Indole derivatives in certain *Panaeolus* species from East Europe and Siberia

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Five species of *Panaeolus* were analysed by thin-layer chromatography for the presence of the indole psychotomimetics psilocybin, psilocin and baeocystin, some tryptophan derivatives and urea.

P. ater, P. rickenii, P. papilionaceous, P. sphinctrinus did not contain psilocybin and its hallucinogenic derivatives. Psilocybin accompanied by baeocystin was found only in two samples of *P. subbalteatus*. Analysis revealed a characteristic distribution of the investigated substances: psilocybin, baeocystin, serotonin and urea were mostly concentrated in pilei of basidiomes, while the concentration of 5-hydroxytryptophan was higher in stipes. Psilocin was not detected. The highest concentration of psilocybin was found among the mushrooms of smaller weight (for basidiomes from the same habitat). All the species contained serotonin and urea. Possible physiological roles of the compounds is briefly discussed.

Species of *Panaeolus* (Fr.) Quél. were included in the group of fungi screened for psilocybin (PSB) production soon after this indole psychotomimetic had been discovered in higher basidiomycetes (Heim & Wasson, 1958). Since then a considerable number of species of this genus have been investigated. However, the results of psilocybin tests obtained using routine experimental methods should be reconsidered after taking into account that techniques in chromatography during the recent 30 years have developed immensely. Some results have not been confirmed and should be referred to with caution. Nevertheless most results, obtained by different means and at different times, are in satisfactory agreement with each other. More than 20 species of *Panaeolus* have been analysed for PSB at the present moment; the vast majority of the studied species do not produce this 4-substituted indole metabolite. The number of PSB-producing *Panaeolus* species discovered by now is rather limited. It includes: *P. cambodginiensis* Ola'h & Heim (Ola'h, 1968; Ott & Guzmán, 1976), *P. cyanescens* Berk. & Br., *P. tropicalis* Ola'h (Ola'h, 1968) and *P. subbalteatus* (Berk. & Br.) Sacc. – the most rich and thoroughly investigated species (Ola'h, 1968; Fiussello & Scurti, 1972; Ott & Guzmán, 1976; Stijve, 1987; *et al.*). Besides, for a number of species the results were doubtful and controversial.

Biosynthesis of 5-hydroxytryptophan derivatives as well as urea is also characteristic of many species of *Panaeolus* (Tyler & Smith, 1963; Tyler, Benedict & Stuntz, 1965; Stijve, 1987).

It should be mentioned that the geography of *Panaeolus* species analysed for PSB is very wide. It embraces a great number of European countries, as well as the U.S.A. and Australia. In the former Soviet Union investigations of this kind have not been conducted until recently. Lately we have published the data for two species of this genus from our country – *P. rickenii* Hora and *P. sphinctrinus* (Fr.) Quél. (Becker *et al.*, 1988).

In this paper the results of investigation of five *Panaeolus* species for psilocybin, related compounds and urea are given. These species were collected in different regions of the former U.S.S.R. One of them – *P. papilionaceus* (Bull. ex Fr.) Quél. – had not been analysed for these compounds elsewhere.

MATERIALS AND METHODS

Fungal material

Basidiocarps of *Panaeolus ater* (Lange.) Kühn. & Rom., *P. papilionaceus, P. rickenii, P. sphinctrinus* and *P. subbalteatus* were collected during 1987–1990 and identified by professional mycologists. The geographical origin of examined specimens are listed in Table 1. Basidiomes were air-dried or dried at 60 °C and then were thoroughly ground to a powder in a mortar directly before the test.

Pilei and stipes of mature *P. subbalteatus* basidiomes were analysed separately; although, in one of the specimens of this species, some whole small basidiomes were analysed (with dry pilei diameter up to 8 mm, stipes length up to 55 mm).

Extraction procedure

Samples of ground mushroom tissue (0·1–1·5 g) were extracted twice at room temperature with portions of 70% water ethanol (1:100, wt/vol.) during the night. The extracts were filtered, combined and concentrated in a Rotavapour apparatus at 40° under slight vacuum. The residue was dissolved in a

