Occurrence of Psilocybin in Various Higher Fungi from Several European Countries

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Abstract: Using high performance liquid and thin-layer chromatographic methods more than 100 species of fungi from Europe belonging to 18 genera were analysed for the hallucinogenic compound psilocybin and for some related tryptamine derivatives. Psilocybin, psilocin and/or baeocystin were found in 3 *Psilocybe* 1 *Panaeolus*, 5 *Inocybes* and one *Pluteus*.

Psilocybe semilanceata and *Panaeolus subbalteatus* proved to be the only psilocybin-containing fungi that can be gathered in Middle and Northern Europe in sufficient quantities to permit abuse.

Introduction

During the last few years recreational use of hallucinogenic mushrooms has been reported in several European countries, including England (1), Norway (2), Finland (3), the Netherlands and the German Federal Republic (4).

The species used for this purpose was virtually always identified as *Psilocybe semilanceata* (Fr.) Kummer, containing psilocybin, 4-phosphoryloxydimethyltryptamine as the active ingredient.

In many publications several other fungi, mostly *Panaeolus, Stropharia* and *Gymnopilus* species are mentioned to be hallucinogenic because of their supposed psilocybin and/or psilocin (4-hydroxydimethyltryptamine) content (5, 6, 7, 8). However, scientific proof for these allegations is lacking. The purpose of this paper is to report the results of a systematic screening of many fungi belonging to several genera for psilocybin and related compounds.

Material and Methods

The investigation was started by collecting European species from genera in which psilocybin has been reported to occur (5, 9), i.e. representatives of *Psilocybe, Panaeolus, Conocybe, Gymnopilus, Stropharia* and *Pluteus*.

At a later stage the search was extended to fungi from other genera exhibiting the bluish colouration of the carpophore, which is said to be indicative of the presence of psilocybin (10).

All material was gathered between 1981 and 1984. Carpophores were cleaned manually, lyophilized and ground to a fine powder, which was stored in glass at 4° C.

In some cases, single dried fruitbodies were obtained from private and university herbaria. This material was often between 2 and 20 years old.

The fungi studied were from Austria, Czechoslovakia, France, the German Federal Republic, Italy, the Netherlands, Spain and Switzerland. A complete list of the more than 100 species analysed is available on request.

- Generally, 100–200 mg of the finely ground material was extracted overnight, at room temperature with 10 ml methanol. Longer extraction times or refluxing with methanol did not increase the yield for psilocybin, psilocin, serotonin, bufotenin and 5-hydroxytryptophan. The extracts were filtered and analysed as such and after 5–10 fold concentration.
- Qualitative and quantitative analysis was performed by high performance liquid chromatography (HPLC) on a 250 × 4 mm LiChrosorb RP 18 column with a mixture of methanol and aqueous phosphate buffer as the mobile phase, using UV absorption detection at 266 nm, as described by Stijve et al. (11).
- Thin-layer chromatography (TLC) on commercially available pre-coated plates was most suitable for screening series of samples. Extract volumes corresponding to 0.05, 0.10 and 1 mg dried material were spotted in order to detect major and minor amounts of tryptamine derivatives. All extracts were analysed in at least two different systems. Tryptamines were detected by spraying with a solution of 0.5 g 4-dimethylamino cinnamaldehyde (DMCA) in a mixture of 10 ml fuming hydrochloric acid and 50 ml methanol. At room temperature this reagent produced different shades of colour with each compound, allowing a high degree of detection selectivity. As little as 25 ng of each tryptamine derivative produced a clearly visible spot (11).
- Semi-quantitative results obtained by TLC by comparison of spot dimensions were generally confirmed by the more accurate HPLC method (11).

All reference compounds used for chromatographic analyses were obtained from Serva, with the exception of psilocin and psilocybin, which were supplied by Sandoz AG, Basle, Switzerland.

Results and Discussion

The analyses of herbarium material were disappointing, since most specimens had been kept in flimsy envelopes, thus exposing the compounds to oxidation reactions. With few exceptions, results for such material have been disregarded. It is interesting to note however, that psilocybin proved to be the most stable of the sought after tryptamines. It was detectable in *Psilocybe semilanceata* and *Panaeolus subbalteatus* specimens gathered in the late sixties, but at concentrations 10–20 times lower than those measured in fresh mushrooms.

Although more than 100 species from 18 genera were analysed, psilocybin, baeocystin and/or psilocin were only found in 3 *Psilocybe* species, 1 *Panaeolus*, 5 *Inocybes* and one *Pluteus* (Table I).

