

Enhancement of Indole Alkaloids Produced by *Psilocybe cubensis* (Earle) Singer (Agaricomycetidae) in Controlled Harvesting Light Conditions¹

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Abstract

Different variables including species, strain, glucose and ammonium succinate concentration in the growth medium, pH, temperature, timing and oxidation have been accounted for the Psilocin (PC) and Psilocybin (PB) content in the "Magic Mushrooms"(MMs). These are but some of the variables in a constellation of factors that complicate consistency in the production of PC and PB. In an attempt to study the effect of light on chemical constitutions, some samples were kept in dark, some samples were kept in dim lighting, whereas others were exposed to natural but indirect light. After picking and drying Mushrooms, a simple one-step extraction involving homogenization of the dried fruit bodies of fungi in chloroform and derivatization with MSTFA was performed. The samples were then analyzed by gas chromatography–mass spectrometry. This investigation shows that the relative PC and PB content of the mushrooms is highly dependent on the light condition. This variation could amount to 100 fold of active components (PB and PC) in samples harvested in dark condition compared to the samples harvested in indirect light condition. Therefore it can be concluded that UV light may have destructive effect on the active components, which would readily explain why sun-struck collections are less potent

Keywords: *Psilocybe cubensis*; psilocybin; psilocin; gas chromatography–mass spectrometry (GC–MS); MSTFA; Psychedelic fungi;

1. Introduction

Psilocybe cubensis is a species of psychedelic mushroom whose primary, pharmacologically active constituents are Psilocybin (PB) and Psilocin (PC). It belongs to the Strophariaceae family with a grey to violet-gray color, and bruise bluish/purplish when crushed or dried (**Bluing Reaction**). The caps are planar when fully mature, and their gills are adnate (horizontally attached to the stem) to adnexed (slightly indented at the attachment point) depending on the subspecies. The gills are closely spaced and drop dark purple spores (1)(Rafati et al., 2009).

The psychedelic effects of some species of the genus *Psilocybe* were first described by Wasson in 1957 (2). Two hallucinogenic components of the tryptamine type, PB (**Psilocybin or 4-phosphoryloxy-*N*, *N*-dimethyltryptamine**), the main psychotropic compound, and PC (**Psilocin or 4-hydroxy-*N*, *N*-dimethyltryptamine**) were then isolated by Hofmann and his colleagues (3). They have structural similarity to the neurotransmitter serotonin, and their highly hallucinogenic potency is thought to occur from their influence on the serotonergic nervous system. (4)

The content of active components of the mushrooms have been reported nearly the same in few references (i.e. 0.75% (14), 0.74% (5)). Different strains have shown a noticeable change in the

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content (from 0.48% in Amazonian strains to 0.65% in Mexican strain (1)). Bigwood and Beug found a fourfold variation in potency in cultivated specimens and up to a tenfold variation in potency from wild specimens (6). Mushrooms grown indoors seem consistently more potent than field-collected specimens, probably due to nutritional factors (precursors) and protection from the damaging effects of ultraviolet radiation (1). Gartz found that raising tryptamine concentrations of cow dung and rice media by 25 millimolars directly increases affected the potency of *P. cubensis* mycelia, specifically in PC content: from 0.09% to 3.3% of the dried mass. (PB content was actually depressed, but not nearly on the same order of magnitude).

