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Biotransformation of tryptamine derivatives in mycelial cultures of *Psilocybe*[1]

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Mycelial cultures of *Psilocybe cubensis* capable of forming psilocybin and psilocin *de novo* display a high capacity for hydroxylation of tryptamine derivatives at the 4-position. A specific biotransformation of added synthetic N,N-diethyl-tryptamine was found. Thus high amounts of 4-hydroxy-N,N-diethyltryptamine (up to 3.3%) and a minor quantity of 4-phosphoryloxy-N,N-diethyltryptamine (0.01—0.8%) were isolated from fruiting bodies of *Psilocybe cubensis* in corresponding experiments. This is the first example of a directed biosynthesis of tryptamine substances by fungi.

An effective biotransformation of N-methyltryptamine was also demonstrated with surface cultures of *Psilocybe semilanceata*. Baeocystin, a possible natural precursor of psilocybin, was detected and quantified in the biomasses.

No alkaloids could be found in the culture medium.

Various secondary metabolites from basidiomycetes are known which have interesting features for medical and biological purposes. But there is a great lack of basis knowledge which would enable strain improvement the basidiomycetes in such a manner as demonstrated for antibiotic-producing actinomycetes or various ascomycetes. By performing a quantitative analysis of the indole alkaloids psilocybin and psilocin in some species of *Psilocybe* and *Inocybe*, we found considerable variations even within one species (SEMERDŽIEVA *et al.* 1986) as well as in cultures (GARTZ 1987). Moreover, fruiting mycelia of *Psilocybe cubensis* (EARLE) SINGER possess a high capacitiy to transform fed tryptamine to psilocin in a methylation and hydroxylation reacti GARTZ 1988). Earlier studies of psilocybin biosynthesis in submerged culture of *Psilocybe cubensis* revealed that tryptophan and tryptamine are precursors of the alkaloids (AGURELL *et al.* 1966, AGURELL and NILSSON 1968a, b).

In the present paper, the biotransformation of fed synthetic tryptamine derivatives by mycelial cultures of *Psilocybe cubensis* and *P. semilanceata* (FR.) KUMM. (MICHAELIS 1977) is described.

Materials and methods

Cultivation of *P. cubensis*: A dried cow dung/rice grain mixture (2:1) suspended into the double amount of water was used to obtain fast fruiting without casing of a *P. cubensis* strain (GARTZ 1987). 0.25 mM N,N-diethyltryptaminehydrochlorid (synthesis: NOGRADI 1957) were added to 13.5 g of this medium. Cultivation without addition of any indole derivative was also tested. The methods for sterile cultivation are described elsewhere (GARTZ 1987). The first mushrooms were produced by the *P. cubensis* cultures with in 4 weeks. The cultures continued to produce sporocarps in five flushes. Each flush was analysed after freeze-drying and storage at —10 °C.

Naturally grown mushrooms: Fruiting bodies of *P. semilanceata* (leg. Dübener Heide, 21. 9. 1985) were analyzed to determine the average alkaloid level in 10 mushrooms. Mycelium obtained from the spores of

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one mushroom (GARTZ 1987) was kept as a stock culture on 6% malt agar.

Surface culture of *P. semilanceata*: Mycelia of *P. semilanceata* were grown in steady culture (50d) on a synthetic medium with 8% glucose (GARTZ 1986). Concentrations up to 10 mM/l of N-methyltryptamine (SIGMA) were added as hydrochloride in to the medium.

Extraction and analysis: The extraction procedures for dried mushrooms and mycelia as well as the analysis of indole alkaloids by using HPLC and TLC are described elsewhere (GARTZ 1985a, b, 1987, SEMERDZIEVA *et al.* 1986). Another mobile phase was also used in the TLC (VANHAELEN-FASTRE and VANHAELEN 1984).

Isolation of indole derivatives: The products of the N,N-diethyltryptamine[1]) biotransformation were isolated from methanolic extracts of the mushrooms by cellulose column chromatography as described for the isolation of psilocybin (KOIKE *et al.* 1981).

Mass spectrometry: Mass spectra were measured in the Varian MAT CH 6 apparatus (ionization energy: 70 eV).

Phosphatase reaction: An alkaline phosphatase from FLUKA was used as described (MICHAELIS 1977).

Results

It was found that in the fruiting mycelia of *P. cubensis* a specific hydroxylation of added synthetic DT in the 4-position of the indole nucleus occured (Fig. 1). 4-Phosphoryloxy-N,N-diethyltryptamine (PT), mp 261—263 °C $C_{14}H_{21}O_4N_2P$, was obtained from mushrooms (0.2% per dry weight) by cellulose column chromatography. Microanalysis suggested the molecular formula for the compound, while the mass spectrum showed fragment ions at m/e 232 (f_1, M^+-HPO_3), 188 (f_1-N $(C_2H_5)_2$, 187 (f_1-NH $(C_2H_5)_2$), 174 (f_1-CH_2N $(C_2H_5)_2$).

