REVIEW

Psychoactive Tryptamines from Basidiomycetes

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Dedicated to the memory of Dr. MARTA SEMERDŽIEVA

ABSTRACT. The review lists natural sources, *i.e.* strains and species of fungi producing predominantly psychoactive tryptamines (indolealkylamines), their chemical structure and properties, toxic effects on the man and psychic symptoms of intoxication. It describes the biosynthesis and production of some tryptamines by the mycelial culture of *Psilocybe bohemica* ŠEBEK, a survey of methods for their analysis and isolation. It evaluates the worldwide use and abuse of psychoactive fungi as sources of drugs in general and in the Czechia in particular during the last two and a half decades.

Abbreviations

BAC	baeocystin	HTA	4-hydroxytryptamine	PDA	photo-diode array (detector)
BUF	bufotenine	4HTR	4-hydroxytryptophan	PSB	psilocybin
DET	N^9,N^9-diethyltryptamine	5HTR	5-hydroxytryptophan	PSC	psilocin
DMT	N^9,N^9-dimethyltrypamine	LSD	(+)-*N,N*-diethyllysergamide	SRT	serotonin
ECD	electron capture detector (GC)	MST	*N*-methylserotonin	TPA	tryptamine
ED	electrochemical detector (HPLC)	MTA	N^9-methyltryptamine	TRP	tryptophan
		NBC	norbaeocystin		

CONTENTS

1 INTRODUCTION

The narcotic effect of some fungi has been known for several thousand years. Small stone statues of fungi from the 13th century B.C., representing very probably *Psilocybe semperviva*, have been found in Guatemala. Medieval books from the 16th and 17th centuries, written by the Spanish monk Sahagoon and by Hernandez, the physician of king Philip II, mention Indians who use in rituals and for medicinal purposes small gill fungi named "teonanacatl" or "the gods' meat" (Hofmann 1960).

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Our current knowledge of species and chemistry of hallucinogenic fungi in Central America has been obtained relatively recently. B.P. Reko confirmed the existence of such fungi in Mexico in 1919 and Weitlauer obtained their samples in 1936.

The physician Wasson obtained first samples of narcotic fungi in 1953–56 during expeditions to the Oaxaco province in southern Mexico (Wasson 1957; Wasson and Wasson 1957). The French mycologist Heim (1957) provided the botanical description of hallucinogenic fungi including 11 species of *Psilocybe* and one species of *Stropharia*.

Hofmann (1958) isolated from fungi of the *Psilocybe* genus psychoactive compounds (compounds causing in certain doses changes in perception, moods and colorful hallucinations in humans and animals; Hoffer and Osmond (1967), which were identified (Hoffmann *et al.* 1959) as indole derivatives of the tryptamine type: psilocybin (PSB) and psilocin (PSC). During the following decade, other tryptamines produced by the genus *Psilocybe* were described: baeocystin (BAC) and norbaeocystin (NBC) (Leung and Paul 1967, 1968).

Psilocybin and similar compounds were found and determined in a number of fungal species of the genus *Psilocybe* and also in the genera *Conocybe*, *Panaeolus*, *Gymnopilus*, *Inocybe*, *Galerina*, and *Pluteus* from South and Central America, South and Southeast Asia, Australia, New Guinea and Europe.

2 SOURCES

The research into indole compounds of the tryptamine type was initiated by the discovery of PSB and PSC in fungi of the genus *Psilocybe* from Central America (Wasson and Wasson 1957; Heim 1957; Hofmann *et al.* 1958a,b; Wasson 1959; Hofmann *et al.* 1959).

The properties of these compounds have been described in a number of studies, reviews and monographs (Wasson and Wasson 1957; Heim and Wasson 1958; Hoffer and Osmond 1967; Ola'h 1969; Ott and Bigwood 1977; Singer 1978; Guzmán 1983; Gartz 1987a; Semerdžieva and Hausner 1992; Stijve 1995; Stamets 1996).

The current literature on the subject lists more than 80 fungal species containing some psychoactive or nonpsychoactive tryptamines. These fungal species belong to 12 genera and 7 families. The best studied in this respect are fungi of the genus *Psilocybe*.

Singer (1978) published a detailed study of 38 hallucinogenic fungi of *Psilocybe* spp. containing both PSB and PSC. The contents of the compounds, however, were not convincingly documented. Allen *et al.* (1992) listed 130 PSB-containing fungal species from 12 genera classed into 6 families. They assumed that 90 out of the 300 recognized *Psilocybe* spp. listed in the monograph by Guzmán (1983) are hallucinogenic.

Stijve (1995) conducted a critical survey of the literature data and concluded that chemical analyses of this group of indole alkaloids had been performed in 40 taxonomically recognized species; when combined with the data by Guzmán (1983), these conclusions give a total number of about 80 hallucinogenic *Psilocybe* species.

The most important psychoactive fungus is still the worldwide spread *Psilocybe semilanceata* (FRIES) KUMMER (Hofmann *et al.* 1963; Semerdžieva and Nerud 1973; Repke and Leslie 1977; Christiansen and Rasmussen 1982; Wurst *et al.* 1984; Stijve 1984; Stijve and Kuyper 1985) known as "liberty cap". Other species, less widely spread and more rarely encountered, are *P. bohemica* ŠEBEK (Wurst *et al.* 1984; Semerdžieva and Wurst 1986), previously recorded as *P. coprinifacies* (ROLL.) POUZ. or *P. mairei* SING. (Šebek 1975, 1983), and in some countries still denoted *P. cyanescens* WAKEFIELD (Benedict *et al.* 1962; Beug and Bigwood 1982) and *P. liniformans* GUZMÁN et BAS (Guzmán and Bas 1977; Stamets *et al.* 1980; Stijve and Kuyper 1985; Kriegelsteiner 1984, 1986).

The other fungal species containing PSB were described mostly in Europe. The genus *Inocybe* was discovered in Germany (Drewitz 1983) due to an accidental poisoning by *Inocybe aeruginascens*, which was inadvertently picked up instead of the edible *Marasmius oreades*. Chemical analyses confirmed the content of PSB and BAC (Stijve *et al.* 1985; Gartz and Drewitz 1985) in *Inocybe aeruginascens* (Semerdžieva *et al.* 1986). Hallucinogenic substances were found also in other four rare *Inocybe* species (Stijve and Kuyper 1985; Stijve *et al.* 1985).

The genus *Gymnopilus* has also been stated to contain the psychoactive substances PSC and PSB (Hatfield *et al.* 1978) but these data were confirmed later only in the exotic *G. purpuratus* (COOKE et MESSE) SING., which was imported to Germany in the eighties (Kreisel and Lindequist 1988; Gartz 1989b).

Many hallucinogenic fungal species are assumed to belong to *Paneolidae*. Popular dictionaries claim that *Paneolina foenisecii* (FRIES) KUEHNER can be hallucinogenic; however, during the last decade, the fungus was repeatedly found to contain no psychoactive substances (Beug and Bigwood 1982; Stijve *et al.*

1984; Allen *et al.* 1991). By contrast, in Europe *Panaeolus subbalteatus* (BERKLEY et BROOME) SACC. was confirmed to contain significant amounts of PSB (Ola'h 1968; Fiussello and Scurti Ceruti 1972; Beug and Bigwood 1982; Stijve 1985). In taxonomic classification, *P. subbalteatus* can be mistaken for the taxonomically superficially similar *P. foenisecii* (FRIES) KUEHNER (= *Paneolina foenisecii*). In USA, both fungal species grow together in well-fertilized lawns, which virtually never happens in Europe (Stijve 1987).

Many species of the genus *Copelandia, e.g.*, among which *C. cyanescens* (BERKLEY et BROOME) SINGER, named also *Panaeolus cyanescens* (BERKLEY et BROOME) SACC., are widely spread in tropical regions. *C. cyanescens* is strongly psychoactive due to a high content of PSB. These species collected in Hawaii show a strongly reduced content of PSB and fungi from Australia and Thailand lack this psychoactive compound completely (Stijve 1992). According to Ola'h (1969) some species of the genus *Panaeolus* from the USA contain PSB latently, *e.g.*, *P. campanulatus* and *P. fimicola.* In Europe, these species were found to contain no PSB whereas collections of both species from southern Brazil were found to contain it (Stijve and Meijer 1993; Stijve 1995).

As published at the beginning of the eighties, some taxonomic species of fungi of the genus *Pluteus* contain PSB, PSC and other unidentified tryptamines (Saupe 1981; Stijve and Bonnard 1986; Stijve and Meijer 1993). A widely spread European species is *Pluteus salicinus* (PERSOON et FRIES) KUMMER, another species, *P. nigroviridis* BABOS is very rare (Stijve and Bonnard 1986). Psychoactive substances were found in *Pluteus glaucus* SINGER and similar species from southern Brazil (Stijve and Meijer 1993).

In a glasshouse of the *Institute of Botany* in Regensburg, Besl (1993) discovered and grew a new species of the genus *Galerina* containing PSB. The species was described as *Galerina steglichii* BESL (family *Cortinariaceae*).

Stijve and Kuyper (1988) pointed out the erroneous data on the content of PSB and related compounds in some fungal species such as *Mycena pura* (Heim 1963; Giacomoni 1984). Some exotic fungi of the family *Hydnaceae,* such as *Sarcodon atroviridis*, were found to contain 4 unidentified tryptamine derivatives (Stijve 1995).

Reports on hallucinogenic fungi of the genus *Lepiota* in Florida are most probably fictitious (*Anonymous* 1983; Stijve 1995).

Psychoactive tryptamine compounds (PSB, PSC, BAC, BUF, *etc.*) and some other inactive indole derivatives (SRT, TRP, 5HTR, *etc.*) are mostly found in scotosporous gill fungi growing in meadows and woods of the subtropic and tropic climatic zone, usually in soils rich in humus and plant debris. The presence of this group of indole alkaloids in a relatively large number of fungal species does not reflect their genetic relatedness but the probability of tryptamine biosynthesis and the simplicity of the biosynthetic pathway (Benedict *et al.* 1962). According to Saupe (1981) PSB and PSC are not suitable chemotaxonomical markers on the level of families and higher, but could play a certain role in studies of the relationships within the families.

Table I gives a survey of *Basidiomycetes* containing largely tryptamine derivatives. It lists both psychoactive and inactive indole derivatives only in fungi in which these compounds have been discovered and proved by physico-chemical methods with sufficient reliability. The number of fungal species, which were proven to contain psychoactive tryptamines, is relatively large. However, only several species of the genus *Psilocybe, Panaeolus subbalteatus, Inocybe aeruginascens* and *Pluteus salicinus* are important in the toxicology, forensic science and psychiatry. The utilization of the relatively high concentrations of bufotenine in *Amanita citrina* and *A. porphyrea* is hampered by the difficulties in the laboratory cultivation of these two species (Wurst *et al.* 1992).

3   CHEMICAL AND PHYSIOLOGICAL PROPERTIES

Aromatics form an important group of metabolites in living organisms. The interaction of π electrons of the conjugated double bonds on the aromatic skeleton is the reason for many specific chemical and biochemical properties of these compounds. Among aromatic metabolites, compounds containing indole ring in their molecule have a special position.

