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OCCURRENCE OF PSILOCYBIN AND BAEOCYSTIN IN THE GENUS INOCYBE (FR.) FR.

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The presence of psilocybin and its monomethyl analogue baeocystin is for the first time demonstrated in five taxa of the genus *Inocybe*, viz. *I. coelestium*, *I. corydalina* var. *corydalina*, *I. corydalina* var. *erinaceomorpha*, *I. haemacta* and *I. aeruginascens*. These taxa are characterized by a glaucous coloration of the stipe. A sixth taxon, *I. calamistrata*, which also has a blue-green stipe was found to be exempt of psilocybin and other methylated tryptamines. Negative results were also obtained for other *Inocybes*. Muscarin is absent in the psilocybin-containing species. However, no relation between taxonomic position and the presence of either compound seems to exist.

INTRODUCTION

After the discovery of psilocybin and related 4-oxygenated indole alkaloids in Mexican representatives of the agaric genus *Psilocybe* (Fr.) Kumm., the possible presence of these hallucinogenic compounds in other genera and families has been subject to various investigations.

To date psilocybin, psilocin and/or baeocystin have been found in the following families of gill-fungi: Strophariaceae (mainly *Psilocybe*; see Guzmán, 1983 for a review), Coprinaceae (*Panaeolus* (Fr.) Quél.; Ola'h, 1969, Stamets, 1978), Bolbitiaceae (*Conocybe* Fay.; Benedict & al., 1962), Pluteaceae (*Pluteus salicinus* (Pers.: Fr.) Kumm.; Saupe, 1981) and Cortinariaceae (*Gymnopilus* P. Karst.; Hatfield & al., 1978).

Recently, Drewitz (1983) published a report on a case of hallucinogenic mushroom-poisoning caused by ingestion of *Inocybe aeruginascens* Babos. Interestingly, no muscarinic syndrome (normally typical for *Inocybe*-poisoning) was observed, but the victims exhibited the symptoms typical for intoxication with psilocybin/psilocin. Additional evidence for the presence of these hallucinogenic compounds was the bluish green colouration of the stipe and the positive indole reaction obtained by a presumptive chemical test. However, the presence of the said 4-oxygenated tryptamines was not unequivocally demonstrated. For this reason, the present authors decided to use modern analytical techniques for the analysis of psilocybin in *I. aeruginascens*. Moreover, this work was extended to a systematic search for hallucinogenic compounds in a representative selection of *Inocybe* species.

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MATERIALS AND METHODS

With very few exceptions specimens of the *Inocybe* species of interest were taken from herbarium-material available at the Rijksherbarium, Leiden, the Netherlands. These collections were made during the last three years and the species form a representative sample of the genus in western and central Europe (Table 1).

Possible present residual water was removed from the carpophores by freeze-drying. The material thus obtained was ground to fine powder and extraction of possible present tryptamin derivatives was performed by shaking 100–200 mg with 10 ml methanol overnight at room temperature. The extract was filtered over a small folded paper filter and concentrated to 2 ml by blowing with a stream of nitrogen.

All extracts were first analysed by thin-layer chromatography (TLC) for muscarin (Stijve, 1981) and for psilocybin and related tryptamines (Stijve & al., 1984). Quantitation was performed by comparing spot dimensions with those given by a suitable range of standards. Presence of muscarin was confirmed by derivatisation to acetyl muscarin and the resulting change in Rf-value (Eugster, 1956). Results obtained by TLC for psilocybin and its precursor were corroborated by subjecting the extracts to high performance liquid chromatography (HPLC) using a reversed phase Lichrosorb RP 18 column and a mobile phase consisting of 15 percent methanol and 85 percent aqueous phosphate citric acid buffer solution (Stijve & al., 1984). Detection was performed by ultra-violet absorption at 266 nm.

The reference compounds of most tryptamine derivatives were obtained from Serva Feinbiochemica GmbH and Co., D-6900 Heidelberg, GFR, whereas psilocin and psilocybin were a gift from Sandoz AG, Basle, Switzerland. Since baeocystin was not commercially available, an extract of *Psilocybe semilanceata* (Fr.) Kumm. containing a known concentration of this compound was co-chromatographed with each series of extracts to serve as a reference.

RESULTS AND DISCUSSION

Psilocybin and its precursor baeocystin were found in the following species: *I. aeruginascens, I. corydalina* var. *corydalina, I. corydalina* var. *erinaceomorpha*[1] and *I. coelestium*[1] and *I. haemacta* (Table 1). Identity of both compounds was confirmed by TLC in three different systems and by HPLC. In the extract of *I. haemacta* the concentration was sufficiently high to permit additional confirmatory procedures as derivatisation to psilocin by treatment with lithium hydroxide (Stahl & Brombeer, 1978), or by measuring the ultraviolet spectrum which showed the maxima at 227, 267, 278 and 291 nm characteristic for psilocybin (Fiussello & Scurti, 1972).