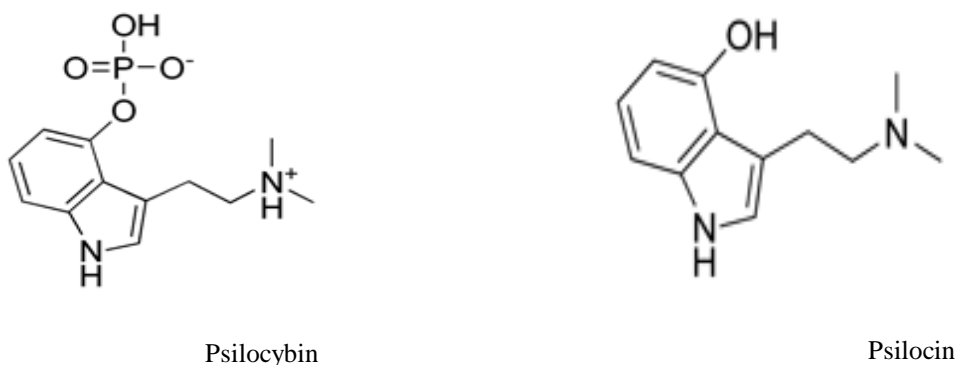


Figure 1. Chemical structures of Psilocin and Psilocybin

Gartz also made the interesting observation in the culturing of *P. cubensis* mycelium that the raising of malt sugars to more than 10% resulted in the complete suppression of PB production (7). These observations underscore that the nutritional content of the substrate significantly affects potency. Additionally, Gartz found that younger specimens are generally more potent than mature ones, an observation many users have also made. These are but some of the variables in a constellation of factors that complicate consistency in the production of PC and PB. Despite the suggestion by P. Stamets (1) that ultraviolet radiation from the Sun markedly lessens the potency of PB-containing mushrooms, but we have not found any reporting the effect of light on the PC/PB content.

The determination of PC and PB has often been studied by thin-layer chromatography (1,5,7,12-13, 15-17), high performance liquid chromatography (HPLC) with ultraviolet detection (1,5,7,12-13,18-19), paper chromatography (14), electrochemical detection (13,18-19) and fluorescence detection (19), gas chromatography (GC) with flame ionization detection and infrared spectrometry (20), ion-mobility spectrometry (21), and gas chromatography-mass spectrometry (GC-MS) with/without trimethylsilyl derivatization (7-8,15-17, 20-21). In this study we have evaluated the effect of cultivation light on the relative amount of PB/PC using GC-MS technique.

2. Sample Preparation:

The mycelium was grown on pasteurized compost consisted of wheat straw, chicken manure and gypsum. During the mycelium growth, temperature and relative humidity were maintained at 25 °C and 95% Rh respectively. After colonization of compost with mycelium, substrate was covered with casing soil. After 10 days case-run the ambient conditions were changed to initiate fructification. A temperature of 18-20°C was maintained during harvest period. In an attempt to study the effect of light on chemical constitutions, some samples were kept in dark, some samples were kept in dim lighting, where as others were exposed to natural but indirect light. Mushrooms were picked and dried for further studies.

3. Sample Analysis:

3.1. Reagents: Chloroform (A.R.). (*N* Methyl- *N*-(trimethylsilyl)-2, 2, 2-trifluoroacetamide (MSTFA)) / obtained from E. Merck (Darmstadt, Germany).

3.2. Equipment: GC/MS (HP 6890/5973), centrifuge, pestle and mortar.

3.3. Procedure for Pure Mushrooms:

Parts of fungal fruit bodies (cap and stem) were placed in oven overnight at 50 °C and grounded to a fine powder in a mortar. 70 mg of powdered samples were accurately weighed and extracted with 1 ml chloroform in an ultrasonic bath for 1 hr. The samples were then Centrifuged at 10,000 rpm for 10 min and filtrated through a cotton filter, resulting a clear solution. The resulting solution was pipetted into a GC vial and evaporated to dryness at 50°C under a gentle stream of nitrogen. Each residue was dissolved in 30 µl MSTFA and heated for 30 min at 70°C. After cooling, 1 µl of the sample was directly used for GC–MS analysis. (21)

4. Results

A mass spectrum of MSTFA derivative of PC/PB is shown in Figs. 3. The drift time for the PC/PB was 17.60 minutes. The dephosphorylation of PB to PC in vivo has been well documented (9,22-23) and is thought to account for most or all of its central nervous system activity (22).Casale described the rapid formation of PC after complete dephosphorylation of PB by heating the dilute acetic acid extract (8). In the present study, reaction of MSTFA with PC and PB results in the same silonized derivative (Bis Trimethylsilyl Psilocin) following complete dephosphorylation of PB , probably due to the heating (Fig 3).

Relative content of PC/PB in dark and light cultivated mushrooms (Fig.2,4-5)can then be calculated using the AUC as an indirect index of the content (Table 1). The statistical analysis (ANOVA) of the results show that content of PC/PB was significantly ($p < 0.001$) different among the samples (Table 2).The results of the LSD analysis shows that The maximum PC/PB content was observed in the Dark sample while minimum was observed in the Light sample. (Table 3).

