

**Occurrence of 5-Hydroxylated Indole Derivatives in
Panaeolina foenicisii (Fries) Kuehner from Various Origin**
(Vorkommen von 5-Hydroxylierten Indolderivaten in *Panaeolina
foenicisii* (Fries) Kuehner verschiedener Herkunft)

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Abstract: We used high performance liquid and thin-layer chromatography to investigate the possible presence of the hallucinogenic compound psilocybin in the mushroom *Panaeolina foenicisii*.

Psilocybin and its hallucinogenic derivatives, such as baeocystin and psilocin were undetectable in all sixteen pooled samples of this mushroom, collected between 1973 and 1982 in various European countries, in Australia, and in the Pacific Northwest of the USA. The limit of detection varied between 0,004 and 0,01 percent. All samples contained 5-hydroxytryptamine (serotonin) and its precursor 5-hydroxytryptophan. In some, the total concentration exceeded 1 percent on dry matter, whereas tryptophan content fluctuated between 0,005 and 0,03 percent. The absence of bufotenin (5-hydroxy-N,N-dimethyltryptamine) suggests that the mushroom is not able to methylate serotonin. Volunteers ingested samples of *P. foenicisii* to test for possible hallucinogenic action. Even the equivalent of 40 g of fresh mushrooms failed to produce any psychotropic effects.

Zusammenfassung: Mit Hilfe der Hochdruckflüssigkeits- und der Dünnschichtchromatographie wurde untersucht, ob *Panaeolina foenicisii* die halluzinogene Verbindung Psilocybin und/oder andere Indolderivate enthält.

Es zeigte sich, daß Psilocybin und seine ebenfalls halluzinogenen wirksamen Derivate Baeocystin und Psilocin in allen sechzehn untersuchten homogenisierten gefriergetrockneten Pilzproben nicht nachweisbar waren. Die Proben wurden zwischen 1973 und 1982 in verschiedenen europäischen Ländern, im Nordwesten der USA und in Australien gesammelt. Die Nachweisgrenze für die untersuchten Verbindungen schwankte zwischen 0,004 und 0,01%, bezogen auf die Trockenmasse. Alle Muster enthielten 5-Hydroxytryptamin (= Serotonin) und seinen Vorläufer 5-Hydroxytryptophan. Manchmal überstieg die Gesamtkonzentration beider Substanzen ein Prozent (bezogen auf Trockenmasse), während der Gehalt an Tryptophan zwischen 0,005 und 0,03 Prozent lag. Die Abwesenheit von Bufotenin (5-Hydroxy-N, N-dimethyltryptamin) läßt vermuten, daß dieser Pilz unfähig ist Serotonin zu methylieren. Versuchspersonen aßen Proben von *P. foenicisii* um eventuelle halluzinogene Wirkungen wahrzunehmen. Sogar eine Menge, entsprechend 40 g frischer Pilze rief keinerlei psychotrope Effekte hervor.

Panaeolina foeniseeii, also known as the haymaker's mushroom, has been variously placed in the genera *Psilocybe* (R i c k e n, 1915), *Coprinarius* (M i c h a e l, 1919) or *Psathyrella* (S m i t h, 1972). There is, however, little doubt that it belongs to the *Panaeoloideae*, although its rough spores and its exclusive habitat in grass distinguish it sufficiently from true *Panaeolus* to justify its being placed in the subgenus *Panaeolina* (S i n g e r, 1975; G u z m á n & P a t r a c a, 1972). The mushroom is widely distributed. It grows scattered to gregariously in grassy areas, but unlike *Panaeolus*, never on dung. It is found most abundantly in early summer and to a lesser extent during the autumn.

In two authoritative older handbooks the mushroom is either classed as edible (R i c k e n, 1915), or as non toxic, but worthless for culinary purposes (M i c h a e l, 1919).