Table 1. Tryptamine derivatives and urea in *Panaeolus* species

Species	Year of collection	Origin of sample	Psilocybin# (±S.E.M.)	Baeocystin# (±S.E.M.)	Serotonin	5-OH-Tryptophan	Urea
P. ater	1988	Penza region, Central Russia	ND*	ND	+ ~	ND	+
P. papilionaceus	1989	Irkutsk region, Middle Siberia	ND	ND	+	Traces	+
P. rickenii	1987	Latvia	ND	ND	+	ND	Traces
P. sphinctrinus	1988	Latvia	ND	ND	+	+	+
P. sphinctrinus	1989	Irkutsk region, Middle Siberia	ND	ND	+	ND	+ +
P. subbalteatus	1989	Irkutsk region, Middle Siberia					
pileus			3·6 ± 0·7 (8)^	1·1 + 0·1 (5)	+ +	ND	+ + +
stipe			1·7 ± 0·2 (10)	Traces	+	+	+
P. subbalteatus	1990	Penza region, Central Russia					
pileus			0·5 ± 0·1 (5)	ND	+	ND	+ + + + +
stipe			0·5 ± 0·2 (5)	ND	+	+	+
small basidiomes			1·2 ± 0·1 (5)	Traces	+ +	Traces	+ + +

\# Content in mg g^{-1} D.W.
* ND = not determined.
~ Intensity of spot colour on chromatograms was estimated on 5-grade scale (volumes of applied extracts correlated the similar weight of the tested tissues).
^ The number in parentheses represents the number of values used in calculating the mean.

suitable volume of 70% ethanol (0·2–0·5 ml). The resulting extracts were investigated using tlc.

Analytical standards

The reference substances, PSB and baeocystin, were isolated from the ethanol extracts of *Psilocybe semilanceata* (Becker *et al.*, 1988) and *Inocybe aeruginascens* (Becker *et al.*, 1989). DL-Tryptophan, tryptamine, serotonin were from 'Reanal' (Hungary), urea – from 'Reachim' (U.S.S.R.). Standard solutions in 96% ethanol or 70% aqueous ethanol (1–2 mg ml^{-1}) were used for tlc.

Tlc analysis

tlc plates. Analysis was performed on commercially available pre-coated plates of Silufol (15 × 15 cm, Kavalier, Czechoslovakia) and HPTLC Kieselgel 60 (10 × 10 cm, Merck 5631). A microsyringe (10 µl, Hamilton, Bonaduz, Switzerland) was used for application of the test solutions onto plates.

Mobile phases for ascending tlc. (*a*) *n*-butanol/acetic acid/water (2:1:1, by vol.), (*b*) ethylacetate/*i*-propanol/19% ammonia (9:7:4, by vol.), (*c*) *n*-propanol/5% ammonia (5:2, by vol.), (*d*) *i*-propanol/5% ammonia (5:4, by vol.).

Chromogenic reagents. (*a*) Ehrlich reagent: solution of 2 g *p*-dimethylaminobenzaldehyde in 100 ml of mixture ethanol/conc. hydrochloric acid (1:1, by vol.). The detection limit for PSB was 0·1 µg (Hatfield, Valdes & Smith, 1978). (*b*) Solution of 0·5 g *p*-dimethylaminocinnamaldehyde in 60 ml of mixture of 10 ml fuming concentrated hydrochloric acid and 50 ml absolute ethanol. The detection limit for PSB was 25 ng (Stijve, Hischenhuber & Ashley, 1984).

Spots of indole alkaloids form colours from lilac to blue, urea produces a yellow-coloured spot.

Semiquantitative tlc. Preparative ascending tlc was used for the purification of *P. subbalteatus* basidiome extracts. Glass plates (12 × 18 cm) with silica gel (100/160 L, Lachema, Czechoslovakia) layers, containing 5% of gypsum, were used; *i*-propanol/5% ammonia (5:4, by vol.) was used as the mobile phase. The zone corresponding to PSB (R_f 0·24–0·30) was taken off the plate, extracted twice at room temperature with 20 ml 70% ethanol during several hours. The filtered extracts were combined and evaporated in a Rotavapour apparatus at 40° under slight vacuum; residues were dissolved in an appropriate volume (0·2–0·5 ml) of 70% ethanol. Extract volumes corresponding to 0·05 mg and 1 mg of dry material were spotted to detect major and minor amounts of indole compounds. PSB and baeocystin contents were estimated using the method of semiquantitative tlc by the simultaneous application of an appropriate volume of the investigated extract and various volumes of standard solutions onto the plate. After having sprayed with detecting reagent the intensities of spots on chromatograms were visually compared. Every test-solution analysis was carried out 5–10 times with the use of several mobile phases. The results were treated statistically.

