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Fungal Hallucinogens: Chemistry, Synthesis, Behavior, Toxicity and Detection Methods in Forensic Perspective

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Abstract

Certain species of the mushrooms contain the Indole Alkaloids such as Psilocybin and Psilocin which exhibit the psychedelic property. These types of mushrooms can be identified by their unique property known as the blue bruising. These alkaloid compounds may cause the hallucinations and other symptoms. The psilocybin and Psilocin are synthesized from the amino acid Tryptophan and resemble the structure of the hormone Serotonin. They also act as an agonist for the 5-HT_{2A} receptors. Intoxication of these compounds can lead to excitation of the nerves which can eventually cause the hallucination, tachycardia, dilated pupils and dysuria in certain cases which may lead to adverse effects. The metabolic product of the Psilocin is psilocin-o-glucuronide which is excreted in the urine within 6-8 hours of ingestion. Psilocybin and psilocin can be analyzed by preliminary color tests with Ehrlich reagent, Marquis reagentand Mandelin's reagent. In this review, brief reports are presented about the chromatographic analysis of these compounds. This articlealso discusses the overview of the psychoactive alkaloids present in certain species of mushrooms.

Key words: Psilocin, Psilocybin, Serotonin, 5 HT 2A receptors, agonist, detection limits.

Introduction

Psilocybin and its active metabolite Psilocin is the prime psychedelic compound in the psychoactive mushrooms such as *Psilocybe, Conocybes, Inocybes*etc[1]. The mushroom producing the Psilocybin and the Psilocin has gained an attention from ancient period. Upon studies Guzzman framed a classification depending on the nature of the compound present in it[2]. The unique characteristic of the mushrooms that produces psilocybin and its active metabolite Psilocinis generally called as the "blue bruising" which may due to their metabolism of action. Because of this unique property, the mushrooms can be easily identified[3]. This bruising property is generally adephosphorylation and an oxidation process, where the dephosphorylation is catalyzed by the PsiP enzyme and oxidation of the Psilocin under the catalysis of the enzyme PsiL present in the mushrooms.

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The mushrooms producing these compounds were later noticed by the scientists and then studied. These compounds were first isolated and identified in 1958 and they were first synthesized in 1959 by Albert Hoffman[4]. Upon studies, the contents vary in range from0.2%- 1% in dry weight[5]. The psilocybin is the major ingested form which is converted into the psilocin by the enzymes present in the body which dephoshorylates the Psilocybin into the active metabolite Psilocin[6]. These compounds present in the mushrooms affect the nervous system and causes neurological effects such as hallucination, auditory hallucination and mental alterations[7]. This is due to their structure which resembles the Serotonin hormone[8]. They differ from each other by functional group, where the Psilocybin contains the phosphoryl group and its active metabolite has a hydroxyl group[9]. Due to their structural similarity, they have higher affinity towards the 5-HT receptors. Their adverse effects are seen within an hour of ingestion. The compounds were metabolized by the body and they are excreted as conjugated compounds. They are eliminated gradually in the urine[10].

The aim of this review article is to bring an updated knowledge on Psilocybin and its active metabolite Psilocin with the historical perspective and their mechanism and metabolism. This also includes the clinical responses to their intake which is staged into three phases. The toxicity of these compounds is also discussed in this article.

Components of Group I Psychoactive Molecules - Psilocybin and Psilocin

Psilocybin and Psilocin are psychoactive molecules that are commonly found in the genus *Psilocybe* predominantly and in other associated species. The ingested form is found to be the Psilocybin which then converted into psilocin by dephosphorylation reaction in the body. This conversion is catalyzed by the enzymes that are generally alkaline phosphatase. These compounds can act very similar to the LSD(Lysergsäurediethylamid) and Mescaline causing the neurological effects[11].

The milestone about the psilocybin and its active metabolite Psilocin was studied by Albert Hoffmann. The Swiss chemist Albert Hoffman initially started to study about the LSD by ingesting it himself during 1930s and for which he was called as Father of LSD. In 1958 Albert Hoffman and his colleagues isolated Psilocybin and trace amounts of Psilocin for the *Psilocybe Mexicana*, which created a mark on psychoactive drugs.This was the first natural phosphorylated compound detected.With references to the study, there was a great development in the understanding of the compound which leads to Psilocybin assisted therapy. Upon the studies, it was found that they resemble the structure and their properties are detailed.

