Arq.Biol.Tecnol.36(2):313-329,jun.,1993

MACROMYCETES FROM THE STATE OF PARANÁ, BRAZIL. 4. THE PSYCHOACTIVE SPECIES (*)

T. Stijve[1] & A.A.R. de Meijer[2]

[1]Nestec Ltd, Nestlé Research Centre, P.O. Box 44 - Vers-chez-les-Blanc, 1000 - Lausanne 26, Switzerland. [2]Sociedade de Pesquisa em Vida Selvagem e Educação Ambiental (S.P.V.S.), Caixa Postal 305, 80001-970 - Curitiba, Brazil

Recebido para publicação em 10 de abril de 1.993

ABSTRACT

Several macromycetes from the Brazilian state of Paraná suspected or reported in literature to contain psilocin and other psychoactive compounds were analysed. Appreciable amounts of psilocin and psilocybin were readily determined in *Psilocybe caeruleoannulata*, *Ps. caerulescens*, *Ps. hoogshagenii*, *Ps.* cf. *subyungensis* and *Ps. uruguayensis*. So far, these taxa were suspected to be hallucinogenic, mainly because of their blueing tissues, and this presumption has now been corroborated by chemical data. The presence of the hallucinogens in *Ps. cubensis*, *Ps. zapotecorum* and *Panaeolus subbalteatus* as reported in literature for collections from elsewhere was amply confirmed.

Upon screening 11 *Pluteus* spp, *Pl. glaucus* Sing. and a closely related taxon were found to contain psilocin and psilocybin in concentrations high enough to class them as psychoactive species. Four collections of *Amanita muscaria* contained muscimol (the active principle) at levels comparable to those measured in the European species. Taxonomic notes on the psychoactive *Psilocybe* and *Pluteus* taxa are included.

Keywords: macromycetes, muscimol, Paraná State, psychoactive fungi, tryptophan derivatives.

INTRODUCTION

During a screening programme for secondary metabolites in higher fungi gathered at various sites around Curitiba, in the Paraná State in Brazil, the present

*The previous parts in these series (1. Introduction; 2. Myxomycetes; 3. Stipitate hydnums) have been published in Arq. Biol. Tecnol., 35(4) : 623-629, 631-633, 635-640, 1992.

authors analysed several taxa suspected or reported in literature to contain psilocybin and other psychoactive compounds.

This investigation included mostly representatives of those genera of gill-fungi reported to contain psilocybin. psilocin and/or baeocystin : *Psilocybe* (Fr.) Kumm.(1), *Panaeolus* (Fr.) Quél. (2,3,4), *Conocybe* Fay. (5), *Pluteus* Fr. (6,7), and *Gymnopilus* P. Karst. (8,9). In addition. several collections of *Amanita muscaria* Hooker were analysed for muscimol, the isooxazole compound responsible for its toxic action (10). The results of this investigation are reported in this paper.

MATERIALS AND METHODS

The specimens of interest were gathered during 1990-1992 at different sites in the Brazilian State of Paraná (11), dried and sent to the laboratory of the senior author in Switzerland. Analyses were performed as described previously (12), with the notable improvement that we used now an optimised extraction procedure (13) : 50 mg test portions were extracted overnight at room temperature with 2 ml methanol-water 3:1 v/v saturated with potassium nitrate for psilocybin, and with ethanol-water 3:1 v/v for psilocin. The extracts thus obtained were filtered and subjected to liquid chromatography and thin-layer chromatography analyses (12).

Muscimol was extracted from *Amanita muscaria* by soaking a 100 mg test portion overnight in 5 ml methanol-water 4:1 v/v containing 1 percent of formic acid, with intermittent shaking. Thin-layer chromatography of the crude extracts was performed as described earlier (14), but with a modification of the detection procedure : after having sprayed the developed and dried cellulose pre-coated nanoplate with ninhydrin reagent, muscimol turned up as a bright yellow spot after reacting about 45 min at room temperature. At normal concentrations this yellow spot is easily observed among the bluish violet zones of the co-extractives. It turns characteristically brownish after a few hours, but its contrast can be augmented by overspraying the chromatogram with a 1 percent solution of p-dimethylaminocinnamylaldehyde in a mixture of concentrated hydrochloric acid and methanol 4:1 v/v. Upon this treatment the muscimol spot turns carmine red whereas the violet co-extractives largely disappear. As little as 0,01 - 0,02 mcg can be distinguished.

RESULTS AND DISCUSSION

Prior to use, the optimised extraction procedure as described by Kysilka and Wurst (13) was tested by comparative analyses of a number of dried collections available in our laboratory. The results obtained as listed in table I indicate that the

Neither Brazilian nor European collections of *Ps. coprophila* contained detectable amounts of the said compounds. *Ps. alnetorum*, *Ps. paupera* and *Ps.* cf. *venezuelana* were also negative in this respect.

Analysis of *Ps. cubensis* revealed a similar composition as that observed in specimens from Mexico and the Amazon. Unlike collections from Koh Samui, Thailand, the carpophores did not contain the newly discovered psilocybin analogue of which the chemical nature is presently being investigated (22).

Panaeoloideae yielded no surprises. Once again, *P. foenisecii* was found to be exempt of psilocybin, as reported earlier (12). All *Panaeolus* species contain serotonin and often also its precursor 5-hydroxytryptophan (4). However, these compounds are not psychoactive when taken orally. The hallucinogenic reputation of *P. subbalteatus* (20) was confirmed.

Negative results for psilocin/psilocybin and related tryptophan derivatives were obtained for all *Gymnopilus-*, *Conocybe-* and *Stropharia* species examined. Interestingly, *Rickenella straminea* (cf. *fibula*) was found to contain a significant amount of 5-hydroxytryptophan in absence of serotonin, as was also reported for *R. fibula* collections from Europe (23).