Indolealkylamines – tryptamines – are one of the twenty classes of indole alkaloids comprising about 600 compounds (Schultes 1976).

The only essential indole amino acid is the tryptophan (2-amino-3-(3-indolyl)propionic acid). Some derivatives of tryptamine are given in Table II. They are mostly hallucinogenic with the exception of serotonin, an important neurotransmitter found in the central nervous system of humans and animals. Most tryptamines have been discovered in microorganisms and plants, some of them also in animals.

Table I. Basidiomycetes sources of tryptamines

Species	Compounds	References
Amanitaceae		
Amanita citrina (SCHAEFF.) GRAY	BUF, SRT BUF, TRP, TPA, 5 HTR, MST	Borner and Brenneisen 1987; Wurst *et al.* 1992
A. porphyria ALB et SCHW.:FR.	BUF, SRT, MST	Tyler and Groeger 1964; Wieland and Mozel 1958; Stijve 1979; Wurst *et al.* 1992
A. rubescens (PERS.:FR.) GRAY	BUF	Wurst *et al.* 1992
Bolbitiaceae		
Agrocybe farinacea HONGO	PSB	Koike *et al.* 1981
Conocybe cyanopus (ATK.) KUEHN.	PSB, PSC, BAC	Benedict *et al.* 1962, 1967; Ohenoja *et al.* 1987; Repke *et al.* 1977a; Christiansen *et al.* 1984; Beug and Bigwood 1982; Gartz 1992
C. kuehneriana SING.	PSC	Ohenoja *et al.* 1987
C. smithii WATLING	PSC, BAC	Benedict *et* al. 1962, 1967; Repke *et al.* 1977a
Coprinaceae		
Copelandia cambodginiensis (OLA'H et HEIM) SING. et WEEKS	PSB	Ola'h 1969
C. chlorocystis SING. et WEEKS	PSB, PSC	Weeks *et al.* 1979
C. cyanescens (BERK. et BR.) SING.	PSB, PSC	Fiussello and Scurti 1972
C. tropicalis (OLA'H) SING. et WEEKS	PSB	Ola'h 1969
Panaeolus acuminatus RICK. (= *P. rickenii* HORA) *P. acuminatus* (SCHAEFF.) QUÉL. sensu RICK.	SRT, TRP, 5HTR	Stijve 1985, 1987
P. africanus (OLA'H)	PSB, PSC	Ola'h 1969
P. antillarum (FR.) DENNIS (= *P. phalaenarum* (FR.) QUÉL., *P. sepulchralis* BERK., *Anellaria sepulchralis* (BERK.) SING.)	SRT	Stijve 1987; Stijve and Meijer 1993
P. ater (LANGE) KUEHN. et ROMAGN.	PSB, SRT	Ola'h 1967; Stijve 1987
P. cambodginiensis OLA'H et HEIM (= *Copelandia cambodginiensis* (OLA'H et HEIM) SING. et WEEKS)	PSB, PSC	Ola'h 1969; Merlin and Allen 1993
P. campanulatus (L.) QUÉL.	TRA, SRT	Stijve 1985
P. castaneifolius (MURRILL) OLA'H	PSB	Ola'h 1969
P. cyanescens (BERK. et BR.) SACC. (= *Copelandia cyanescens* (BERK. et BR.) SACC.; *C. papilionacea* (BULL.:FR.) BRES.)	PSB, PSC, BAC, SRT	Stijve and Meijer 1993; Stamets 1996
P. fimicola (FR.) QUÉL. (= *P. ater* (LANGE) KUEHN. et ROMAGN.)	TPA, SRT	Ola'h 1967; Stijve 1987
P. foenisecii (FR.) KUEHN. (= *Panaeolina foenisecii* (FR.) KUEHN.)	SRT	Robbers *et al.* 1969; Stijve *et al.* 1984, 1985; Gartz 1985a; Ohenoja *et al.* 1987; Borner and Brenneisen 1987
P. foenisecii (PERS.:FR.) SCHORET ap. COHN (= *Panaeolina foenisecii* (PERS.:FR.) R. MAIRE)	SRT, 5HTR	Stijve and Meijer 1993
P. guttulatus BRES.	TPA, SRT	Stijve 1985, 1987
P. nirimbii WATLING et YOUNG	SRT	Stijve 1987
P. olivaceus MOELLER	PSB, SRT	Ohenoja *et al.* 1987; Stijve 1987

P. papilionaceous (BULL.:FR.) QUÉL. (= *Psilocybe callossa* (FR.:FR) QUÉL., *Panaeolus campanulatus* (FR.) QUÉL., *P. retirugis* (FR.) QUÉL., *P. sphinctrinus* (FR.) QUÉL.)	TPA, SRT	Stijve 1985; Stamets 1996
P. phalaeonarum (FR.) QUÉL.	SRT	Stijve 1987
P. semiovatus (FR.) LUNDELL (= *P. separatus* GILLET, *Anellaria separata* KARST.)	TPA, SRT	Stijve 1985. 1987
P. sphinctrinus (FR.) QUÉL.	TPA, SRT	Stijve 1985, 1987
P. subbalteatus (BERK. et BR.) (= *P. venenosus* MURRIL)	PSB, PSC, BAC, SRT, TPA	Beug and Bigwood 1982; Stijve and Kuyper 1984; Stijve 1985, 1987; Ohenoja *et al.* 1987; Gartz 1989*a*
P. subbalteatus (BERK. et BR.) SACC.	5HTR, PSB, SRT	Stijve and Meijer 1993
Psathyrella candoleana (FR.) SMITH	TPA	Agurell *et al.* 1969; Koike *et al.* 1981

Cortinariaceae		
Galerina steglichii BESL	PSB	Besl 1993
Gymnopilus liquiritiae (FR.) KARST.	PSB	Koike *et al.* 1981
G. purpuratus (COOKE et MASS.) SING. (= *Flammula purpurata*)	PSB, PSC, BAC	Kreisel and Lindequist 1988; Gartz 1989*b*, 1993, 1994
G. validipes (PECK) HESLER	PSB	Hatfield *et al.* 1978
Inocybe aeruginascens BABOS	PSB, PSC, BAC	Drewitz 1983; Stijve *et al.* 1985; Stijve and Kuyper 1985; Gartz and Drewitz 1985; Semerdžieva *et al.* 1986; Gartz 1986*a*, 1987*c*
I. coelestium KUYP.	PSB, BAC	Stijve *et al.* 1985; Stijve and Kuyper 1985
I. corydalina QUÉL. (= *I. corydalina* var. *corydalina* QUÉL., *I. corydalina* var. *erinaceomorpha* (STANGEL et VESELSKÝ) KUYP.)	PSB, BAC	Stijve and Kuyper 1985; Stijve *et al.* 1985
I. haemacta (BERK. et COOKE) SACC.	PSB, PSC, BAC	Stijve and Kuyper 1985; Gartz 1986*c*; Stijve *et al.* 1985

Pluteaceae		
Pluteus atricapillus	PSB	Ohenoja *et al.* 1987
P. glaucus SING.	PSB, PSC	Stijve and Meijer 1993
P. nigroviridis BABOS	PSB	Stijve and Bonnard 1986
P. salicinus (PERS.: FR.) KUMM.	PSB, PSC, BAC	Saupe 1981; Christiansen *et al.* 1984; Stijve and Kuyper 1985; Stijve and Bonnard 1986; Ohenoja *et al.* 1987

| Strophariaceae		
Psilocybe argentipes YOKOHAMA	PSB	Koike *et al.* 1981
P. atrobrunnea (LASCH) GILLET	PSB	Hoiland 1978
P. aztecorum HEIM emend. GUZMÁN	PSB	Heim and Hofmann 1958
P. azurescens STAMETS et GArtz	PSB, PSC, BAC	Stamets 1996
P. baeocystis SING. et SMITH	PSB, PSC, BAC	Leung and Paul 1967, 1968; Beug and Bigwood 1981, 1982
P. bohemica ŠEBEK	PSB, PSC, BAC	Semerdžieva and Nerud 1973; Wurst *et al.* 1984; Kysilka *et al.* 1985; Stijve and Kuyper 1985; Semerdžieva and Wurst 1986; Kriegelsteiner 1984, 1986; Gartz 1993*b*, 1994
P. bonetii GUZMÁN	PSB	Ott and Guzmán 1976

Table I – *continued*

P. caeruleoannulata SING.: GUZMÁN	PSB, PSC	Stijve and Meijer 1993
P. caerulescens MURR. (= *P. caerulescens* var. *mazatecorum* HEIM, *P. caerulescens* MURR. var. *caerulescens*)	PSB, PSC	Heim and Hofmann 1958; Heim and Wasson 1958; Stijve and Meijer 1993
P. caerulipes (PECK) SACC.	PSB	Leung *et al.* 1965
P. callosa (FR.: FR.) QUÉL.	PSB	Guzmán 1983
P. candidipes SING. et SMITH	PSB	Ott and Guzmán 1976
P. cubensis (EARLE) SING. (= *P. cubensis* var, *caerulescens* (MURR.) SING et SMITH, *Stropharia cubensis* EARLE, *S. cyanescens* MURR., *S. caerulescens* (PAT.) SING.)	PSB, PSC, BAC	Heim and Hofmann 1958; Repke *et al.* 1977*b*; Bigwood and Beug 1982; Gartz 1987*a*, 1989*c*, 1994; Stijve and Meijer 1993
P. cyanescens WAKEFIELD	PSB, PSC, BAC	Beug and Bigwood 1982; Stijve and Kuyper 1985; Kriegelsteiner 1984, 1986; Gartz 1994
P. cyanofibrillosa GUZMÁN et STAMETS (= *P. rhododendronensis* STAMETS nom.prov.)	PSB, PSC	Stamets *et al.* 1980
P. eucalypta GUZMÁN et WATLING	PSB	Margot and Watling 1981
P. fimentaria HEIM, *P. fimentaria* (ORTON) WAT. (= *P. fimentaria* (ORTON) SING., *P. caesio annulata* SING., *Stropharia fimentaria* ORTON)	PSB	Benedict *et al.* 1967; Stamets 1996
P. hoogshagenii HEIM var. *hoogshagenii* (= *P. caerulipes* var. *gastonii* SING., *P. zapotecorum* HEIM sensu SING., *P. semperviva* HEIM et CALLIEUX)	PSB, PSC, BAC	Heim and Hofmann 1958; Heim and Wasson 1958; Stijve and Meijer 1993; Stamets 1996
P. liniformans GUZMÁN et BAS	PSB, PSC, BAC	Stamets *et al.* 1980; Stijve and Kuyper 1985; Kriegelsteiner 1984, 1986
P. mexicana HEIM	PSB, PSC	Hofmann *et al.* 1958*b*; Heim and Hofmann 1958
P. muliericula SING. et SMITH (= *P. wassonii* HEIM, *P. mexicana* var. *brevispora* HEIM)	PSB, PSC	Heim and Hofmann 1958
P. nataliensis GARTZ, REID, SMITH et EICKER (nom.prov.)	PSB, PSC	Gartz *et al.* 1996
P. pelliculosa (SMITH) SING. et Smith	PSB, BAC	Repke *et al.* 1977*a*; Beug and Bigwood 1982
P. quebecensis OLA'H et HEIM	PSB, PSC	Ola'h 1967
P. samuiensis GUZMÁN, ALLEN et MERLIN	PSB, PSC, BAC	Guzmán *et al.* 1993*a,b*; Stijve and Meijer 1993; Gartz *et al.* 1994
P. semilanceata (FR.) KUMM.	PSB, PSC, BAC	Repke and Leslie 1977; Christiansen *et al.* 1981*a,b*; Vanhaelen-Fastre and Vanhaelen 1984; Wurst *et al.* 1984; Stijve and Kuyper 1985; Kriegelsteiner 1984, 1986; Gartz 1986*b*, 1993, 1994; Brennaisen and Borner 1988*a*
P. semperviva HEIM et CAILLEUX	PSB, PSC	Heim and Hofmann 1958; Heim and Wasson 1958
P. silvatica (Peck) SING. et SMITH	PSB, BAC	Repke *et al.* 1977*a*
P. strictipes SING. et SMITH (= *P. callosa* (FR.: FR.) QUÉL. sensu AUCT. sensu GUZMÁN 1983, *P. semilanceata* var. *obtusa* BON., *P. semilanceata* var. *microspora* SING.)	PSB	Leung *et al.* 1965; Stamets 1996
P. stuntzii GUZMÁN et OTT (= *P. pugetensis* HARRIS)	PSB, PSC, BAC	Repke *et al.* 1977*a*; Beug and Bigwood 1982; Stamets 1996
P. subaeruginascens HOEHNEL (= *P. aerugineomaculans* (HOEHNEL) SING. et SMITH	PSB, PSC	Koike *et al.* 1981; Stamets 1996
P. subaeruginosa CLELAND	PSB	Perekal *et al.* 1980
P. cf. *subyungensis* GUZMÁN	PSB, PSC, BAC	Stijve and Meijer 1993