Structure of Psilocybin and its active metabolite Psilocin

The Psilocybin and its active metabolite Psilocin are Tryptamine derived Indole alkaloids isolated from the mushroomsexhibiting the hallucinogenic properties[12]. The psilocybin is also called asO-phosphoryl-4-hydroxy-N, N-dimethyltryptamine and the psilocin is called as 4-hydroxy-N, N-dimethyltryptamine. The Psilocybin and its active metabolite Psilocin have the backbone structure of Tryptophan. Hence they resemble the structure of Serotonin hormone and being an agonist of it[13]. From the Figure1, the structural difference of thecompounds can be noted by the presence of Phosphoryl functional group in Psilocybin and a hydroxyl group at the functional site of Psilocin. The psilocybin when ingested it rapidly dephosphorylated toPsilocin[14].

Physico-chemical Characteristics

The Psilocybin usually tastes like ammonia. The Psilocybin is found in the form of colorless crystals in boiling water and it is sensitive to temperature. Its melting point is found to be 224.0º C. It is fairly soluble in boiling methanol, water and difficultly soluble in ethanol and insoluble in chloroform and benzene[15]. Its molecular weight is found to be 284.27 g/mol[16]. They appear as green crystals when extracted[17]. Psilocin islipid soluble and hence it is diluted by acidified aqueous solution and very slightly soluble in water[18]. Its melting point is found to be 173-176ºC. Its molecular weight is to be 204.3g/mol[19].

Biosynthesis of Psilocybin and its active metabolite Psilocin

The biosynthesized Psilocybin and its active metabolite Psilocin synthesized from the mushrooms

were studied by using radio isotopes. Later, these compounds have been synthesized using dibenzylphosphoryl chloride by Hoffmann which was replaced by Tetrabenzyl pyrophosphate[20]. Later Kargbo initiated direct phosphorylation of Psilocin by using Phosphoryl chloride[21]. The first published synthetic event which included the labelling of radiotracer has explained the derivation of Psilocybin and psilocin by Agurell and Nilsson in 1968 by ^{14}C and ^{3}H[22].

Later Fricke et. al.[23]made a refined biosynthetic pathway by sequencing the genomes of *Psiocybecubensis* and *Psilocybecyanescens*. They used the 4- hydroxy-L-tryptophan as the substrate and identified enzymes that are involved in the biosynthesis. The enzymes were named as PsiD, a decarboxylase enzyme and PsiH a monooxygenase, Psik a kinase that transfer the phosphoryl group and a PsiM an S-Adenosyl dependent Methyl Transferase which mainly catalyzes the terminal Methylation step.

In this synthesis pathway the L-tryptophan is decarboxylated as in the Agurell[20] description leading to the formation 4- hydroxy Tryptamine. The 4-hydroxy tryptaine is further phosphorylated to Norbaeocystin by the action of PsiK enzyme. This Norbaeocystin is then converted to Baeocystin with PsiM enzyme upon methylation, upon further methylation the psilocybin is formed in the presence of PsiM enzyme. Further the dephosphorylation converts the psilocybin to psilocin. The genome and the loci identified by Fricke and the loci is seen in figure.1. The psiR represents the Putative Transcriptional Regulator. The $psiT_1$ and $psiT_2$are the major facilitator type transporter. The psiK has Kinase enzyme activity. The psiH is a P_{450}monoxyenase enzyme locus. The psiM is for the encodation of Methyl transferase enzyme. The psiD is responsible for the decarboxylase enzyme[24].

