# Bioactive Alkaloids from Fungi: Psilocybin 18

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## Contents



**Abstract**

Studies on bioactive alkaloids have gained considerable importance in recent years and have been given prominent position in the field of medicine, both with respect to their biological activity and role played in the introduction of new pharmaceuticals. The biotechnological approach has further enhanced their industrial applications. Some specific groups of bioactive alkaloids have been ignored and not been given much attention because of various reasons, including their legal status. The tryptophan-based indole alkaloids, psilocybin and psilocin, are the best examples in this category which have been identified as most attractive bioactive alkaloids in large number of mushrooms, especially of the genus *Psilocybe*, but waiting since long for more control studies to ascertain

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their therapeutic role in some other conditions, apart from psychiatry. These alkaloids have long history of association with mankind for their use as sacraments in religious ceremonies along with medical and recreational purposes and thus need more attention to explore their therapeutic role.

**Keywords**
Hallucinogenic mushroom • magic mushroom • neurotropic fungi • psilocin • psilocybe • psilocybin • psychedelic

**Abbreviations**

| | |
|---|---|
| 5HT | 5-Hydroxytryptamine |
| CNS | Central Nervous System |
| DMACA | Dimethylaminocinnamaldehyde |
| DMT | Dimethyltryptamine |
| GC/MS | Gas chromatography coupled with mass spectrometry |
| INCB | International Narcotics Control Board |
| LSD | Lysergic acid diethylamide |
| OCD | Obsessive-compulsive disorders |

## 1 Introduction

Alkaloids have occupied a unique and prominent position in both science and culture due to their diversified source of availability, biochemical properties, and applications. It represents one of the largest groups of natural products which almost all phyla are capable to synthesize at any stage of their life cycle. Fungi (mushrooms), in particular, are able in common along with the higher plants and bacteria to produce alkaloids. The indoleamines (four-substituted compounds) found in mushroom have a long history of investigation and research topic for various laboratories and institutions all over the world, and as a result, more than five decades back in 1958, Dr. Albert Hofmann, the discoverer of LSD, further reported two most fascinating bioactive alkaloids – Psilocybin and Psilocin – from mushroom *Psilocybe mexicana*. Both alkaloids are derived from L-tryptophan. Later, he also developed a synthesis technique for the production of psilocybin and psilocin [1, 2]. The chemical synthesis of psilocybin opened a new vista which ended with the introduction of first synthetically developed psilocybin under the brand name of "Indocybin" marketed by Sandoz for experimental and psychotherapeutic purposes in the 1960s. Psilocybin, also known as psilocybine, is classified as neurotropic or psychedelic indole alkaloid of the tryptamine family, with mushrooms of the genus *Psilocybe*, commonly known as hallucinogenic mushroom or magic mushroom [3] as the most common source. Various species of genus *Psilocybe* are found all over the world in most terrestrial (land) biomes, and more than 100 species have been so far identified and reported, but their potential to produce psilocybin is still to be determined because in some

species the level of psilocybin and psilocin are reported as nondetectable. *Psilocybe cubensis* is considered as the most widely known species capable of producing psilocybin and psilocin in considerable quantity, though originally both alkaloids were isolated from *Psilocybe mexicana* [4]. The capability to produce psilocybin and psilocin varies among species on dry weight basis of mushroom and dependent upon various biochemical factors and the parts (mycelium or fruit bodies) of the mushroom used for processing and extraction. The fruit bodies have more alkaloid content than the mycelium. The rate limiting factors or the critical steps in the analysis of psilocybin and psilocin in mushrooms are the techniques used for the extraction, separation, and purification. The biotechnological approach and its utilization have greatly affected the production capacity of these alkaloids using submerged fermentation technology by manipulating media composition and growth parameters as well as with advanced extraction and purification techniques.