P. uruguayensis SING.: GUZMÁN	PSB, PSC, BAC	Stijve and Meijer 1993
P. tampanensis GUZMÁN et POLLOCK	PSB, PSC	Gartz *et al.* 1994
P. wassonii HEIM	PSB, PSC	Heim and Wasson 1958
P. weilii (nom.prov.)	PSB, PSC, BAC, TRP	Stamets 1996
P. zapotecorum HEIM (= *P. zapotecorum* HEIM emend. GUZMÁN)	PSB, PSC	Heim and Hofmann 1958; Heim and Wasson 1958; Stijve and Meijer 1993; Stamets 1996

Tricholomataceae		
Rickenella straminea (PETCH) PEGLER	5HTR	Stijve and Meijer 1993

Table II. Tryptamines – indolalkylamines
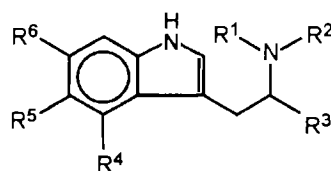
Compound	R^1	R^2	R^3	R^4	R^5	R^6
Tryptamine	H	H	H	H	H	H
N-Methyltryptamine	Me	H	H	H	H	H
N,N-Dimethyltryptamine	Me	Me	H	H	H	H
4-Hydroxytryptamine	H	H	H	OH	H	H
4-Hydroxy-*N*-methyltryptamine	Me	H	H	OH	H	H
4-Hydroxy-*N,N*-dimethyltryptamine (psilocin)	Me	Me	H	OH	H	H
4-Phosphoryloxytryptamine (norbaeocystin)	H	H	H	O–P	H	H
4-Phosphoryloxy-*N*-methyltryptamine (baeocystin)	Me	H	H	O–P	H	H
4-Phosphoryloxy-*N,N*-dimethyltryptamine (psilocybin)	Me	Me	H	O–P	H	H
5-Hydroxytryptamine (serotonin)	H	H	H	H	OH	H
5-Hydroxy-*N*-methyltryptamine (*N*-methylserotonin)	Me	H	H	H	OH	H
5-Hydroxy-*N,N*-dimethyltryptamine (bufotenin)	Me	Me	H	H	OH	H
5-Hydroxy-*N*-acetyltryptamine (*N*-acetylserotonin)	Ac	H	H	H	OH	H
5-Methoxy-*N*-acetyltryptamine (melatonin)	Ac	H	H	H	OMe	
5-Methoxy-6-hydroxy-*N*-acetyltryptamine (6-hydroxymelatonin)	Ac	H	H	H	OMe	OH
6-Hydroxytryptamine	H	H	H	H	H	OH
6-Hydroxy-*N*-methyltryptamine	Me	H	H	H	H	OH
6-Hydroxy-*N,N*-dimethyltryptamine	Me	Me	H	H	H	OH
N-Ethyltryptamine	Et	H	H	H	H	H
N,N-Diethyltryptamine	Et	Et	H	H	H	H
Tryptophan	H	H	COOH	H	H	H
5-Hydroxytryptophan	H	H	COOH	H	OH	H

Bekker *et al.* (1985) found that positional isomers of tryptamines containing a phosphate or hydroxyl group in positions 5, 6, 7, and structural analogues containing various alkyl radicals on the side chain amino group nitrogen have a markedly lower physiological efficiency than PSB and PSC.

The psychoactive effects increase in the series 4-hydroxytryptamine, 4-hydroxy-*N*-methyltrypt-amine, 4-hydroxy-*N*,*N*-dimethyltryptamine (PSC). The phosphate group is assumed to protect the PSB mole-cule from oxidative decomposition but it has no effect on physiological properties, *i.e.* the intensity of the hallucinations. In equimolar amounts, PSB and PSC cause an identical hallucinogenic effect (Hoffer and Osmond 1967).

In contrast to 5-hydroxy substituted indole derivatives, which can be easily obtained by chemical synthesis, the preparation of 4-hydroxy derivatives is more complicated. The synthesis of PSB includes ten steps with very low yield (Hofmann *et al.* 1958*a*). Higher physiological activity makes 4-hydroxytryptami-nes more suitable for various applications (Hoffer and Osmond 1967; Bekker *et al.* 1985) than 5-hydroxy compounds. 4-Hydroxy- and 4-phosphoryloxytryptamine derivatives can be also isolated from fruit bodies and from mycelia obtained by laboratory cultivation.

The effect on molecular level classes PSB, PSC, BUF and other tryptamines among antiserotogenic compounds. Due to their structural similarity with tryptamines, they exhibit a high affinity to serotonin receptors. Binding to these receptors results in lower transfer of SRT in interneuron synapses (Chilton *et al.* 1979). This causes an unregulated activity of the neurons associated with the processing of visual and emo-tional information, and leads to hallucinations.
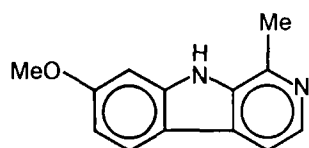
Hallucinations are illusory perceptions that, for the afflicted person, have the character of real per-ceptions (Grof 1993). Hallucinogens are natural and synthetic compounds, which can evoke in humans and in animals marked changes or disturbances of perception, emotions, experience and thus also behavioral changes. Thinking, fantasy, and attention are also very often affected. Prolonged abuse can lead to conside-rable disturbances of moral and ethical values (Miovský 1996). Hallucinogens are defined as compounds that cause transiently fairly deep psychic changes without altering the state of consciousness. They have sympathomimetic effects, which antagonize the action of SRT (Miovský 1996).

## 4   TOXIC EFFECTS, PSYCHIC SYMPTOMS

The fifties was the period of an explosive development of psychopharmacology and so-called "gol-den era of hallucinogens", which resulted in growing interest in the study of compounds produced by some fungi. During this period, the team including R. Wasson, P. Wasson, R. Heim, A. Hofmann and the psy-chiatrist J. Delay discovered the first natural phosphorylated indole and synthesized the hallucinogen PSB (Hofmann *et al.* 1958*a,b*).

4.1   Psilocybin and psilocin

These compounds are chemically related to BUF, harmine and LSD. The toxicity of PSB is rela-tively low and the same effect on intoxicated persons requires a multiple amount of the compound per kg body mass than with other hallucinogens. The low toxicity of PSB and PSC provided the basis for using them in psychodiagnostics and psychotherapy as substitutes for the toxic and strongly habit-forming LSD and mescaline (a substance isolated from the cactus *Lophophora williamsii* from Central America (Leuner and Schlichting 1986). PSB is 2.5-fold less toxic than mescaline and its LD_{50} for mice is 250 mg/kg i.v. (Weid-

harmine
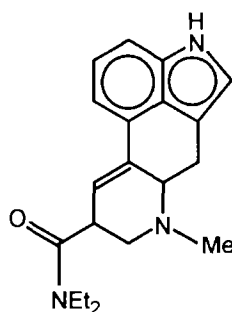
LSD
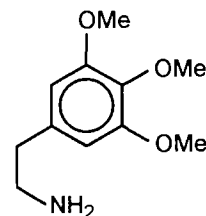
mescaline

mann *et al.* 1958), its psychotomimetic efficacy in man is about 50 times higher (Hoffer and Osmond 1967) than that of mescaline and an effective dose of PSB is about 5–15 mg (Benedict 1972).

The course of PSB intoxication is highly specific. A period of latency (several minutes to 1 h) is followed by the appearance of somatic symptoms: reddening of the face, lowering of the heart activity, extension of pupils, sweating, headache, tremor, *etc.* The intoxicated person often experiences euphoria. The overall length of intoxication is 4–6 h depending on the dose and the individual condition of the intoxicated person. The period of intoxication is about 4 h for a 10 mg dose (Miovský 1996).

The psychic symptoms of intoxication vary widely. They exhibit many similarities and some differences with the other known hallucinogens, especially mescaline and LSD. The criteria for psychotic disturbances with hallucinations for LSD and PSB (Table III) are generally identical (Miovský 1996).

Table III. Basic signs and symptoms of PSB (10 mg subcutaneously) and LSD (0.15 mg) intoxication[a]

Intoxication	PSB	LSD
Time course	latency period – minutes to 1 h, duration of psychosis 4–6 h, intoxication peak 2–3 h	latency period 40–50 min, duration of psychosis 5–6 h, intoxication peak 5–6 h
Somatic signs	increased heartbeat and blood pressure, mydriasis, vertigo and nausea, sweating, shivers, excitation and agitation leading sometimes to aggressive behavior	mostly at the onset of intoxication – nausea, urge to vomit, sensation of empty head, feebleness and fatigue, inner tremor, skin reddening, sweating, motoric discoordination
Perception disturbances	illusions, hallucinations, increased tendency to pareidolia, ornamentalization of objects seen	illusions, pseudohallucinations, hallucinations, eidetism
Affectivity disorders	euphoria, laughter to ecstatic mood–disphoria– anxiety, tearfulness–stronger depression–panic anxiety	at the onset often strong euphoria, later depression, shiftlessness, frequent ambivalence feelings
Thinking disorders	sensitive huffiness, paranoid delusions, persecution mania, incoherent thinking	all disorders depend on the individual's sensitivity; paranoid delusions, huffiness
Memory disorders	confusion, amental states, delirious states, confabulation	memory is usually retained
Judgement	marked disorders of space and time perception and assessment	frequent disturbances of time perception, strongly disturbed orientation in space
Attention disturbances	disturbances in concentration	ability to concentrate depends on willingness
Personality disturbances	depersonalization and derealization	depersonalization and derealization
Consciousness disorders	somnolence, at higher doses sopor to coma	transient and mild, marked self centering
Special features	disorders of basic instincts: self-damage, suicidal actions, self-mutilation	geometrization, architectural structures

[a]According to Miovský (1996).