Although we screened 11 *Pluteus* species, we only detected psilocin and psilocybin in *Pl. glaucus* Sing. and in a closely related taxon. This has not been reported earlier and our finding adds two more taxa to the growing list of psychoactive mushrooms. Some of the *Pluteus* species, notably *Pl. xylophilus* that gave negative results for psilocybin, were found to contain some as yet unidentified tryptophan metabolites, which had been earlier observed in *Pl. ephebeus* (7). Finally, four collections of *Amanita muscaria* contained about the same muscimol or ibotenic acid concentrations as measured in specimens from Europe (24).

During screening of other taxa from Europe, North America and Brazil it became apparent that there is still a wide field of investigation as far as tryptophan metabolism of higher fungi is concerned. So far, formation of 4-substituted tryptophan derivatives, i.e. psilocin/psilocybin seems to be widespread since the compounds are encountered in various unrelated genera. To a lesser extent, this also applies to 5-substituted derivatives, such as serotonin and bufotenin (4), but nothing is yet known about tryptophan metabolites which are substituted at other positions in the indole nucleus. Such compounds may well occur in other genera as suggested by the often highly

optimised method is definitely superior to classic methanol extraction. The differences are especially dramatic for psilocin and serotonin, which are really very poorly extracted with pure methanol. For psilocybin, the discrepancies observed are not as great, but still significant.

Obviously, a certain percentage of water is necessary to obtain a good extraction yield. Optimal yields for psilocybin and baeocystin are obtained with 75 % methanol, saturated with potassium nitrate, where as 75 % ethanol is the solvent of choice for psilocin. Serotonin and 5-OH-tryptophan are about equally well extracted by both solvents, provided the extraction time is longer than 10 hours.

Clearly, these observations cast some doubt on literature data for psilocin/psilocybin and related compounds. Hofmann et al. (15) recommended the use of methanol for extraction, and since then, many authors (3,7,12,16,17,18,19), have used this solvent with little or no modification. It may, therefore, be assumed that the majority of reported concentrations for psilocin, serotonin, and to a lesser extent also for psilocybin, are too low.

The results obtained for the Brazilian species analysed by the optimised procedure are listed in table II. Not surprisingly, the *Psilocybe* taxa belonging to the section *Stuntzae* Guzmán contain enough psilocin/psilocybin to class them as hallucinogenic fungi. *Ps. uruguayensis*, a slightly blueing species reported from Uruguay by Guzmán (1), is taxonomically very close to *Ps. stuntzii*, a species of which the recreational use is widespread in the Pacific Northwest of the USA (20). Another related blueing species is *Ps. caeruleoannulata*, which was already known from Southern Brazil and Uruguay (1). So far, no chemical analysis was reported, but its suspected hallucinogenic properties are now amply confirmed.

Ps. zapotecorum has long been known as a hallucinogenic species. It was discovered in Mexico by Heim & Hofmann who isolated psilocybin from it as early as 1958 (21).

Ps. caerulescens and *Ps. hoogshagenii*, both belonging to section *Cordisporae* Guzmán, were already a long time known as hallucinogenic species. The distributional range of the first was known as south-eastern U.S.A. to northern South America (Venezuela) and our records from southern Brazil extend this range considerably. The second was known from Mexico and Argentina, and thus its' occurrence in southern Brazil was to be expected.

coloured spots observed during spraying of thin-layer chromatograms with pDMCA reagent.

Such spots were readily detected in extracts of some *Psathyrella* species, notably in that of *Ps. spadicea* (Schaeff. ex Fr.) Sing. in which the amount of the metabolite present must be as high as 1-2 percent on dry weight. Similarly, *Leucoagaricus* extracts are characterised by the presence of two intense blue spots that probably represent 6- or 7-substituted tryptophan metabolites. This possibility is under investigation in the senior author's laboratory. Apparently, these metabolites are neither hallucinogenic nor acutely toxic, since they were also encountered in an edible species, *Leucoagaricus pudicus* (Bull.) ss. str. That these as yet unidentified compounds should be tryptophan derivatives is strongly suggested by the virtually total absence of the free amino acid in the said fungi, and by their chromatographic behaviour.

On the other hand, there are fungi in which tryptophan is rather a final metabolite than a precursor e.g. representatives of *Agaricus* and *Micropsalliota* have a free tryptophan content that is significantly higher than that of other melanosporea (25).

In this paper are included all the psychoactive macrofungi encountered in Paraná State. Other species of *Psilocybe* and *Panaeolus* encountered in Paraná and not treated in this paper are presumably non-psychoactive. The non-treated species are : *Panaeolus ater* and the following species of *Psilocybe* : *P.* cf. *chilensis* Sing., *P.* cf. *crobula* (Fr.) M. Lange : Sing., *P. dunicola* (Speg.) Sing., *P.* cf. *phyllogena* (Peck) Peck, *P. pseudobullacea* (Petch) Pegler, *P.* cf. *xeroderma* Huysman, *P.* cf. *valdiviensis* Sing., *P.* sp. a (aff. *andina* Guzmán) and *P.* sp. b (sect. *Chrysocystidiatae*).

ACKNOWLEDGEMENTS

The junior author would like to express his sincere gratitude towards his colleagues in S.P.V.S. and A.D.E.A., particularly towards Drs. Clovis R.S. Borges and Dr. João J. Bigarella. He also wishes to thank Dr. G. Guzmán, for having identified his earlier (period 1979-80) collections of *Psilocybe*. Last but not least, the authors are indebted to Karima Ziaoullah for the preparation of the manuscript.

coloured spots observed during spraying of thin-layer chromatograms with pDMCA reagent.

