

Occurrence of 5-Hydroxylated Indole Derivatives in *Panaeolina foeniseeii* (Fries) Kuehner from Various Origin

(Vorkommen von 5-Hydroxylierten Indolderivaten in *Panaeolina foeniseeii* (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 foeniseeii*.

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. foeniseeii* 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 foeniseeii* die halluzinogene Verbindung Psilocybin und/oder andere Indolderivate enthält.

Es zeigte sich, daß Psilocybin und seine ebenfalls halluzinogen 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. foeniseeii* um eventuelle halluzinogene Wirkungen wahrzunehmen. Sogar eine Menge, entsprechend 40 g frischer Pilze rief keinerlei psychotrope Effekte hervor.

Panaeolina foenisecii, 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 foenisecii* 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, & a l. 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. foenisecii* 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 o l e s, 1980). The mushrooms involved were not examined by a competent mycologist, so it is still not clear whether *P. foenisecii* 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. foenisecii* 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