The chemistry of psilocybin and psilocin is not complex. Chemically, psilocybin is 4-phosphoryloxy-*N*,*N*-dimethyl-tryptamine, whereas psilocin is 4-Hydroxy-*N*, *N*-dimethyl-tryptamine. Psilocybin is regarded as prodrug which is finally converted into psilocin – the pharmacologically active form of the drug in the body by a dephosphorylation reaction and thus acts as a partial agonist at the serotonin receptor (5-$HT_{2A}$), with high affinity in the brain where it mimics the effects of serotonin (5-HT). Based on this mechanism of action, psilocin is classified as a 5-$HT_{1A}$, 5-$HT_{1D}$, 5-$HT_{2A}$, and 5-$HT_{2C}$ agonist, and accordingly its psychotomimetic effect can be blocked by 5-$HT_{2A}$ antagonists, such as ketanserin and risperidone in a dose-dependent pattern [4].

In view of its nature and classification as a neurotropic or psychedelic, psilocybin is capable of acting on CNS, crossing the blood–brain barrier and consequently altering perception, mood, consciousness, cognition, and behavior in a dose-dependent fashion. The use of *Psilocybe* (mushroom) and its two constituents, psilocybin and psilocin, remains very popular in the USA and Europe till the mid-1960s for its recreational and therapeutic purposes when finally in 1968 all three (mushroom and its two alkaloids) were declared illegal in the USA, and as a result psilocybin and psilocin were added to the new "Drug Abuse Prevention and Control Act of 1970" (commonly known as Controlled Substance Act), which came into force in 1971 and both listed in Schedule I drugs under the United Nations Convention on Psychotropic Substances. Despite all these, research actives continued till mid-1970s, and research activities continued to be halted throughout the 1980s and 1990s due to strict governmental control worldwide. The restrictions imposed worldwide during these periods have greatly affected the research activities on psilocybin and psilocin, preventing researchers to investigate the biological activities to ascertain biochemical and pharmacological properties of these two psychedelic indole alkaloids. So far, psilocybin has not been recognized as drug but has been extensively investigated in the treatment of various psychological disorders. However, findings of various investigations are not only highlighting the possible role and importance of psilocybin but also suggesting and demanding more organized and controlled studies to explore its

role in the treatment of various mental conditions, including chronic cluster headaches and obsessive-compulsive disorders (OCD) and OCD-related clinical depression.

## 2 Occurrence

The archaeological evidences available throughout different cultures indicate history of psilocybin mushrooms as quite ancient. Though it is really difficult to correlate and extend any scientific note on such a diversified and scattered subject, yet the mushroom-shaped statuettes or mushroom-formed stones and several Mesolithic rock paintings reported at different archaeological sites, such as Guatemala, El Salvador, Mexico, and Tassili n'Ajjer (a prehistoric North African site identified with Caspian culture), and anthropological literatures further support the presence and history of traditional use of these mushrooms that dates back as far as 2000 BC [5, 6]. The period between fourteenth and sixteenth century remains much popular with respect to use of psilocybin mushrooms when Aztecs (ruler Moctezuma II) and Chichimecas (Nahua people of Mexico) were also reported as users of such mushrooms which they called "teonanacatl" (good mushrooms or flesh of the gods or wondrous mushroom, etc.). In the Western medical literature, the first scientific report appeared in 1799 when a critical article was published in London Medical and Physical Journal with the title "On a poisonous species of agaric." There is hardly any scientific study or report appeared between 1799 and 1899 on psilocybin mushrooms. Some activity was finally been reported by scientists working on psilocybin mushrooms in Harvard University, USA, during the end of the 1930s and 1940s [7] followed by the introduction of the word "magic mushrooms," appeared for the first time in Western literature in 1957 to describe the hallucinogenic property of psilocybin mushrooms [3]. The subsequent studies resulted finally in the discovery of both psilocybin and psilocin in 1958 [1].

Psilocybin mushrooms are quite diversified in nature and thus geographically have a wide range of distribution worldwide which covers both tropical and subtropical regions. These include Mexico, Cuba, Guatemala, Colombia, Bolivia, Brazil, Chile, Argentina, the USA (Florida, Vancouver, Washington, Oregon, California), Canada, England, Norway, Finland, the Netherlands, Germany, Austria, Spain, Thailand, Vietnam, India, Sri Lanka, Nepal, Cambodia, Indonesia, the Philippines, Japan, and Australia. Some species of Psilocybe are reported exclusively in Mexico and Guatemala. Mexico has the highest number of *Psilocybe* species (44 out of total 76 neurotropic fungal species), representing 39% of the total species reported worldwide [8–10]. Being classified as saprotrophs, psilocybin mushrooms can grow on various kinds of decaying organic matters in most biomes, with the exception of high deserts. Based on extensive work of various researchers and their findings reported elsewhere, presence of psilocybin has been reported in large number of species belonging to about 25 different genera [11–13]. These include *Agrocybe, Amanita, Conocybe, Copelandia, Coprinus, Entoloma,*