Depersonalization due to PSB intoxication is not so intensive but the changes in space perceptions are more marked. More conspicuous is the feeling of well being or even an exquisite bliss, while vegetative symptoms are milder. Drawings made by intoxicated persons have characteristic geometric and ornamental features, with motifs of concentric belts. The patterns are interspersed by whirling, poorly decipherable changes in the figures, with disproportions of figure motifs (cephalopods); decomposition of the body patterns and with signs of interlacing and association of images (Roubíček and Drvota 1960).

Other disorders encountered in intoxicated persons apart from those mentioned above include maladaptive behavior, sometimes considerable anxiety or depressions, dread of getting mad, irascibility, lack of judgement and failures in social interactions. These conditions can aggravate and the afflicted person may become convinced that the deranged perception and thinking reflects reality. The affected persons sometimes display haughtiness, lowered need of sleep, inappropriate magnitude of emotions, absentmindedness, hyperactivity and garrulousness. The ideas in these cases have not the character of delusions.

In the past, those monitoring mostly medically controlled PSB intoxications stated that in the days following the experiment none of the experimental subjects expressed the wish to repeat the unambiguously pleasant experience or the tendency of being intoxicated again. Most authors therefore assumed that PSB is not potentially dangerous in terms of addiction formation. However, the experimental persons were nearly always psychologists, psychiatrists, biologists or artists, *i.e.* persons with high probability of creative life

style, addiction to work, life orientation mostly to positive values and the ability to achieve satisfaction in a natural way. In many criteria, the experimental subjects were therefore markedly above standard. A physician-monitored PSB intoxication was conducted also in Czechia (Auert *et al.* 1980).

A completely different view of the matter may be held by a person suffering wrongs from others, a person with psychic disorders, incapable of a normal sexual contact, or suffering mentally or physically; such a person could experience the intoxication as a peak of pleasantness and bliss. The problem of possible drive for repeated PSB intoxication in such a person may not be as straightforward as formerly seen (Miovský 1996).

4.2 Bufotenine

BUF is a component of many animal toxins. It is also found in the seeds of the *Cohoba* plant growing on Haiti, which is used by the Otomaco Indians in their rituals for evoking delirant condition. Recently, this hallucinogen was found also in fungi of the genus *Amanita* (Wurst *et al.* 1992). Fabing (1955) used the drug in therapy. Intravenous doses of 4–16 mg cause hallucinations, disturbances of time and space perception, depersonalization and unquiet. High doses of 8–16 mg cause shivers, hallucinations of purple and black color, and again time and spatial control disorders. Somatic symptoms often include mydriasis, vasodilatation in the face and motoric discoordination.

DMT found in the plants *Prestonia amazona* and *Pitadena perigrina* from South America and DET are compounds very closely related to BUF and can be formed also in the animal organism under certain conditions. A prolonged use of these two substances could bring about a possible adverse impact and functional damage to human psychic processes (Miovský 1996).

5   BIOSYNTHESIS

An important group of aromatic metabolites comprises compounds containing indole ring in the molecule. These include growth hormones of plants and some microorganisms (auxins), human and animal neurohormones (serotonin, melatonin) and one group of indole alkaloids (tryptamines).

Biosynthesis of aromatic compounds was thoroughly investigated in different mutants of the bacterium *Escherichia coli* and of the fungus *Neurospora crassa*. In plants and microorganisms, the biosynthesis of aromatics follows two main pathways.

The oligoketide (acetate–malonate, acetate, acetogenic) pathway of aromatic ring formation is basically an enzymic (*via* a specific ligase) condensation of acetate units. Aromatic compounds synthesized in the oligoketide pathway are characteristic products of ascomycetes and deuteromycetes (fungi imperfecti).

An alternative pathway of aromatic production derives from aromatic amino acids (phenylalanine, tyrosine and tryptophan). These amino acids are synthesized from saccharides *via* the chorismate and shikimate pathways. In basidiomycetes, the oligoketide pathway of synthesis of aromatics is relatively rare, metabolites and intermediates of the chorismate and shikimate pathways being more common.

Tryptophan is a potential precursor of tryptamines (PSB, PSC, BUF, DMT, SRT), ergot alkaloids and other substances. A number of indole substances can also arise in the metabolic pathway of the aromatic amino acid tyrosine (*e.g.*, 5,6-dihydroxyindole).

Leung and Paul (1967, 1968) showed that TRP is the precursor in the biosynthesis of the tryptamines PSB and PSC. They belong to a small unique group of natural products containing hydroxy or phosphate groups in position 4 of the indole ring. Laboratory cultivations of saprophytic *Psilocybe* cultures showed that the metabolism of TRP involves the following enzyme reactions: decarboxylation, N^9-methylation, 4-hydroxylation and O-phosphorylation (Brack *et al.* 1961; Agurell and Blomkvist 1966; Agurell and Nilsson 1968*a,b*; Repke *et al.* 1977*a*; Repke and Leslie 1977). The sequence of the reactions has not yet been unambiguously determined.

The biosynthesis of PSB is similar to the biosynthesis of SRT (5-hydroxytryptamine) and gramine (3-dimethylaminomethylindole). Gramine is formed from TRP by dealkylation of 3-aminoethylindole (TPA) and subsequent N^9-methylation. SRT is synthesized from TRP *via* 5-hydroxytryptophan (5HTR), the decarboxylation following hydroxylation (Harborne 1962). According to Agurell and Nilsson (1968*b*), 4-hydroxytryptophan (4HTR) is not a precursor of PSB and PSC.

A number of reports appeared during the eighties on species of the genus *Panaeolus* capable, under certain conditions, of synthesizing both 4- and 5-hydr-

gramine
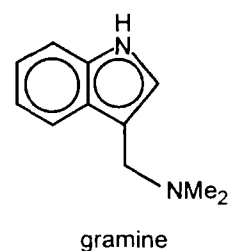
oxy derivatives of N^9,N^9-dimethyltryptamine (DMT). Based on these data, the existence of a common biogenetical precursor, 4–5 epoxide, in which a cleavage of the oxirane ring can lead to the formation of both 4- and 5-hydroxy derivatives, is assumed (Chilton *et al.* 1979). Literature data point to the probable existence of several biosynthetic pathways leading from TRP to PSB and PSC.

Agurell and Blomkvist (1966) and Agurell and Nilsson (1968*a,b*) studied PSB biosynthesis in cultures of *Psilocybe cubensis* by measuring incorporation of radioactively (^{3}H, ^{14}C) labeled potential intermediates of indole derivatives. In a culture of *P. cubensis*, TRP gives readily rise to TPA, which is better utilized for PSB biosynthesis than TRP. Addition of 4-hydroxytryptamine (HTA) to the culture led to the production of a small amount of two other compounds similar to PSB. Leung and Paul (1967, 1968) have identified these demethylated PSB derivatives as BAC and NBC.

According to Stijve (1984) the PSB biosynthesis in *Psilocybe semilanceata* proceeds differently from *P. cubensis* (Fig. 1). This would also explain the fact that *P. cubensis* cultures produce relatively large amounts of PSC but virtually no BAC. Analyses of *P. semilanceata* showed that in this organism N^9-methylation is preceded by O^4-phosphorylation. Many other authors (Repke *et al.* 1977*a*; Repke and Leslie 1977; Stijve *et al.* 1985; Gartz 1985*a*; Stijve and Bonnard 1986; Brenneisen and Borner 1988*a,b*) also assume BAC to be a probable PSB precursor. Some authors explain the very low PSC contents as being an artifact due to either enzymic (Levine 1967; Repke *et al.* 1977*a*; Repke and Leslie 1977) or, more frequently, thermal (Brenneisen and Borner 1988*a,b*) PSB dephosphorylation caused by unsuitable drying or storage of the biological material.
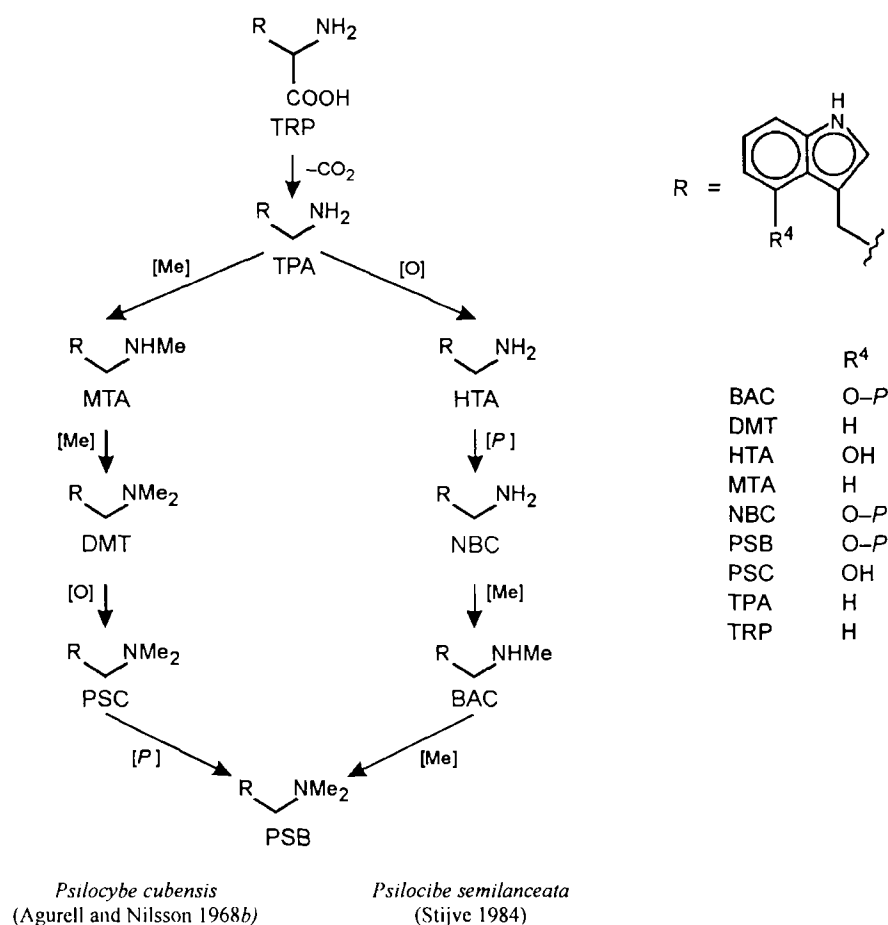
Fig. 1. Biosynthesis of PSB and PSC in *Psilocybe* spp.

A number of authors (Repke and Leslie 1977; Repke *et al.* 1977*a*; Stijve *et al.* 1985; Gartz 1987*c*) ascribe the relatively high BAC content and comparable PSB content in *Psilocybe* species to a probable O^4-phosphorylation preceding N^9-methylation during the biosynthesis. On the other hand, in the fungus *Gymnopilus purpuratus* Gartz (1986b) presumes an inverse sequence.