The measured UV maxima in methanol at 221, 267, 280 and 290 nm were very similar to reported data of psilocybin and could be attributed to a 4-substituted tryptamine derivative (KOIKE *et al.* 1981, REPKE and LESLIE 1977, WURST *et al.* 1984). 4-Hydroxy-N,N-diethyltryptamine (HT), mp 105 °C, $C_{14}H_{20}ON_2$, was isolated in high amounts (1.5% per

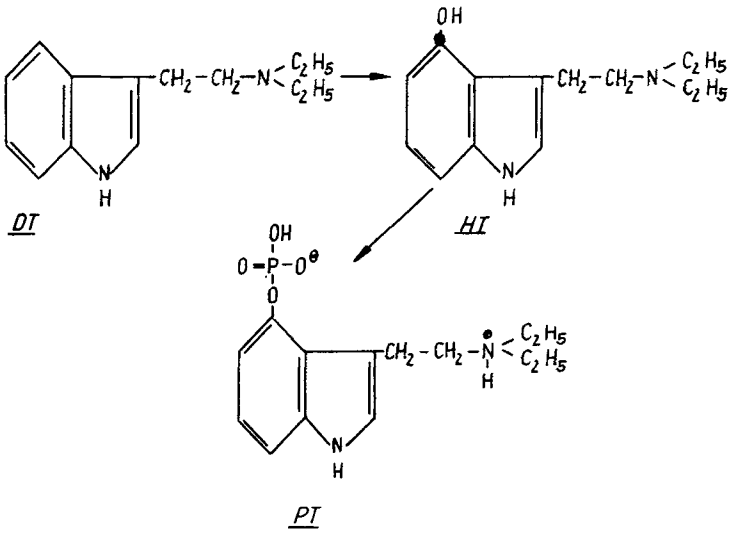

Fig. 1
Biotransformation of DT to HT and PT in fruiting mycelia of *P. cubensis*

[1]) Abbreviations: DT = N,N-diethyltryptamine, HT = 4-hydroxy-N,N-diethyltryptamine, PT = 4-phosphoryloxy-N,N-diethyltryptamine, MT = N-methyltryptamine

dry weight) from fruiting bodies of *P. cubensis*. It displayed the same mass spectrum as phosphoric ester (PT). The UV spectra of the compound showed very similar maxima as psilocin (CHRISTIANSEN and RASMUSSEN 1982, WURST *et al.* 1984): 223, 260, 267, 282 and 293 nm.

By using alkaline phosphatase a complete cleavage of PT and a subsequent formation of HT could be observed.

The levels of DT were always zero in the mushrooms as well in the mycelia. No additional amounts of psilocin or psilocybin could be detected in the mushrooms.

P. cubensis contains indole alkaloids in both naturally grown fruiting bodies and cultivated mushrooms (GARTZ 1987). The mushrooms containing the ethyl-compounds PT and HT (Fig. 2) had smaller caps than the fruit bodies from the cultivation without tryptamine derivative (Fig. 3). However, the spores of the mushrooms with HT and PT were normal in size and germinated well on malt agar. These mushrooms developed a blue-green colour handling but not the deep blue colour characteristic for the naturally grown mushrooms (GARTZ 1987). This observation is in good agreement with model reactions concerning the oxydation of pure psilocin and HT reported in the literature (WEBER and HORITA 1963).

Table 1 shows that the levels of HT and PT from a second mushroom cultivation varied from one flush to the next. The standard deviation of the determination by HPLC was $\pm$ 3.4% (relative). The detection limits for PT and HT were 20 and 30 ng, respectively, when measured by the UV detector.

The TLC analysis of PT shows the same red-violet colour arising after the reaction of psilocybin with EHRLICH's reagent (GARTZ 1985b). On silica gel, *n*-propanol-water-acetic acid (10:3:3) allowed a clear resolution of PT (Rf 0.17), psilocybin (Rf 0.23), DT (Rf 0.30), psilocin (Rf 0.36) and HT (Rf 0.45). Butanol-water-acetic acid-isopropanol (8:5:2:1) (GARTZ 1985b, MICHAELIS 1977) resolved also these indole derivatives (Rf 0.10, 0.16, 0.18, 0.21, 0.27, respectively). EHRLICH's reagent produced a differently shaded colour

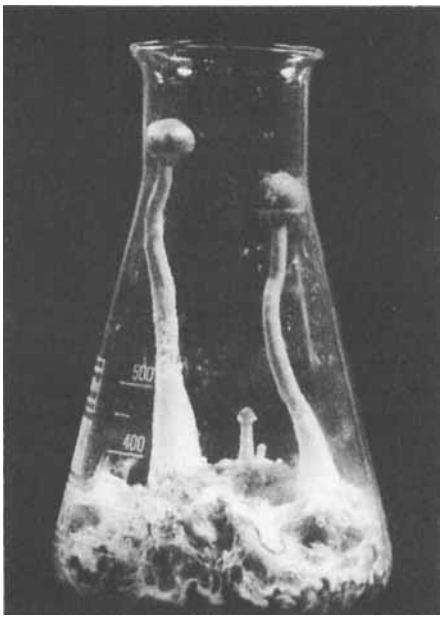

Fig. 2
Psilocybe cubensis on cow dung/rice grain mixture containing the ethyl-compounds PT and HT (photo: THIEL)