Such spots were readily detected in extracts of some *Psathyrella* species, notably in that of *Ps. spadicea* (Schaeff. ex Fr.) Sing. in which the amount of the metabolite present must be as high as 1-2 percent on dry weight. Similarly, *Leucoagaricus* extracts are characterised by the presence of two intense blue spots that probably represent 6- or 7-substituted tryptophan metabolites. This possibility is under investigation in the senior author's laboratory. Apparently, these metabolites are neither hallucinogenic nor acutely toxic, since they were also encountered in an edible species, *Leucoagaricus pudicus* (Bull.) ss. str. That these as yet unidentified compounds should be tryptophan derivatives is strongly suggested by the virtually total absence of the free amino acid in the said fungi, and by their chromatographic behaviour.

On the other hand, there are fungi in which tryptophan is rather a final metabolite than a precursor e.g. representatives of *Agaricus* and *Micropsalliota* have a free tryptophan content that is significantly higher than that of other melanosporea (25).

In this paper are included all the psychoactive macrofungi encountered in Paraná State. Other species of *Psilocybe* and *Panaeolus* encountered in Paraná and not treated in this paper are presumably non-psychcactive. The non-treated species are : *Panaeolus ater* and the following species of *Psilocybe* : *P.* cf. *chilensis* Sing., *P.* cf. *crobula* (Fr.) M. Lange : Sing., *P. dunicola* (Speg.) Sing., *P.* cf. *phyllogena* (Peck) Peck, *P. pseudobullacea* (Petch) Pegler, *P.* cf. *xeroderma* Huysman, *P.* cf. *valdiviensis* Sing., *P.* sp. a (aff. *andina* Guzmán) and *P.* sp. b (sect. *Chrysocystidiatae*).

ACKNOWLEDGEMENTS

The junior author would like to express his sincere gratitude towards his colleagues in S.P.V.S. and A.D.E.A., particularly towards Drs. Clovis R.S. Borges and Dr. João J. Bigarella. He also wishes to thank Dr. G. Guzmán, for having identified his earlier (period 1979-80) collections of *Psilocybe*. Last but not least, the authors are indebted to Karima Ziaoullah for the preparation of the manuscript.

Table I : Comparison of results obtained by classic (a) and optimised (b) extraction procedures in the analysis of various fungi

Species		Psilocin	Psilocybin	Baeocystin	Serotonin	5-OH-tryptophan
Psilocybe cubensis (Earle) Sing.						
cultivated in the laboratory	a	0,05	0,12	n.d.	-----	-----
1. from a Mexican strain	b	0,50	0,15	n.d.	-----	-----
2. from an Amazonian strain	a	0,10	0,12	n.d.	-----	-----
	b	0,33	0,15	n.d.	-----	-----
Psilocybe thailandensis Guzmán &	a	0,10	0,055	n.d.	-----	-----
Allen,from Koh Samui, Thailand	b	0,60	0,075	n.d.	-----	-----
Ps semilanceata (Fr.) Quél.						
var. semilanceata, from Ondallaz	a	n.d.	0,39	0,088	-----	-----
(VD) Switzerland	b	n.d.	0,47	0,14	-----	-----
Inocybe haemacta B.&Br., from	a	n.d.	0,020	0,003	-----	-----
La Tour-de-Peilz (Switzerland)	b	n.d.	0,042	0,008	-----	-----
Inocybe corydalina Quél., from,	a	n.d.	0,023	0,025	-----	-----
Jura neuchâtelois, Switzerland	b	n.d.	0,030	0,060	-----	-----
Panaeolus foenisecii (Pers. :Fr)						
Schroet. ap. Cohn, from Vevey,	a	n.d.	n.d.	n.d.	0,22	0,33
Switzerland	b	n.d.	n.d.	n.d.	0,50	0,45
Panaeolus cyanescens (B.&Br.)						
Sacc.(= *Copelandia cyanescens*	a	0,10	0,05	n.d.	0,02	n.d.
(B.&Br.) Sing.), from Oahu, Hawaii	b	0,33	0,09	n.d.	0,06	n.d.

All values expressed in percentage on dry matter

n.d. = not detectable

----- = not analysed for

Table II a: Psychoactive compounds in some Brazilian fungi

Species	N	Psilocin	Psilocybin	Baeocystin	Serotonin	5-OH Tryptophan	Muscimol
Amanitaceae							
Amanita muscaria (L.:Fr.) Hooker	4	n.d.	n.d.	n.d.	n.d.	n.d.	0,08-0,13
Coprinaceae							
Panaeolus subbalteatus (Berk. & Br.) Sacc.	3	n.d.	0,033-0,080	n.d.	0,058-0,097	0,10-0,21	-----
Pluteaceae							
Pluteus glaucus Sing.	1	0,12	0,28	n.d.	n.d.	n.d.	-----
Pluteus sp. (aff. glaucus Sing.)	1	0,10	0,15	n.d.	n.d.	n.d.	-----
Strophariaceae							
Psilocybe caeruleoannulata Sing. : Guzmán	2	0,20-0,23	0,055-0,30	n.d.	n.d.	n.d.	-----
Psilocybe caerulescens Murr. var. caerulescens	2	n.d. - 0,25	0,10 - 0,22	n.d.	n.d.	n.d.	-----
Psilocybe zapotecorum Heim, emend. Guzmán	5	0,05-1,0	0,06-0,30	n.d. - 0,02	n.d.	n.d.	-----
Psilocybe cubensis (Earle) Sing.	4	0,20-0,60	0,10-0,36	n.d. - 0,025	n.d.	n.d.	-----
Psilocybe hoogshagenii Heim var. *hoogshagenii*	2	0,20-0,30	0,15-0,30	n.d. - 0,014	n.d.	n.d.	-----
Psilocybe cf. *subyungensis* Guzmán	1	0,40	0,50	0,033	n.d.	n.d.	-----
Psilocybe uruguayensis Sing. : Guzmán	4	n.d. - 0,01	0,085 - 0,14	0,015 - 0,020	n.d.	n.d.	-----

