtained from the sample simply by washing the gills in the 10% ammonia.

2.4. Microchemical examination

Melzer's reagent when placed on tissues or spores may turn them blue or bluish-black, purple-brown, or golden to yellowish. The colour change is characteristic for the fungi under study. If they become blue, bluish-grey or blue-black they are amyloid, and if they are unchanged or only become slightly yellowish they are termed inamyloid or non-amyloid. Mushrooms of the genus Psilocybe are always inamyloid.

Treatment of dried mushroom samples with Ehrlich's reagent for 30 min may results in a purple or greyish-purple colour reaction in the presence of primary aromatic amines, i.e. the hallucinogenic alkaloids of the *Psilocybe* genus.

2.5. Toxicological analysis

For the extraction of psilocybin and psilocin from mushrooms 100 mg of dried and pulverized plant material are added to 9 ml methanol. This mixture is ultrasonicated for 120 min (maximum temperature of the mixture 50°C). Then the volume is brought to 10.0 ml with methanol; the mixture is centrifuged and the supernatant is used for the HPLC determination. A Hewlett-Packard Series II 1090 liquid chromatograph equipped with a Hewlett-Packard Series 1050 variable wavelength detector (266 nm) was used. Chromatographic separation was performed with a LiChrospher 60 RP-select B (250×4 mm, 5 μm) analytical column (Merck) and gradient elution was carried out at a constant flow-rate of 1 ml/min. Solvent A consisted of 20 mM KH_2PO_4 and solvent B of acetonitrile. Initial conditions were 5% B for 2.5 min increasing to 25% B at 15 min. For the calibration aqueous standard solutions of psilocybin and psilocin in methanol in the concentration range 0, 10, 50 and 100 μg/ml were used.

3. Results

Using Melzer's reagent all samples tested were inamyloid. Further microchemical investigation of our material resulted in most cases in a positive reaction using Ehrlich's reagent. By toxicologic analysis it was confirmed that all positive samples contained psilocybin or psilocin.

Photographs, descriptions of morphologic characteristics, and toxicologic analysis are summarized in Figs. 1–3 and Table 1. Microscopic examination revealed in most cases fungal tissues consisting of parallel hyphae in which adhering cells were connected to clamp connections. The spores we recognized measured 3–10 μm by 12–20 μm and were

Fig. 1. *Panaeolus cyanescens*.

elliptic or lemon-shaped, brown to black in color with markedly thick walls, smooth surfaces and an easily visible germ-pore (Fig. 4).

The results of the toxicological analysis are summarised in Table 2. The calibration curves are linear in the range 0–100 μg psilocybin and psilocin/ml

Fig. 2. *Psilocybe semilanceata*.

Fig. 3. *Psilocybe cubensis.*

(limit of quantification, 0.01%; limit of detection, 0.003%). The simple and rapid extraction procedure results in chromatograms without interfering peaks (Fig. 5).

4. Discussion

Macroscopic examination of dried hallucinogenic mushrooms is extremely difficult, because the samples often consist of broken parts or powders. In extreme instances microscopic examination can reveal further results. The microscopic evaluation of tissues consisting of parallel hyphae in which adhering cells are connected to clamp connections are a definite proof that the material is of fungal origin. In addition spores are of interest for identification purposes. All spores of Psilocybe mushrooms are of similar shape, brownish, and smooth. The validity of microchemical reactions is limited. The reaction with Melzer's reagent may serve only as an exclusion diagnosis. A positive reaction with Ehrlich's reagent depends on the alkaloid content, which can be decreased in stored material.

Morphologic examination revealed *Psilocybe cubensis* as the most popular mushroom in our sample collective, followed by *Psilocybe semilanceata*, *Panaeolus cyanescens,* and *Psilocybe tampanensis*. The alkaloid content was determined with $<$0.003–1.15% of psilocybin and 0.01–0.90% psilocin. *Panaeolus cyanescens* was the mushroom with highest levels of psilocybin and psilocin.