More recently, *Panaeolina foeniseeii* from Canada and the USA have been reported to contain the hallucinogenic compound psilocybin (4-phosphoryloxy-N,N-dimethyl-tryptamine) (O l a ' h, 1968; R o b b e r s, & al. 1969), although a more recent investigation yielded negative results for the species gathered in the Pacific Northwest (B e u g & B i g w o o d, 1982). These reports of psilocybin in the species have found their way to the popular mushroom guides and *P. foeniseeii* is now frequently listed as a hallucinogenic mushroom (M i l l e r, jr., 1978; P a c i o n i, 1981; C o o p e r, 1980; S t e v e n s & G e e, 1978). Ingestion of this species for its presumed hallucinogenic properties has recently been described (C o l e s, 1980). The mushrooms involved were not examined by a competent mycologist, so it is still not clear whether *P. foeniseeii* is a hallucinogenic mushroom or not.

Often, contradictory reports on the toxicity of a mushroom species are explained in terms of differences in chemical composition due to geographical origin. In order to investigate this possibility, we analysed fifteen samples of *P. foeniseeii* of various origin for indole derivatives, including the hallucinogens psilocin and psilocybin.

The results of this investigation and a detailed description of the analytical methods used are presented in this paper.

Materials and methods

All carpophores were gathered and identified by competent mycologists at several sites in Switzerland, Austria, the USA, Australia, France, Spain, England and the Netherlands (Prof. Dr. R. Seeger, Institut f. Pharmakologie und Toxikologie der Universität Würzburg, FRG; Dr. E. Kits van Waveren, Amsterdam, The Netherlands; Prof. Dr. C. Andary, Laboratoire de Botanique et de Cryptogamie, Montpellier, France; J. Bigwood, The Evergreen State College, Olympia, Washington, USA; Dr. A. Young, Quakershill, New South Wales, Australia). None of the freshly collected fungi turned bluish upon bruising as is generally the case with psilocybian species (S t a m e t s, 1978). Some of the fungi had been preserved as herbarium species in air-dried state, but most of the samples consisted of several more or less developed fresh fruitbodies which were cleaned mechanically, lyophilised, ground to fine powder, and sealed in glass bottles at 4 ° C until analysis.

Extraction of any indole compounds present was performed by shaking 100–200 mg of the lyophilised material with 10 ml of methanol overnight at room temperature. The extract was filtered over a small folded paper filter and concentrated to a suitable volume (usually 2 ml) by blowing with a stream of clean air.

Initially, tryptophan, 5-hydroxytryptophan and serotonin were simultaneously determined in the crude extracts using High Performance Liquid Chromatography (HPLC) with electrochemical detection (L e a t h w o o d & A s h l e y, 1983). A Bondapak C-18 reversed-phase column and a mobile phase consisting of 7.5% methanol in an aqueous ammonium acetate buffer of pH 4,7 yielded a rapid separation and quantitation of nanogram amounts of the amino acid and its metabolites.

As psilocybin could not be detected by this system, the extracts were also run in two other HPLC systems, using the same column and similar mobile phases. The column effluent was monitored with a Perkin Elmer LC-55 variable wavelength UV detector, operated at 266 nm. The operation conditions

for the separation of the various indoles and their retention times in two mobile phases are listed in Table I.

Most samples were analysed in system A because it allowed a more precise evaluation of both 5-hydroxytryptophan and psilocybin.

Injection volumes of both standard solutions and extracts were kept at 10 microlitres. If significant larger volumes were injected, the difference in composition between mobile phase and the injected liquid caused a peak-splitting phenomenon which led to elution of 5-hydroxytryptophan and serotonin as broadened double peaks.

Results obtained by HPLC were confirmed by thin-layer chromatography (TLC) on commercially available pre-coated plates using the mobile phases listed in Table II and a number of indole derivatives as reference compounds (Psilocin and Psilocybin were gifts from Sandoz AG, Basle, Switzerland). For this purpose, extract volumes corresponding to 0,05 and 1 mg of lyophilised material were spotted in order to detect major and minor amounts of possible present indolic constituents.