It should be kept in mind that conclusions about the biosynthetic pathways based solely on the determination of concentrations of intermediates in fungal fruit bodies are questionable and the final judgement should be reserved.

6   LABORATORY CULTIVATION OF SOME *Psilocybe* FUNGI

The first sterile cultures of *Psilocybe* fungi were obtained from basidiospores and from fruit body tissue from collections acquired in Mexico (Heim and Cailleux 1957; Singer 1958). At present, the laboratory collections worldwide house several tens of hallucinogenic fungi of genera *Psilocybe* and *Panaeolus*. Some species fructify readily even under the conditions of laboratory culture (*Psilocybe cubensis*, *Panaeolus sphinctrinus*; Singer 1958; Ott and Bigwood 1977).

The fruit bodies of basidiomycetes containing psychoactive tryptamines were obtained by cultivation on cereals, straw, horse manure, compost, sweet wort agar and some other substrates (Heim and Wasson 1958; Singer 1958; Ott and Bigwood 1977). Fructification requires high air humidity of about 95 % (Heim *et al.* 1958), good aeration (Heim and Wasson 1958) and light, especially from the short wavelength region of the visible spectrum (Heim *et al.* 1958; Badham 1980).

The onset of fructification of different fungal cultures is highly variable – from 3 to 8 weeks in *Psilocybe cubensis* to 12 weeks in *P. bohemica* and *P. semilanceata* (Repke *et al.* 1977a; Gartz 1987a; Gartz and Mueller 1989). A number of authors have proved that fruit bodies in culture retain their ability to biosynthesize PSB, PSC and BAC (Heim and Wasson 1958; Catalfomo and Tyler 1964; Leung *et al.* 1965; Agurell and Blomkvist 1966; Scurti *et al.* 1972; Repke *et al.* 1977b; Chilton *et al.* 1979; Beug and Bigwood 1982; Gartz 1986a; 1987a; Gartz and Mueller 1989). The content of TPA derivatives depends on cultivation conditions and on individual physiological and biochemical properties of production strains (Bekker 1985). Catalfomo and Tyler (1964) found that intensive PSB biosynthesis is associated with active mycelial growth in an acidic nutrient medium. Studies of the effects of carbon and nitrogen sources (Leung and Paul 1969; Scurti *et al.* 1972; Gartz 1986a) and phosphorus (Neal *et al.* 1968) show that the requirements of individual species and genera for production of tryptamines differ widely. The synthetic nutrient medium designed for the cultivation of *Psilocybe cubensis* and *Panaeolus subbalteatus* is unsuitable for PSB production by *P. cyanescens* and *P. pelliculosa* (Catalfomo and Tyler 1964; Scurti *et al.* 1972). The addition of TRP into the culture medium of *P. cubensis* resulted in decreased production of PSB (Catalfomo and Tyler 1964).

The concentrations of PSB in the fruit bodies of *P. cubensis* are in the range of 0.17–0.59 % dry mass, whereas the mycelium was found to contain 0.01–2 % PSB (Catalfomo and Tyler 1964; Agurell and Blomkvist 1966; Neal *et al.* 1968).

Psilocybe pelliculosa and *P. cyanescens* do not produce PSB in laboratory cultivations (Catalfomo and Tyler 1964; Neal *et al.* 1968), in contrast to *P. baeocystis* which produces PSB in concentrations up to 1.87 % dry mass (Leung and Paul 1969) as compared with the maximum of 0.85 % dry mass in fruit bodies collected in nature (Beug and Bigwood 1982).

7   PRODUCTION OF PSB AND PSC BY MYCELIAL CULTURES
OF *Psilocybe bohemica* ŠEBEK

Three strains of *P. bohemica* were used to study growth and tryptamine production in mycelial cultures (Wurst *et al.* 1992).

According to Agurell and Nilsson (1968b) the precursor of tryptamines is TRP. *P. bohemica* showed no significant differences in intracellular PSB levels in the range of TRP concentrations of 0–4 mmol/L in nutrient and complex nutrient medium Sekyko A and B (Kysilka 1989).

Cultivation of three strains of *P. bohemica* and one strain of *P. cyanescens* showed that the ability to produce PSB varies widely (Table IV). *P. cyanescens* exhibits rich mycelial growth (nearly 10 g/L) and the PSB content is a mere 0.01 % dry mass. The highest PSB production, nearly 1.0 % dry mass, was found in *P. bohemica* strain III.

Table IV. Growth and PSB production of different *Psilocybe* strains[a]

Strain	Mycelium g/L	PSB %
P. bohemica ŠEBEK (1967)	5.5	0.02
P. bohemica II (1971)	6.2	0.11
P. bohemica III (1982)	7.6	0.93
P. cyanescens WAKEFIELD	9.5	0.01

[a]Submerged cultivation (25 d) on medium Sekyko A enriched with 1 mmol/L TRP.

Submerged culture of *P. bohemica* strain III ceases growing after reaching dry mass of about 6 g/L, *i.e.* between 13 15 d of cultivation. Static culture of this strain exhibits mild growth throughout the cultivation period and the maximum dry mass does not exceed 1.9 g/L (Fig. 2). Substances of probably polysaccharide character accumulate in the medium and the culture broth becomes poorly filtratable when the length
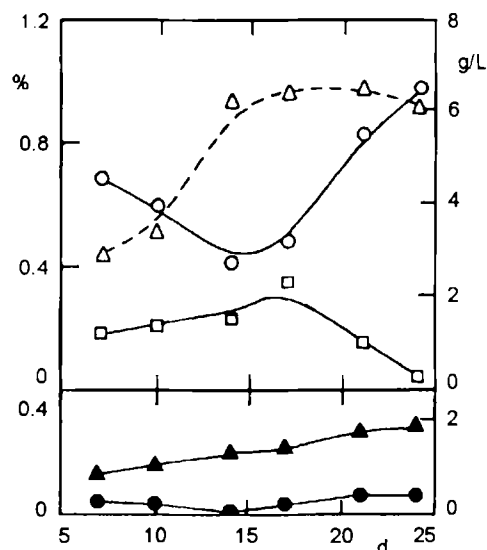
of both static and submerged cultivations exceeds 28–30 d. This results in a markedly decreased amount of isolated PSB in the late period of cultivation. The concentration of PSB under static cultivation conditions remains more or less constant, in contrast to submerged culture, where the PSB concentration decreases in the exponential phase of mycelial growth and intensive biosynthesis starts again after the cessation of culture growth.

The concentration of PSC in a mycelium grown under static conditions was below the detection limit of the analytical method used. A submerged culture of *P. bohemica* strain III has a maximum PSC content of 0.3 % dry mass after 16–17 d of cultivation (Fig. 2) and its content markedly drops with increasing amount of PSB, which may be ascribed to PSC being a precursor of PSB.

An important finding in terms of the biotechnological production of PSB and PSC is that the cultivation should be discontinued after 16-17 d for isolation of PSC, and after 25–26 d for isolation of PSB. The theoretical yield of PSB from mycelium grown in 1 L nutrient medium could exceed 50 mg, with PSC it would be almost 20 mg (Bekker *et al.* 1991; Semerdžieva and Kysilka 1992).

Fig. 2. Growth (*triangles*, g/L dry mass), PSB (*circles*) and PSC (*squares*) content (%, *W/W*) in submerged (*above*) and static (*below*) culture of *P. bohemica* strain III (no PSC was produced in static cultivation).

8   ANALYSIS AND ISOLATION

The considerable interest in the analysis of tryptamines was initiated by the isolation of PSB and PSC from fungi of the genus *Psilocybe* (Hofmann *et al.* 1958b, 1959). Analysis of psychoactive tryptamines in microbiological materials has always included a combination of separation methods with gravimetric, spectral or electrochemical methods, the combination being necessitated by the complex composition of the biological samples.

8.1   Extraction

During the more than 4 decades from the discovery of PSB and PSC, several tens of studies were published concerning their separation and determination, mostly in fruit bodies of macromycetes.

The key step in any analytical procedure is the quantitative extraction of the active compound from the sample. Hofmann *et al.* (1959) found that PSB and PSC are readily soluble in methanol, in aqueous ethanol, and virtually insoluble in chloroform and petroleum ether. In their subsequent studies they used solely methanol and this solvent was used with some modifications also in other studies (Table V).

Beug and Bigwood (1981) verified the yields of methanol extraction by using the fruit bodies of *Panaeolus foenisecii* and *Psilocybe baeocystis* enriched with PSB and PSC. The recovery was 90 % for PSB and 60 % for PSC from *P. foenisecii*, and about 90 % for both compounds from *P. baeocystis*. Christiansen *et al.* (1981b) used methanol with 10 % of 1 mol/L ammonium nitrate solution for PSB extraction. In a single-step extraction the PSB yield was a mere 91 %, in repeated extraction the minimum yield was 98 %. According to Vanhaelen-Fastre and Vanhaelen (1984) a 2-h extraction in a micropercolator with 50 % ethanol and 50 mmol/L 1-heptanesulfonic acid at pH 3.5 is the procedure of choice. Large differences exist between the results of individual authors (Table V).

We found that the composition of the extraction medium has a crucial effect on the yield of tryptamine extraction (Kysilka and Wurst 1990) and a new procedure was developed for the extraction of these compounds. This procedure was later verified and confirmed in an independent study (Stijve and Meijer 1993; Table VI).

Table V. Methods of extraction of PSB and PSC from samples of the genus *Psilocybe*

Species	PSB[a]	PSC[a]	Extraction[b]	Time, h	References
P. semilanceata (FR.) KUMM. *P. bohemica* ŠEBEK	0.2 0.1	traces	methanol; 1 : 1	16	Semerdžieva and Nerud 1973
P. subaeruginosa CLELAND	0.01–0.02	traces	methanol; 3 : 100	2[c]	Perekal *et al.* 1980
P. baeocystis SING. et SMITH	0.15–0.85	0 0.59	methanol; 7 : 250	12	Beug and Bigwood 1981
P. semilanceata (FR.) KUMM.	0.20–2.00	0.002	methanol–1 mol/L ammonium nitrate (9 : 1); 2–3 : 300	3 × 0.5	Christiansen *et al.* 1981
P. semilanceata (FR.) KUMM. *P. bohemica* ŠEBEK	0.33–1.05 0.25–1.14	0.04–0.12 0.02–0.07	methanol; 1 : 10	16	Wurst *et al.* 1984
P. semilanceata (FR.) KUMM.	1.17–1.19	traces	ethanol–water (1 : 1) with 50 mmol/L 1-heptanesulfonic acid; 3 : 20	3 × 2	Vanhaelen-Fastre and Fanhaelen 1984
P. bohemica ŠEBEK	0.58	0.06	methanol; 3 : 100	16	Kysilka *et al.* 1985
P. semilanceata (FR.) KUMM. *P. cyanescens* WAKEFIELD *P. bohemica* ŠEBEK *P. liniformans* GUZMÁN et BAS	0.05–1.70 0.20–0.85 0.28–0.80 0.16	0–0.02 0.04–0.36 0–0.02 0	methanol; 1 : 10	12	Stijve and Kuyper 1985

[a] %, *W/W*.   [b] Extraction solvent (or solvent mixture); the relation (g/l.) of dry mass of extracted fungus to volume of extraction solvent used.   [c] In min (steel blender).

