

OCCURRENCE OF PSILOCYBIN/PSILOCIN IN *PLUTEUS SALICINUS* (PLUTEACEAE)

STEPHEN G. SAUPE

Department of Botany, University of Illinois, Urbana, Illinois 61801

The development of blue color in a basidiocarp after bruising is a reliable, although not infallible, field character for detecting the presence of the N-methylated tryptamines, psilocybin and psilocin (1, 2, 8). This color results from the stepwise oxidation of psilocybin to psilocin to a blue pigment (3). *Pluteus salicinus* (Pers. ex Fr.) Kummer (Pluteaceae) has a grey pileus with erect to depressed, blackish, spinulose squamules in the center. It is distinguished from other species in section *Pluteus* by its bluish to olive-green stipe, the color intensifying with age and bruising (10, 11). This study was initiated to determine if the bluing phenomenon exhibited by this fungus is due to the presence of psilocybin/psilocin.

Pluteus salicinus (sgs-230, ILL) was collected on decaying wood in Brownfield Woods, Urbana, Illinois, a mixed mesophytic upland forest. Carpophores were solitary and uncommon. Although Singer (10) reported that this fungus is common in some areas of North America and Europe, it is rare in Michigan (5). All specimens exhibited a marked bluing of bruised regions of the stipe and pileus. Putrescent specimens were olive-green.

A combination of chromatographic and spectral techniques were used to analyze *Pl. salicinus* for psilocybin/psilocin. Freeze-dried carpophores (1.5 g) were pulverized in a mortar with pestle and then extracted with methanol on a rotary shaker at 20 C for 8 h. The methanolic extract was removed from the marc by vacuum filtration, concentrated *in vacuo* at 35 C and the concentrated extract taken up in a minimum volume of 80% methanol. Air-dried carpophores (5 g) of *Psilocybe cubensis* (Earle) Singer obtained from rice cultures (sgs-189, ILL) were treated identically to serve as a reference since authentic samples of psilocybin and psilocin were unavailable.

Methanol extracts of *Pl. salicinus* and *Ps. cubensis* were spotted singly and in admixture on Whatman 3MM sheets and developed (ascending) in several solvents (see TABLE I). Indoles were visualized

TABLE I
PAPER CHROMATOGRAPHIC (WHATMAN 3MM, ASCENDING) ANALYSES OF
Pluteus salicinus AND *Psilocybe cubensis* EXTRACTS

	$R_f \times 100$ (color with Ehrlich's reagent*)		
	1	Solvents** 2	3
<i>Pl. salicinus</i> (sgs-230)	72 (BP) 42 (RV)	44 (BP) 07 (RV)	85 (BP) 05 (RV)
<i>Ps. cubensis</i> (sgs-189)	72 (BP) 43 (RV)	44 (BP) 07 (RV)	85 (BP) 05 (RV)
psilocin***	75 (BP)	50 (BP)	86 (BP)
psilocybin***	44 (RV)	04 (RV)	05 (RV)

* Color abbreviations: BP, bluish-purple; RV, reddish-violet.

** Solvents: 1. *n*-butanol:acetic acid:water (12:3:5); 2. water-saturated *n*-butanol; 3. *n*-propanol:1.0 N NH_4OH (5:1).

*** Literature values from Ott and Guzmán (9).

with Ehrlich's reagent (13). Psilocin produces a bluish-purple color and psilocybin a reddish-violet color that eventually changes to purple with this reagent.

Psilocin and psilocybin were isolated from the *Pl. salicinus* extract by preparative paper chromatography (PC) on Whatman 3MM sheets developed ascendingly in solvent 3 and by preparative thin-layer chromatography (TLC) on silica gel G thin-layer plates (1,000 μ ; 20 $\times$ 20 cm) developed in benzene: methanol: 5% ammonium hydroxide (10: 15: 2), respectively. A small strip cut from the edge of a paper chromatogram and an uncovered edge of a thin-layer plate were sprayed with Ehrlich's reagent to visualize indoles. The appropriate bands [$R_f \times 100$: psilocybin, 05-10 (TLC); psilocin, 82-85 (PC)] were removed, eluted with methanol (80%) on a rotary shaker for 4 h, vacuum filtered and concentrated *in vacuo* at 35 C to dryness. The extracts were dissolved in a minimum volume of methanol, and ultraviolet spectra (UV) were determined with a Coleman 124 double-beam spectrophotometer.

Chromatographic analyses showed that *Ps. cubensis* and *Pl. salicinus* extracts contained two Ehrlich-reactive compounds with R_f values and color reactions consistent with those of psilocybin and psilocin (TABLE I). Co-chromatography of these extracts indicated that the compounds in these two fungi were identical. The UV spectral data for these compounds (λ max: psilocin, 293,282,267,260; psilocybin, 291,278,267,227) were identical to those of psilocin and psilocybin (4, 7). Although quantitative determination of these compounds

was not made, visual examination of the size and intensity of spots on chromatograms indicated that psilocin occurred in greater concentration than psilocybin in *Pl. salicinus*.

These data indicate that psilocybin and psilocin are synthesized in carpophores of *Pl. salicinus*. This represents the first reported occurrence of 4-oxygenated indole alkaloids in the Pluteaceae. According to Singer's (10) classification, these compounds have previously been isolated from agarics in six genera representing four families: Bolbitiaceae (*Conocybe*, *Pholiotina*); Coprinaceae (*Copelandia*, *Panaeolus*); Cortinariaceae (*Gymnopilus*) and Strophariaceae (*Psilocybe*) (3, 6, 8, 12).

The widespread distribution of psilocybin and psilocin in numerous unrelated species suggests that these compounds are not valuable chemosystematic markers at the family level or above. Benedict et al. (1) predicted that the relatively simple biogenetic pathway and uncomplicated structures of these compounds precluded a restricted distribution. Although they are of apparently limited systematic value for higher taxa, these compounds do appear useful for circumscribing infrafamily taxa. For example, the section *Caerulescentes* in the genus *Psilocybe* is characterized by production of psilocybin/psilocin (11). Further, Singer (10) recognizes a non-bluing (perhaps non-psilocybin/psilocin synthesizing) variety of *Pl. salicinus* (var. *achloes* Sing.).

Since *Pl. salicinus* contains hallucinogenic compounds it should be added to the list of psychoactive fungi occurring in North America.

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