All extracts were analysed in at least two TLC systems, using 20 x 20 cm plates and ascending migration in pre-saturated all glass chambers over a distance of 15 cm.

The developed plates were placed under a hood in a stream of air to allow complete evaporation of the adherent solvent and subsequently sprayed with a suitable chromogenic reagent.

Ehrlich's reagent (Révélateurs pour la chromatographie en couches minces et sur papier, E. Merck, Darmstadt 1975, no 91, p. 32) was initially used because it yielded brightly coloured spots with most of the compounds of interest. However, detection of psilocybin required a few minutes heating at 100 ° C and under these circumstances co-extracted urea yielded a brightly yellow zone which interfered (in system I) with the evaluation of serotonin, a major constituent. In addition, sensitivity for tryptophan was poor.

Better results were obtained using 4-dimethylamino cinnamaldehyde (DMCA) (Fluka no 39421) which was used as a solution of 0,5 g in 10 ml fuming concentrated hydrochloric acid, mixed with 50 ml methanol. This reagent was more sensitive than its benzaldehyde analogue and did not need heating to react with the various indoles. In addition, it produced a different shade of colour with each compound. For example, psilocin turned greenish gray, psilocybin reddish, bufotenin violet, 5-hydroxyindole acetic acid green, serotonin and 5-hydroxytryptophan bright blue, tryptophan purple and tryptamine purple red.

It should be pointed out that these colours may vary with the chemical nature of the TLC adsorbent. For example, psilocybin spots are reddish on SiO₂ and on SilCel layers, but violet on cellulose.

The reagent proved to be remarkably sensitive: the detection limit for serotonin and 5-hydroxytryptophan was 10 ng and for psilocybin and tryptophan 25 ng. At room temperature psilocybin was the last of the indolic compounds to become visible. Usually, optimal visibility was obtained after 10–15 min. The reaction could be accelerated by slightly heating with a stream of warm air from a hair-dryer.

Interestingly, the DMCA reagent reacted only very slowly with urea, which yielded a reddish spot only after a few hours, and thus did not interfere with the determination of serotonin.

Possible psychotropic effects of lyophilised *P. foenicicium* were tested by ingestion of 1–2 g amounts of material from both Switzerland and the USA of which we had an ample supply.

Among the five volunteers involved in these subjective appreciations three had previous experience with hallucinogenic mushrooms and two did not.

Results

All *P. foenicicium* examined were found to be free of the hallucinogens psilocin and psilocybin. The limit of detection by both HPLC and TLC varied between 0,004 and 0,01 percent on dry weight for both compounds. In all samples varying amounts of serotonin (5-hydroxytryptamine) and its precursor 5-hydroxytryptophan were found (Table III), in some cases the sum of both indole derivatives exceeded 1 percent. The levels as determined by HPLC were confirmed by TLC analysis.

Herbarium material such as samples 12 and 13 were rather low in both compounds, presumably due to advanced decomposition during storage.

It is unlikely that initially present psilocybin in these samples underwent the same fate, because by virtue of its phosphoryloxy group psilocybin is far more stable than the hydroxylated tryptamine derivatives. In samples of *Panaeolus subbalteatus* and *Psilocybe semilanceata* which were more than 10 years old, psilocybin was readily detected (Stijve, unpublished), and in one paper the presence of this compound was reported even in 30-year-old herbarium material (Christiansen & Rasmussen, 1982).

In addition to serotonin and its precursor, all *P. foeniseeii* samples contained low tryptophan levels varying from 0,005 to 0,03 percent on dry weight.

Freshly gathered material was found to contain traces (< 0,02%) of at least two other indolic compounds. One of these had the same Rf value and chromogenic reaction as 5-hydroxyindole acetic acid in two TLC systems, but this result could not be confirmed by HPLC analysis, which indicated the presence of a minor constituent having a somewhat longer retention time. Special attention was paid to the possible presence of other 5-substituted indoles, such as bufotenin (dimethyl 5-hydroxytryptamine), but this compound was definitely absent (limit of detection 0,01%).

