Ann. Mus. civ. Rovereto	Sez.: Arch., St., Sc. nat.	Suppl. vol. 8 (1992)	107-124	1993

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NEW ASPECTS OF THE OCCURRENCE, CHEMISTRY AND CULTIVATION OF EUROPEAN HALLUCINOGENIC MUSHROOMS

Abstract - J. GARTZ - New aspects of the occurrence, chemistry and cultivation of European hallucinogenic mushrooms.

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 be also described.

In 1963, the appearance of an article of 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 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 that *Psilocybe semilanceata* is the most potent European hallucinogenic mushroom.

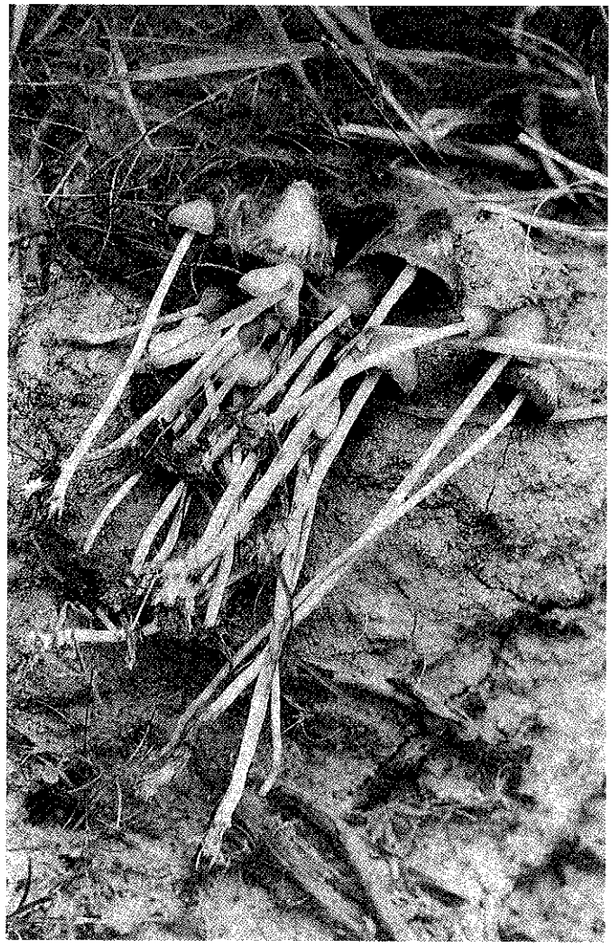

Fig. 1 - Naturally grown fruit bodies of *Psilocybe semilanceata* from the Duebener Heide, Germany (9/21/1989).

Samples show a considerable variation in the psilocybin levels of single fruit bodies (0.2 to 2.37% dry weight). But about 1% psilocybin was always determinated if the average content from 10 to 20 mushrooms was analysed. Baeocystin was found to occur along with small amounts of 4-phosphoryloxytryptamine (norbaeocystin).

I have cultivated some fruit bodies of *Psilocybe semilanceata* to determinate the alkaloid levels in comparison to the 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 month at 20°C.