RESULTS

Results of chromatograph analysis show the absence of PSB and baeocystin in most tested species except *P. subbalteatus*. All the species, including the newly tested *P. papilionaceus*, contained urea and serotonin. Besides, traces of unidentified Ehrlich-positive compounds were noticed in the extracts from *P. papilionaceus*, *P. rickenii* and *P. sphinctrinus*. Psilocin was not detected in either sample. The main experimental data are listed in Table 1.

Two samples of dried *P. subbalteatus* basidiomes (from

European and Asian parts of the former U.S.S.R.) contained considerable amounts of PSB. The Siberian sample proved to be richer in this psychotomimetic than the European one. The biosynthetic precursor of PSB – baeocystin, was detected in noticeable amounts in pilei of the sample, richer in PSB, whereas in stipes and smaller basidiomes only traces were found. In the sample, poorer in PSB, baeocystin was not detected.

It should be noted that both specimens of *P. sphinctrinus* (from European and Asian parts of the former U.S.S.R.) demonstrated very similar chromatographic patterns of the extracts. At the same time it is of interest that, in one of *P. sphinctrinus* samples (from Latvia), a compound was detected with chromatographic mobility (R_f) on Silufol plates (mobile phase *a*) close to that of PSB. However, having analysed an extract of this specimen more thoroughly, using mobile phases of alkaline character (*b*, *c*, *d*), we have confirmed the absence of PSB in this sample. Tryptophan and tryptamine were not detected in either sample of this species.

DISCUSSION

Rather new are the data for *P. papilionaceus*, about which we only knew that Central American Indians had used it as a non-toxic drug (Tyler & Smith, 1963). Since we have not found PSB in *P. papilionaceus* fruit bodies, it is quite possible that its narcotic effect may be related to the presence of other indole derivatives and serotonin in particular.

The negative result of the analysis for PSB content for *P. ater* is in agreement with the data obtained by Stijve (1987) who had tested 3 samples of this species. Data on PSB presence in *P. ater*, published earlier (Ola'h, 1968) should be considered as an artifact. Detection of serotonin and urea in basidiomes of this species confirms the data of Stijve (1987).

Chromatographic analyses of samples of *P. rickenii* show that they do not contain PSB either, which is in agreement with our earlier data (Becker *et al.*, 1988, 1989) and with Christiansen & Rasmussen's results (1983).

P. sphinctrinus is interesting because different authors gave contradictory results of the analysis for PSB content in this species: in earlier papers results were reported as positive (Heim & Wasson, 1958; Ola'h, 1968), whereas in later works (Fiussello & Scurti, 1972; Ott & Guzmán, 1976; Stijve, 1987) negative results were given. We have previously investigated two samples of this species (Becker *et al.*, 1988) from different geographical origins – from Penza district (Central Russia) and from Krasnoyarsk region (Middle Siberia). In the present paper two more samples were analysed, which were collected both in European and Asian parts of the former U.S.S.R. Our previous data, as well as the results given in the present paper, are undoubtedly negative and are in agreement with other authors' data (Fiussello & Scurti, 1972; Ott & Guzmán, 1976; Stijve, 1987). Positive results reported for this species in 1950–60s could be explained by the fact that authors had not used very specific or precise methods of chromatographic analysis. Besides, material for analysis could have been contaminated with basidiomes of morphologically similar basidiomycetes of other species, producing PSB. Therefore, the total range of information, obtained up to the present moment, shows that the ability of *P. sphinctrinus* fruit bodies to produce PSB is doubtful. Presence of urea and 5-hydroxytryptophan is in agreement with information published earlier (Fiussello & Scurti, 1972; Stijve, 1987).

P. subbalteatus had been thoroughly investigated by earlier authors, as it is one of the most effective PSB-producers. It is known that basidiomes of this species are used for narcotic intoxication (Weil, 1976).

PSB and baeocystin concentrations detected in *P. subbalteatus* samples from the former U.S.S.R. (Table 1) are within the range of concentrations, known for this species; PSB, 0·1–7·0 mg g^{-1} of dried material (Ohenoja *et al.*, 1987; Gartz, 1989*a*); baeocystin, 0·08–4·6 mg g^{-1} of dried material (Stijve, 1987; Gartz, 1989*a*). Considerable variability in PSB concentrations is also characteristic of basidiomycetes of other species (even for the basidiomes of the same habitat and similar weight) (Beug & Bigwood, 1981, 1982; Christiansen, Rasmussen & Hoiland, 1981; Gartz, 1986).