It is noteworthy that all material analysed contained appreciable levels of urea, in some cases as much as 1,5 percent.

During the self-experiments, *P. foeniseeii* failed to elicit any psychotropic effects, even when up to 40 g of fresh mushrooms was ingested.

In the same persons, a 5 times lower dose of the well-known hallucinogenic mushroom *Psilocybe semilanceata* produced after 30–45 min the typical symptoms of heavy feeling in the limbs, yawning, followed by a feeling of slight euphoria, expanded perception and, when the subject was in the dark, vivid colour visions.

Discussion

This investigation involving sixteen samples of *P. foeniseeii* from eight different countries strongly suggests that the species is not hallucinogenic. When ingested it did not produce any psychotropic effects and our analyses indicated absence of psilocin and psilocybin. Furthermore, earlier analyses performed in this laboratory were negative for GABA inhibitors such as muscimol.

In the majority of the samples examined we found surprisingly high levels of serotonin and 5-hydroxytryptophan. Taken orally, these compounds do not produce any psychotropic effects (Garattini & Valzelli, 1965).

At least two authors (Robbers et al., 1969; Fiu ssello & Scurti, 1972) have found serotonin and its precursor in *P. foeniseeii*, but no attempt at quantitation was made.

The results reported in this paper confirm that *P. foeniseeii* is related with the genus *Panaeolus*, in that it contains high levels of both urea and 5-hydroxylated indoles like all members do. *Psilocybe* and *Psathyrella* species do not possess these characteristics (Stijve, unpublished).

The analytical methods used in this investigation are similar to those already reported in literature. Among the numerous methods used in clinical chemistry for the determination of tryptophan metabolites we chose an HPLC technique (Koch & Kissinger, 1979), which we slightly modified. The TLC systems used are largely the same as those described by earlier authors (Fiu ssello & Scurti, 1972; Beug & Bigwood, 1981), but we introduced the use of mixed cellulose and silica gel layers, because these

were found to be superior to either of the two adsorbents separately in terms of loading capacity, separation characteristics and detection limit.

Furthermore, in our hands the 4-dimethylamino-cinnamaldehyde spray was superior to the classic Ehrlich reagent, because it was more selective and more suitable for detection of low levels of psilocybin and tryptophan.

Serotonin and its precursor have been found in other mushrooms notably in *Amanita citrina*, but only at low concentrations, as a rather transient metabolite in the biosynthesis of bufotenin (Tyler, jr. & Gröger, 1964; Andary, 1978; Stijve, 1979).

Like other *Panaeolus* species *P. foenicicii* is not able to methylate serotonin, as indicated by the absence of 5-hydroxylated methyl- and dimethyltryptamines.

Considering the results of this investigation, the findings of psilocybin in earlier collections (Ola'h, 1968; Robbers, & al. 1969) are hard to explain.

If is, of course, possible that the material examined was accidentally mixed with a small amount of a true psilocybian mushroom. One carpophore of such a species mixed with twenty *P. foenicicii* would be sufficient to give a positive result on chemical analysis. This, in fact, is quite a strong probability since identifying small brownish mushrooms with dark lamellae is notoriously difficult, and the various species are hard to distinguish even for experienced mycologists.

During gathering of these kinds of mushrooms for scientific purposes, the senior author has observed that even people who were more or less familiar with the species of interest, frequently misidentified individual carpophores. It was always necessary to check the whole collection thoroughly, each mushroom separately, before it could be lyophilised.

In collections of *Psilocybe semilanceata* we frequently found small fruitbodies of *Stropharia semiglobata*, *Conocybe tenera* and even once an *Inocybe* species! A crop of *Stropharia semiglobata* gathered in alp meadows contained some *P. semilanceata* and small *Panaeolus semiovatus*, although to the experienced mycologist these two fungi bear little resemblance to the species sought.

