New Aspects of the Occurrence, Chemistry and Cultivation of European Hallucinogenic Mushrooms

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Abstract

The analysis of fruit bodies from species of the genera *Psilocybe, Gymnopilus, Inocybe, Conocybe* and *Pluteus* revealed the presence of psilocybin, psilocin and baeocystin in various collections from different countries.
Some investigations about the main synthesis of psilocybin and its derivatives were also carried out. Fruiting of various species could be demonstrated for the first time.
The spontaneous blueing of many of these mushrooms and mycelia as well as some directed biotransformation reactions will also be described.

In 1963, the appearance of an article by HOFMANN *et al.* [1] about the occurrence of psilocybin in European *Psilocybe semilanceata* (Fr.) Kumm. spurred renewed scientific investigation into psychotropic indole derivatives in mushroom species of Europe.

Psilocybe semilanceata (figure 1) is growing wild in Middle and North Europe as well as in other European countries, North- and South-America, Australia and very probably Asia.

The main alkaloids are psilocybin and baeocystin (4-phosphoryloxy-N-methyltryptamine) and in some cases only traces of psilocin [2-5].

For the first time baeocystin was isolated from this species in 1979 [5].

Habitats of the mushrooms are wet grassy fields and uncultivated pastures. The mushrooms grow on manured soil but not directly on dung. It seems than *Psilocybe semilanceata* is the most potent European hallucinogenic mushroom.

Fig. 1 - Naturally grown fruit bodies of *Psilocybe semilanceata* from the Duebener Heide, Germany (9/21/1989).

Samples show a considerable variation in the psilocybin levels of single fruit bodies (0.2 to 2.4% dry weight). But about 1% psilocybin was always determined if the average content from 10 to 20 mushrooms was analyzed. Baeocystin was found to occur along with small amounts of 4-phosphoryloxytryptamine (norbaeocystin).

I have cultivated some fruit bodies of *Psilocybe semilanceata* to determine determinate the alkaloid levels in comparison to naturally grown mushrooms.

Mycelium obtained from the spores of one mushroom was kept as a stock culture on 6% malt agar [6]. The spores can germinate after a storage of the dried mushrooms for 9 months at 20°C.

Fig. 2 - Fruiting of *Psilocybe semilanceata*. [in Erlenmeyer flask]

No indole derivatives have been detected in the mycelia from a surface culture of *P. semilanceata* on liquid malt extract media. In contrast to these results a fruiting of the species (figure 2) yielded fruit bodies with a similar high level of alkaloids as the naturally grown mushrooms (table 1).

This strain fruited after 3 to 4 months but other mycelia failed to form fruit bodies at all.

Table 1
Indole derivatives in fruit bodies of *Psilocybe semilanceata* from the cultivation on a sterile substratum with of compost, rice grain, grass seed, and water (12:3:4:22) after casing with peat/chalk.[6]
Content of dried mushrooms (%)

Flush No.	Psilocybin	Baeocystin
1	0.91	0.15
2	1.04	0.20
3	0.92	0.21
4	1.12	0.19

Fruiting of the subtropical species *Psilocybe cubensis* (Earle) Singer on a dung/rice grain substratum (figure 3) begins earlier than the formation of fruit bodies of *P. semilanceata* (3 to 4 weeks versus 3 to 4 months). But the former species failed to accumulate significant amounts of baeocystin. So both species are important for studying biochemical pathways of the formation of the tryptamine derivatives [7].

P. semilanceata is characterized by a blue-green coloration especially at the stem but blues inconsistently, mostly after several minutes to hours.

The use of this species ("liberty caps") as a hallucinogen is popular in Great Britain, Norway and other European countries for about 20 years. It seems that this mostly moderate use does not cause significant health problems.

Because of the high content of psilocybin dysphoric experiences were described in some cases as of overdoses. It is also well known that one's experiences with hallucinogens depend upon a host of experimental, cultural and psychological factors (setting and set) [8, 9].

Another interesting European species with strong psychoactivity is *Psilocybe bohemica* Sebek [9, 10].

