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Extraction and analysis of indole derivatives from fungal biomass

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The occurrence and extraction of indole derivatives in six species from four genera of higher fungi were investigated. By using pure methanol for extraction of the mushrooms analysis revealed the highest concentrations of psilocybin and baeocystin. The psilocin content of the species was higher by using aqueous solutions of alcohols than with methanol alone but was an artificial phenomenon caused by enzymatic destruction of psilocybin. The extraction with dilute acetic acid yielded better results than with the water containing alcohols.

The simple one-step procedure with methanol for the quantitative extraction is still the safest method to obtain the genuine alkaloids from fungal biomass.

In the last 15 years many papers have been published about the occurrence and determination of psychotropic tryptamine derivatives like psilocybin, psilocin and baeocystin in fungi (GARTZ 1992, 1993).

Various extraction procedures of these substances from mushrooms have been used mainly with methanol as solvent (BEUG and BIGWOOD 1982, GARTZ 1987, SOTTOLANO and LURIE 1983).

In 1985 an aqueous-organic extraction method with acetic acid for these compounds was described (CASALE 1985). Recently, Czech analysts have used aqueous solutions of methanol and ethanol (pure or in presence of potassium-nitrate) for extraction of the indole derivatives in *Psilocybe bohemica* SEBEK (KYSILKA and WURST 1990, WURST *et al.* 1992). They claimed that it was possible to found more psilocin with aqueous ethanol extraction than with pure methanol and that a dissimilar extraction of the alkaloids by using both new systems could be achieved. In this work the extraction procedures of psilocybin, psilocin and baeocystin from various mushroom species including *P. bohemica* SEBEK were studied by using methanol and the recommended mixtures of solvents (CASALE 1985, KYSILKA and WURST 1990, WURST *et al.* 1992), respectively.

Materials and methods

Fungal material: Cultivated mushrooms: *Psilocybe semilanceata* (FR.) KUMM. from horse manure compost (GARTZ 1991) (Fig. 1); *Psilocybe cubensis* (EARLE) SINGER grown on cow dung/rice grain mixture (GARTZ 1989a); *P. bohemica* from rice grain/water (GARTZ and MÜLLER 1989); *Gymnopilus purpuratus* (COOKE & MASS.) SINGER from rice grain/saw dust medium (GARTZ and MÜLLER 1990, GARTZ 1991).

Naturally grown mushrooms: *Panaeolus cyanescens* (BK. & BR.) SACC. (leg. Oahu, Hawaii 13. 11. 88); *Inocybe aeruginascens* BABOS (leg. Potsdam 20. 5. 1987) (Fig. 2); *P. bohemica* SEBEK (leg. near Sazava, Czech Republic 15. 11. 89).

All basidiocarps were dried at room temperature. Possible present residual water was removed from the mushrooms by freeze-drying. Voucher speciments of each species have been deposited in the herbarium of the University of Leipzig (LZ).

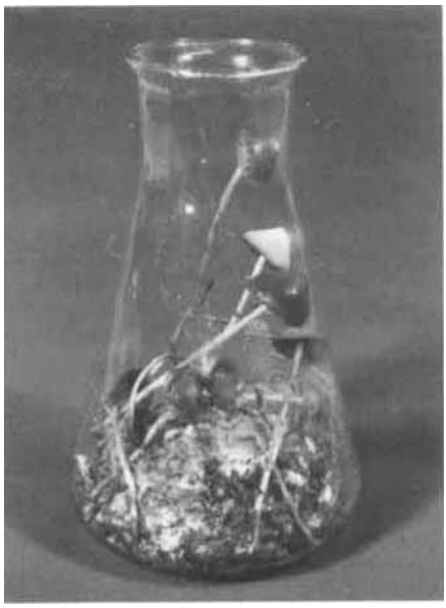

Fig. 1
Psilocybe semilanceata on compost (photo: THIEL)

Fig. 2
Inocybe aeruginascens on sand (photo: DREWITZ)

Extraction: Samples (0.01 – 0.1 g) of dried ground mushrooms were extracted with 5 to 20 ml of methanol for 0.5 to 12 hours by using a magnetic stirrer at room temperature. Under equal conditions the mixtures with aqueous acetic acid (CASALE 1985) and aqueous ethanol (psilocin) and methanol (psilocybin) (KYSILKA and WURST 1990, WURST *et al.* 1992) were used for extraction of the same batch of mushrooms. In the cases with aqueous alcohols as solvent a different extraction time for psilocybin (10 min) and psilocin (160 min) was performed (KYSILKA and WURST 1990). By using of dilute acetic

acid the solution was placed in a boiling water bath for 10 min after extraction and analysis and was then analysed 10 min after extraction and analysis and was then analysed again (CASALE 1985).

The filtration and analysis of the indole derivatives by using HPLC and TLC were described elsewhere (GARTZ 1987, SEMERDZIEVA *et al.* 1986, WURST *et al.* 1992). An analysis of the extracts for enzymes of the phosphatase type was also carried out (WEBER and HORITA 1963).

Results

In this investigation the extraction of psilocin, psilocybin and baeocystin with pure methanol was not completely after 30 min in all species and even 6 hours in analysis of *P. cubensis* and *G. purpuratus*. But the full extraction of the alkaloids from all mushrooms was reached after 12 hours. After this time no traces of indole derivatives could be detected after subsequent extraction of the fungal material with aqueous solutions of ethanol/methanol or acetic acid as well as with chloroform for psilocin. Baeocystin as incompletely methylated counterpart and possible precursor of psilocybin (GARTZ 1989a) was found in all species by using methanol but in some cases only in very small amounts (Table 1).

