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

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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 Erhlich's reagent*)		
	Solvents**		
	1	2	3
Pl. salicinus (sgs-230)	72 (BP) 42 (RV)	44 (BP) 07 (RV)	85 (BP) 05 (RV)
Ps. cubensis (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 Erhlich'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 × 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 Erhlich'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 Erhlich-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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