All values expressed in percentage on dry matter

n.d. = not detectable ---- = not analysed for

Arq.Biol.Tecnol.36(2):313-329,jun.,1993

Table Ii b: Non-psychoactive Brazilian species containing serotonin and 5-OH-tryptophan

Species	N	Psilocin	Psilocybin	Baeocystin	Serotonin	5-OH Tryptophan	Muscimol
Coprinaceae							
Panaeolus antillarum (Fr.) Dennis (= *Anellaria sepulchralis* (Berk.) Sing.	1	n.d.	n.d.	n.d.	0,035	n.d.	-----
Panaeolus foenisecii (Pers. ex Fr.) Schroet. ap. Cohn (=*Panaeolina foenisecii* (Pers. ex Fr.) R. Maire)	1	n.d.	n.d.	n.d.	0,25	0,58	-----
Tricholomataceae							
Rickenella straminea (Petch) Pegler (cf. fibula (Bull. : Fr.) Raith.	1	n.d.	n.d.	n.d.	n.d.	0,10	-----

All values expressed in percentage on dry matter

n.d. = not detectable

---- = not analysed for

Arq.Biol.Tecnol.36(2):313-329,jun.,1993

TABLE III

Brazilian fungi found to be exempt of psilocin/psilocybin and serotonin

Bolbitiaceae :

- *Conocybe brunneola* (Kühn.) Watl.
- *Conocybe mesospora* (Kühn.) Kühn. & Watl.
- *Conocybe plicatella* (Peck) Kühn. (= *Galerella plicatella* (Peck) Sing.)

Cortinariaceae :

- *Gymnopilus chrysopellus* (Berk. & Curt.) Murr.
- *Gymnopilus peliolepis* (Speg.) Sing.
- *Gymnopilus pampeanus* (Speg.) Sing.
- *Inocybe curvipes* Karst. (= *I. lanuginella* (J. Schroet. ap. Cohn) Konr. & Maubl.)

Pluteaceae :

- *Pluteus* cf. *albostipitatus* (Dennis) Sing.
- *Pluteus* cf. *beniensis* Sing.
- *Pluteus chrysophlebius* (Berk. & Rav.) Sacc. subsp. *bruchii* (Speg.) Sing. var. *bruchii*
- *Pluteus cubensis* (Murr.) Dennis
- *Pluteus fibulatus* Sing. in Sing. & Digilio
- *Pluteus fluminensis* Sing.
- *Pluteus pulverulentus* Murr., var. *pseudonanus* Sing.
- *Pluteus xylophilus* (Speg.) Sing. var. *tucumanensis* (Sing.) Sing.
- ditto var. *xylophilus*

Strophariaceae :

- *Psilocybe alnetorum* Sing. (= *Naematoloma alnetorum* (Sing.) Guzmán)
- *Psilocybe coprophila* (Bull. : Fr.) Quél.
- *Psilocybe paupera* Sing. ss. Guzmán
- *Psilocybe* cf. *venezuelana* Dennis
- *Stropharia coronilla* (Bull. : Fr.) Quél.
- *Stropharia rugosoannulata* Farl. : Murr.

Remarks :

Pluteus cubensis contains 0,05 percent tryptamine.

Pluteus xylophilus var. *tucumanensis* contains several unidentified tryptamine derivatives.

TAXONOMIC COMMENTS

The colour names used in the descriptions are based on ref. 26. For the identification of the *Psilocybes* the main source has been Guzmán's monograph (1). Finding localities are indicated by a two- or three-letter code as is explained in ref. 11.

For substrate plants and substrates the following abbreviations are used: dicot. = dicotyledonous, dec. = decayed.

The descriptions are based exclusively on the collections included in Table II; data from Paraná State collections referable to the same species but not chemically analysed, are not incorporated.

The species are treated in alphabetical order and the collections are listed on chronological sequence of the collecting data.

Amanita muscaria

Collections examined: COb - 16 June 1990, CL - 8 July 1991, CUe - 20 July 1991, COa - 21 July 1991. All four collections are from Pinus-plantations.

Panaeolus antillarum

Collection examined: CUa - 12 March 1991 (on horse excrement).

Panaeolus foenisecii

Collection examined: Cua - 21 March 1991 (on soil between grass)

Panaeolus subbalteatus

Collections examined: PAb - 14 Jan. 1991 (on cattle excrement), CUa - 5 November 1991 (on cattle excrement and on soil between grass), MA - 8 July 1992 (on buffalo excrements and on soil between grass).

In the first two collections the spore sizes are: 10-12x(7-)8(-8,7)x5,8-6,5 /um. In the third collection the spore size is 14-15x9-10x7,5-8 /um. The spores are distinctly lentiform, the difference between broadness and thickness being 1,5-2 /um. Chrysocystidia are absent.

Pluteus glaucus

Fruitbody solitary. Pileus 27 mm broad, margin slightly decurved, with slightly depressed centre, hygrophanous, already half-dry when collected and striate only at margin, greenish grey (28E2), centre grey (28F1), surface smooth, dry. Gills 5 mm broad, close, free and 1 mm remote from stipe, orange white (5A2), short gills abundant. Stipe 30x2,5(-3,5 at base) mm, slightly tapering upwards, narrowly fistulose, grey (28B1), smooth, dry, minutely innately striate. Context in pileus and stipe grey (28B1). Smell indistinct.