**Table 18.1** Psilocybin and psilocin content of some major species of *Psilocybe*

| Species | Alkaloidal content (%)[a] | | | References |
|---|---|---|---|---|
| | Psilocybin | Psilocin | Baeocystin | |
| *P. azurescens* | 1.70 | 0.38 | 0.35 | [15] |
| *P. baeocystis* | 0.85 | 0.59 | 0.10 | [16, 17] |
| *P. bohemica* | 1.34 | 0.11 | 0.02 | [18, 19] |
| *P. cubensis* | 0.63 | 0.60 | 0.025 | [19, 20] |
| *P. cyanescens* | 0.85 | 0.36 | 0.03 | [16, 21] |
| *P. cyanofibrillosa* | 0.21 | 0.04 | 0.00 | [22] |
| *P. hoogshagenii* | 0.60 | 0.10 | 0.00 | [23] |
| *P. liniformans* | 0.16 | 0.00 | 0.005 | [21] |
| *P. pelliculosa* | 0.12 | 0.00 | 0.00 | [24] |
| *P. samuiensis* | 0.36 | 0.21 | 0.02 | [25] |
| *P. semilanceata* | 0.98 | 0.02 | 0.36 | [19] |
| *P. semperviva* | 0.30 | 0.07 | 0.00 | [23] |
| *P. subcubensis* | 0.80 | 0.02 | 0.00 | [26] |
| *P. stuntzii* | 0.36 | 0.12 | 0.02 | [16, 24] |
| *P. tampanensis* | 0.68 | 0.32 | 0.00 | [19] |
| *P. weilii* | 0.61 | 0.27 | 0.05 | [6] |

[a]Average content and may vary in different regions due to environmental conditions and parts used

*Galerina, Gerronema, Gymnopilus, Hygrocybe, Hypholoma, Inocybe, Marasmius, Mycena, Naematoloma, Panaeolina, Panaeolopsis, Panaeolus, Pholiota, Pluteus, Psathyra, Psathyrella, Psilocybe, Rickenella, and Stropharia.* Out of 216 known species of neurotropic fungi reported elsewhere, most (116 species) fall in the genus *Psilocybe* [9], and out of 190 different mushrooms tested, 90 species have been identified to contain either psilocybin or psilocin or both with genus *Psilocybe*, showing the highest numbers (41 out of 55 species or varieties) in terms of psilocybin or psilocin content [14]. Few species of genus *Psilocybe* are quite prominent in producing psilocybin and psilocin, such as *P. azurescens, P. baeocystis, P. bohemica, P. cubensis, P. cyanescens, P. cyanofibrillosa, P. hoogshagenii, P. liniformans, P. pelliculosa, P. samuiensis, P. semilanceata, P. semperviva, P. subcubensis, P. stuntzii, P. tampanensis*, and *P. weilii* (Table 18.1).

Mushroom species with high psilocybin and psilocin content belonging to genera other than *Psilocybe* include *Agrocybe praecox, Conocybe cyanopus, Copelandia cambodginiensis, Gymnopilus purpuratus, Inocybe aeruginascens, Panaeolus subbalteatus*, and *Pluteus salicinus* (Table 18.2).

The quantity of psilocybin as mentioned in Tables 18.1 and 18.2 is quite variable (approximately 0.5–2.0% dry weight) and dependent on several factors, including age (mature mycelium contains more than the young one), growing and drying conditions (heat and light can affect alkaloidal content), and specific part of the mushroom used for estimation of psilocybin and other alkaloids. In some cases, variation in psilocybin content may be recorded up to 4x for species grown under controlled condition and as much as 10x for ones that are not.