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

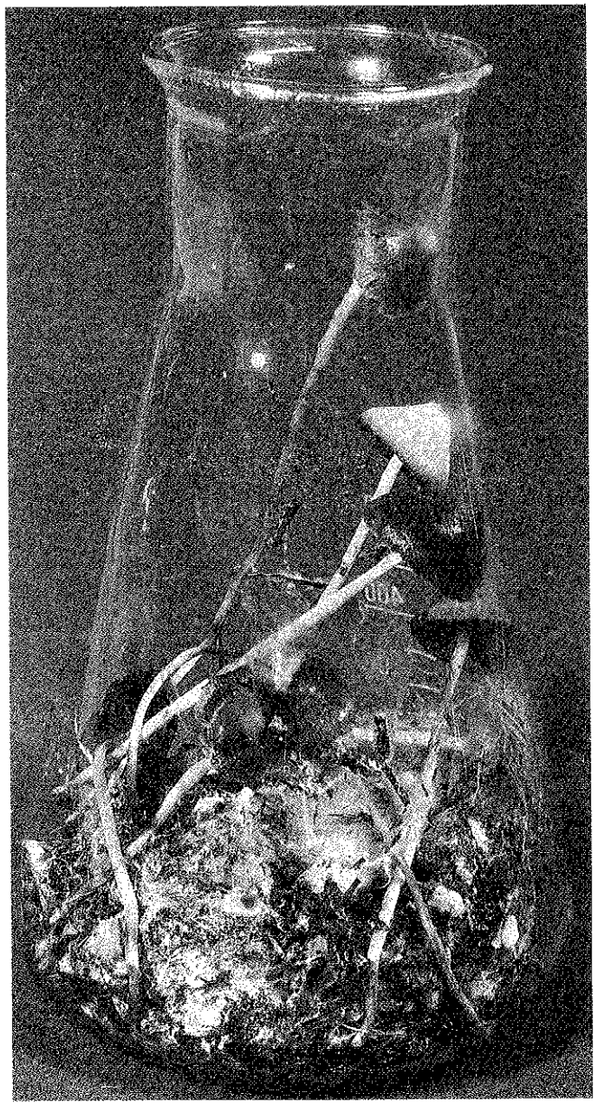

Fig. 2 - Fruiting of *Psilocybe semilanceata*.

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 month but other mycelia failed to form fruit bodies at all.

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 month). But the former species failed to accumulate significant amounts of baeocystin. So both species are important for study some 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 hallucinogen is popular in Great Britain, Norway and other European countries for about 20 years. It seems that this mostly moderate use do not cause significant health problems.

Because of the high content of psilocybin dysphoric experiences were described in some cases as overdoses. It is also well know that one's experience with hallucinogens depends 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 and blues 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.

INDOLE DERIVATIVES IN FRUIT BODIES OF *PSILOCYBE SEMILANCEATA* FROM THE CULTIVATION ON A STERILE SUBSTRATUM WITH COMPOST, RICE GRAIN GRASS SEED AND WATER (12:3:4:22) AFTER CASING WITH PEAT/CHALK (6)

Table 1

Flush No.	Content of the dried mushrooms (%)	
	Psilocybin	Baeocystin
1	0.91	0.15
2	1.04	0.20
3	0.92	0.21
4	1.12	0.19

The species is widespread in Czechoslovakia (9).

It seems that in next years the species will be find in many other European countries. 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).

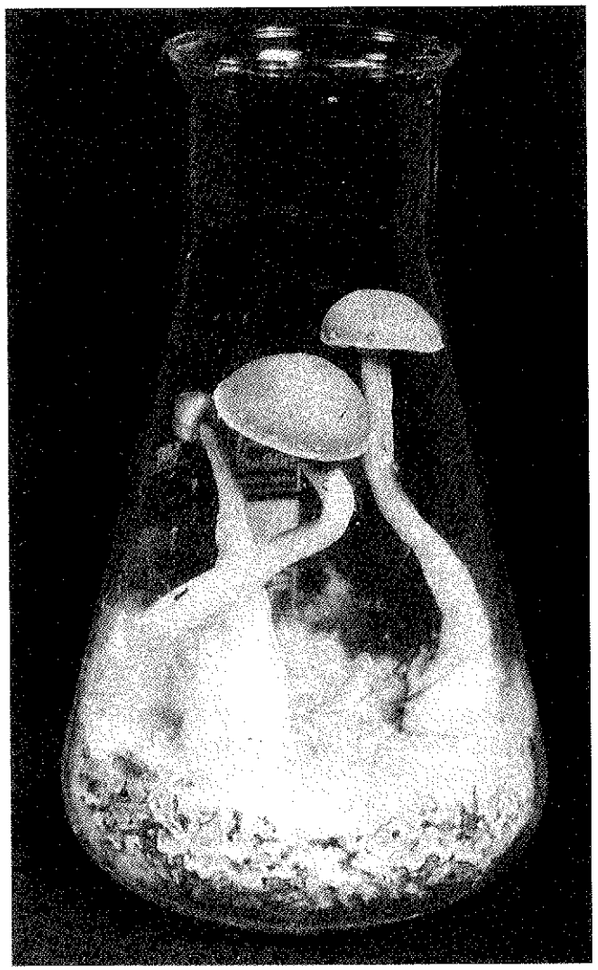

Fig. 3 - The subtropical mushroom, *Psilocybe cubensis* on grain/dung without casing.