Fig. 3
Fruiting bodies of *Psilocybe cubensis* on cow dung/rice grain mixture (photo: THIEL)

Table 1
Variation of PT and HT levels (HPLC) in dried fruit bodies of *Psilocybe cubensis* as a function of flush number

Flush No.	content of the mushrooms	
	HT (%)	PT (%)
1	2.5	—
2	0.2	0.8
3	3.1	0.01
4	3.3	—
5	2.1	0.02

with psilocin and HT (blue) and DT (violet to deep blue after storage). The detection limit for the indole derivatives was approximately 0.01 percent on dry matter in the TLC analysis.

The spores of *P. semilanceata* 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 the surface culture of *P. semilanceata* without addition of a precursor.

In contrast to these results, a biotransformation of MT to 4-phosphoryloxy-N-methyltryptamine (baeocystin) in the mycelial cultures of the species was observed (Table 2, Fig. 4).

An increasing concentration of the tryptamine derivative also raised the content of baeocystin in the mycelia. Only small amounts of psilocybin could be found in the mycelia. This is in contrast to the relation between the amounts of the alkaloids in the naturally

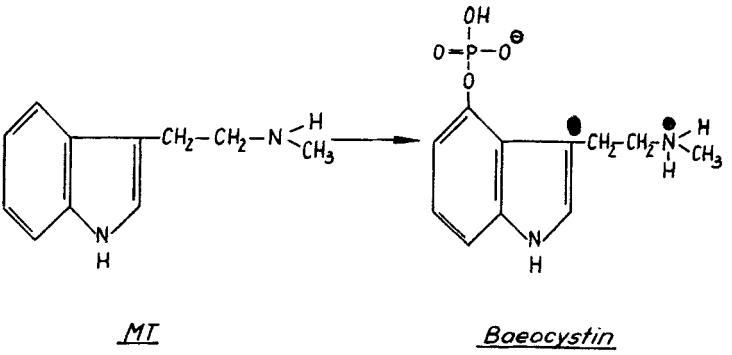

Fig. 4
Biotransformation of MT to baeocystin by surface culture of *P. semilanceata*

Table 2
Levels of baeocystin and psilocybin (HPLC) in dried mycelia of *Psilocybe semilanceata* (surface culture: 50 d)

concentration of MT (mM/l)	baeocystin (%)	psilocybin (%)	mass of mycelia (g)
2.5	0.3	0.02	20.2
5.0	0.4	0.05	19.1
10.0	0.9	0.08	21.3

grown fruiting bodies. An average of 0.94% psilocybin and 0.19% baeocystin was detected in these dried mushrooms. No alkaloids could be detected in the nutrient media.

Discussion

TROXLER *et al.* (1959) synthesized the two ethyl-analogs PT and HT of psilocybin and psilocin as well as many other tryptamine derivatives. These substances were not found in any naturally growing mushrooms. The reported biotransformation of DT to PT and HT is the first evidence of a directed biosynthesis of such substances in fungi. In comparison with the levels of psilocybin and psilocin in naturally growing fruiting bodies (CHRISTIANSEN and RASMUSSEN 1982, KOIKE *et al.* 1981, GARTZ 1987, SEMERDŽIEVA *et al.* 1986, WURST *et al.* 1984), the amounts of PT and HT are higher in the cultivated fruiting bodies of *P. cubensis*.

By performing quantitative analysis of the indole alkaloid levels in laboratory-grown *P. cubensis*, large variations among different flushes were also found (GARTZ 1987). It is interesting to note that the size of the mushrooms was affected by the addition of DT to the nutrient media. As shown in earlier investigations, cultures on malt agar and rice substrate also produced mushrooms in various amounts and with different appearance (GARTZ 1987).

Baeocystin was first detected by LEUNG and PAUL (1968), who isolated this incompletely methylated counterpart of psilocybin from *Psilocybe baeocystis* SINGER and SMITH grown in submerged culture. The alkaloid was subsequently detected in various collections

of *P. semilanceata* (GARTZ 1985a, CHRISTIANSEN and RASMUSSEN 1982, REPKE and LESLIE 1977, VANHAELEN-FASTRE and VANHAELEN 1984).

This investigation shows that even baeocystin as possible precursor in the psilocybin biosynthesis occurs in significant amounts in *Psilocybe* species. The high amount of baeocystin as product of the biotransformation of MT indicates a high hydroxylation and phosphorylation capacity of the surface culture of *P. semilanceata* but a low ability in methylation of tryptamine derivatives.

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