**Table 18.2** Psilocybin and psilocin content of some genera other than *Psilocybe*

| Species | Alkaloidal content (%)[a] | | | References |
|---|---|---|---|---|
| | Psilocybin | Psilocin | Baeocystin | |
| *Agrocybe praecox* | 0.80 | 0.00 | 0.00 | [26] |
| *Conocybe cyanopus* | 0.93 | 0.70 | 0.03 | [16, 17, 27] |
| *Copelandia cambodginiensis* | 0.30 | 0.13 | 0.02 | [28] |
| *Gymnopilus purpuratus* | 0.34 | 0.29 | 0.05 | [19] |
| *Inocybe aeruginascens* | 0.40 | 0.11 | 0.20 | [25] |
| *Panaeolus subbalteatus* | 0.16 | 0.00 | 0.08 | [17, 21] |
| *Pluteus salicinus* | 1.20 | 0.00 | 0.00 | [29] |

[a]Average content and may vary in different regions due to environmental conditions and parts used

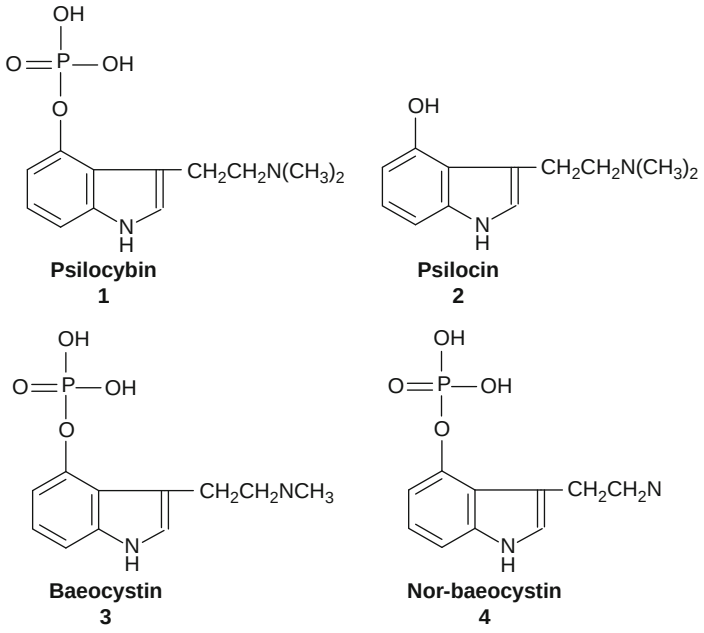
The word *Psilocybe* is derived from Greek roots – *psilos* and kube – which means "bald head" or "bare headed." *Psilocybe* is moderately large genus, with a variety of species. The characteristic macroscopic and microscopic features and taxonomy all are well documented elsewhere comprehensively [5, 6, 8–10, 15, 22, 30] and thus are beyond the scope of the present title for discussion. Precisely, the species of the genus *Psilocybe* are small brown to tan mushrooms with a primary distinguishable feature that they bruise blue when handled or injured. Though *Psilocybe* is placed taxonomically in the agaric (*Agaricales*) family *Strophariaceae* based upon spore and pileipellis morphology, yet the genus is quite complex in terms of classification and thus difficult to separate from genera *Stropharia* and *Hypholoma*, and all three are no doubt closely related. According to some authors, the relationship is close enough to combine these genera into one genus, *Psilocybe* [31]. It has long been suspected that the mushrooms in *Pholiota, Stropharia, Hypholoma, and Psilocybe* share a common ancestor. The current interpretation of DNA has resulted in a different way, suggesting *Psilocybe* comprising of two groups (one producing psilocybin and other does not) that are only distantly related to each other and both are distantly related to *Stropharia*. Presently, both groups are classified under *Psilocybe* but in fact resembling more to *Hypholoma* and *Pholiota* than to *Stropharia* [32, 33]. The DNA studies of mushrooms, no doubt, are revealing some new findings and relationships that were previously unsuspected, but the technology is still unable to answer a number of hidden questions which mycologist and molecular biologists may be interested to know to reach to final conclusion. The overall distribution of psilocybin among various genera has been reported as *Psilocybe* (116 species), *Gymnopilus* (14 species), *Panaeolus* (13 species), *Copelandia* (12 species), *Hypholoma* (6 species), *Pluteus* (6 species), *Inocybe* (6 species), *Conocybe* (4 species), *Panaeolina* (4 species), *Gerronema* (2 species), and *Agrocybe*, *Galerina*, and *Mycena* (1 species each) [9]. The mushroom caps contain more psilocybin or psilocin than the stem, while spores do not contain either psilocybin or psilocin [34, 35]. Most references cited elsewhere [10, 36–39] suggest that the distribution of psilocybin mushrooms may have their origin in southern hemisphere, mainly in South America and Australia and to some extent in South Africa because of the highly diversified geographical and climatic conditions in these regions.