Figure 1: Map of loci of the Psilocybin and its active, metabolite Psilocin biosynthesis

Mechanism of action

The psilocin, active metabolite targets at the $5HT_{2A}$ receptors. These receptors are adversely expressed in the apical dendrites of the cortical pyramidal cells[25]. In humans, the active compound psilocin is present in a significant amount in the plasma within 20-40 minutes after ingestion[26]. The maximum concentrations were found to reach in 80-100 minutes. The effects are completely eliminated within 4-6 hours. The elimination half-life of Psilocybin in the plasma is found to be 160 minutes and the active metabolite Psilocin half-life is found to be 50 minutes[27, 28]. The pharmacokinetic and forensic studies on the compounds revealed that psilocin is mostly (80%) eliminated as psilocin-O-glucuronide as a result of conjugation process[29]. The conjugated compound further undergoes enzymatic hydrolysis extends their detectability in the urine samples[30]. The figure 2 describes the mechanism of action of Psilocin.

The mechanism of action is by activation of prefrontal network and glutamate release by the intoxication of compound. The compound may increase the extracellular glutamate levels by prefrontal cortex through the stimulation of post synaptic serotonin especially $5HT_{2A}$ receptors that are located on the large glutamatergic pyramidal cells which are seen in the cortical layer V and layer VI layer of cortical cells, where layer V projects to the neurons.

When the glutamate is released it leads to the activation of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolle propionic acid) and NMDA (N-methyl-D-aspartate) receptors. The psilocin can also directly activate the 5 HT_{2A} receptors. This activation is thought to ultimately lead to increase of brain derived neurotrophic factor (BDNF).

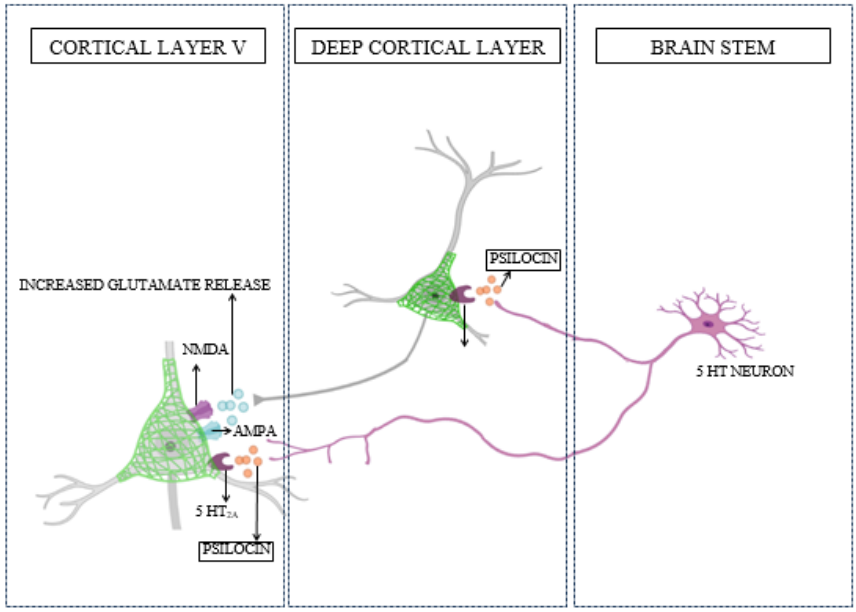

Figure 2: The mechanism of Psilocin[31]

The mechanism of action is very closely related with other compounds such as dopamine, epinephrine and other bio amines. The psilocybin increases the 5-HT_{2A} receptors in thepost synaptic membranes and stimulates neural excitatory effects[32]. The dissociation constant (K_i) of psilocybin is >10,000nM whereas the psilocin has (K_i) 107.2 nM which means the psilocin can bind more strongly to the receptor. The pharmacokinetics of Psilocybin and Psilocin is well established[31]. After ingestion, the rapid dephosporylation of Psilocybin occurs under acidic environment of the stomach or by alkaline phosphatase (and other nonspecific esterase) in intestine andkidney. This may cause to generate the Psilocin and the Psilocin in turn which can easily cross the blood brain barrier. The inhibition of the enzyme alkaline phosphatase can cause competitive substrates such as β- glycerophosphate which prevents the symptoms of intoxication.