5. Discussion

PB/ PC -containing mushrooms are commonly called "magic mushrooms"(MMs) or simply "shrooms". They are naturally occurring and have been used as a god-like traditional medicine for centuries in the religious ceremonies by shamans in Central and South America. Currently, they have been used extensively for recreational purposes as hallucinogenic substances in various countries in Europe, America, Japan and elsewhere. (10). *Convention on Psychotropic Substances* has defined the PB and PC, and the mushrooms containing these substances as the *Schedule I* drugs, which includes dangerous drugs claimed to create a serious risk to public health, and whose therapeutic value is doubtful.They act on the central nervous system to produce changes in perception, mood, and thinking ability. The effects produced by PC and PB are similar to those produced by LSD and mescaline (11)

In the US, an FDA-approved study supported by "Multidisciplinary Association for Psychedelic Studies" (MAPS) began in 2001 to study the effects of PB on patients with obsessive-compulsive disorder (OCD). MAPS has also proposed studying PB's potential application for the treatment of cluster headaches based on anecdotal evidence presented to them by a cluster headache sufferer.

Different variables including species, strain, glucose and ammonium succinate concentration in the growth medium, pH, temperature, timing and oxidation have been accounted for the PC and PB content in the "Magic Mushrooms"(MMs). For example oxidation, absence of glucose and low

levels of ammonium succinate will all give poor yields of PC/PB production. Whereas, adjustment of pH, temperature and timing reported to increase the PC/PB content (8). These are but some of the variables in a constellation of factors that complicate consistency in the production of PC and PB. To the best of our knowledge, no data on the effect of the light on the relative PC/PB content in *Psilocybe Cubensis* mushroom has been published so far. From the results presented here, the ultraviolet radiation from the Sun could be considered to markedly lessen the potency of MMs.