The lower level of industrialization, smaller landmasses, and overall low population densities may have contributed well in the growth and propagation of enormous number of mushroom species in these regions. The historical findings also suggest the traditional users of psilocybin mushrooms among various ethnic groups located in Mexico and Colombia (South America), New Guinea (greater Indo-Australian Archipelago) and in Africa. From South America, it is believed to have further migrated to northern parts (North America, Canada, and Europe), while from Australia, it may have reached to Southeast Asia, South Asia, and other parts of Asia Pacific (Table 18.3).

## 3 Phytochemistry

The primary bioactive alkaloids reported in most *Psilocybe* mushrooms include psilocybin and psilocin and to a lesser extent baeocystin and norbaeocystin **(1–4)**.

**Psilocybin:** 4-phosphoryloxy-*N*,*N*-dimethyltryptamine;
3-[2-(dimethylamino)ethyl]-1 H-indol-4-ol-dihydrogen phosphate
**Psilocin:** 4-hydroxy-*N*,*N*-dimethyltryptamine;
3-[2-(dimethylamino)ethyl]-1-H-indol-4-ol
**Baeocystin:** 4-phosphoryloxy-N-methyltryptamine
**Norbaeocystin:** 4-phosphoryloxytryptamine.




Apart from these four bioactive indole alkaloids, *Psilocybe* mushrooms are capable of producing a number of indole derivatives (Table 18.4). Psilocybin has the molecular formula $C_{12}H_{17}N_2O_4P$ (mol. weight = 284.27). It is a prodrug that is

**Table 18.3** Occurrence of psilocybin and psilocin in various species of mushrooms

| Genus/Species | 1 | 2 | Country | References |
|---|---|---|---|---|
| ***Agrocybe*** | | | | |
| *Agrocybe farinacea* Hongo | + | – | Japan | [40] |
| *Agrocybe praecox* (Pers.) Fayod | + | – | Austria | [26] |
| ***Amanita*** | | | | |
| *Amanita muscaria* (L.: Fr.) Hooker | + | | Brazil | [20] |
| ***Conocybe*** | | | | |
| *Conocybe cyanopus* (Atk.) Kuhner | + | + | Finland, Norway, USA | [24, 41, 42] |
| *Conocybe kuehneriana* (Sing.) Kuhner | – | + | Finland | [41] |
| *Conocybe smithii* Watling | + | – | USA | [43] |
| ***Copelandia*** | | | | |
| *Copelandia anomala* Murrill | + | – | USA (Hawaii) | [28] |
| *Copelandia bispora* (Malencon and Bertault) Singer and Weeks | + | – | USA (Hawaii) | [28] |
| *Copelandia cambodginiensis* (Ola'h and Haim) Singer and Weeks | + | + | USA (Hawaii) | [28] |
| *Copelandia chlorocystis* sp. Nov. Singer and Weeks | + | + | Brazil | [44] |
| *Copelandia cyanescens* (Berk. and Br.) Singer | + | + | Italy | [45] |
| *Copelandia cyanescens (Berk. and Br.)* Singer | + | – | USA (Hawaii) | [28] |
| *Copelandia tropicalis* (Ola'h) Singer | + | – | USA (Hawaii) | [28] |
| *Copelandia* sp. | + | + | Japan | [46] |
| ***Galerina*** | | | | |
| *Galerina steglichii* Besl spec. nov | + | + | Germany | [47] |
| ***Gerronema*** | | | | |
| *Gerronema fibula* (Bull) Singer | + | – | Germany | [48] |
| *Gerronema swartzii* (Fr.) Kreisel | + | – | Germany | [48] |
| ***Gymnopilus*** | | | | |
| *Gymnopilus aeruginosus* (Peck) Sing | + | – | USA | [49] |
| *Gymnopilus liquintiae* (Pers.) P.Karst | + | – | Japan | [40] |
| *Gymnopilus luteus* (Peck) Hesler | + | – | USA | [49] |
| *Gymnopilus purpuratus* (Cooke and Mass.) Sing. | + | – | Germany | [50] |
| *Gymnopilus purpuratus* (Cooke and Mass). Sing | + | – | Thailand | [25] |
| *Gymnopilus sapineus* (Fr.) Maire. | + | – | USA | [49] |
| *Gymnopilus spectabilis* (Fr.) A.H.Sm. | + | – | USA | [49] |
| *Gymnopilus validipes* (Peck) Hesler | + | – | USA | [49] |
| *Gymnopilus viridans* Murr. | + | – | USA | [49] |
| ***Hygrocybe*** | | | | |
| *Hygrocybe psittacina* (Schff.ex.Fr.) Wunsche (f.optima R. Schultz). | + | + | Germany | [48] |