Upon further metabolism, the Psilocin is demethylated and deaminatedoxidatively by the liver. This process is catalyzed by the Monoamine Oxidase enzyme (MAO) or by an alcohol dehydrogenase. The intermediate formed was 4-hydroxyindole-3-acetaldehyde which may yield 4-hydroxy-indole-3-acetic acid, 4- hydroxy-indole-3-acetaldehyde and 4-hydroxytryptophol[33]. The Psilocin may cause the competitive inhibition of the MAO. Italso may metabolize the Serotonin which in turn elevates their levels and simultaneously decreases the 5-HIAA[34].It can also undergo minor oxidative pathway which leads to the formation of the O-quinone or Immunoquinone compounds. They are oxidized either enzymatically with hydroxyindol oxidase enzymes such as ceruloplasmin etc., or non-enzymatically with Fe^{3+}.

In the analysis of the serum sample obtained after 5 hours of intoxication, it showed that 80% of the Psilocin in the conjugated form of Psilocin-O-glucuronide and it is eliminated in the urine[35].This glucuronidation of the hydroxyl group is the important detoxification step. The extensive glucuronidation is achieved by the UDP Glucuronysl Transferase (UGT)1A10 in the small intestine. The UGT1A9 is the main contributor to the glucuronidation when it is absorbed in the circulation.

Toxicity and LD_{50}

As discussed in the clinical response, the Psilocin binds to the 5 HT_{2A} receptors and causes the neuronal excitation and exhibits the symptoms. In the case of the toxic effects, the hyper activation of the receptor causes the symptoms such as drowsiness, increases in mood changes, altered state of consciousness, weakness, dizziness, nausea and vomiting in humans. When the dose exceeding 15mg may create severe

effects such as acute agitation, shift in emotions, sensory modalities and visual perceptions. Upon the study of the lethality of these compounds, the LD_{50} for animals has been reported in Table 1[32].

Table 1: Toxicity level of Psilocybin

Animal Model	Route of administration	LD_{50}
Mouse	Intraperitoneal	196 mg/kg
	Intravenous	74 mg/kg
Rat	Intravenous	75 mg/kg
Rabbit	Intravenous	7 mg/kg

Forensic analysis from bodily fluids and hair

In the analysis of the bodily fluids, generally the active metabolite Psilocin is present rather than Psilocybin. During the analysis the blood samples, the samples were centrifuged and the plasma is collected for the analysis. The proteins present in the plasma were precipitated by the addition of methanol or by adding 20% of PEG 6000 on ice. In some cases, to prevent the oxidation of Psilocin, ascorbic acid may be used and this is essential to pre column in the HPLC separation of the compounds[36].

Once after the separation of precipitated proteins at a slight acidic condition, the solid phase extraction is performed. In the cation exchange sorbent separation, the precipitated proteins were adjusted to the pH 6.8 with lithium acetate where it aids in protonating the compound which is eluted with methanol[37]. In the reverse phase, Bond Elut C18 cartridges were used. The blood and urine samples up to 2-1 ml can be treated with magnesium carbonate buffer and at a pH of 9.3. Then, the product is eluted with methanol and acetic acid[38]. The enzymatic reaction with glucuronidase will yield a relatively high concentration of psilocin from plasma, serum and urine samples[39].

In detecting the Psilocin and Psilocybin in the bodily fluids, the capillary electrophoresis is a common method in qualitative and quantitative analysis. Their limit of detection is found to be a 13 ng/ml of urine (direct measurement) and 7 ng/ml of urine (with a system of preliminary Sequential Injection -SPE)[40]. When it comes to the Gas chromatography along with spectrometry generally electron ionization mode is applied in the detection of the Psilocin and Psilocybin[41]. Among these techniques, it is believed that the more prominent detection of Psilocin and Psilocybin is achieved by Chemiluminescence methods[41].