Spores (measured from pileal surface) 6,8-8(-9)x 5,6-7 /um, broadly ellipsoid, smooth. Basidia 8,5 /um broad, ventricose, 4-spored. Cheilocystidia 30x12-20 /um, crowded, hyaline, thinwalled, broadly clavate (rarely slightly ventricose and then up to 50 /um long). Pleurocystidia 50-90x16-20 /um, abundant, hyaline, constantly thickwalled (wall 1-2 /um thick, at apex 2-3 /um thick), ventricose, apex broadened and always with conical to acute prongs or hooks, the last occurring only apically, not laterally (metuloids of the "Cervinus-type"). Pileipellis composed of up to 10 /um broad hyphae with intracellular pigment. Clamps easily found and abundant in the stipitepellis. Collection examined: MA - 5 December 1992 (on dec. dicot. branch).

This material was collected in the "Serra do Mar" mountain range, at an altitude of 1400 m. Horak (27) distinguishes in this species two varieties; one (var. glaucus) with pleurocystidia up to 80 /um long, and the other (var. chilensis Horak) with pleurocystidia up to 110 /um long. In my collection the length of the pleurocystidia is intermediate between these two varieties.

Pluteus sp. (aff. glaucus)

Fruitbodies loosely gregarious. Pileus 30-45 mm broad, convex, striate up to 3/4R, centre olive brown (4E4), else also olive brown but becoming paler (4D3) on drying, surface smooth, dry, centre minutely flocculose. Gills up to 5 mm broad, very close, free and remote from stipe, first white, then orange white (5A2), edge concolourous. stipe 40-60x3,5-4 mm, almost cylindrical, solid, white, base with extremely weak greenish blue hue but finally becoming more distinctly blue, surface smooth, dry. Smell fungoid.

Spores 6,5-7x5-5,5 /um, smooth. Cheilo- and pleurocystidia all hyaline, thinwalled, ventricose or clavate, pleurocystidia sometimes utriform. Cheilocystidia 30-40x12-15 /um, pleurocystidia 68-75x18-30 /um. Pileipellis strictly hyphous, hyphae 10-17 /um broad, with obtuse apices, pigment intracellular. Clamps present. Collection examined: SJa - 9 Feb. 1992 (on forest soil).

In this collection all hymenial cystidia are thinwalled, but in other collections made by me (all in locality CUa, situated at only 1 km distance of SJa, at the same altitude, viz. 900 m, and certainly belonging to the same species, a part of the pleurocystidia sometimes has a locally slightly thickened wall (Meijer 287) or, more often, has a subcapitate apex with few very short, obtuse excrescensies (not really horn- or pronglike) (Meijer 426, 426b and 287b). In the collections from locality CUa the size of the pleurocystidia is variable, in most collections reaching a length up to 80 /um, but in one up to 95 /um (Meijer 426) and in yet another (Meijer 287b) up to 110 /um long. In all collections the basidia are 4-spored, with spores reaching a size of, at most, 8x6.2 /um, but in one collection (Meijer 426b) there are both 2- and 4-spored basidia and the spore size varies between 5,3-13x4,8-8/um. Clamps are present and easily found in all collections.

Psilocybe caeruleoannulata

Fruitbodies gregarious. Pileus 7-20 mm broad, semiglobose or campanulate to convex, sometimes with a small papilla, strongly hygrophanous, when fresh striate up to centre, dark brown, between striae white, when dry astriate and entirely yellowish white, surface smooth, pellicle viscid, no veil. Gills up to 5 mm broad, close, broadly adnexed, ventricose, soot brown, edge pale. Stipe 25-70x0,8-2,2 mm cylindrical, hollow, yellowish, smooth, non-striate, dry, basal mycelium white. Annulus 1 mm broad, superior, membranous. Context concolorous with the surfaces, drying whitish. Blueing distinct but faint, strong in annulus. Smell fungoid. Taste mild.

Spores 9,5-12 (-13)x6,5-7,5x6-7 /um, sublentiform, ovoid in face view, ellipsoid in lateral view, wall 0,5 /um thick, germ pore truncate, 1,5 /um broad. Basidia 20-26x9 /um, clavate, 4-spored. Cheilocystidia 16-25x4,5-7 /um, crowded, hyaline, thinwalled, lageniform, often with two necks, neck tapering (2 /um broad at base, 1 /um broad at apex). Pleurocystidia not found. Chrysocystidia absent. Pileipellis a thin ixocutis, composed of strictly cylindrical, smooth, 2-5 /um broad, non-encrusted hyphae. Collections examined: CUa - 11 October 1991 (in pasture), TI - 16 September 1992 (in pasture). This species occurs in graminoid savanna, between grass and on cattle excrement. For comments see under *Ps. uruguayensis*.

Psilocybe caerulescens var. *caerulescens*

Fruitbodies gregarious. Pileus 22-80 mm broad, first strongly convex with incurved margin, becoming fully expanded, thinfleshy, strongly hygrophanous, when fresh dark brown and striate up to 1/2R, when dry astriate and much paler, surface smooth, dry, no veil. Gills 3-8 mm broad, close to subdistant, narrowly adnate, first greyish brown, when mature dark brown, edge white. Stipe 40-75x4-7 mm, cylindrical, hollow, light yellow to light brown, arachnoid veil zone present in young carpophores, but soon disappearing, lower half of stipe densely covered with white fibrills and flocci. Blueing strong on stipe and pileus surfaces. Smell strongly farinaceous.

Spores lentiform, ovoid in face view and ellipsoid in side view, wall 0.6-0.8 /um thick, germ pore broad, in material from 1June 1992 measuring 6,5-7x5-6x4.7-5.2 /um,

in material from 8 November 1991 measuring 8-9x6-6,8x5 /um. Basidia 25x7 /um, 4-spored. Cheilocystidia 25-30x6-8 /um, crowded, hyaline, thinwalled, lageniform, neck always simple, slightly attenuated, 2-2,5 /um broad at apex. Pleurocystidia not found (if present then very small, e.g. 17x6 /um). Chrysocystidia absent. Collections examined: CUa - 8 November 1991 (under solitary *Pinus*, between grass), CUk - 1 June 1992 (under dicot. trees, between grass).

