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THE OCCURRENCE OF PSILOCYBIN AND PSILOCIN IN FINNISH FUNGI

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The use of hallucinogenic fungi containing psilocybin and or psilocin (1) has, during the past few decades, spread from Central America to the whole western world (2-4). Psilocybin (4-phosphoryloxy-*N*, *N*-dimethyltryptamine) and psilocin (4-hydroxy-*N*,*N*-dimethyltryptamine) were first isolated from *Psilocybe mexicana* (5) but have later been reported from more than 30 species of the genus and from several species of other genera.

In the Scandinavian countries, the most common psilocybin-containing fungus is *Psilocybe semilanceata* (6,7); in all, about ten active species have been reported from Norway and Denmark (8,9). In this study, a variety of Finnish fungi were screened for their psilocybin and psilocin content using two methods of hplc (10,11).

MATERIALS AND METHODS

The fungus specimens were collected for analysis from different parts of the country, most of them, however, from eastern and northern Finland. A few specimens from Denmark, Scotland, and Germany were also analyzed. The material was collected partly randomly, but an emphasis was placed on the fungi that are assumed to be hallucinogenic on the basis of the literature, and, in addition, on blue species and species that turn blue or black.

A total of 61 species belonging to 30 genera were analyzed, and the whole screening procedure involved about 450 analyses. Most of the fungi studied belong to the order Agaricales, and three species belong to the order Boletales. The fungal material was collected mainly in the autumn of 1983. Some older samples were also analyzed, because not all the desired species were found during the season. A series of specimens of *P. semilanceata* collected in the years 1843, 1869, 1954, and 1976 was analyzed in order to test the stability of psilocybin and psilocin in the fruiting body.

The analyses were performed on fresh, deep-frozen, or dried material. The identification was, in some cases, made in the field with the aid of fresh characteristics, but several species were also determined microscopically. Some species were identified or confirmed by outside specialists. Parts of the samples analyzed are preserved in the herbarium of the University of Oulu (Herb. OULU).

The fresh samples (each about 500 mg) were frozen right after harvesting until analysis. They were ground in an homogenizer (Ultra-Turrax) with 2 ml of MeOH; whereafter 5 ml of MeOH was added, and the mixture was agitated for 60 min.

The dry samples, including the herbarium specimens, were dried overnight at 45°, ground into powder, weighed, and shaken for 60 min with 7 ml of MeOH. Both samples were then centrifuged for 15 min (3000 rpm). The supernatant (5 ml) was stored at −70° until analysis.

The hplc-system used (excluding the stationary and mobile phases) has been described in an earlier publication (7). The stationary phase consisted of a prepacked μBondapak RP-C 18 column (Waters, Milford, Mass.). All the samples were screened with method A, and the positive and suspected ones were reanalyzed with method B. In method A the mobile phase consisted of MeOH-H_2O (60:40), and the paired ion

TABLE 1. Psilocybin and Psilocin Contents (% of weight) Found in the Studied Fungus Species

Species	Number of positive /studied specimen	(dry) or (fresh) specimen	psilocybin (%)	psilocin (%)
Pluteus atricapillus[a]	2/5	d	0.004	—
		d	0.005	—
Pluteus salicinus[a]	2/2	d	0.21	—
		d	0.30	0.05
Panaeolus olivaceus[b]	1/3	f	0.005	—
Panaeolus subbalteatus[b]	4/4	d	0.11	0.004
		f	0.01	—
		d	0.06	—
		d	0.14	—
Panaeolina foenisecii	2/19	d	0.03	—
		d	0.03	—
Psathyrella candolleana	1/7	d	0.004	0.005
Conocybe cyanopus[c]	1/1	f	0.45	0.07
Conocybe kuehneriana[c]	1/1	d	—	0.004
Psilocybe semilanceata[d]	5/5	f	0.80	0.003
		f	0.19	0.004
		d	0.87	—
		f	0.82	0.025
		d	0.20	—
Agrocybe[c] sp.	1/1	d	0.003	—
Herbarium specimens of *Psilocybe semilanceata*				
Collecting Year				
1843	−/1	d	—	—
1869	1/1	d	0.014	—
1954	1/1	d	0.67	—
1976	1/1	d	0.84	—

[a]Identification confirmed by E. Vellinga.
[b]Identified or confirmed by E. Gerhardt.
[c]Identified or confirmed by R. Watling.
[d]Identified or confirmed by G. Guzmán and E. Rald.