The concentrations of the psychoactive principles found in *Ps. semilanceata* amply confirm earlier published data (3, 12, 13, 14). Baeocystin was always found to occur along with small amounts of 4-phosphoryloxytryptamine (norbaeocystin), which could not be quantitated for lack of a standard. Some carpophores were found to contain traces of psilocin, though never in concentrations over 0.02 percent, and generally even much lower.

Ps. cyanescens Wakefield is quite abundant in the Pacific Northwest of the USA, where young people use it for recrea-

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Table I. Psilocybin and related compounds in various mushroom species

Species	Number of Samples	Psilocybin	Psilocin	Baeocystin
Psilocybe semilanceata (Fr.) Kummer	30	0.05–1.70	n.d.–0.02	n.d.–0.36
Ps. cyanescens Wakefield ex USA	3	0.20–0.85	0.04–0.36	0.01–0.03
Ps. bohemica Sebek ex Czechoslovakia	3	0.28–0.80	n.d.–0.02	0.01–0.03
Ps. liniformans Guzman and Bas	1	0.16	n.d.	0.005
Panaeolus subbalteatus Berkeley and Broome	5	0.08–0.14	n.d.	0.008–0.033
Inocybe aeruginascens Babos	2	0.085–0.28	n.d.–0.008	0.02–0.08
In. corydalina Quél. var. *corydalina*	2	0.011–0.032	n.d.	0.007–0.034
ditto var. *erinaceomorpha* (Stangl and Veselsky) Kuyp.	1	0.10	n.d.	0.04
In. coelestium Kuyp.	1	0.035	n.d.	0.025
In. haemacta (B. and C.) Sacc.	1	0.17	n.d.	0.034
Pluteus salicinus (Pers. ex Fr.) Kummer	2	0.05–0.25	n.d.	n.d.–0.008

Percentage calculated on dry weight
n.d. = not detectable, i.e. < 0.005 %

tional purposes. However, reports on its occurrence in Europe are scarce. According to Krieglsteiner (15) *Ps. bohemica* Sebek is in all probability identical with *Ps. cyanescens* Wakefield. The data listed in Table I confirm that the species is a potent hallucinogenic mushroom (14), but in Europe its occurrence seems to be restricted to Sazava in Central Bohemia (CSSR). Among the other Psilocybes analysed, only the rare *Ps. liniformans* contained psilocybin.

The search for 4-substituted tryptamines in 10 members of the genus *Panaeolus* has been most unrewarding, in spite of the fact that these fungi have been claimed to be hallucinogenic (6, 7, 8). However, only *P. subbalteatus* contained psilocybin and baeocystin and, indeed, this species is considered a hallucinogenic mushroom in the USA (5).

It could well be that the alleged psychotropic properties of Panaeoli can be traced to the appreciable amounts of serotonin and its precursor 5-OH-tryptophan that were invariably observed during chromatographic analysis. These non-toxic compounds may have been mistaken for psilocybin during earlier investigations using non-selective spot tests.

The presence of these 5-substituted tryptamines seems to be characteristic for *Panaeolus*, since they were not found in members from other genera.

Recently, Drewitz published a report on a case of mushroom poisoning caused by ingestion of *Inocybe aeruginascens*, in which the victims exhibited the symptoms typical for intoxication with psilocybin/psilocin (16). Evidence for the presence of the said hallucinogens was the glaucus green colouration of the mushroom's stipe and the positive indole reaction obtained in a spot test. These observations prompted us to analyze a representative selection of 20 *Inocybe* species, including *In. aeruginascens* and other members of the genus having a greenish-greyish colour.

Five *Inocybes* were found to be positive for psilocybin. The hallucinogen was accompanied by its precursor baeocystin, indicating that during biosynthesis phosphorylation precedes methylation, just as is the case in *Psilocybe semilanceata* (13). The psilocybin concentrations are rather low, but almost all carpophores analysed were 2–5 years old herbarium material.

Saupe (9) and Christiansen et al. (2) reported the occurrence of psilocybin/psilocin in *Pluteus salicinus* from respectively Illinois, USA, and from Norway. The same species gathered in Switzerland in the Rhone valley was found to contain 0.25 percent psilocybin, but no psilocin. Analysis of herbarium material of *Pl. salicinus* found in the Netherlands was also positive for psilocybin. Another dozen *Pluteus* species were screened for the hallucinogen, but with negative results. However, in *Pl. villosus* (Bull.) ss Decary, Romagn. there was a significant amount of a tryptamine derivative closely related to psilocybin. Unfortunately, not enough material was available to permit its isolation and identification. It is interesting to note that the carpophores of *Pl. salicinus* were faintly bluish green at the lower part of the stripe, whereas those of *Pl. villosus* had a definite bluish to violet colour.