Table VI. Comparison[a] of classical (*first lines*) and optimized (*second lines*) extraction in the analysis of various fungi[b]

| Species	PSB	PSC	BAC	SRT	5HTR
Psilocybe cubensis (EARLE) SING.[c], from a Mexican strain	0.05 0.50	0.12 0.15	0 0	– –	– –
Psilocybe cubensis (EARLE) SING.[c], from an Amazonian strain	0.10 0.33	0.12 0.15	0 0	– –	– –
P. thailandensis GUZMÁN et ALLEN, from Samui, Thailand	0.10 0.60	0.055 0.075	0 0	– –	– –
P. semilanceata (FR.) QUÉL. var. *semilanceata*, from Ondallaz (VD), Switzerland	0 0	0.39 0.47	0.088 0.14	– –	– –
Inocybe haemacta B. et BR., from La Tour-de-Peilz, Switzerland	0 0	0.02 0.042	0.003 0.008	– –	– –
Inocybe corydalina QUÉL., from, Jura Neuchatelois, Switzerland	0 0	0.023 0.03	0.025 0.06	– –	– –
Panaeolus foenisecii (PERS.:FR)SCHROET. APOD. COHN, from Vevey, Switzerland	0 0	0 0	0 0	0.22 0.50	0.33 0.45
Panaeolus cyanescens (B. et BR.) SACC. (= *Copelandia cyanescens* (B. et BR.) SING.), from Oahu, Hawaii	0.10 0.33	0.05 0.09	0 0	0.02 0.06	0 0

[a] Percentage of dry matter.   [b] According to Stijve and Meijer (1993).   [c] Cultivated in the laboratory.

An important finding was that the highest yield of PSB was obtained by the extraction of fungi with 75 % methanol saturated with potassium nitrate; by comparison, methanol gives only 80 % of the maximum value, and aqueous ethanol giving only 36 % of this value. The highest yield of PSC was obtained with 75 % aqueous ethanol whereas methanol gave less than 10 % of this amount (Kysilka and Wurst 1990; Fig. 3), which means that optimum conditions for the extraction of PSB and PSC are different. The critique of this new extraction method raised by Gartz (1994) is questionable.

8.2   Spectral methods

The methods used for identification and determination of tryptamine derivatives included visible (VIS), ultraviolet (UV), infrared (IR), mass (MS) and nuclear magnetic resonance (NMR) spectroscopy.

Agurell and Blomkvist (1966) and Gartz (1986b) used colorimetric determination of tryptamines after a TLC separation. They measured the intensity of color of a reaction product of these tryptamines with 4-dimethylaminobenzaldehyde.
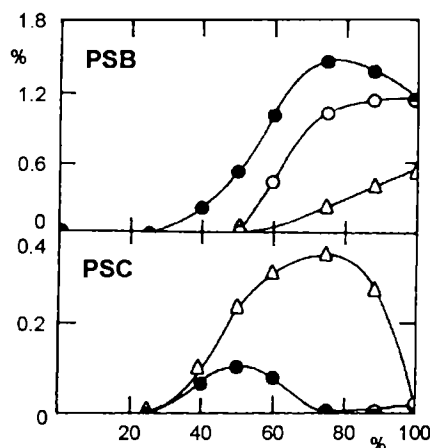
Fig. 3. Dependence of the determined amount (%, *W/W*) of PSB and PSC on the composition of the extraction solvent (*abscissa*, % of alcohol, *W/W*); *closed circles* – methanol–water saturated with KNO₃, *open circles* – methanol–water, *triangles* – ethanol–water.

UV spectrometry was used to verify the identity of PSB and PSC in synthetic and natural materials (Hofmann *et al.* 1958b; Christiansen and Rasmussen 1982; Wurst *et al.* 1984). PSB exhibits characteristic absorption maxima at 220, 267. and 290 nmand PSC at 222, 260, 267, 283, and 293 nm (Budavari 1996). Spectroscopy is most important as a detection technique in HPLC of tryptamines. IR spectrometry was used only for elucidating the structure of these compounds (Hofmann *et al.* 1958a,b); in the analysis of samples from natural materials it is considerably less important than other analytical methods.

Mass spectrometry was employed for verifying the structure of compounds isolated from hallucinogenic fungi (Repke *et al.* 1977b; Koike *et al.* 1981; Christiansen and Rasmussen 1982; Wurst *et al.* 1984). The mass spectrum of PSB is characterized by the ion at m/z 58 (C_3H_8N) which is formed by the cleavage of the side chain in the β-position relative to the nitrogen, and by the ion $[M-H_2PO_3]^+$ at m/z 204 ($C_{12}H_{16}N_2O$). The mass spectrum of PSC is practically the same. Spectroscopic characteristics of PSB and PSC are surveyed in Table VII.

Table VII. Some spectral characteristics of PSB and PSC

Compound	UV[a]		IR[b] $\max$ v, cm⁻¹	FL[c], nm		MS[d]	
	λ_{max}, nm	logε		λ_{ex}	λ_{em}	m/z	%
PSB	221	4.27	2350	267	335	204	21
	268	3.84				160	3
	280	3.74				159	4
	290	3.64				146	5
						130	3
						117	4
						58	100
PSC	222	4.63	2300–2400	260	312	204	21
	260	3.72				160	2
	268	3.77				159	5
	285	3.67				146	8
	294	3.64				130	4
						117	4
						58	100

[a]Heim and Wasson (1958), Fiusclo and Scurti (1972), Koike *et al.* (1981).
[b]Hofmann *et al.* (1958b).
[c]Fluorescence; Perekal *et al.* (1980).
[d]Wurst *et al.* (1984).

8.3   Chromatographic methods

Gas chromatography has also been widely used as a separation method for assays of tryptamine mixtures. However, its application was not free of problems. The separation of these compounds in native state on commonly used stationary phases has often been complicated by peak tailing (Fales and Pisano

1962) which lowered the resolution and often prevented the separation of some tryptamines (Agurell *et al.* 1969). Derivatization of tryptamines with pentafluoropropionanhydride (Blessington and Fiabe 1973) yields several reaction products. Acetate (Brooks and Horning 1964), enamine (Horning *et al.* 1964) and isothiocyanate (Narasimhachari *et al.* 1971) derivatives give simple symmetrical peaks. Later studies (Cattabeni *et al.* 1972) made use of halogenated derivatives, which permitted the detection of very low tryptamine concentrations by ECD. PSB and PSC were determined also as free bases or as trimethylsilyl derivatives (Holmstedt *et al.* 1964; Lerner and Katsiaficas 1969; Wurst *et al.* 1992; Fig. 4). A combination of GC and MS was used for analyzing bis(trimethylsilyl) derivatives of BUF (Narasimhachari *et al.* 1971), PSB and PSC (Repke *et al.* 1977b). The disadvantage of GC is the necessity of derivatization, which poses additional demands on the time of analysis and causes a lower accuracy of the results.
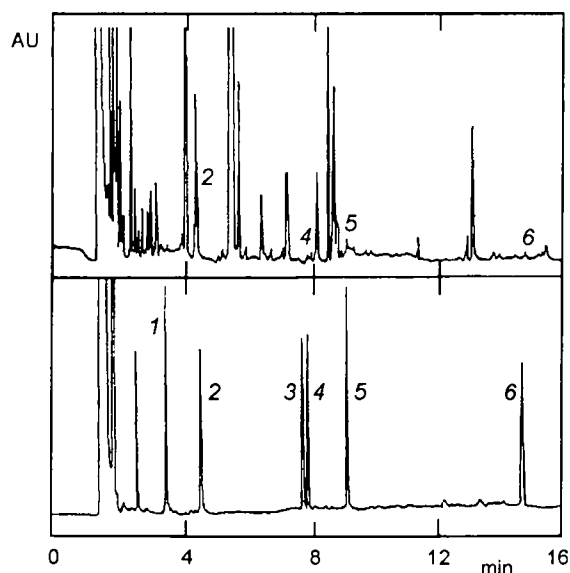

Fig. 4. GC analysis of the extract from fruit bodies of the fungus *Amanita citrina* (SCHAEFF.)GRAY (*above*) and a mixture of the hallucinogen standards (*below*) (as t-butyldimethylsilyl derivatives; Wurst *et al.* 1992); *1* – TPA, *2* – BUF, *3* – TRP, *4* – SRT, *5* – 5-hydroxy-TPA, *6* – 5HTR; capillary column SPB-1 fused silica (30 m × 0.25 mm); temperature program linear from 250 to 300 °C at 3 K/min; carrier gas helium at 42 cm/s; AU – detector response (arbitrary).

Thin layer chromatography on silica gel is very often used for screening of tryptamines and for their semiquantitative determination (Semerdžieva and Nerud 1973; Repke *et al.* 1977a; White 1979; Koike *et al.* 1981; Saupe 1981; Gartz 1986b).

PSB in an alkaline mobile phase (ammonia–propanol) is characterized by a low mobility (R_F = 0.10–0.15), which is slightly higher (R_F = 0.15–0.25) in an acid mobile phase (butanol–acetic acid). In the same solvent systems, PSC has respective R_F values of 0.76 and 0.46–0.55 (Leung *et al.* 1965; Hatfield *et al.* 1978; Beug and Bigwood 1981). The detection in TLC is based on a color reaction with 4-dimethylaminobenzaldehyde (Ehrlich or van Urk reagent), which has a high sensitivity of ca. 0.1 µg (Hatfield *et al.* 1978; Weeks *et al.* 1979; Beug and Bigwood 1981). This reaction was used for quantitative analysis of the tryptamines after a TLC separation (Agurell and Blomkvist 1966; Gartz 1986b; Gurevich 1993). MS and NMR (Koike *et al.* 1981) were used to identify the tryptamines separated by TLC. The HPTLC method has recently been in use due to its high sensitivity of detection.

Liquid chromatography. Separation of tryptamines on a preparative scale has been performed by using low-pressure liquid chromatography on cellulose (Heim and Hofmann 1958; Picker and Richards 1970) on ion exchangers (Agurell and Blomkvist 1966; Hatfield *et al.* 1978; Koike *et al.* 1981) and on silica gel (Weeks *et al.* 1979). In view of its low efficiency and high time demands of separation, this chromatographic technique has no practical importance in analytical separation of tryptamines.

High-performance liquid chromatography is the current method of choice for separation and identification of tryptamines. Important studies concerning the analysis and isolation of tryptamines by HPLC over the last two decades are surveyed in Table VIII.

A number of systems have been designed for the HPLC separation of tryptamines, with silica gel as stationary phase (White 1979; Christiansen *et al.* 1981b; Christiansen and Rasmussen 1982; Sitaram *et al.* 1983; Christiansen and Rasmussen 1983; Sottolano and Lurie 1983). Today, chemically untreated silica gel is mostly replaced by reversed phase (Balandrin *et al.* 1978; Sitaram *et al.* 1983; Wurst *et al.* 1984; Kysilka *et al.* 1985; Kysilka and Wurst 1988–1990; Wurst *et al.* 1992) or by ion pair reversed phase chromatography (Beug and Bigwood 1981; Vanhaelen-Fastre and Vanhaelen 1984; Borner and Brenneisen 1987). Separation of tryptamines has also been done on a strongly acid cation exchanger (Perekal *et al.* 1980).