These mushrooms were already found in Czechoslovakia near Sazava in 1942. They blue consistently after bruising and spontaneously in the age. The fruit bodies grow up to 15 cm high on humus and wood chips in the woods.

The species is widespread in Czechoslovakia [9] and it will probably be reported from many other European countries in the next years. Recent finds in Austria and Germany support this claim.

The analysis of fruit bodies revealed psilocybin, baeocystin and in some cases psilocin. Psilocybin levels varied from 0.11% up to 1.34% by dry weight [9]. The content of psilocybin and baeocystin was highest in the caps of the mushrooms (table 2).

Fig. 3 - The subtropical mushroom, *Psilocybe cubensis* [fruiting] on grain/dung without casing. [in Erlenmeyer flask]

Table 2
Amount of indole alkaloids in dried fruit bodies of *Psilocybe bohemica* (%)

Sample	Part of mushroom	Psilocybin	Psilocin	Baeocystin
1	fruit body	0.96	0.02	0.03
2	fruit body	1.34	-	0.01
3	fruit body	0.29	-	0.008
4	fruit body	1.12	-	0.02
5	fruit body	0.94	0.01	0.01
6	fruit body	0.22	0.01	0.02
7	cap	0.42	0.02	0.02
7	stem	0.12	-	0.01
8	cap	1.02	-	0.03
8	stem	0.50	-	0.01

Psilocybin was also found to be contained in the cultivated mycelia of this species. The amount ranged from 0.15% to 0.21% by dry weight in 6 different mycelia grown on 6% malt agar over 4 weeks. No other alkaloids were detected in the mycelial extracts.

A rhizomorphic to closely linear growth on the blueing mycelia was observed on soaked unsterilized cardboard (figure 4).

Fig. 4 - Mycelia of *Psilocybe bohemica* on soaked cardboard

Fruiting of the mycelia on rice grain/water mixture occurred without casing 12 weeks after inoculation (figure 5) but only if a temperature of 4°C was maintained for 3 days at the end of cultivation. This observation is in agreement with the occurrence on the naturally grown fruiting bodies in late autumn and early winter. Wild mushrooms of this species, differing from the cultivated mushrooms mainly by the absence of the 2 rings and the less robust habitat, have very similar other features, microscopic ones and the blueing in particular. The species required diffuse day light for pinhead initiation. Growth of the vegetative mycelia was observed on malt agar at 4°C.

Fig. 5 - Fruit body of *Psilocybe bohemica* on rice grain/water (1:2) 12 weeks after inoculation. [in Erlenmeyer flask]

In 1984 and 1986 Krieglsteiner had classified *P. bohemica* and the similar *Psilocybe serbica* Moser & Horak as *Psilocybe cyanescens* Wakefield [12, 13]. But some doubt still exists about the taxonomic conformity of different collections from Europe and North America.

For example, I have found, that the monokaryotic mycelia on *P. bohemica* and *P. cyanescens* do not form dicaryotic mycelia. Additionally, fruit bodies of *Psilocybe cyanescens* (U.S.A.) generally contained high amounts of psilocin [13, 14].

The recent discovery on hallucinogenic *Inocybe* species was a sensation in the study of the distribution of psilocybin and its derivatives in the fungal world. Until the 80s only muscarin was the classical toxin detected in a great number of the about 160 species of the genus *Inocybe*.

In 1965 J. FERENCZ found a new *Inocybe* species in Hungary. M. BABOS described these blueing mushrooms as *Inocybe aeruginascens* BABOS in 1968 (figure 6). Since 1980 *I. aeruginascens* has caused over 20 accidental hallucinogenic poisonings in East Germany because of some similarities with edible mushrooms like *Marasmius oreades* (Bolt.: Fr.) Fr. [9].

In all of these cases only euphoric experiences with hallucinations and illusions were reported. The mushrooms contained relative constant amounts of psilocybin, baeocystin and in some cases also tryptophan (table 3).

A new indole derivative, which I called aeruginascin, with a still unknown structure was also detected in this species [15-17]. We found only traces of psilocin in a few mushrooms but no muscarin at all.