The spore sizes in both collections are slightly different and might even suggest different taxa, but apart from the spore sizes no differences were found and the author has made other collections in which the spore sizes where intermediate between these two extremes; e.g. in the collection from 31 May 1987, not treated here, but certainly identical, the spores measured 7-8x6-7x5 /um.

Three other species known from southern South America come close to this species but differ in the following characteristics:

- *Ps. acutipilea* (Speg.) Guzmán , has much smaller carpophores with a different habit
- *Ps. farinacea* Rick: Guzmán, has spores which are narrower than 4 /um in side view
- *Ps. wrightii* Guzmán, has slightly shorter and narrower cheilocystidia.

Psilocybe cubensis

Collections examined: PAb - 14 January 1991 (on cattle excrement), CUl - 1 June 1992 (on (cattle ?) excrement), MA - 8 July 1992 (on buffalo excrement), TI - 16 September 1992 (on cattle excrements). The spore length in these four collections is 13-16 /um. This species is strictly coprophilous, just as the very similar *Ps. subcubensis*, which differs in shorter spores. From all species of psychoactive mushrooms collected by me the blueing is strongest in this one and in *Ps. zapotecorum*.

Psilocybe hoogshagenii var. *hoogshagenii*

Fruitbodies loosely gregarious. Pileus 4-35 mm broad and 3-12 mm high, conico-papillate, finally more or less expanded but remaining papillate, strongly hygrophanous, when fresh striate up to 3/4R, mature carpophores dark brown, when dry astriate and pale yellow to brownish yellow, surface smooth, dry, in young unopened carpophores with white arachnoid veil between pileus margin and stipe, veil soon disappearing. Gills up to 4,5 mm broad, subclose, varying from free to broadly adnate, in unopened pilei white, soon greyish yellow, finally dark brown, edge white. Stipe 20-120x1-3 mm, almost cylindrical, hollow, white in very young carpophores, soon darkening from base upward, becoming reddish brown and finally almost black, entire surface white fibrillose-striate, veil only present in young buttons. Blueing distinct in stipe and pileus (particularly in the papilla). Smell strongly farinaceous.

Spores lentiform, (sub)rhomboid in face view, wall 0,5-0,8 / um thick, germ pore broad, spores measuring 6-7,2x5-5,5x4,5 /um in material from 21 February 1992 and 7-8,5(-9)x5,8-6,5x5 /um in mat. from 14 May 1992. Cheilocystidia 20-30x4-7 /um, crowded, hyaline, thinwalled, lageniform, with long, simple (rarely bifurcate) thin neck 1,2-2 /um broad. Pleurocystidia abundant, 30-40x12-13 /um, lageniform, neck 3-5 /um broad. Chrysocystidia none. Pileipellis hyphous, no subcellular hypodermium. Collections examined: PIb - 21 February 1992 (along forest edge between grass), GUP - 14 May 1992 (along forest edge between grass).

Two other species known from southern South America come close but differ in the following characteristics :

- *Ps. acutipilea* (Speg.) Guzmán, has slightly larger spores and is possibly devoid of pleurocystidia,

- *Ps. brasiliensis* Guzmán, has shorter cheilocystidia and also shorter pleurocystidia.

Psilocybe cf. *subyungensis*

Fruitbodies gregarious. Pileus 10-20 mm broad, 7-10 mm high, campanulate to convex, always with prominent rounded umbo, thinfleshy, hygrophanous, when fresh closely striate up to 3/4 R, brown (5D5), centre yellowish brown (5D5), when dry yellowish to orange white (4-5A2) with greyish orange centre (5B4), surface dry, smooth, glabrous, no veil. Gills up to 2,2 mm broad, very close, narrowly adnexed to broadly adnate, first pale, when mature greyish brown (8E3), edge white. Stipe 33-50x1,8-3 mm, cylindrical, hollow, dark brown (7F4), smooth, dry, lower half, particularly base, densely covered with small white fibrills which might be veil remnants, no other veil present. Context in pileus and stipe apex greyish brown (5D3), drying pure white, elsewhere in stipe dark reddish brown (8EF6), no blueing or blackening. Smell more or less strongly farinaceous.

Spores (measured from pileal surface) 5,5-6.2x5-6x4 /um, lentiform, rhomboid-triangular, wall 0,5-0,7 /um thick, germ pore 1-1,2 /um broad. Basidia 4-spored. Cheilocystidia 45-60x5-10 /um, crowded, hyaline, thinwalled, cylindric-flexuous and mostly somewhat strangulated, often with one or a few lateral, "finger"-like (2 /um broad, cylindrical, obtuse) appendages. Pleurocystidia not found. Chrysocystidia absent. Hymenophoral trama composed of 5-50 /um broad, cylindrical to strongly inflated hyphae. Pileipellis composed of 3-7 /um broad, cylindrical, non-encrusted hyphae, with clamps. Collection examined: MA - 5 December 1992 (on forest humus and on dec. dicot. branch).

I have collected this species at three earlier occasions, always in the dense ombrophilous forest of the "Serra do Mar" mountain range, the substrate being wood and also humus. In two of these earlier findings the carpophores showed a distinct blueing which, however, was limited to the context of the stipe apex. The present fungus certainly belongs to section Cordisporae Guzmán, in which the following three species come closest : *Ps. subyungensis* Guzmán (from Venezuela), *Ps. yungensis* Sing. & Smith (from Mexico south to Bolivia) and *Ps. plutonia* (Berk. & Curt.) Sacc. (from Cuba, Venezuela and the Brazilian State of Amazonas). *Ps. subyungensis* seems to fit best with my material, especially because of the exact shape and broadness of the cheilocystidia, although Guzmán indicates shorter cheilocystidia (up to 25 /um long only) for the species.