AMOUNT OF INDOLE ALKALOIDS IN DRIED FRUIT BODIES OF *PSILOCYBE BOHEMICA* (%)

Table 2

Sample	Part of the mushroom	Psilocybin	Psilocin	Baeocystin
1	fruit body (fb)	0.96	0.02	0.03
2	fb	1.34	—	0.01
3	fb	0.29	—	0.008
4	fb	1.12	—	0.02
5	fb	0.94	0.01	0.01
6	fb	0.22	0.01	0.02
7	cap	0.42	0.02	0.02
	stem	0.12	—	0.01
8	cap	1.02	—	0.03
	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 malt agar (6%) over 4 weeks. No other alkaloids were detected in the mycelial extracts.

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

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 of the naturally grown fruit 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 habit have other features very similar, the microscopic and the blueing in particular. The species required diffuse day light for pinhead initiation. Growth of the vegetative mycelia was even observed on malt agar at 4°C.

In 1984 and 1986 Krieglsteiner had been 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 of Europe and North America.

For example, I have found that the monocaryotic mycelia of *P. bohemica* and *P. cyanescens* (U.S.A.) do not form dicaryotic mycelia. Additionally, fruit

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

bodies of *Psilocybe cyanescens* (U.S.A.) generally contained high amounts of psilocin (13, 14).

The recent discovery of 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 Hungaria.

M. Babos described these blueing mushrooms as *Inocybe aeruginascens* Babos in 1968 (figure 6). Since, 1980 *I. aeruginascens* has caused over 20 accidental

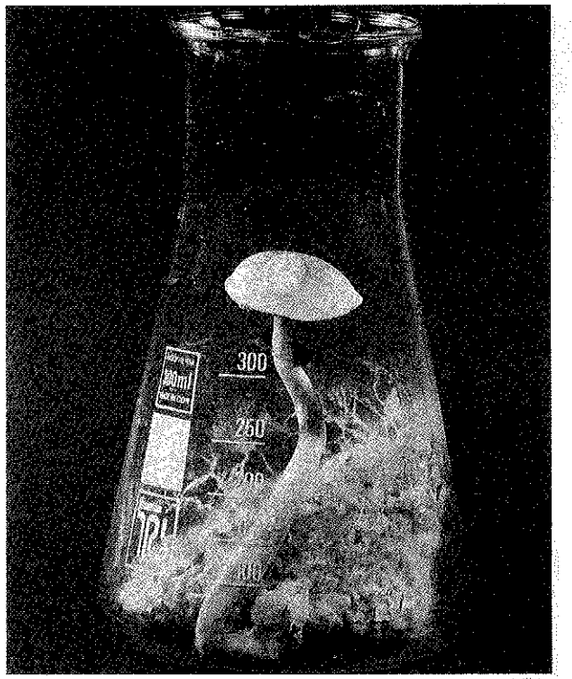

Fig. 5 - Fruit body of *Psilocybe bohemica* on rice grain/water (1:2) 12 weeks after inoculation.

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 also in some cases 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 month after my initial paper (17) other groups (for example (18)) have published about the occurrence of psilocybin in *I. aeruginascens*.

I. aeruginascens is characterized by a blue-green coloration of the stem after bruising or in a few cases spontanously 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 ofter grows in colonies of great numbers in parks of Germany and Hungaria in grass on sand mainly in May or June these species are very uncommon, growing in woods

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

CONTENT OF INDOLE DERIVATIVES IN DRIED MUSHROOMS OF *INOCYBE AERUGINASCENS* (%)

Table 3

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

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 from *I. aeruginascens* which degenerated already after a few month of cultivation completely without further growth.

INDOLE ALKALOIDS IN *GYMNOPILUS PURPURATUS*
(% DRY WEIGHT)

Table 4

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

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 Japon 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 Rostock, Germany. It seems that this blueing species was introduced with grain from Argentina 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 a own quantitative study of 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 *G. purpuratus*. The spores of *G. purpuratus* germinated fastly on malt agar.