(*continued*)

**Table 18.3** (continued)

| Genus/Species | 1 | 2 | Country | References |
|---|---|---|---|---|
| ***Hypholoma*** | | | | |
| *Hypholoma aurantiaca* | + | – | Australia | [50] |
| ***Inocybe*** | | | | |
| *Inocybe aeruginascens* Babos | + | – | Germany | [51] |
| *Inocybe aeruginascens* Babos | + | – | Germany | [19] |
| *Inocybe aeruginascens* Babos | + | – | Germany | [52] |
| *Inocybe aeruginascens* Babos | + | – | Germany | [53] |
| *Inocybe aeruginascens* Babos | + | – | Germany | [54] |
| *Inocybe aeruginascens* Babos | + | – | Germany | [55] |
| *Inocybe aeruginascens* Babos | + | – | Hungary | [54] |
| *Inocybe aeruginascens* Babos | + | – | Germany | [56] |
| *Inocybe aeruginascens* Babos | + | + | Switzerland | [21] |
| *Inocybe calamistrata* Gillet | + | + | Germany | [48] |
| *Inocybe corydalina* Quel | + | – | Switzerland | [21] |
| *Inocybe corydalina* Quel | + | – | Germany | [48] |
| *Inocybe corydalina* Quel | + | – | Switzerland | [20] |
| *Inocybe corydalina* Quel. var. erinaceomorpha (Stangl and Veselsky) | + | – | Switzerland | [21] |
| *Inocybe coelestium* Kuyp | + | – | Switzerland | [21] |
| *Inocybe haemacta* (Berk.et Cooke) | + | + | Germany | [48] |
| *Inocybe haemacta* (Berk.et Cooke) | + | – | Switzerland | [20, 21] |
| *Inocybe haemacta* (Berk.et Cooke) | + | + | Czech Republic | [57] |
| ***Panaeolina*** | | | | |
| *Panaeolina foenisecii* (Pers.ex.Fr.) | + | – | Finland | [27] |
| *Panaeolina foenisecii* (Pers.ex.Fr.) | + | – | Austria | [51] |
| ***Panaeolus*** | | | | |
| *Panaeolus africanus* Ola'h | + | + | Sudan | [58] |
| *Panaeolus antillarum* | + | + | Thailand | [59] |
| *Panaeolus ater* (J.E. Lange) Bon | + | + | India | [58] |
| *Panaeolus cambodginiensis* Ola'h and Heim | + | – | USA | [60] |
| *Panaeolus campanulatus* (Fr.) Gillet | + | – | Italy | [45] |
| *Panaeolus castaneifolius* (Murr) | + | + | Canada, France | [58] |
| *Panaeolus cyanescens* (Berk and Broome) Sacc. | + | + | Germany | [61] |
| *Panaeolus cyanescens* (Berk and Broome) Sacc. | + | + | USA (Hawaii), Thailand | [19, 20, 59] |
| *Panaeolus fimicola* (Pers.) Gillet | + | + | United Kingdom | [58] |
| *Panaeolus foenisecii* (Pers.) J. Schrot | + | + | Canada, France | [58] |
| *Panaeolus foenisecii* (Pers.) J. Schrot | + | – | Italy | [45] |
| *Panaeolus olivaceus* F.H. Moller | + | – | Finland | [27] |
| *Panaeolus retirugis* (Fr.) Gillet | + | – | Italy | [45] |
| *Panaeolus sphinctrinus* | + | + | Canada | [58] |