Psilocybe uruguayensis

Fruitbodies solitary, in twos or gregarious. Pileus 15-40 mm broad, convex or flat, with or without prominent umbo, rather thinfleshed, hygrophanous, when fresh striate up to 3/4R, centre yellowish brown to light yellow, striae yellowish brown to olive brown, between striae greyish yellow to beige, when dry astriate and pale yellow, surface smooth, dry, no veil. Gills up to 4 mm broad, close, narrowly adnexed to narrowly adnate, yellowish brown, when fully mature olive brown, edge white. Stipe 30-59x1,7-4 mm, cylindrical, solid or hollow, white, striate, surface smooth, dry. Annulus 2 mm broad, superior, membranous. Context concolourous with surfaces, drying white, Blueing particularly distinct in the annulus.

Spores 7,5-10,5x6-7,5x5,5-6 /um, lentiform, ovoid-subangular in face view, ellipsoid in lateral view, wall 0,5-0,8 /um thick, germ pore 1 /um broad. Basidia 21-24x8-9 /um, clavate, 4-spored. Cheilocystidia 17-32x6-10 /um, crowded, hyaline, thinwalled, versiform : utriform, clavate-pedicellate, lageniform, neck cylindrical or sometimes with subcapitate apex, neck always simple. Pleurocystidia not found. Chrysocystidia absent. Pileipellis composed of narrow, cylindrical hyphae which are not encrusted. Collections examined : CUc - 16 April 1991 (on forest humus), CUd - 11 March 1992 (on dec. dicot. twigs), CUi - 18 March 1992 (on dec. woody material), CUb - 9 December 1992 (on woody litter).

I have collected this species on a wide variety of substrates : on decayed dicotyledonous wood and humus in forest, on naked soil of a dried up river bed in forest and also several times on cattle excrements in pastures. Guzmán (1) mentions this species only from excrements.

Psilocybe caeruleoannulata is very close to *Ps. uruguayensis* but might have narrower cheilocystidia. Guzmán's description suggests that the cheilocystidia are slightly shorter than in *Ps. uruguayensis* and the spores slightly narrower, but these observations are not confirmed by those of mine. I am not fully convinced that both taxa really are specifically different.

Psilocybe zapotecorum var. *zapotecorum*

Fruitbodies solitary or gregarious, sometimes caespitosely aggregate. Pileus 9-100 mm broad, first campanulate with an obtuse papilla, - 15 mm high, finally fully expanded and flat, strongly hygrophanous, when fresh striate up to centre, light to dark brown, between striae light brown to greyish orange, when dry astriate and pale yellow to orange white, surface smooth, dry, veil only present in youngest, nearly closed pilei and leaving a very narrow white edge which soon disappears. Gills up to 7 mm broad, subclose to very close, narrowly adnexed to broadly adnate, brownish orange, becoming brown, edge white. Stipe 15-70 x (1,5-) 3-10 mm, cylindrical, hollow, first pale, finally dark brown with white striation, surface particularly in upper half white fibrillose-squamulose, dry, no trace of annular veil. Context concolourous with the surfaces, drying white except in lower half of stipe where brown. Blueing very distinct, in all parts. Smell strongly farinaceous.

Spores (4,8-)5,8-6,8(-7,5)x(3,2-)3,5-4 /um, not distinctly lentiform, ellipsoid or slightly ovoid in face view, ellipsoid in lateral view, wall 0,3-0,4 /um thick, germ pore 0,8-1 /um broad. Basidia 25x6-7 /um, 4-spored. Cheilocystidia 15-25x4,5-6 /um, very crowded, hyaline, thinwalled, lageniform with tapering neck, neck somewhat irregular and mostly simple, rarely bifurcate, at apex 2 /um broad. Pleurocystidia 22-40x7-12 /um, abundant and often conspicuous (particularly when stained in cottonblue), hyaline, slightly ventricose or constricted in the middle, mostly broadly rounded and simple, sometimes similar to the cheilocystidia but much larger. Chrysocystidia absent. Pileipellis composed of cylindrical, smooth, non-encrusted, 2-6 /um broad hyphae. Pileocystidia very numerous and conspicuous, similar to the cheilocystidia but often larger and sometimes with bifurcate neck, 28-45x5-8 /um, apex of tapering neck 2-3,5 /um broad. Collections examined : MAN - 24 February 1991 (on dec. dicot. branch), CUb - 31 December 1991 (on forest litter), PIb - 29 February 1992 (along forest edge between grass), CUc - 16 march 1992 (on dec. (*Araucaria* ?) branch), CUb - 22 March 1992 (on forest humus).

Psilocybe zapotecorum var. *ramulosum* (28) differs from var. *zapotecorum* in the frequently branched cheilocystidia.

Rickenella straminea (Petch) Pegler, cf. *fibula* (Bull. : Fr.) Raith.

Collection examined : TB - 2 June 1992 (on earth-bank, between moss). Pegler (29) states that *R. straminea* resembles *R. fibula* closely, differing merely in the persistently convex pileus and in the slightly larger spores. According to the same author do the spores of *R. straminea* measure 6-8x2,5-3 /um (29), whilst those of *R. fibula* measure 5-6,5x2,5-3,5 /um (30).

In my material the pileus centre is slightly depressed, which would suggest *R. fibula*, whilst the narrow spores (three spores, all measuring 7x2,5 /um) would suggest *R. straminea*.

REFERENCES

(1) GUZMÁN, G. (1983). The genus *Psilocybe*. **Beih. Nova Hedwigia, 74** : p.1-439.

