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PSILOCIN, PSILOCYBIN, SEROTONIN AND UREA IN PANAEOLUS CYANESCENS FROM VARIOUS ORIGIN

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The occurrence of tryptamine derivatives and urea in *Panaeolus cyanescens*, also known as *Copelandia cyanescens*, from Australia, Hawaii and Thailand was investigated. All 70 collections contained psilocin, serotonin and urea. Those from Hawaii were also relatively rich in psilocybin, whereas the species from Australia and Thailand were virtually exempt of this compound. Many collections also contained detectable amounts of precursors as tryptophan, tryptamine and baeocystin, but 5-hydroxytryptophan – widely encountered in many other Panaeoloideae – was found to be absent in all samples. The role of these 4- and 5-hydroxylated tryptamine derivatives in the metabolism of the fungus and their possible chemotaxonomic significance is briefly discussed. Volunteers ingesting samples of *Panaeolus cyanescens* reported a stronger psychotropic effect than that experienced with the same amount of *Psilocybe semilanceata*.

Panaeolus cyanescens (Bk. & Br.) Sacc., also known as *Copelandia cyanescens* (Bk. & Br.) Sing., is a primarily coprophilic *Panaeolus* of average size encountered in the tropics and neotropics of both hemispheres (Singer, 1960). It is characterised by the blue-staining reaction of its flesh on exposure to air, and by the fact that the gills possess honey-coloured ventricose metuloids (Gerhardt, 1987; Young, 1989).

Panaeolus cyanescens was already recognised as a hallucinogenic mushroom about 25 years ago (Heim, 1967). Its recreational use on the Samoan islands has been reported (Cox, 1981), and, more recently, also on the island of Bali in Indonesia, and in the Hawaiian archipelago (Allen & Merlin, 1989).

The mushroom is reported to contain both psilocin and psilocybin in unusually high quantities (Schultes & Hoffmann, 1980), but published analytical data are few. Panaeoloideae contain appreciable levels of both urea and serotonin, 5-hydroxytryptamin (Stijve, 1985), and it has been suggested that their presence could have chemotaxonomic significance, since these compounds are absent in other Coprinaceae, such as *Psathyrella* and *Coprinus* (Stijve, 1987). It was therefore considered of interest to check whether *P. cyanescens* would share these chemical characteristics.

Since the chromatographic techniques used for the assay of urea, serotonin and their precursors permit the simultaneous determination of psilocin and psilocybin, the possible fluctuation in the concentrations of the latter hallucinogens according to geographical origin could also be monitored. The results of the investigation are reported in this paper.

MATERIAL AND METHODS

The available *P. cyanescens* collections had been gathered and identified by competent mycologists. Material from Queensland, Australia was supplied by Dr. A. Young, Black

butt. Three abundant collections from Hawaii were sent by John Allen, who was also able to provide us with some dried carpophores from the island Koh Samui in Thailand. Carpophores from the three locations were sent to Dr. E. Gerhardt in Berlin, who confirmed their identity as *P. cyanescens* (Bk. & Br.) Sacc. The material was received in air-dried condition and stored at 5 °C in air-tight containers. Considering that some of the possible present compounds such as psilocin are sensitive to oxygen and enzymatic activity, the fungi were analyzed soon after receipt. Just prior to extraction, the individual carpophores were weighed and ground to a fine powder. When large, a single fruit-body was taken for analysis, when small, several specimens. Methanol extraction and subsequent TLC and HPLC analyses were performed as reported earlier (Stijve, 1985).

Psychotropic effects of the dried powdered material from Hawaii (of which an ample supply was available) were tested by ingestion of 1 g amounts of the finely ground material. Among the four volunteers involved in this experiment all had previous experience with hallucinogenic mushrooms, mainly *Psilocybe semilanceata*.

RESULTS

The results of the chromatography analyses of the individual carpophores are listed in Table I. In all samples varying amounts of psilocin, serotonin and urea were found using two chromatographic systems.

There was no indication of significant degradation of the material having occurred between collection and analysis. In fact, the most labile compound, psilocin, was invariably found to be accompanied by its primary oxidation product observed as a greenish spot just below psilocin during TLC in the cellulose/BAW system (Stijve et al., 1984) – but its concentration rarely exceeded 0.02%, which added little to the total psilocin content. The limit of detection for psilocybin and its precursors tryptophan, tryptamine, and baeocystin fluctuated somewhat with the sensitivity of the chromatography systems, but it was always adequate. No other tryptamine derivatives such as methylserotonin or bufotenin were observed, although the limit of detection was often better than 0.01%.

During the self-experiments, taking 1 g of the powdered pooled Hawaiian carpophores (containing 0.6 percent psilocin and 0.2 percent psilocybin) produced a most powerful psychotropic effect. The initial symptoms were felt within 20 minutes: accentuation of visual patterns, slight euphoria and intensifying of colour perception. These effects became stronger for about 4 hours, but since the session was held indoors, the participants experienced more a soulsearching trip with much introspection than a strongly visual adventure.

DISCUSSION

The results of the investigation (Table I) indicate that the tryptamine derivative composition of *P. cyanescens* varies appreciably according to origin. The dimensions and thus the weight of the carpophores fluctuated widely, even within a collection. However, a small specimen weighing about 20 mg is not necessarily in the primordial stage. It can be adult and sporulating as witnessed by an often high urea content. Not surprisingly, all carpophores contained psilocin, the predominant tryptamine derivative. Collections from Australia and Thailand were found virtually exempt of psilocybin, which can perhaps be

Table. I. Tryptamine derivatives and urea in *Panaeolus cyanescens* from various origin.