(*continued*)

**Table 18.3** (continued)

| Genus/Species | 1 | 2 | Country | References |
|---|---|---|---|---|
| *Panaeolus subbalteatus* (Berk and Br.) | + | + | Finland | [27] |
| *Panaeolus subbalteatus* (Berk and Br.) | + | – | Italy, Mexico | [45, 60] |
| *Panaeolus subbalteatus* (Berk and Br.) | + | + | Canada, USA | [58] |
| *Panaeolus subbalteatus* (Berk and Br.) | + | – | Brazil, Russia | [20, 62] |
| ***Pluteus*** | | | | |
| *Pluteus atricapillus* Singer | + | – | Finland | [27] |
| *Pluteus glaucus* Singer | + | + | Brazil | [20] |
| *Pluteus nigroviridis* Babos | + | – | Switzerland | [63] |
| *Pluteus salicinus* (Pers.) P Kumm | + | + | USA, Finland | [27, 64] |
| *Pluteus salicinus* (Pers.) P Kumm | + | – | Germany, Switzerland | [21, 29, 63] |
| *Pluteus salicinus* (Pers.) P Kumm | + | + | Czech Republic | [57] |
| *Pluteus salicinus* (Pers.) P Kumm | + | – | Norway | [65] |
| ***Psathyrella*** | | | | |
| *Psathyrella candolleana* (Fr.) Maire | + | + | Finland | [27] |
| *Psathyrella candolleana* (Fr.) Maire | + | – | Japan, Germany | [40, 48] |
| ***Psilocybe*** | | | | |
| *Psilocybe argentipes* K. Yokohama | + | – | Japan | [40] |
| *Psilocybe argentipes* K. Yokohama | + | + | Japan | [66] |
| *Psilocybe arcana* Borovicka and Hlavacek | + | + | Czech Republic | [57] |
| *Psilocybe atrobrunnea* (Lasch) Gillet | + | – | Norway | [65] |
| *Psilocybe aztecorum* Hem var. aztecorum emend. Guzman | + | – | Mexico | [67] |
| *Psilocybe aztecorum* var. bonetii Guzman | + | – | Mexico | [60] |
| *Psilocybe azurescens* Stamets and Gartz | + | + | USA | [15] |
| *Psilocybe baeocystis* Singer and A.H.Sm. | + | + | USA | [17, 24, 68] |
| *Psilocybe bohemica* Sebek | + | + | Czech Republic | [18, 19, 57, 69–71] |
| *Psilocybe bohemica* Sebek | + | + | Switzerland | [21] |
| *Psilocybe bonetii* Guzman | + | – | Mexico | [60] |
| *Psilocybe caeruleoannulata* Sing | + | + | Brazil | [20] |
| *Psilocybe caerulescens* Murrill | + | + | Brazil | [20] |
| *Psilocybe caerulipes* Peck | + | + | USA | [72] |
| *Psilocybe candidipes* Singer and A.H.Sm | + | – | Mexico | [60] |
| *Psilocybe copninifacies* (Roll). Pouz | + | – | Czech Republic, Slovenia | [73] |
| *Psilocybe cubensis* (Earle) Singer | + | + | Japan, Germany, Mexico, Brazil | [20, 46, 61, 74] |
| *Psilocybe cyanescens* Wakef | + | + | Czech Republic, Switzerland, USA | [17, 21, 57, 75] |
| *Psilocybe cyanofibrillosa* Guzman and Stamets | + | + | USA | [22] |
| *Psilocybe fimetaria* (P.D. Orton) Watling | + | – | United Kingdom | [43] |
| *Psilocybe hoogshagenii* Heim | + | + | Brazil | [20] |
| *Psilocybe liniformans* Guzman and Bas | + | – | USA | [21, 22] |