The psilocybin and psilocin content was in the same order of magnitude as that found earlier (GARTZ 1987, 1989c, 1991, GARTZ and MÜLLER 1989, SEMERDZIEVA *et al.* 1986).

I. aeruginascens also contained an indole derivative with a still unknown structure which was designated as aeruginascin and has a very similar behavior in chromatorgraphy as psilocybin (GARTZ 1992, 1993). This substance seems to be a phosphoric acid ester like psilocybin and baeocystin. Similar concentrations of psilocin were detected in the extracts of *P. cubensis* and *G. purpuratus* by using an aqueous solution of acetic acid versus pure methanol (Table 2).

Table 1
Amount of indole alkaloids in fruiting bodies of different species by using pure methanol as solvent

Species	Psilocybin	Psilocin	Baeocystin
	(%, dry weight)		
P. semilanceata	0.98	–	0.34
P. bohemica	0.85	0.02	0.04
P. bohemica (cultivated)	0.93	0.04	0.02
P. cubensis	0.63	0.11	0.02
G. purpuratus	0.34	0.29	0.05
I. aeruginascens	0.40	–	0.21
P. cyanescens	0.32	0.51	0.02

Table 2
Concentration of alkaloids by using acetic acid for extraction of the dried mushrooms

Species	Psilocybin	Psilocin	Baeocystin
	(%)		
P. semilanceata	0.97	–	0.33
P. bohemica	0.80	0.05	0.03
P. bohemica (cultivated)	0.91	0.07	0.01
P. cubensis	0.55	0.16	–
G. purpuratus	0.25	0.33	0.03
I. aeruginascens	0.40	–	0.22
P. cyanescens	0.30	0.54	0.01

Table 3
Results of the mushroom extraction of six species by using aqueous mixtures of methanol and ethanol

Species	Psilocybin	Psilocin	Baeocystin
	(% dry weight)		
P. semilanceata	0.80	0.15	0.11
P. bohemica	0.60	0.21	–
P. bohemica (cultivated)	0.65	0.28	–
P. cubensis	0.45	0.25	–
G. purpuratus	0.24	0.35	0.01
I. aeruginascens	0.32	0.05	0.15
P. cyanescens	0.20	0.61	–

By using the new solvent mixtures containing ethanol and methanol for extraction it was found that more psilocin could be detected in extracts of every species but always smaller amounts of psilocybin than with pure methanol (Table 3).

Additionally, a high activity of enzymes of the phosphatase type could be detected in these aqueous solutions from all species. In contrast to these results only the extracts of *P. cubensis* and *P. cyanescens* showed a significant enzymatic activity by using acetic acid as solvent. In these cases psilocybin was completely dephosphorylated to psilocin by heating the acid extracts and no baeocystin could be detected in *P. cyanescens*.

Discussion

It is well known that an extraction procedure with methanol needs much time (up to 12 hours) at room temperature (BEUG and BIGWOOD 1982, GARTZ 1987, SEMERDZIEVA *et al.* 1986) or one hour at 45 °C (SOTTOLANO and LURIE 1983) for complete extraction.

In our investigations psilocin could be found in high concentrations as well as psilocybin after simple extraction with methanol from various species (GARTZ 1987, 1989c, 1991). When undertaking quantitative analysis of levels of indole derivatives after biotransformation of tryptamine and similar compounds in fruiting mycelia of *P. cubensis* the highest concentrations of psilocin in every mushroom for example could be detected by using methanol (GARTZ 1989a, b). By using aqueous methanol and ethanol as solvent for analysis of *P. bohemica* the Czech analysts have not always analyzed the same batch of mushrooms during their comparative study of extraction methods (KYSILKA, pers. communication 1989).

We generally found variations from one mushroom to another in every species even within *P. bohemica* from a single location (GARTZ and MÜLLER 1989) and also in controlled cultures (GARTZ 1991). Additionally, the high activity of enzymes of the phosphatase type in the aqueous solutions of alcohols was already described in aqueous mycelial extracts of *P. cubensis* and other psilocybin containing mushrooms many years ago (BOCKS 1968, GARTZ 1993, WEBER and HORITA 1963). These enzymes were also extracted with the water containing solvents and caused a partial dephosphorylation of psilocybin to psilocin (Tables 1 and 3). By using these aqueous solutions it was also observed that in some cases bluish mixtures have been resulted after extraction as a sign of partial oxydation of psilocin (BOCKS 1968, GARTZ 1989a, WEBER and HORITA 1963). It is also interesting that most of the baeocystin was destroyed during the extraction procedure with water containing alcohols (Tables 1 and 3).

CASALE (1985) described the rapid formation of psilocin after complete dephosphorylation of psilocybin by heating the dilute acetic acid extract. It is now quite clear that the

decomposition under these conditions is an enzymatic reaction and was not caused by the acid alone. For example the phosphoric acid ester psilocybin, baeocystin and aeruginascin in these acidic extracts from *I. aeruginascens* were stable during heating in contrast to the behavior of the same alkaloids in solutions of *P. cubensis* and *P. cyanescens*. It seems that active enzymes of the phosphatase type could be extracted with aqueous acetic acid only in these two species in contrast to water containing alcohols as extraction method. In the past attempts at the separation of psilocybin and psilocin simply using mixtures of organic solvents and water were also unsatisfactory (THOMSON 1980).

This investigation shows that the high percentage of psilocin detected in *P. bohemica* (KYSILKA and WURST 1990, WURST *et al.* 1992) and not found earlier (GARTZ and MÜLLER 1989) was an artificial phenomenon caused by enzymatic destruction of psilocybin which is common in different species by using water containing organic solvents. Extraction with pure methanol is the safest method to obtain the genuine indole derivatives from mushroom species of various genera.

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