Psychoactive mushrooms are either eaten fresh, which supposedly produces the most powerful and intense visual experience, or dried and consumed at a later date [8]. Some users freeze mushrooms for later use, while others put them in capsules for resale. Mixing the psychoactive fungi in milk shakes or tea or cooking the mushrooms in a soup, stew or omelette has also been reported. Boiling the mushrooms in water to remove the active, hot water-soluble ingredients and use of the water to prepare foods such as rice or others is another ingestion form. Chocolate and honey are also employed by recreational users for use with mushrooms and is a form for transport and export. Mushrooms in honey preserve their potency much better than those that are dried or frozen, especially if placed in honey immediately after having been picked.

Symptoms produced by eating psychoactive mus-

Table 1
Morphologic characteristics of hallucinogenic mushrooms

	n	Description
Psilocybe cubensis	18	Macroscopic features Cap 1.5–8 cm broad; conic-campanulate to convex shape whitish to pale yellow color; smooth surface; whole mushrooms with a stem of 40–150 mm×5–15 mm; whitish to yellowish color, smooth surface; gills attachement adnate to adnexed; gray-purplish gray to nearly black color Microscopic features Spores dark purplish brown to violet brown; subellipsoid; 8–10 μm×11–17 μm Psilocybin n.d.–1.07%; psilocin 0.01–0.23%
Psilocybe semilanceata	9	Macroscopic features Cap 0.5–2.5 cm broad and about twice as high as wide; mostly conical with a pointed top and rarely convex or plane with an incurved margin; dark chestnut brown to pale yellow color; smooth surface; whole mushrooms with a stem of 30–90 mm×0.5–2 mm; whitish color, pallid to more brownish towards the base; smooth surface; gills attachement adnexed; cream to purple black color Microscopic features Spores dark purplish brown; ellipsoid; 10–14 μm×6–8 μm Psilocybin 0.01–0.91%; psilocin 0.01–0.90%
Psilocybe tampanensis	4	Macroscopic features Cap 1–2 cm broad; convex to plane; ochraceous-straw brown to yellowish gray color; smooth surface; whole mushrooms with a stem of 20–60 mm×1–2 mm; yellowish brown to reddish brown color; smooth surface; gills attachement adnexed; brownish to dark violet brown color Microscopic features Spores purplish brown; subellipsoid-subrhomboid; 7.5–10 μm×4–8 μm Psilocybin n.d.–0.19%; psilocin 0.01–0.03%
Panaeolus cyanescens	6	Macroscopic features Cap 1–4 cm broad; hemispheric to convex with decurved expanding margin; pale gray to brownish color; smooth or cracked surface; whole mushrooms with a stem of 50–70 mm×2.5–5 mm; whitish to darkening brown color, smooth surface covered with a white powder; gills attachement adnate; mottled grayish black color Microscopic features Black nontransparent spores; 11–14 μm×7–10 μm Psilocybin 0.02–1.15%; psilocin 0.14–0.90%

hrooms begin to occur within 20–40 min after ingestion or from 5 to 10 min when prepared in the form of soup or tea. Symptoms persist for up to 4–8 h after ingestion. The hallucinogenic effects are similar to those observed after LSD intake. However, the dose of psilocybin/psilocin required for the same effect is approximately 200 times higher. The usual dose of psilocin required to induce psychedelic effects ranges from 8 to 10 mg [9].

In Germany psilocybin and psilocin are classified substances (§1, Abs.1, Anl.1 BtmG). The cultivation or possession of whole *Psilocybe* mushrooms and its spores are restricted by German law since 1998 (10. BtmÄndV).