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).

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 found later 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.

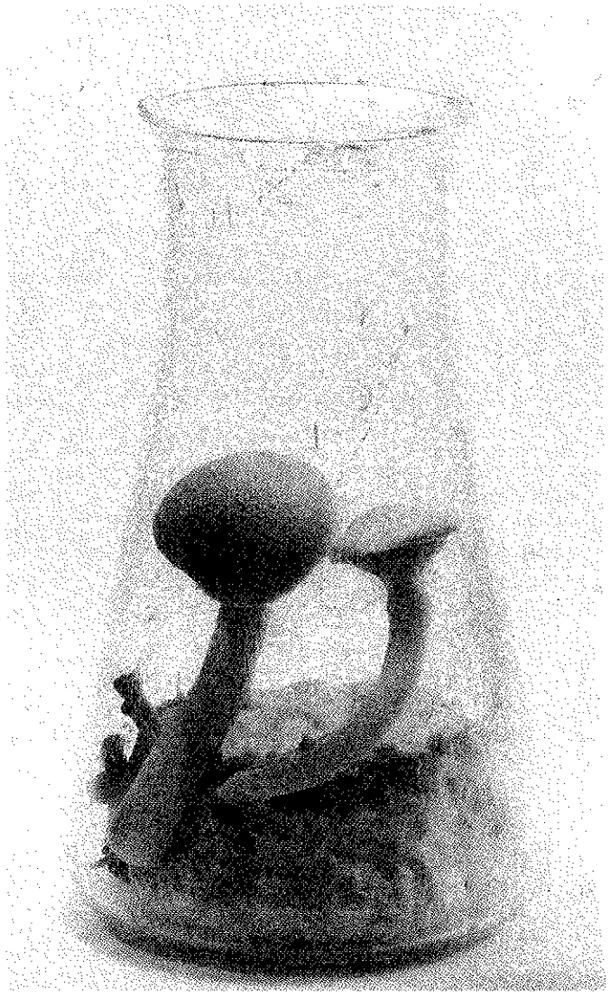

Fig. 7 - Fruiting of *Gymnopilus purpuratus* on a rice grain medium.

ALKALOIDS IN CULTIVATED DRIED FRUIT BODIES OF *GYMNOPILUS PURPURATUS* (%)

Table 5

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

Often, contradictory reports on hallucinogenic effects of species from 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 investigation fo 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 detected psilocybin and its derivatives in one single mushroom of *Panaeolina foenisecii* (Fr.) Kühn.

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 *P. subbalteatus* were significant differences between the amounts and nature of the metabolites (table 6). Additionally, it seems that much more work must be done to study the taxonomy and chemistry of *Panaeolus* species in Europe and even in the world.

Only a very few fruit bodies of *P. subbalteatus* show a blueing feature (26). In model reactions the oxydation of pure psilocin yielded to bluish products (27). Many enzyms can remove the phosphoric acid from psilocybin to form psilocin before oxydation.

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 well as other authors later (28). In Europe some mushrooms of this species contained psilocybin and traces of psilocin (for example in the first analysis from Norway (29)).

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

INDOLE DERIVATIVES IN *PANAEOLUS SUBBALTEATUS*
(%) DRY WEIGHT

Table 6

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

I found in 5 naturally grown mushrooms from East Germany (1989) about 1% psilocybin and similar amounts of baeocystin as *P. semilanceata* can accumulate. The nonblueing mycelia grows on malt agar very slowly under 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 a 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 biomasses during the process of the formation of fruit bodies or sclerotia (32). The total content of the alkaloids in the mycelia without fruiting or differentation was comparatively low. In physiological old mushrooms of *P. cubensis* the spontaneous strong blueing is a sign of a significant decomposition of the alkaloids (32).

But in *P. semilanceata, I. aeruginascens* and *P. subbalteatus* this oxydation process with the sign of a slight blueing does not cause a significant destruction of psilocybin and baeocystin (5, 16, 26).

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Acknowledgements

The author thanks G. Drewitz, M. Semerdžieva and G. K. Mueller, who generously supplied valuable information and mushrooms for the chemical analysis.

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