The four psilocybin-containing species are all characterized by a glaucous discolouration at the lower half of the stipe, and often also at the centre of the pileus. This greenish-greyish colour is sometimes rather indistinct, but it may become more manifest on bruising and/or ageing. This phenomenon is reminiscent of the typical blue col-

[1]See Kuyper on pp. 479–482 of this fascicle of Persoonia.

ouration observed in many psilocybian mushrooms, which is caused by the stepwise oxidation of psilocybin to psilocin to a blue pigment (Chilton, 1978).

Inocybe calamistrata also has a blue-green stipe, but its colour does not change on bruising, and this species does indeed not contain any psilocybin.

In the psilocybin-positive *Inocybes* the hallucinogen was found to be accompanied by appreciable amounts of baeocystin. In one sample of *I. corydalina* var. *corydalina* the concentration of the latter compound even exceeds that of psilocybin. This phenomenon has not yet been observed in other psilocybin-containing fungi. However, the data reported in this paper concern mostly herbarium material and should, therefore, be interpreted with caution. It is well-known that psilocybin slowly disappears from exsiccates during conservation, especially if the latter are exposed to air. This can be concluded from a comparison between dried material of *I. corydalina* var. *corydalina* collected in 1982 which showed much higher concentrations of psilocybin and baeocystin than another collection of the same taxon gathered in 1977. Moreover, a fresh carpophore of *I. aeruginascens* picked in 1984 in the Rhone valley was also found to contain significantly more of the two psychotropic compounds than the two year old herbarium collection. For the moment, no definite conclusions can be drawn about the biochemical pathway of the synthesis of psilocybin in *Inocybe*. However, the presence of relatively high concentrations of baeocystin suggests that phosphorylation preceded methylation, just as is the case in *Psilocybe semilanceata*, implying the following reactions (Repke & Leslie, 1977; Stijve, 1983):

tryptophan → tryptamin → 4-hydroxytryptamin → norbaeocystin → baeocystin → psilocybin.

Making allowance for appreciable decrease during storage in the herbarium, it can be concluded that the psilocybin content of these *Inocybe* species is sufficiently high to include them in the rapidly growing list of European psychotropic fungi.

Since the occurrence of hallucinogenic compounds in certain mushrooms generally invites their recreational use, it is interesting to note that the psilocybin containing *Inocybes* were found to be exempt of muscarin, the toxic principle of many species of the genus. Unlike psilocybin, muscarin does hardly degrade during storage, and the levels listed in Table 1 for such species as *I. griseolilacina, I. napipes* and *I. trechispora* are in agreement with those reported in literature (Malone et al., 1962). The absence of muscarin also confirms Drewitz's (1983) observations on the behaviour of poisoned patients after their consumption of *I. aeruginascens*.[1]

The occurrence of psilocybin in the genus *Inocybe* seems to be restricted to only two sections, viz. *Lactiferae* Heim and *Fibrillosae* Heim. Moreover, only some species within these sections are psilocybin-positive, whereas others are not. The presence of psilocybin in the said *Inocybe* species has therefore no chemotaxonomic relevance.

[1]Absence of muscarin in *I. corydalina* var. *corydalina, I. haemacta* and *I. calamistrata* was also demonstrated in vivo with rats (after Malone & al., 1962).

The absence of muscarin in psilocybin-containing species of *Inocybe* is interesting, but can hardly be considered characteristic, since many *Inocybes* do not contain detectable levels of either compound.

Table 1. Concentrations of muscarin, psilocybin, psilocin and related compounds in selected *Inocybe* species.

Species	Provenance	Year	% Muscarin	% Psilocybin	% Baeocystin	% Psilocin
I. terrigena (Fr.) Kuyp.	I	1982	–	–	–	–
I. calamistrata (Fr.: Fr.) Gill.	NL	1979	–	–	–	–
I. bongardii (Weinm.) Quél.	D	1980	–	–	–	–
I. cervicolor (Pers.) Quél.	D	1980	–	–	–	–
ditto	A	1982	–	–	–	–
I. adaequata (Britz.) Sacc.	NL	1982	–	–	–	–
I. haemacta (B. & Cooke) Sacc.	A	1982	–	0.17	0.034	0.02
I. corydalina Quél. var. *corydalina*	F	1977	–	0.011	0.007	–
ditto var. *corydalina*	A	1982	–	0.032	0.092	–
ditto var. *erinaceomorpha* (Stangl & Veselský) Kuyp.	D	1982	–	0.1	0.04	–
I. coelestium Kuyp.	D	1982	–	0.035	0.025	–
I. incarnata Bres.	D	1982	–	–	–	–
I. appendiculata Kühner	D	1982	–	–	–	–
I. pudica Kühner	A	1982	0.027	–	–	–
I. aeruginascens Babos	NL	1980	–	0.085	0.02	–
ditto	CH	1984	–	0.28	0.08	0.008
I. flocculosa (Berk. →) Sacc.	NL	1982	0.19	–	–	–
I. griseolilacina J. Lange	NL	1982	0.063	–	–	–
I. napipes J. Lange	NL	1982	0.55	–	–	–
I. trechispora (Berk.) P. Karst	NL	1982	0.25	–	–	–

A = Austria; CH = Switzerland; D = Federal Republic of Germany; F = France; I = Italy; NL = Netherlands; – = below detection limit, i.e. less than 0.005 %.

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