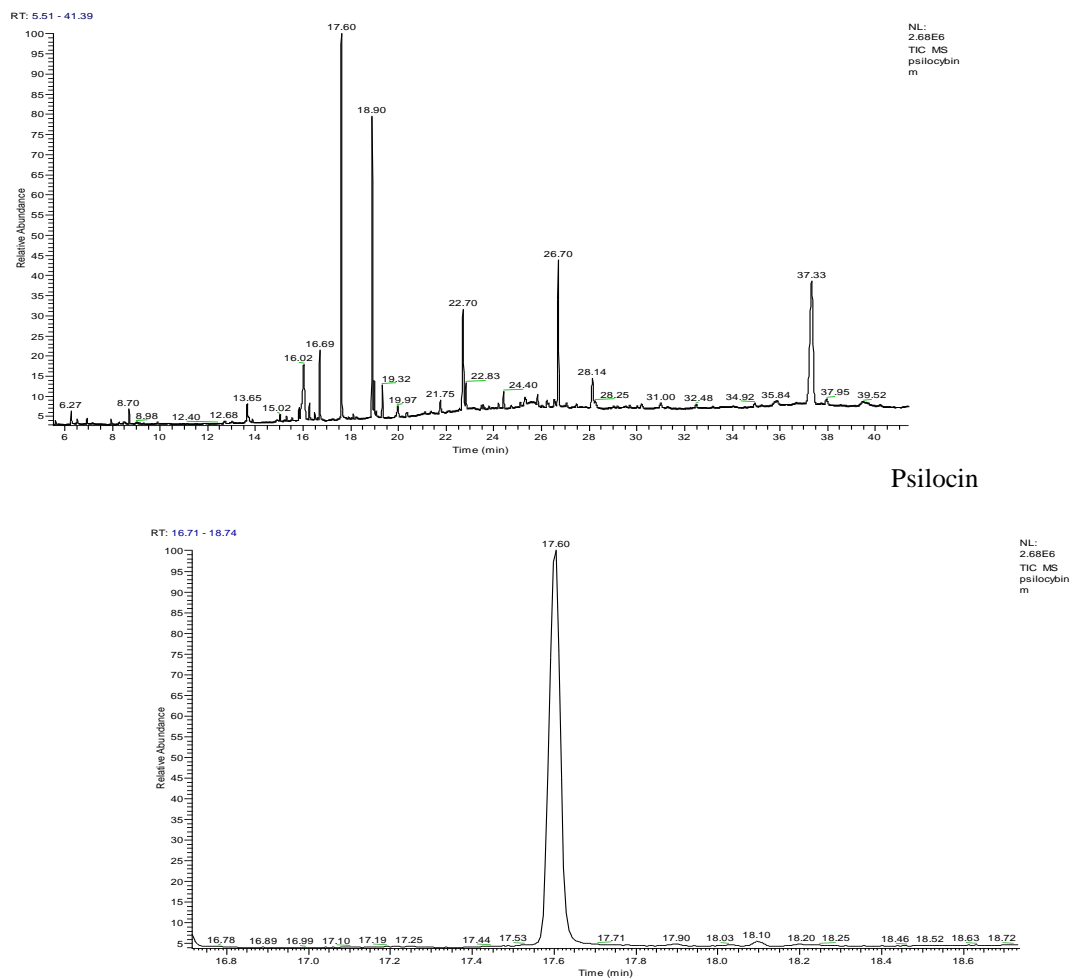


Figure 2: Dark sample Plasmogram of *Psilocybe Cubensis* (DTime =17.60 ms (Pc)) : (A) Total (B) Expanded

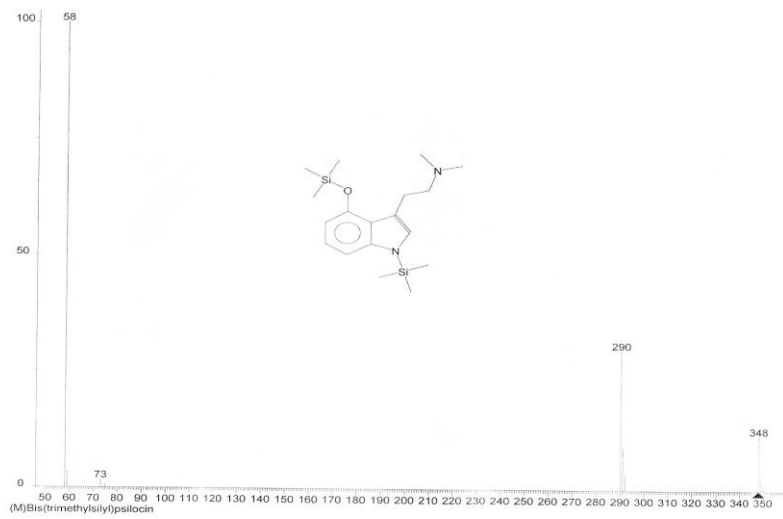


Figure 3. Mass Spectrum of MSTFA derivative of PC/PB

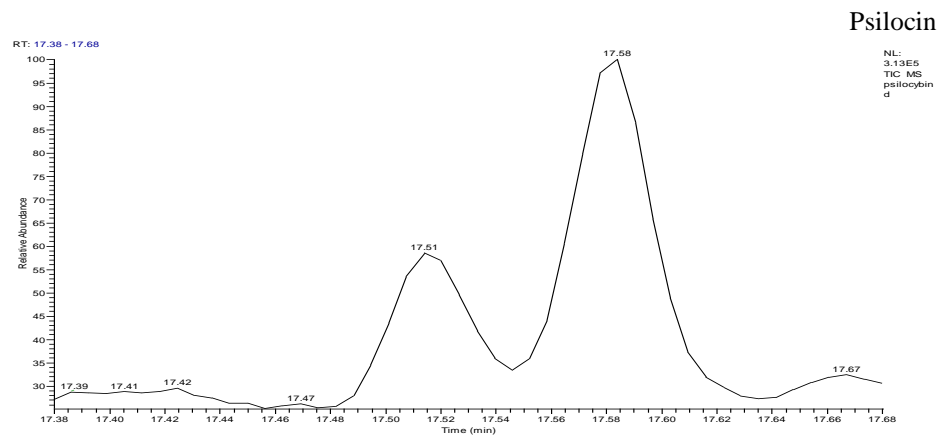


Figure 4: Dim lighting sample Plasmogram of *Psilocybe Cubensis* (DTime =17.60 ms (Pc))