Information on the distribution of metabolites in different parts of basidiomata (pileus/stipe), as well as on how their concentration changes with the growth and development of fruit bodies, is of interest, since it enables us to discuss the role played by these compounds in the life cycle of organisms producing them. Reported data of this kind are rather limited. Increased concentrations of PSB, baeocystin, serotonin and urea in pilei of fruit bodies, whereas 5-hydroxytryptophan – in stipes, as well as increased level of PSB in small basidiomes (Table 1) are in agreement with published data for *P. subbalteatus* (Stijve, 1987, Gartz, 1989*a*). Several papers describe the investigation of other species of basidiomycetes. For example, cultivated fruit bodies of *Psilocybe cubensis* (Earle) Sing. did not show any change in PSB concentration during basidiome development (Beug & Bigwood, 1981). At the same time, it was shown that natural fruit bodies of *P. semilanceata* (Fr.) Quél. (from the same habitat) contain more PSB and other indoles in smaller basidiomes than in larger ones (Christiansen *et al.*, 1981; Gartz, 1986). Analogous results were obtained for *Gymnopilus purpuratus* (Cooke & Moss.) Sing. (Gartz, 1989*b*) and *Inocybe aeruginascens* Babos (Gartz, 1989*c*). Data showing the highest concentration of PSB in pilei have been obtained for *Pluteus salicinus* (Pers. ex Fr.) Kummer (Gartz, 1987*a*), *Psilocybe semilanceata* (Beug & Bigwood, 1982; Wurst, Semerdžieva & Vocoun, 1984), *P. cubensis* (Beug & Bigwood, 1981), *P. bohemica* Šebek (Wurst *et al.*, 1984), *I. aeruginascens* (Gartz, 1987*b*), *Gymnopilus purpuratus* (Gartz, 1989*b*). Similar data were obtained for baeocystin distribution in basidiomes of *Inocybe corydalina* var. *corydalina* (Gurevich & Nezdoiminogo, 1992). It has been reported for several *Panaeolus* species that urea and serotonin are mainly localized in pilei of basidiomes, whereas the biosynthetic precursor of serotonin – 5-hydroxytryptophan – is mostly concentrated in stipes (Stijve, 1987); our present data (Table 1) are in agreement with these results. It must be noted that distribution type of urea, serotonin and 5-hydroxytryptophan does not depend on the ability of basidiomycetes to produce PSB simultaneously; moreover, urea accumulation is not related to the substrate on which mushrooms grew (Stijve, 1987).

It is believed that the increase of urea concentration during basidiome development, as well as its prevailing localization in pilei, especially in old ones, can be connected with the role played by this compound in the process of spore formation (Stijve & Bonnard, 1986; Stijve, 1987). This supposition is confirmed by the fact that substrate mycelium of *P. subbalteatus*, unlike the basidiomes, developed on it, does not contain urea (Gartz, 1989*a*); urea was also not found in old but sterile basidiomes of some species (Stijve & Bonnard, 1986; Stijve, 1987).

Serotonin is an indole metabolite, characteristic of the Panaeoloideae (Tyler & Smith, 1963; Stijve, 1987). It is also interesting that sterile basidiomes (with no spores) contain more serotonin than normal ones (Stijve, 1987). The role of this metabolite seems not to be connected with spore formation but it is possible that serotonin plays a role in the process of basidiome formation, as its concentration is higher in smaller basidiomes of *P. subbalteatus* than in substrate mycelium (Gartz, 1989*a*). Stijve (1987) believes that active serotonin biosynthesis is one of the means to neutralize the surplus of toxic ammonia (the main pathway is urea biosynthesis). It is quite possible that in some species psilocybin biosynthesis can also play an analogous role. The latter supposition needs further experimental corroboration.

To conclude, we consider biochemical investigation of Panaeoloideae to be rewarding because of the heterogeneity of this genus and due to the fact that it is difficult to identify the species precisely, using only morphological characters. Since many species of this genus contain indole derivatives it might be useful to exploit them as chemotaxonomical markers, as Ola'h (1968) supposed. Among five species, investigated in this paper, *P. subbalteatus* is most clearly distinguished for its ability to produce PSB and baeocystin. Since data on the biosynthesis of 4-hydroxytryptamine derivatives discussed obtained by different authors for certain *Panaeolus* species as well as for representatives of other genera, are consistent and do not depend on geographical or ecological factors (Gurevich & Becker, 1990; Gurevich & Nezdoiminogo, 1992), they can be used as additional biochemical criteria in taxonomic identifications.

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