A few months after my initial paper [17] other groups (for example [18]) have published about the occurrence of psilocybin in *I. aeruginascens*.

Table 3
Content of indole derivatives in dried mushrooms of *Inocybe aeruginascens* (%)

Sample	Psilocybin	Baeocystin	Aeruginascin
1	0.29	0.19	0.15
2	0.40	0.20	0.30
3	0.30	0.18	0.21
4	0.26	0.24	0.35
5	0.36	0.52	0.32

I. aeruginascens is characterized by a blue-green coloration of the stem after bruising or in a few cases spontaneously in the age.

After the detection of psilocybin in *I. aeruginascens* other alkaloid containing mushrooms like *Inocybe corydalina* Quel. or *Inocybe haemacta* (B. & Cooke) Sacc. were found. But in contrast to *I. aeruginascens* , which often grows in colonies of great numbers in parks of Germany and Hungary in grass on sand mainly in May or June, these species are very uncommon, growing in woods, and contain only small amounts of the psychotropic tryptamine derivatives. For example, *I. corydalina* accumulates about 0.01% to 0.03% psilocybin in the dried fruit bodies [18].

So only *I. aeruginascens* can cause significant accidental intoxications. I have isolated a mycelial strain of *I. aeruginascens* which degenerated completely after a few months of cultivation without further growth.

The greenish sclerotia and the brownish mycelia from the surface cultivation also contained about 0.1% psilocybin in the dried biomass.

Fig. 6 - Fruit bodies of *Inocybe aeruginascens* from Potsdam (Germany).

Another very interesting field of research is the occurrence of psilocybin in *Gymnopilus* species. Despite some old stories about possible intoxications with *Gymnopilus spectabilis* (Fr.) A. H. Smith from JapanJapon and some other hallucinogenic experiences with other species from North America no psilocybin could be detected in the genus *Gymnopilus* in Europe until 1988 [19].

In 1978 psilocybin was found in some North American species [19]. Since 1983 *Gymnopilus purpuratus* (Cooke & Mass.) Singer has been observed on heapes of mixtures of pig dung and wood chips in the district of Rostock, Germany. It seems that this blueing species was introduced from Argentina with grain used for forage in pig-breeding during last years. Recently the qualitative detection of psilocybin in extracts of *G. purpuratus* has been described [19]. In this investigation no quantitative analysis of the indole alkaloids was carried out.

In 1989 our own study on the amounts of psilocybin and its derivatives was published (table 4) [20]. It was found that the level of psilocin decreased appreciably during storage. No other species found in Europe contained such high amounts of psilocin like as *G. purpuratus*. Spores of *G. purpuratus* germinated quickly on malt agar.

Table 4
Indole alkaloids in *Gymnopilus purpuratus* (% dry weight)

Sample	Psilocybin	Psilocin	Baeocystin
1	0.29	0.28	0.05
2	0.31	0.29	0.04
3	0.21	0.20	0.03
4	0.28	0.31	0.04
5	0.33	0.28	0.05

The whitish mycelia blues consistently after bruising and after about 3 weeks of cultivation also spontaneously like old naturally grown fruit bodies. Fruiting of the mycelia on rice grain or sawdust and even mixtures occurred after 8 to 12 weeks after inoculation (figure 7). These cultivated mushrooms contained similar amounts of psilocybin and its derivatives as the naturally grown fruit bodies (table 5).

Table 5
Alkaloids in cultivated dried fruit bodies of *Gymnopilus purpuratus* (% dry weight)

Flush No.	Psilocybin	Psilocin	Baeocystin
1	0.13	0.15	0.03
2	0.15	0.18	0.02
3	0.23	0.21	0.04
4	0.18	0.21	0.05
5	0.15	0.14	0.02

Fig. 7 - Fruiting of *Gymnopilus purpuratus* on a rice grain medium. [in Erlenmeyer flask]

Psilocybin has also been found in *Pluteus salicinus* (Pers.: Fr.) Kumm. but this species is uncommon and grows on dead wood. STIJVE *et al*. detected only a mean concentration of 0.25% psilocybin in dried fruit bodies [21]. In my own investigations one collection contained about 1% psilocybin and traces of baeocystin as well as high amounts of urea [22]. In other dried samples I later found 0.4 to 0.6% psilocybin but no psilocin. I think much more investigations should be done to determinate the variation of the alkaloid levels in this species.