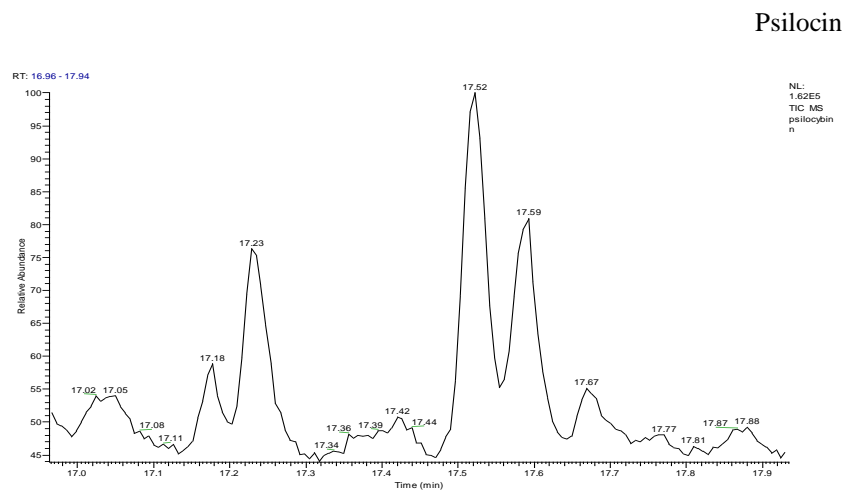
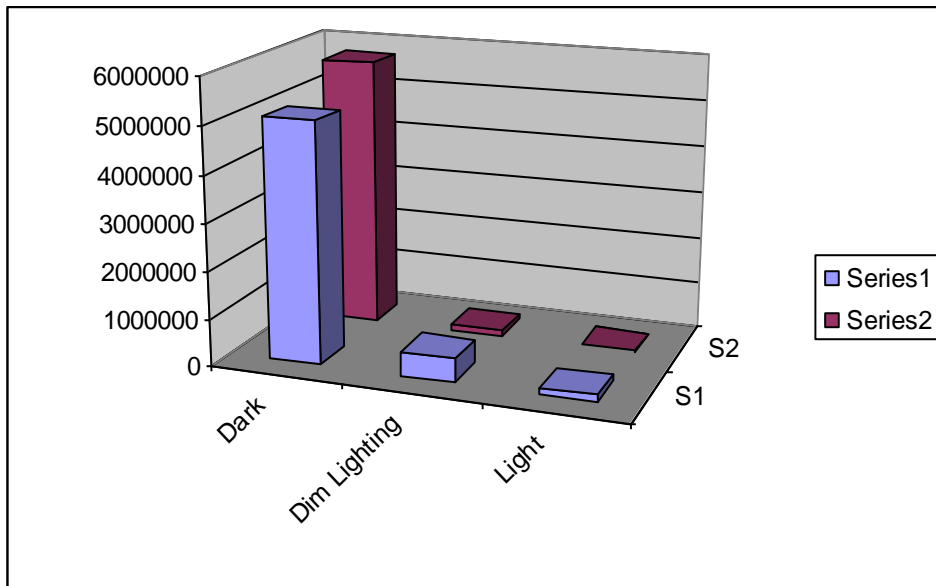


Figure 5: Light sample Plasmogram of *Psilocybe Cubensis* (DTime =17.60 ms (Pc))



Treat (I)	Treat (J)	Mean Difference (I-J)	Std. Error	Sig.
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Groups	Replica 1	Replica 2
Dark	5083237.00	5740283.00
Dim Lighting	505846.40	103905.50
Light	133300.20	17296.11

Table 1. Results of the analysis

		SS	DF	MS	F	P
x * treat	Between Groups	4E+013	γ	1.820 E+013	180.019	.001
	Within Groups	3E+011	ϛ	1.011 E+011		
	Total	4E+013	δ			

Table 2 : ANOVA showing comparison of samples

* The mean difference is significant at the .001 level.

Table 3: Multiple Comparisons of samples (LSD)

Dark	Dim Lighting	5106884.09650(*)	317994.49658	.001
	Light	5336461.88750(*)	317994.49658	.000
Dim Lighting	Dark	-5106884.09650(*)	317994.49658	.001
	Light	229577.79100	317994.49658	.523
Light	Dark	-5336461.88750(*)	317994.49658	.000
	Dim Lighting	-229577.79100	317994.49658	.523

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