(2) OLA'H, G.M. (1969). Le genre *Panaeolus*. **Rev. Mycol. Mém. h.S. 10** : 1-273.

(3) STIJVE, T. & KUYPER, TH. W. (1985). Occurrence of psilocybin in various higher fungi from several European countries. **Planta Medica 5** : 385-387.

(4) STIJVE, T. (1987). Vorkommen von Serotonin, Psilocybin und Harnstoff in *Panaeoloideae*. **Beiträge zur Kenntnis der Pilze Mitteleuropas III** : 229-234.

(5) BENEDICT, R. G., BRADY, L. R., SMITH, A. H. & TYLER, JR., V. E. (1962). Occurrence of psilocybin and psilocin in certain *Conocybe* and *Psilocybe* species. **Lloydia 25** : 156-159.

(6) SAUPE, S. G. (1981). Occurrence of psilocybin/psilocin in *Pluteus salicinus (Pluteaceae)*. **Mycologica 73** : 781-784.

(7) STIJVE, T. & BONNARD, J. (1986). Psilocybine et urée dans le genre *Pluteus*. **Mycologica Helvetica 2** : 123-130.

(8) HATFIELD, G. M., VALDES, L. J. & SMITH, A. H. (1978). The occurrence of psilocybin in *Gymnopilus* species. **Lloydia 41** : 140-144.

(9) GARTZ, J. (1989-1). Occurrence of psilocybin, psilocin and baeocystin in *Gymnopilus purpuratus*. **Persoonia 14** : 19-22.

(10) MÜLLER, G. F. R, & EUGSTER, C. H. (1965). Muscimol, ein pharmakodynamisch wirksamer Stoff aus *Amanita muscaria*. **Helv. Chimica Acta 48** : 910-926.

(11) MEIJER, A. A. R. (1992). Macromycetes from the state of Paraná, Brazil.I. Introduction. **Arq. Biol. Tecnol. 35** : 623-629.

(12) STIJVE, T., HISCHENHUBER, C. & ASHLEY, D. (1984). Occurrence of 5-hydroxylated indole derivatives in *Panaeolina foenisecii* (Fries) Kühner from various origin. **Z. Mykol. 50** : 361-366.

(13) KYSILKA, R. & WURST, M. (1990). A novel extraction procedure for psilocybin and psilocin determination in mushroom samples. **Planta Medica 56** : 327-328.

(14) STIJVE, T. (1981). High performance thin-layer chromatographic determination of the toxic principles of some poisonous mushrooms. **Mitt. Gebiete Lebensm. Hyg. 72** : 44-54.

(15) HOFMANN, A., HEIM, R., BRACK, A., KOBEL, H., FREY, A., OTT, H., PETRZILKA, TH. & TROXLER, F. (1959). Psilocybin und psilocyin, zwei psychotrope Wirkstoffe aus mexikanischen Rauschpilzen. **Helv. Chimica Acta XLII, 167-168** : 1557-1572.

(16) BEUG, M. W. & BIGWOOD, J. (1981). Quantitative analysis of psilocybin and psilocin in *Psilocybe baeocystis* (Singer and Smith) by high-performance liquid chromatography and thin-layer chromatography. **J. of Chromatography, 207** : 379-343.

(17) CHRISTIANSEN, A. L., RASMUSSEN, K. E. & HOYLAND, K. (1984). Detection of psilocybin and psilocin in Norwegian species of *Pluteus* and *Conocybe*. **Planta Medica 4** : 341-343.

(18) WURST, M., SEMERDZIEVA, M. & VOKOUN, J. (1984). Analysis of psychotropic compounds in fungi of the genus *Psilocybe* by reversed-phase high-performance liquid chromatography. **J. of Chromatography, 286** : 229-235.

(19) GARTZ, J. (1989-II). Analyse der Indolderivate in Fruchtkörpern und Mycelien von *Panaeolus subbalteatus* (Berk. & Br.) Sacc. **Biochem. Physiol. Pflanzen 184** : 171-178.

(20) OTT, J. & BIGWOOD, J. (1978). Teonanacatl. Hallucinogenic mushrooms of North America. **Madrona Publishers Inc., Seattle.**

(21) HEIM, R. & HOFMANN, A. (1958). Isolement de la psilocybine à partir du *Stropharia cubensis* Earle et d'autres espèces de champignons hallucinogènes mexicains appartenant au genre Psilocybe. **Comptes Rendus Acad. Sci. 262** : 519-523.

(22) STIJVE, T. (1992) Work in progress.

(23) STIJVE, T. & KUYPER, TH. W. (1988). Absence of psilocybin in species of fungi previously reported to contain psilocybin and related tryptamine derivatives. **Persoonia 13** : 463-465.

(24) STIJVE, T. (1982). Het voorkomen van muscarine en muscimol in verschillende paddestoelen. **Coolia 25 (4)** : 94-100.

(25) STIJVE, T. Unpublished work.

(26) KORNERUP, A. & WANSHER, J. H. (1978). Methuen handbook of colour. **3 Ed. Eyre Methuen, London.**

(27) HORAK, E. (1964). Fungi austroamericani. II. *Pluteus* Fr. **Nova Hedw. 8** : 163-199.

(28) GUZMÁN, G., BONONI, V. L. & GRANDI, R. A. P. (1984). New species, new varieties, and a new record of *Psilocybe* from Brazil. **Mycotaxon 19** : 343-350.

(29) PEGLER, D.N. (1986). Agaric Flora of Sri Lanka. **Kew Bull. Addit. Ser. XII** : 1-520.

(30) PEGLER, D.N. (1983). Agaric flora of the Lesser Antilles. **Kew Bull. Addit. Ser. IX** : 1-668.