The mobile phases for RP HPLC include mixtures of different organic modifiers with water and acid or with buffers, sometimes with additions of an ion-pairing reagent. Tryptamine separations have mostly been performed isocratically, gradient elution being employed only exceptionally (Borner and Brenneisen 1987).

The literature concerned with optimization of HPLC mobile phase composition is very extensive; separation of indole compounds has been the subject of a number of studies (cf., *e.g.*, Parker *et al.* 1985; Gertz and Fellmann 1986; Foleye 1987; Kysilka and Wurst 1988).

The eluate monitoring of during HPLC analysis has mostly been done using spectrometric detection in the UV region at 254 or 267 nm either alone (Balandrin *et al.* 1978; White 1979; Beug and Bigwood 1981; Christiansen and Rasmussen 1982; Vanhaelen-Fastre and Vanhaelen 1984; Gartz 1989) or in combination with fluorimetric detection (Perekal *et al.* 1980; Christiansen *et al.* 1981*b*; Sitaram *et al.* 1983; Christiansen and Rasmussen 1983; Sottolano and Lurie 1983; Wurst *et al.* 1984; Semerdžieva *et al.* 1986). A combination of UV and ED was used by Christiansen and Rasmussen (1983), Kysilka *et al.* (1985), Semerdžieva *et al.* (1986), Kysilka and Wurst (1988, 1990) and Wurst *et al.* (1992). The advantages inherent in using ED detection have been described in other, more general studies (Kissinger *et al.* 1981; Edmons 1985; Štulík and Pacáková 1986, 1987; Selavka and Krull 1987).

Combinations of detection techniques based on different physical and chemical principles make it possible to check the "peak purity", *i.e.* to confirm the identity of the eluted compound with a standard substance, and to eliminate the overlapping of elution zones of several substances. This has a considerable importance in quantitative analysis (Christiansen and Rasmussen 1983; Kysilka and Wurst 1989; Fig. 5). PDA detector (Borner and Brenneisen 1987; Kysilka and Wurst 1990; Kysilka 1990; Wurst *et al.* 1992) permits an operative control of peak homogeneity as well as confirmation of the identity of the analyzed substance.
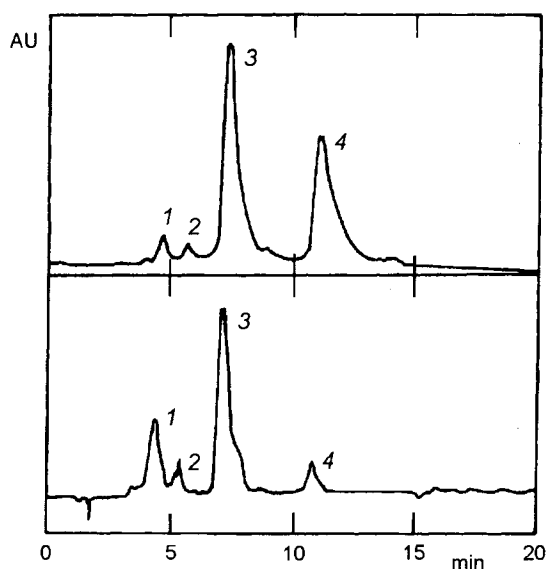
Fig. 5. HPLC analysis of the extract of *Psilocybe bohemica* strain III with UV photometric (*above*) and electrochemical detection (*below*) (Kysilka *et al.* 1985); column Partisil ODS; mobile phase 0.1 mol/L citrate–phosphate buffer (pH 3.8) and various amounts of methanol or ethanol; flow rate 1.0 mL/min; detection UV 268 nm or ED +1.0 V (Ag/AgCl); *1* – methanol, *2* – BAC, *3* – PSB, *4* – PSC; AU – detector response (arbitrary).

A method allowing absolute identification used in HPLC separation of tryptamines is taken to be MS (White 1979; Christiansen and Rasmussen 1982; Wurst *et al.* 1984, 1992).

More than twenty analytical papers concerning separation, identification, determination and isolation of mixtures of psychoactive tryptamines in biological materials, *i.e.* in fungi of the genera *Psilocybe*, *Inocybe*, *Panaeolus* and *Amanita* have been published over the last two decades (Table VIII). Some studies are devoted to the analysis of tryptamine mixtures in biological fluids (urine). The method used as a rule in these studies for analyzing a mixture of tryptamines is HPLC with isocratic elution, rarely gradient elution. Some papers concern semipreparative isolation of PSB and PSC. Almost all HPLC separations of tryptamine mixtures have been performed on chromatographic columns with reversed stationary phase, ion exchanger being used as the stationary phase only in isolated cases. The main elution solvents were mixtures of methanol or ethanol, water and aqueous solutions of ammonium nitrates, carbonates, phosphates, acetates and citrates at pH ranging from 2 to 10. Acetic acid is also often used, whereas acetonitrile is used rarely, and 1-heptanesulfonic acid or tetrasodium ethylenediaminetetraacetate have been employed in isolated cases.

In addition to the generally used UV detector, some authors employed also electrochemical and/or fluorimetric detectors for increasing detection sensitivity and for tryptamine identification.

Table VIII. HPLC of tryptamines

Stationary phase, column (length × inner diameter, mm)	Mobile phase, elution type	Flow rate mL/min	Detector[a]	References Comments
Partisil 5 (250 × 4.6)	methanol–water–1 mol/L ammonium nitrate (24 : 5 : 1), pH 9.7 isocratic	2.0	UV 254	White 1979; *Psilocybe semilanceata*; PSB, PSC, BAC, MS, TLC
Partisil SCX-10 (260 × 4.6) pellicular beads pre-column (30 × 2.8)	methanol–water (2 : 3) with 0.2 % ammonium phosphate and 1 % potassium chloride, pH 4.5; isocratic	1.0	UV 267 fluorimetric: 267 ex., 335 em. 260 ex., 312 em.	Perekal *et al.* 1980; *P. subaeruginosa*; PSB, PSC, DMT
µBondapak C18 (300 × 4)	1,4-dioxane–0.1 mol/L ammonium carbonate (4 : 5) isocratic	1.0	UV 280	Balandrin *et al.* 1978; plant material *Accacia podalyriaefolia*, urine; TPA, MTA, DMT, BUF, MS, TLC
µBondapak C18	methanol–water (1 : 3) with 50 mmol/L 1-heptanesulfonic acid, pH 3.5; PIC reagent B-7; isocratic	2.0	UV 254	Beug and Bigwood 1981; *P. baeocystis*; PSB, PSC; TLC
Partisil 5 (250 × 4.6)	methanol–water–1 mol/L ammonium nitrate (22 : 7 : 1), pH 9.6 2 mol/L ammonium hydroxide; isocratic	1.0	UV 254 fluorimetric: 267 ex., 335 em.	Christiansen *et al.* 1981a,b; *P. semilanceata*; PSB
Partisil 5 (250 × 4.6)	methanol–water–1 mol/L ammonium salt (22 : 7 : 1 – for analytical purposes, 24 : 5 : 1 – for preparative purposes); pH 9.6 2 mol/L ammonium hydroxide; isocratic	1.0	UV 254	Christiansen and Rasmussen 1982; *P. semilanceata*; PSB, BAC; TLC, MS
Partisil 5 (250 × 4.6)	methanol–water–1 mol/L ammonium nitrate (22 : 7 : 1), pH 9.6 2 mol/L ammonium hydroxide with 1 mmol/L Na2EDTA; isocratic	1.0	UV 254 fluorimetric: 267 ex., 320 em. ED +0.65 V (Ag/AgCl)	Christiansen and Rasmussen 1983; *P. semilanceata*; PSB, PSC, BAC
Partisil 10 PAC (250 × 4.6)	acetonitrile–water–phosphoric acid (10 : 189 : 1) pH 5.5 2 mol/L sodium hydroxide	2.0	UV 254, 267	Sottolano and Lurie 1983; PSB
Zorbax (250 × 4.6)	methanol–0.4 % ammonium hydroxide–1 mmol/L cysteine hydrochloride	1.5	UV 219–225 fluorimetric: 265–279 ex. 332–350 em.	Sitaram *et al.* 1983; psychotomimetic indolealkylamines and analogous tetrahydro-β-carbolines from body fluids (urine, plasma)
Partisil 10 SCX (250 × 4.6)	methanol–(53 mmol/L phosphoric acid–ammonia buffer); pH 4.0 (3 : 7)	1.5		
Zorbax ODS (250 × 4.6)	acetonitrile–(53 mmol/L phosphoric acid–ammonia buffer); pH 7.0 (7 : 3) isocratic	2.0		
LiChrosorb RP18 (250 × 2), (500 × 8)	ethanol–water–acetic acid (20 : 79.2 : 0.8); isocratic	0.33	UV 267 fluorimetric: 280 ex., 360 em.	Wurst *et al.* 1984; *P. semilanceata, P. bohemica*; PSB, PSC; TLC, MS
LiChrosorb RP 18 (250 × 2)	ethanol in 0.1 mol/L aqueous citrate–phosphate buffer, pH 3.8 (1 : 9); isocratic	1.0	UV 267	Gartz 1989a; *Panaeolus subballeatus*; PSB, PSC, BAC, SHTR, SRT, TPA, TRP, TLC