chromatography (pic) reagent was heptanesulfonic acid buffered to pH 3.5 with HOAc (10). In method B the mobile phase consisted of MeOH-H_2O-cetrimoniumbromide (40:60:0.15, v/v/w). The buffer was 0.25% Na_2HPO_4+0.15% $NaH_2PO_4 \cdot H_2O$ (w/v), pH 7.6 (11). The mobile phases were degassed with an ultrasonic bath and filtered through a 0.45 μm filter (Millipore). The flow rate was 2 ml/min, the injection volume 10 μl, and the wavelength of the detector 280 nm (bandwidth 2nm). The pure standards for psilocybin and psilocin were from Sandoz AG (Basel).

RESULTS AND DISCUSSION

Altogether ten species belonging to seven genera were found to contain psilocybin and/or psilocin (Table 1). The highest concentrations (over 0.5% of dry weight) of psilocybin were detected in *Conocybe cyanopus* (Atk.) Kühn. and *Psilocybe semilanceata* (Fr.) Kumm., and fairly high ones in *Pluteus salicinus* (Pers. ex Fr.) Kumm. and *Panaeolus subbalteatus* (Berk. & Br.) Sacc. Smaller amounts (below 0.1% of dry weight) were measured in *Pluteus atricapillus* Sing., *Panaeolus olivaceus* Möller, *Panaeolina foenisecii* (Pers. ex Fr.) R. Maire, *Psathyrella candolleana* (Fr.) R. Maire, and *Conocybe kuehneriana* Sing. and in a species of *Agrocybe*. *C. cyanopus*, *C. kuehneriana* and *Pa. olivaceus* have not been recorded earlier from Finland. *Pl. atricapillus*, *C. kuehneriana* and *Pa. olivaceus* are not reported in the literature

to contain these compounds. One reason might be that the concentrations of psilocybin and psilocin are very low and cannot be detected from dry material. Of the specimens that contain psilocybin and/or psilocin, *Pl. atricapillus, Panaeolina foenisecii, Ps. semilanceata,* and *Psathyrella candolleana* are common species in Finland.

The difficulties in the taxonomy of fungus species may be one source of notable confusion and error in the literature; in addition, our material has included problems. We analyzed a specimen, identified as *Psilocybe atrobrunnea* by Guzmán, which contains much psilocybin and psilocin. Its morphological characteristics agree fairly well with those given by Guzmán (12), but the ecology is different. *Ps. atrobrunnea* was described from *Sphagnum* vegetation; our specimen grew on lawn. In this study it is included with *Ps. semilanceata*. We have seen also the specimens analyzed by Hoiland (13) and reported as *Ps. atrobrunnea*. They have smaller and paler spores, for example, than *Ps. atrobrunnea* has.

Rald (14) considers *Pa. fimicola* and *Pa. olivaceus* synonymous, but Gerhardt (15), who identified our material, has found those two species distinctly different. Stijve *et al.* (16) are of the opinion that *Panaeolina foenisecii* cannot contain psilocybin or psilocin at all. Two of our analyses were, however, positive.

The genus *Conocybe* is very little known in Finland and even in whole Fennoscandia. According to Watling (17), *C. kuehneriana* is fairly common in the British Isles, but it is not known to be hallucinogenic.

The genus *Agrocybe* is considered psychoactive according to Koike *et al.* (18), who found psilocybin in *A. farinacea*. Our collection was inadequate for exact identification.

During the course of this study, some old herbarium samples were also analyzed in order to detect the length of time that psilocybin and psilocin can persist in fungal fruit bodies. The specimens were from the year 1843, 1869, 1954, and 1976, all being of *Ps. semilanceata*. Psilocybin was found to be very stable in dried fruit bodies. Even the 115-year-old collection still showed a measurable amount of psilocybin, namely 0.014% of dry weight (Table 1). The oldest specimen, on the other hand, did not show any activity. The concentration of psilocybin had a linear negative correlation with the age of collections. Psilocin seemed to be much less stable, and it was only detected in fresh specimens or in species that contained high concentrations of psilocybin.

The chromatography column used was found to be reliable and stable; about 450 samples were analyzed without significant changes in the retention volume or the peak configuration. Because interfering peaks of method A were not present in method B, we consider both methods essential for reliability. The same selectivity can also be obtained by using simultaneous multiple detection (19).

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