Making allowance for appreciable decrease during storage in the herbarium, it can be concluded that the psilocybin content of the 5 *Inocybe* species and of *Pluteus salicinus* is sufficiently high to add them to the list of European hallucinogenic mushrooms.

However, it is unlikely that these fungi will find much recreational use, since said *Inocybes* are rare and easily confused with toxic, muscarin-containing members of the genus. *Pluteus salicinus* which grows solitary on decaying wood is easily recognisable, but far from common.

In summary, *Psilocybe semilanceata* and to a lesser extent *Panaeolus subbalteatus* are the only psilocybin-containing mushrooms that can be gathered in Middle and Northern Europe in sufficient quantities to permit abuse.

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Untersuchungen zum Turnover von Cardenoliden in *Convallaria majalis*[1],*

Studies on the Turnover of Cardenolides in *Convallaria majalis*

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Abstract: In leaves of *Convallaria majalis* L. the cardenolide content is known to decrease towards the end of the vegetation period. However, at this stage the rate of biosynthesis is even higher than at the onset of flowering. This has been revealed by photosynthesis experiments in the presence of $^{14}CO_2$ and the subsequent isolation of 15 labelled cardenolides using PC, TLC and HPLC. *In vitro* and *in vivo* experiments showed that the first step in the degradative pathway of convalloside is deglucosidation yielding convallatoxin, followed by conversion into several yet unidentified degradation products other than known cardenolides of *C. majalis*. This indicates that the cardenolides of *C. majalis* are subjected to a turnover at least towards the end of the vegetation period.

Einleitung

Aus *Convallaria majalis* L. (Liliaceae) sind bereits 38 herzwirksame Glykoside vom Cardenolidtyp strukturell bekannt, die sich von insgesamt 9 Geninen ableiten und überwiegend als Mono- oder Diglykoside vorliegen (1). Untersuchungen über Cardenolidgehalt und Zusammensetzung des Wirkstoffkomplexes im Laufe der Vegetationsperiode zeigten, daß der Gesamtcardenolidgehalt in den Blättern von der Hochblüte an stetig bis zum Einziehen der oberirdischen Pflanzenteile sinkt (2,3). Auch im Wirkstoffspektrum ist eine signifikante Schwankung der prozentuellen Beteiligung einiger Hauptglykoside zu beobachten, wobei in fortgeschrittenem Alter der Pflanze vor allem Convallosid gespeichert wird (3). In den unterirdischen Organen bleiben sowohl Glykosidspektrum als auch Absolutgehalt das ganze Jahr über annähernd konstant (3). Es ist aber nunmehr eindeutig geklärt, daß nur photosynthetisch aktive Gewebe zur Biosynthese der Cardenolide befähigt sind (4). Die Verminderung des Gesamtgehalts in den oberirdischen Pflanzenteilen kann somit nur schwerlich durch den bereits beobachteten Abtransport der Cardenolide in Wurzeln und Rhizome (5, 6) bei gleichzeitigem Nachlassen der Syntheseleistung erklärt werden. Eine überzeugendere Hypothese ist die, daß die Cardenolide einem ständigen Turnover unterliegen, wobei in der späteren Wachstumsperiode der enzymatische Abbau überwiegt.

Für die Verfolgung dieser dynamischen Prozesse bietet sich die Radioisotopentechnik an; die Versuchsanordnung wurde so gewählt, daß dabei auch radioaktiv markierte Herzglykoside für weitere biogenetische Arbeiten (4) gewonnen werden konnten.

Material und Methoden

Pflanzenmaterial

Für die Photoassimilation wurden im Institutsgarten gezogene „Einzelpflanzen" in den beiden Entwicklungsstadien zu Ende der Hochblüte bzw. mit bereits vergilbenden Blatträndern herangezogen. Zur Gewinnung der Enzymfraktionen und für die Abbauversuche *in vivo* verwendeten wir Blätter mit schon deutlich braunen Stellen, wobei für die Homogenatgewinnung die Mittelrippe und bereits abgestorbene Gewebeteile entfernt wurden.

Photosynthese mit $^{14}CO_2$

„Einzelpflanzen" wurden in eine nach (7) modifizierte Glasapparatur eingebracht. Die Freisetzung von $^{14}CO_2$ erfolgte durch Übergießen

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