Column	Mobile phase	Flow	Detection	Reference
μBondapak alkylphenyl (300 × 4.6)	(a) ethanol–water (7 : 13) with 50 mmol/L 1-heptanesulfonic acid, pH 3.5; PIC reagent B-7 (b) water with 50 mmol/L 1-heptanesulfonic and 0.05 mol/L acetic acid; pH 3.5; PIC reagent B-7; isocratic	1.0	UV 254, 267, 290	Vanhaelen-Fastre and Fanhaelen 1984; *P. semilanceata*; PSB, PSC, BAC; preparative TLC
Partisil ODS (250 × 4.6) Separon SIC18 (250 × 4)	10 % ethanol or methanol in 0.1 mol/L aqueous citrate–phosphate buffer, pH 3.8; isocratic	1.0	UV 267 ED +1.0 V (Ag/AgCl)	Kysilka et al. 1985; *P. bohemica*, cerebrospinal fluid; TRP, SRT, BUF, MTA, TPA, DMT, PSB, PSC, N-acetyl-SRT, 5-methoxy-TPA, 5-methoxy-DMT
LiChrosorb RP-18 (250 × 2)	ethanol–water–acetic acid (20 : 79.2 : 0.8)	0.33	UV 267 fluorimetric: 280 ex. – 360 em.	Semerdzieva et al. 1986; *P. bohemica*, *P. semilanceata*, *Inocybe aeruginascens*; PSB, PSC; TLC, MS
	ethanol and 0.1 mol/L aqueous citrate–phosphate buffer, pH 3.8 (9 : 1); isocratic	1.0	ED +1.0 V (Ag/AgCl)	
Spherisorb ODS-1 (250 × 4.6)	(a) water–0.3 mol/L ammonium acetate buffer– ammonium hydroxide, pH 8 (b) methanol–0.3 mol/L ammonium acetate; gradient: 0–2 min – 0 % *b* in *a* (isocratic), 2–14 min – 0–95 % *b* in *a* (linear)	2.0	PDA 269max.	Borner and Brenneisen 1987; *P. semilanceata*, *P. cubensis*, *Amanita citrina*, *Panaeolina foenisecii*; PSB, PSC, BAC, BUF, TPA, DMT, SRT, HTA
Separon SGX C18 (250 × 4)	varying ethanol contents (10–30 %) in 0.1 mol/L aqueous citrate–phosphate buffer, pH 3.8; isocratic	1.0	UV 267 ED +1.0 V (Ag/AgCl)	Kysilka and Wurst 1988; PSB, PSC, TPA, SRT, BUF, MTA, MST, N-acetyl-SRT, 5-methoxy-TPA
Separon SGX C18 (250 × 4)	ethanol in 0.1 mol/L aqueous citrate–phosphate buffer, pH 3.1 (1 : 9); isocratic	1.0	UV 267 ED +0.6 V and 0.8 V (Ag/AgCl)	Kysilka and Wurst 1989; *P. bohemica*; PSB, PSC, SRT, TRP, BUF, TPA
Silasorb SPH C18 (250 × 4)	methanol–water–acetic acid (10 : 90 : 1) for PSB, (35 : 65 : 1) for PSC; isocratic	1.0	PDA 269max ED +0.6 V (Ag/AgCl)	Kysilka and Wurst 1990; *P. bohemica*; PSB, PSC
Silasorb SPH C18 (250 × 4)	methanol–water–acetic acid (5 : 95 : 1) for PSB, (10 : 90 : 1) for PSC; isocratic	1.0	PDA 269max ED +1.2 V (Ag/AgCl)	Kysilka 1990; *P. bohemica*, *P. cyanescens*, *P. semilanceata*, *Inocybe aeruginascens*; PSB, PSC
μBondapak C18 (300 × 3.9) with guard pack C18 cartridge	10 % of ethanol in 1.37 % aqueous citric acid monohydrate–0.47 % potassium dihydrogenphosphate buffer, pH 2.8; isocratic	1.0	ED +0.65 V Ag/AgCl	Kysilka 1990; PSC; urine
μBondapak C18 (300 × 3.9) with guard pack C18 cartridge	ethanol in 0.1 mol/L aqueous citrate–posphate buffer, pH 2.8 (5 : 95); isocratic	1.0	PDA 269max ED +1.2 V (Ag/AgCl)	Wurst et al. 1992; *P. bohemica*, *P. cyanescens*, *P. semilanceata*, *Inocybe aeruginascens*, *Amanita citrina*, *A. porphyrea*, *A. rubescens*; PSB, PSC, TRP, TPA, BUF, SRT, MST, 5HTR; GC, TLC, MS
Silasorb SPH C18 (250 × 4) Separon SGX C18 (250 × 8)	methanol–water–acetic acid (5 : 95 : 1) methanol–water–acetic acid (10 : 90 : 1); isocratic			
Partisil 5 ODS-3 (110 × 4.7)	acetonitrile–0.1 mol/L ammonium acetate buffer (2 : 3); isocratic	1.0	PDA 254	Lurie et al. 1993; PSB, PSC; on-line post-elution photoirradiation technique, MS

aSpectrometric, fluorimetric – λ, nm; ED – electrochemical; PDA – photo-diode array.

8.4 Isolation and preparation of pure substances

The preparative isolation of PSB and BAC from the fruit bodies and mycelia of fungi of the genus *Psilocybe* has been described in the literature (Hoffmann et al. 1958b; Gartz 1985, 1986b; Brenneisen and Borner 1988a). Since PSC is a minor component, it was isolated only for identification.

The procedures for PSB isolation consist of separation of lipidic material by extraction with non-polar solvents petroleum ether and chloroform. Active compounds are extracted from the fruit bodies with methanol and the extract is further separated by low-pressure liquid chromatography on a column of micro-crystalline cellulose (Hofmann et al. 1958b) or silica gel (Gartz 1985b, 1986b). Pure compounds are obtained by recrystallization from a saturated aqueous or methanolic solution. The yield of PSB isolation from *Psilocybe mexicana* was about 0.4 % dry fruit body mass (Hofmann 1958b) while an alternative procedure in *P. semilanceata* gave 0.2 % (Gartz 1985b, 1986b).

BAC isolation was described by Brenneisen and Borner (1988a). Methanolic extract from fruit bodies was subjected to repeated flash chromatographic separation on silica gel columns. The yield was 0.3 % of dry fruit body mass of *P. semilanceata*.

Pure compounds PSB and PSC were also isolated from the mycelium and fruit bodies of *P. bohemica* (Kysilka et al. 1989; Fig. 6) by reversed phase HPLC. An amount of 7 mg for PSB and 2.5 mg for PSC was isolated from 1 g of dry mycelium.

Fig. 6. *Psilocybe bohemica* ŠEBEK (photo J. Beier).

9   USE AND ABUSE OF PSYCHOACTIVE FUNGI

Popular articles (Oss and Oeric 1976; Stamets 1978; Cooper 1980; Scheibler 1987) have triggered the present abuse of psychoactive fungi containing PSB and PSC in America and Europe. Alarming medical reports (Young et al. 1982; Jansen 1988) and other articles published on the subject did not prevent this misuse (Stijve 1995).

The most abused fungus in Europe, from the Pyrenean peninsula to Scandinavia, is *Psilocybe semilanceata* (Semerdžieva and Nerud 1973; Michaelis 1977; Christiansen et al. 1981b; Flammer and Horak 1983;

Wurst *et al.* 1984; Samorini and Festi 1988; Ohenoja *et al.* 1987; Gartz 1993). In large cities, dry fungi can often be bought for sums equivalent to 2 3 SFR (Turberg 1984; Unsigned 1990) – a negligible sum compared with the price of heroin. Both professional and state authorities in many European countries are well aware of the risk.

Still, the data published by Ott (1978) on the abuse of 21 fungal species seem to be exaggerated in view of the actual extent of popular abuse of PSB and PSC containing fungi in the USA. Widely abused are wildly growing or cultivated *Psilocybe cubensis* (ERLE) SINGER often called "golden tops", *P. stuntzii* GUZ-MÁN et OTT discovered in the seventies and an equally popular *P. cyanescens* WAKEFIELD growing both in the woods and in glasshouses (Stamets and Chilton 1983). The fungus in popular use in the Pacific Northwest of USA 20 years ago was *Panaeolus subbalteatus* (Ott 1978) but its popularity has declined. Stomach troubles and nausea were described in many individual cases as symptoms accompanying the use of this fungus (Bigwood 1984). Merlin and Allen (1993) have described the popular abuse of the species *Copelandia* (*Panaeolus*) *cyanescens* on Hawaii.

Psilocybe cubensis and *Copelandia* sp. are sold to tourists in several regions of Thailand, especially on Koh Samui and Koh Pha-ngan islands (Allen and Merlin 1992). Restaurants on these islands offer hallucinogenic soups, omelets, meat stews and risottos with "magic fungi" (*P. cubensis*). Similar tourist attractions with hallucinogenic fungi, albeit to a smaller extent, are offered by the natives on the Indonesian island Bali (Hollander 1981).

Possession of hallucinogenic fungi in Japan is illegal and even the transport of botanical specimens of these fungi has to be done with care. *Gymnopilus spectabilis* (FR.) SINGER is known as the first hallucinogenic fungus from Japan (Romagnesi 1964). It does not contain PSB and its psychoactive properties are due to a group of neurotoxic oligoisoprenoids (Tanaka *et al.* 1993).

In Australia and New Zealand, the public is well informed about the PSB containing fungi growing in these regions (Shepherd and Hall 1973; Hall 1973). Allen *et al.* (1991) mention the fungal species *Psilocybe cubensis*, *P. subaeruginosa* CLELAND and *Copelandia cyanescens*, which are misused by the populace in Australia.

No "pandemic fungi" are found in South America (Pollock 1975). The interest in hallucinogenic fungi and their psychoactive effects in the local population in Brazil, Uruguay and Argentina is negligible (Guzman 1983; Stijve and Meijer 1993).

In 1994 Gartz *et al.* (1996) also found the first indigenous blueing *Psilocybe* species from South Africa on a field trip in the province Natal (*Psilocybe natallensis* nom.prov.). The concentration of tryptamines (PSB, PSC) in *P. natalensis* was similar to the amount in the subtropical and somewhat similar *P. cubensis* and in *P. samuiensis* from Thailand.

Three main species of the genus *Psilocybe* grow in our latitudes: *P. semilanceata* (FR.) KUMMER, *P. cyanescens* WAKEFIELD, and *P. bohemica* ŠEBEK. These species contain PSB and other tryptamines as psychoactive substances. In former Czechoslovakia, the information on these fungi has long been marginal, the assumption being that they are rather rare.

This previous underrating of the situation is now the more tragic because the recent frequent occurrence of these fungi, together with a more detailed current information on their hallucinogenic properties, resulted in *P. bohemica* ŠEBEK becoming during the autumn 1995 a drug more widely used than many other narcotics. The available information about these fungi of the genus *Psilocybe* is often incorrect, sketchy and fragmentary.

The practice of abuse of these fungal species in the past was rather limited, similar to other psychoactive substances. In view of its limited occurrence, this drug was for most addicts more of a legend than reality. The relatively large similarity of hallucinogenic fungi with other species containing no toxic substances, and the more glamorous offers of other "tested" hallucinogenic substances limited their use.

Owing to the specific occurrence and type of use of this drug, the circle of "consumers" did not include the classical drug addicts in the current sense of the word but, rather, persons with artistic or philosophical inclinations, with tendencies to eccentricity and to alternative lifestyle and music (Miovský 1996). The present situation, however, is different - the abuse of *Psilocybe* fungi has spread rapidly and successfully to discos and rock clubs. The consumers know hardly anything about the fungus, and may be shocked by its effects.

Psilocybe fungi are processed by drying of fruit bodies, mostly their pileus parts. They are sometimes consumed raw or are extracted into various decoctions or mixed with other substances.

PSB and PSC are not addictive and few individuals use them after their first experience with taking hallucinogenic fungi. PSB is a highly dangerous substance, especially in combination with other drugs.

10 CONCLUSION

The last two decades saw the discovery of many fungal species containing PSB. These include the genera *Psilocybe*, *Panaeolus*, *Inocybe*, *Gymnopilus*, *Pluteus*, *Conocybe* and others. Popular abuse of psychoactive fungi has spread to Europe from the USA, but in this and other parts of the world psychoactive fungi remain a marginal phenomenon. The best-known European species is *P. semilanceata* whereas in the USA the most frequently used species is *P. cubensis*. Locally used species include *P. stuntzii* and *Panaeolus subbalteatus*. The abuse of fungi in South America and Asia is practically unknown although psychoactive fungi are readily available there.

The business with "magic fungi", *e.g.*, *P. cubensis* and *Copelandia cyanescens*, on the Thai island Koh Samui and the Indonesian island Bali serves merely to attract tourists from Europe and America. The young populations, as well as the members of various drug-abusing communities in Australia and New Zealand, are well aware that psychoactive fungi grow in these regions (Stijve 1995).

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