In the cases of forensic evaluation, the DNA study plays a major role. Generally, the Psilocin and the Psilocybin were determined using the PCR (polymerase chain reaction) techniques specifically by the Amplified Fragment Length Polymorphism. One of the prominent screenings is found to be the Internal Transcribed Spacer region analysis which leads a great analysis method by reaching the relationships between the genius and species. In a recent study, there was a method developed for the detection of Psilocin from hair by using LC-MS/MS method. Initially 10 mg of cut hair is taken in 1 ml of acid methanol in the presence of Psilocin-d_{20} for 2 hours at 4^0C. The chromatographic separation was performed with reverse phase column HSS C18 with a gradient elution of 8 mins. The estimated limit of detection was 0.4 pg/mg. When a person is exposed to these compounds repetitively, then the concentration would be 2.5 pg/mg for segment of 0-1 cm, 4.4 pg/mg for segment 1-2 cm and 5.4 pg/mg for segment 2-3 cm[42].

Detection techniques

There are preliminary test that helps to detect the presence of the psilocybin and the psilocin. The test includes the reagents such as the Ehrlich reagent and the Marquis reagent which are commonly used in identifying the Indole compounds. This color test is only to detect the presence of compounds further for confirming the compounds, chromatography is required.

Ehrlich test

In the Ehrlich test, p-dimethylaminobenzaldehyde along with methanol under acidic condition with the help of concentrated orthophosphoric acid leads to the electrophilic substitution reaction producing grey violet – violet color[43].

Marquis test

In the Marquis test, 40% formaldehyde with glacial acetic acid under acidic condition maintained

by concentrated sulfuric acid causes the green-brown color which indicates the possible presence of Psilocin[44].

Mandelin's test

The reagent is composed with 1% ammonium vanadate in sulfuric acid which determines a wide range of colors where reacting with psilocybin produces a green color.

Thin Layer Chromatography (TLC)

In the TLC, the solvent system consists of n-Butanol: acetic acid: water is used. For visualization, the UV and spray agents can be used. In the UV visualization, the compounds can be visualized at 254 nm and 365 nm and appears as dark blue spots. There are two spray reagents such as Ehrlich Reagent and Paradimethylaminocinnamaldehyde. The paradimethylaminocinnamaldehyde produces blue and Ehrlich reagents produce violet – grey violet color. The detection limit of paradimethylaminocinnamaldehyde is 20ng of Psilocybin and 10ng of Psilocin. The retention factor (R_f) is found to be 34 for Psilocybin and 59 for Psilocin[43].

Detection limits

In the detection of Psilocybin and Psilocin, there are various techniques involved and each of the techniques has its own detection limits. Table 2 explores the overall techniques used and their detection limits.

Table 2: Overall techniques and their Detection limits[45]

TECHNIQUES		DETCTION LIMITS	
		PSILOCIN	PSILOCYBIN
Ion mobility spectrometry		–	1.4×10^{-4}
HPLC	UV Absorption	3.9×10^{-6}	3.5×10^{-6}
	Fluorescence	9.7×10^{-8}	1.7×10^{-6}
	Electrochemical	3.7×10^{-8}	1.7×10^{-6}
	ESI-MS	6.2×10^{-7}	–
GC-MS		4.9×10^{-4}	3.5×10^{-4}
TLC		1.5×10^{-4}	1.1×10^{-4}
Capillary Electrophoresis		–	3.1×10^{-6}
Chemiluminescence		9.0×10^{-10}	3.5×10^{-10}

Conclusion

The psychedelic compounds present in magic mushrooms maycause neurological discomforts in humans because of their oxidizing behaviour. The fungal hallucinogenic compounds, such as, Psilocybin and Psilocin can play a major role in the therapeutic applications of the neuronal disorders. These compounds seemed to be efficient in the therapeutic studies and they also have an effective response in treatments. Furthermore, they also possess psychedelic effects because of which they can also cause toxic effects. Upon the action, the molecules increase the 5HT 2A receptors at the synaptic membranes. This leads to neuronal excitation and leads to hallucination in severe cases. The metabolic fate of the psilocin is eliminated as psilocin-O-glucuronide. Due to the higher stability,they may extend the time of detection in the urine samples. These can be detected using various techniques such as capillary electrophoresis, gaschromatography, HPLC and also with chemiluminescence technique. Biosensors can be used in the detection of the mycotoxins and ergot alkaloids. Future studies focusing in these perspectives may help in better detection of these compounds in the human samples and also in the forensic analysis.

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