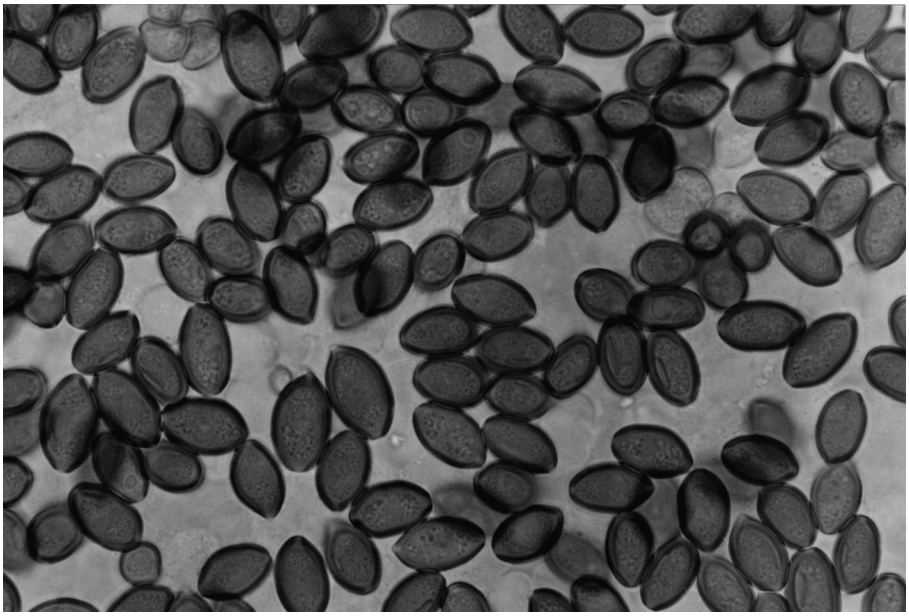

Fig. 4. Spores of *Psilocybe cubensis* (magnification×1250).

Table 2
Results of toxicological analysis

Psilocybe cubensis		*Psilocybe semilanceata*		*Panaeolus cyanescens*		*Psilocybe tampanensis*	
n=18		*n*=9		*n*=6		*n*=4	
Psilocybin (%)	Psilocin (%)	Psilocybin (%)	Psilocin (%)	Psilocybin (%)	Psilocin (%)	Psilocybin (%)	Psilocin (%)
n.d.	0.14	0.01	0.48	0.02	0.56	n.d.	0.02
n.d.	0.05	0.16	0.13	0.44	0.14	0.01	0.03
n.d.	0.10	0.25	0.08	0.47	0.22	0.03	0.03
n.d.	0.10	0.27	0.24	0.51	0.64	0.19	0.01
n.d.	0.11	0.30	0.03	0.54	0.09		
0.01	0.05	0.42	0.04	1.15	0.90		
0.02	0.09	0.51	0.12				
0.02	0.06	0.72	0.01				
0.03	0.06	0.91	0.90				
0.05	0.19						
0.15	0.09						
0.17	0.09						
0.31	0.23						
0.41	0.13						
0.50	0.12						
0.87	0.04						
0.98	0.03						
1.07	0.01						

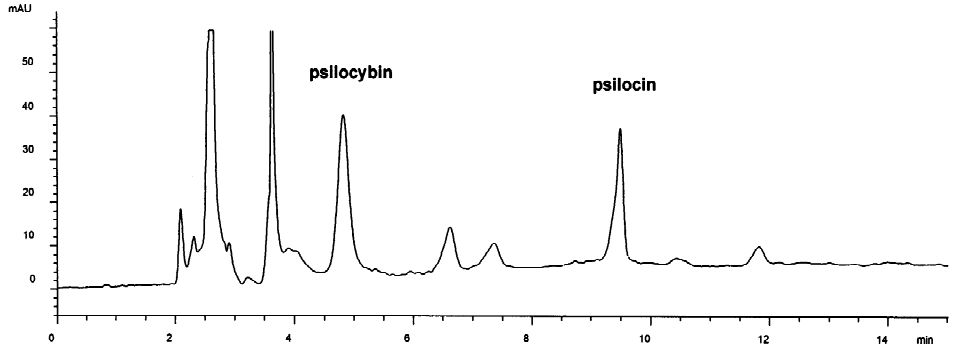

Fig. 5. Chromatogram of an extract from a *Psilocybe cubensis* mushroom, containing 0.31% psilocybin and 0.23% psilocin.

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