Collections	Australia Queensland 1989 N = 11	Thailand Koh Samui Isl. 1990 N = 5	Hawaii Sept. 1989 N = 13	Hawaii Dec. 1989 N = 14	Hawaii Coral Kingdom Oahu, July 1990 N = 27
Weight of carpophores (mg)	65–340 (125)	–	24–260 (154)	15–300 (103)	6–250 (56)
Psilocin	0.025–0.71 (0.31)	0.40–1.05 (0.95)	0.055–0.33 (0.17)	0.38–1.30 (0.71)	0.04–0.60 (0.26)
Psilocybin	< 0.012–0.04	< 0.025	0.03–0.28 (0.16)	0.07–0.44 (0.19)	0.01–0.73 (0.16)
Baeocystin	< 0.01	< 0.025	< 0.01–0.025 (0.020)	< 0.005–0.026 (0.016)	< 0.005–0.035 (0.015)
Tryptophan	< 0.01–0.03	< 0.01	0.006–0.014 (0.011)	< 0.01–0.02	< 0.01
Serotonin	0.023–0.45 (0.17)	0.026–0.038 (0.031)	0.025–0.11 (0.062)	0.02–0.24 (0.064)	0.005–0.10 (0.035)
Tryptamine	< 0.004–0.02	0.002–0.008 (0.005)	< 0.005	< 0.005	
Urea	0.20–4.5 (1.65)	1.8–3.3 (2.66)	0.40–2.50 (1.33)	0.53–1.87 (1.20)	0.07–3.0 (1.97)

All values in ~~mg/kg~~ percentage on dry weight. Mean values in brackets.

explained by a lack of a phosphorylating enzyme. On the other hand, contrary to our observations for the Hawaiian material, we found often small but detectable amounts of tryptamine which may be a precursor both in the biosynthesis of psilocin and serotonin. Interestingly, the alternate serotonin precursor, 5-hydroxytryptophan, which is present in substantial amounts in most Panaeoloideae, was found to lack completely in *P. cyanescens*. Although we paid special attention to its possible presence it was even found absent in the stipes of the carpophores in which it is usually concentrated (Stijve, 1987). The detection limit was 0.005% or better.

When analyzing separately stipe and pileus it was found that psilocin was about equally distributed between both parts of the carpophore. Serotonin was found exclusively in the pileus, but the stipe contained 3 × more psilocybin than the pileus. That most of the urea (80–90%) is concentrated in the cap was already known (Stijve, 1987).

The collections rich in psilocybin often contained detectable amounts of the mono-methyl analogue baeocystin, indicating that in the biosynthesis phosphorylation precedes methy-

lation, although to only a modest extent. The specificity of the biosynthesis routes for psilocin and serotonin is highlighted by the total absence of bufotenin (dimethylserotonin), the 5-substituted analogue of psilocin. Similarly, no phosphate ester of serotonin was observed either.

It has been suggested (Stijve, 1987) that the biosynthesis of both urea and serotonin could be ways of neutralising toxic ammonia which is invariably present in the nitrogen-rich substrate of the Panaeoloideae. It was already noticed that some of the species low in urea had often, although not always, a higher than average serotonin content. It could well be that psilocin and psilocybin are similar waste products. Not seldom a high concentration of the said compounds in *P. cyanescens* was accompanied by a low serotonin content in the individual carpophores. Even the average values point in that direction (Table 1). For example, the Australian collections are low or average in psilocin, but definitely higher in serotonin than the psilocin-rich material from Thailand and Hawaii.

It is not unthinkable that the ability to biosynthesize psilocin in a number of *Panaeolus* is a consequence of a genetical accident: initially, these members of the genus probably produced more or less important quantities of serotonin until a mutation conferred the ability to produce 4-hydroxylated tryptamines in addition to the 5-substituted ones. So far, no other genus or species has been found to accomplish this feat.

The ability to produce both urea and serotonin is even shared by members of the genus that grow on rotten wood, an unusual substrate for *Panaeolus*. We found 0.18 percent serotonin, 0.05 percent 5-hydroxytryptophan and 0.5 percent urea in a collection of *P. bernicii* Young, only known from the Bunya pine rainforest in Queensland, Australia, where it grows gregarious to subcaespitose on very rotten wood (Young, 1989). This remarkable species did not contain any psilocin or psilocybin.

Singer (1986) created the genus *Copelandia* for those Panaeoloideae that have characteristic coloured metuloid cystidia and blueing tissues. Gerhardt (1987) has rightly pleaded for a broader generic concept, since the metuloids are also found in many Inocybes, and species with blue- or blue green-staining flesh occur in many genera (Stijve & Kuyper, 1985).

The results presented in this paper show that *P. cyanescens* has also the chemical characteristics (urea, serotonin) of a true *Panaeolus*. In addition, recent studies on the bioconcentration of metals indicate that *P. cyanescens* shares the ability to accumulate manganese with the other members of the genus. No other dark brown to black spored agarics seem to have a marked affinity for this metal (Stijve, 1990).

The four volunteers testing the psychotropic effects of *P. cyanescens* agreed that the 1 g portion ingested proved it to be more potent than *Psilocybe semilanceata*. The effects were felt sooner after ingestion, but the duration of the trip was shorter, which can be explained by the high psilocin content of the *Panaeolus*. Unlike psilocybin, which has first to be hydrolysed, psilocin works directly on the neurotransmitter receptors. (*Psilocybe semilanceata* contains much psilocybin, but no psilocin.) No disagreeable side-effects were noted. Initially, one person got slightly nauseous when told that the fungi had been growing on cow dung, but she recovered real soon and later qualified the experience as 'rewarding'.

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