Often contradictory reports on hallucinogenic effects of species of the genus *Panaeolus* are explained in terms of differences in chemical composition due to geographical origin ("latent ability to form psilocybin").

Ola'h described such behavior during his investigations of the genus *Panaeolus* [23]. I think that this claim failed completely. All modern analytical investigations with HPLC and TLC including competent mycological examination yielded to a definitive composition of the species.

For example, STIJVE *et al.* [24] and my own investigation [25] could not detect psilocybin and its derivatives in one single mushroom of *Panaeolina foenisecii* (Fr.) Kü.

It seems that only *Panaeolus subbalteatus* (Berk. &Br.) Sacc. (figure 8) contains significant amounts of psilocybin in the genus *Panaeolus* in Europe [26]. All *Panaeolus* species are able to accumulate serotonin and its precursor 5-hydroxy-L-tryptophan as well as urea. I think that Ola'h has misinterpreted these compounds as psilocin because of his use of nonselective analytical methods.

In naturally grown mycelia and fruit bodies of *Panaeolus subbalteatus* there were significant differences in amounts and nature of the metabolites (table 6). Additionally it seems that much more work must be done to study the taxonomy and chemistry on *Panaeolus* species in Europe and even in the world.

Only a very few fruit bodies of *P. subbalteatus* show blueing [26]. In model reactions the oxidation of pure psilocin yielded bluish products [27]. Many enzymes can remove the phosphoric acid from psilocybin to form psilocin before oxidation.

Table 6
Indole derivatives in *Panaeolus subbalteatus* (% dry weight)

Substance	**Mycelium**	**Fruit bodies**
Psilocybin	0.07	0.08-0.70
Baeocystin	-	0.05-0.46
5-OH-L-tryptophan	-	qualitativ
Serotonin	0.10	0.08-0.30
L-Tryptophan	0.20	traces
Psilocin	-	-
Urea	-	1.8-2.3

The very rare species *Conocybe cyanopus* (Atk.) Kühn has been found only twice in East Germany in a period of 50 years. BENEDICT *et al*. found psilocybin in this species from North America, as did other authors later [28]. In Europe some mushrooms of this species contained psilocybin and traces of psilocin (for example the first analysis from Norway [29]).

In 5 naturally grown mushrooms from East Germany (1989) I found about 1% psilocybin and similar amounts of baeocystin as *P. semilanceata* can accumulate. The nonblueing mycelia grow on malt agar very slowly with formation of sclerotia, which also contained about 0.2% psilocybin.

The widespread occurrence of baeocystin in all European psilocybian species and in many other mushrooms from all over the world supports the hypothesis of REPKE *et al*. about the central role of this compound in the biosynthesis of psilocybin [30]. Mycelial cultures of *Psilocybe* species display a high capacity for hydroxylation of synthetic tryptamine derivatives at the 4-position. So specific biotransformations of *N,N*-diethyltryptamine and N-methyltryptamine were found [31].

In model investigations I have also found that the main synthesis of psilocybin and psilocin takes place in the biomass during the process of the formation of fruit bodies or sclerotia [32]. The total content of the alkaloids in the mycelia without fruiting or differentiation was comparatively low. In physiological old mushrooms of *Psilocybe cubensis* the spontaneous blueing is a sign of a significant decomposition of the alkaloids [32].

But in *P. semilanceata* , *I. aeruginascens* , and *P. subbalteatus* this oxidation process with slight blueing does not cause a significant destruction of psilocybin and baeocystin [5, 16, 26].

Fig. 8 - Cultivated fruit bodies of *Panaeolus subbalteatus* from dung/rice grain 92 days after inoculation. [in Erlenmeyer flask]

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