These observations emphasize the importance of correct botanical identification of a mushroom before subjecting it to chemical analysis. In fact, many controversial reports on the occurrence of certain compounds probably result because the different authors have not analysed the same species.

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Tabelle 1: Retentionszeiten, in min, von verschiedenen Indolverbindungen nach HPLC auf einer Umkehrphasensäule RP 18, 250 x 4 mm, mit zwei verschiedenen mobilen Phasen, Fluss : 1 ml/min.

Table 1: Retention times, in min, of various indolic compounds during HPLC on a reversed-phase Lichrosorb RP 18, 250 x 4 mm column using two mobile phases at a flow rate of 1 ml/min.

	<u>A</u>	<u>B</u>
	15 percent methanol + 85 percent aqueous buffer solution consisting of 200 ml 0,2 M K_2HPO_4 and 350 ml 0,1 M citric acid v/v	15 percent methanol + 85 percent 0,5 M aqueous ammonium acetate solution v/v
Psilocybin	4,2	5,1 (tailing peak)
5-Hydroxytryptophan	4,7	4,8
Serotonin	5,9	7,2
Bufotenin	7,7	9,4
Psilocin	—	19,2 (tailing peak)
Tryptophan	10,1	—
5-Hydroxyindole acetic acid	13,9	—

Tabelle 2: Rf-Werte von verschiedenen Indolderivaten in drei dünnschichtchromatographischen Systemen.

Table 2: Rfvalues of various indole derivatives in three thin-layer chromatographicsystems.

- I SilCel Mix, Macherey-Nagel, 5160 Düren, FRG, Butanol – acetic acid – water 60 : 15 : 25 v/v.
 II Cellulose F, Merck 5718, Propanol – 25% ammonia 5 : 1 v/v.
 III Silica gel 60, Merck 5715, Methanol – acetic acid – water 75 : 10 : 15 v/v.

	<u>I</u>	<u>II</u>	<u>III</u>
Psilocybin	0,28	0,03	0,40
Baeocystin	0,31	0,02	0,51
5-Hydroxytryptophan	0,36	0,15	0,70
Bufotenin	0,40	0,90	0,54
Psilocin	0,46	0,90	0,55
Serotonin	0,50	0,65	0,65
Tryptophan	0,49	0,30	0,68
Urea	0,53	0,36	—
Tryptamine	0,65	0,85	0,65
5-Hydroxyindole acetic acid	0,72	0,18	0,75

Tabelle 3: Gehalte an Serotonin und 5-Hydroxytryptophan (in Prozent, bezogen auf Trockengewicht) in Karpophoren von *Panaeolina foenicisecii* verschiedener Herkunft.

Table 3: Serotonin and 5-hydroxytryptophan levels (percent on dry weight) in carpophores of *Panaeolina foenicisecii* from various origin.

Sample nr	Origin	Serotonin		5-OH-tryptophan	
		HPLC	TLC	HPLC	TLC
1	La Tour-de-Peilz CH June '82	0,36	0,40	0,67	0,80
2	Clies CH Juni '82	0,33	0,40	0,30	0,26
3	Clies CH July '81	0,37	0,50	0,28	0,32
4	Clies CH September '81	0,36	0,48	0,30	0,22
5	Clies CH August '82	—	0,20	—	0,45
6	Villeneuve CH August '82	—	0,25	—	0,22
7	Fischl Tirol September '82	—	0,42	—	0,60
8	Olympia USA September '82	0,29	0,36	0,85	1,10
9	ditto	0,18	0,16	1,07	1,20
10	ditto	0,24	0,22	0,79	1,02
11	ditto	0,24	0,25	0,94	1,15
12	Kortenhoef Netherlands 1973	—	0,005	—	0,04
13	Lake Vyrnwy Wales UK 1977	0,015	0,01	0,016	0,006
14	Bedarieux France 1982	—	0,08	—	0,17
15	Barcelona region Spain 1980	—	0,51	—	1,27
16	New S. Wales Australia '83	—	0,53	—	0,40



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