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Occurrence of psilocybin and psilocin in *Psilocybe pseudobullacea* (Petch) Pegler from the Venezuelan Andes

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Abstract

Using thin-layer chromatographic and spectroscopic (UV) methods two *Psilocybe* species from the Venezuelan Andes were analysed for the hallucinogens psilocybin and psilocin. These species are: *P. montana* (Pers. ex Fr.) Kumm. and *P. pseudobullacea* (Petch) Pegler. Both hallucinogens were found in *P. pseudobullacea*, while *P. montana* was found to be exempt of these compounds.

Keywords: Andes; Fungi; Indoles

1. Introduction

In recent years recreational use of hallucinogenic mushrooms has been reported in the Venezuelan Andes, mainly in Mérida State (Llorca-Izquierdo, 1972; Guzmán, 1978). The species used for this purpose was identified as *Psilocybe subcubensis* Guzmán, containing psilocybin (4-phosphoryloxydimethyltryptamine) and psilocin (4-hydroxydimethyltryptamine) (Llorca-Izquierdo, 1972). The genus *Psilocybe* in the Venezuelan Andes comprises 6 species (Guzmán, 1983): *P. andina* Guzmán, *P. argentina* (Speg) Singer, *P. coprophila* (Bull. ex Fr.) Kumm., *P. montana* (Pers. ex Fr.) Kumm., *P. pseudobullacea* (Petch) Pegler and *P. subcubensis* Guzmán. *P. montana* has already been analysed for psilocybin in Norwegian specimens (Hoiland, 1978) with negative results. In the Venezuelan Andes various *Psilocybe* species are thought to lack hallucinogenic compounds (Guzmán, 1983), but chemical proof for these claims is lacking.

The purpose of the present paper is to report the results of a screening of two *Psilocybe* species from the Venezuelan Andes for psilocybin and psilocin.

2. Material and methods

Twenty-two specimens of both *P. montana* and *P. pseudobullacea* were collected, between 1991 and 1993. *P. montana* was collected on mosses near Mucubaji Lake at Sierra Santo Domingo, Mérida

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State, a mixed mesophytic upland forest of *Pinus* with *Espeletia* (Frailejón), while *P. pseudobullacea* and *P. subcubensis* were found on cow dung in El Chorotal, near La Carbonera, Mérida State. Voucher samples of all collected specimens have been deposited in the Ruiz Terán Herbarium (MERF), Faculty of Pharmacy, University of the Andes, and duplicates were sent to the Institute of Ecology (XAL) at Xalapa, México.

A combination of chromatographic and spectral techniques were used to analyse the *Psilocybe* species for psilocybin and psilocin. Carpophores (12 g per species) were cleaned manually, lyophylized, pulverized in a mortar and extracted with methanol on a rotary shaker at 22°C for 1 day. The methanolic extract was removed from the marc by vacuum filtration, and the residue was extracted again with MeOH. This procedure was repeated three times. The solution was concentrated in vacuo at 35°C and the concentrated extracts taken up in a minimum volume of 80% methanol. Carpophores (8 g) of *P. subcubensis* were treated identically to serve as a reference since authentic samples of psilocybin and psilocin were unavailable.

Methanol extracts of *P. montana*, *P. pseudobullacea* and *P. subcubensis* were spotted singly and in admixture on six preparative, 0.5–1.0-mm, silica G gel, TLC plates (20 × 20 cm) and developed (ascending) in several mobile phases: (1) *n*-butanol/acetic acid/water (12:3:5) and (2) *n*-propanol/1.0$_N$ NH_4OH (5:1). The indole derivatives were visualized with Ehrlich's reagent and under UV light (254 nm and 366 nm). The zones of interest were scraped off, 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 their UV spectra were measured using a Coleman 124 double-beam spectrophotometer.

3. Results and discussion

During TLC analysis psilocin produced a bluish-purple color and psilocybin a reddish-violet color that eventually changes to purple with Ehrlich's reagent, while UV light (254 nm) showed both indole compounds were visible as obscure bluish-purple spots. Chromatographic analyses showed that *P. pseudobullacea* and *P. subcubensis* extracts contained two Ehrlich-rective compounds with R_f values and color reactions consistent with those of psilocybin and psilocin (Table 1). Co-chromatography of these extracts indicated that the compounds in those two fungi were identical. *P. montana* lacks indoles and their results agree with the data obtained by Hoiland (1978). The values obtained for *P. pseudobullacea* and *P. subcubensis* are similar to those reported in other *Psilocybe* species (Ott and Guzmán, 1976; Koike et al., 1981; Stijve and Kuyper, 1985).

The UV spectral data for these compounds were identical to those of psilocybin and psilocin (Hoffman et al., 1958, 1959; Koike et al., 1981; Saupe, 1981): psilocybin UV λMax(MeOH) nm: 221, 227,

Table 1
TLC analyses of *Psilocybe montana*, *P. pseudobullacea* and *P. subcubensis* extracts

Species	R_f × 100 Solvents		Color with Ehrlich Reagent[a]	UV Color[a]
	n-butanol/acetic acid/water	n-propanol/1.0$_N$ NH_4OH		
P. montana	—	—	—	—
P. pseudobullacea	75	86	BP	+ OBP
	44	05	RV	+ OBP
P. subcubensis	72	85	BP	+ OBP
	42	05	RV	+ OBP

[a]Color abbreviations: BP, bluish-purple; RV, reddish-violet; OBP, obscure bluish-purple.

268, 279, 290; psilocin UV λMax(MeOH) nm: 222, 260, 267, 282, 293. Although a quantitative determination of these compounds was not made, visual examination of the size and intensity of the spots on the chromatograms indicated that psilocin occur in greater concentration in *P. pseudobullacea*, while in *P. subcubensis* psilocybin was more abundant. Gartz (1989) reported highest amounts of psilocin in *P. cubensis* (Earle) Singer. According to several authors (Christiansen et al., 1981; Jokiranta et al., 1984; Gartz 1987, 1989; Kysilka and Wurst, 1990), psilocybin is the major constituent in *Psilocybe* species.

These data indicate that psilocybin and psilocin are synthesized in carpophores both of *P. subcubensis* and *P. pseudobullacea*. This represents the first reported occurrence of 4-oxigenated indole alkaloids in *P. pseudobullacea*. According to Guzmán (1983), these compounds have previously been isolated from 66 *Psilocybe* species. These species grows on different substrats: on horse, cow, rabbit dungs, rich soils, grassy soil, mosses, clay soil and humus.

P. subcubensis occurs widely in Mérida State, where young people use it for recreational purposes but *P. pseudobullacea* is less abundant. However, since *P. pseudobullacea* contains hallucinogenic compounds it should be added to the list of psychoactive organisms occurring in the Venezuelan Andes.

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