(*continued*)

**Table 18.3** (continued)

| Genus/Species | 1 | 2 | Country | References |
|---|---|---|---|---|
| *Psilocybe mexicana* Heim | + | + | Mexico | [1, 2, 76] |
| *Psilocybe mulliericula* Singer and AH Sm | + | + | Mexico | [1] |
| *Psilocybe pelliculosa* Singer and AH Sm | + | + | USA | [24, 77, 78] |
| *Psilocybe pseudobullacea* (Patch) | + | + | Venezuela | [79] |
| *Psilocybe samuiensis* Guzman | + | + | Thailand | [19] |
| *Psilocybe semilanceata* (Fr.) Kumm | + | + | Czech Republic | [54, 55, 70, 73] |
| *Psilocybe semilanceata* (Fr.) Kumm | + | + | Finland, Germany | [27, 54, 80, 81] |
| *Psilocybe semilanceata* (Fr.) Kumm | + | – | Germany, Norway | [82–85] |
| *Psilocybe semilanceata* (Fr.) Kumm | + | – | Sweden, United Kingdom | [43, 86, 87] |
| *Psilocybe semilanceata* (Fr.) Kumm | + | + | Switzerland, USA, Germany, Czech Republic | [57, 61, 77, 88] |
| *Psilocybe semilanceata* (Fr.) Kumm | + | – | Switzerland, Netherlands, USA | [57, 89] |
| *Psilocybe semperviva* Heim and Cailleux | + | – | Mexico | [23, 90] |
| *Psilocybe serbica* Mos. and Horak | + | – | Serbia | [91] |
| *Psilocybe strictipes* A.H. Sm. | + | – | USA | [72] |
| *Psilocybe stuntzii* Guzman and Ott | + | + | USA | [24, 92] |
| *Psilocybe subaeruginosa* Cleland | + | – | Australia | [93, 94] |
| *Psilocybe subaeruginosa* Cleland | + | + | Australia | [51] |
| *Psilocybe subcubensis* Guzman | + | – | Venezuela | [79] |
| *Psilocybe subcubensis* Guzman | + | + | Japan | [66] |
| *Psilocybe subyungenis* Guzman | + | + | Brazil | [20] |
| *Psilocybe tampanensis* Guzman and Pollock | + | + | Thailand | [19] |
| *Psilocybe tampanensis* Guzman and Pollock | + | + | Germany | [61] |
| *Psilocybe thailandensis* Guzman and Allen | + | + | Thailand | [20] |
| *Psilocybe uruguayensis* Sing | + | + | Brazil | [20] |
| *Psilocybe wasanii* Heim | + | + | Mexico | [76] |
| *Psilocybe weilii* Guzman | + | + | USA | [6, 22] |
| *Psilocybe zapotecorum* Heim | + | + | Mexico | [2, 20, 23] |
| ***Stropharia*** | | | | |
| *Stropharia cubensis* Earle | + | + | Mexico | [76] |
| *Stropharia cubensis* Earle | + | + | Cambodia | [23, 67] |
| *Stropharia cubensis* Earle | + | + | Thailand | [23, 67] |

*1* Psilocybin, *2* Psilocin, + Detected, – Not detected

converted by the mechanism of dephosphorylation into psilocin (another pharmacologically active compound). Psilocin has the molecular formula $C_{12}H_{16}N_2O$ (mol. weight = 204.27).

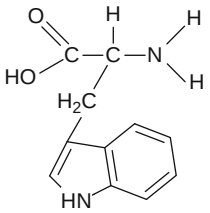
Psilocybin is regarded as tryptamine compound based on its biosynthesis route and precursor. Being a tryptamine compound, the chemical structure is derived from the amino acid tryptophan **(5)** which has a ring configuration, simply known as

**Table 18.4** List of indole derivatives reported in various *Psilocybe* mushrooms

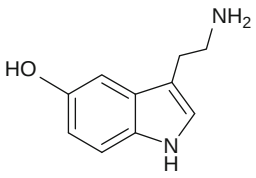
| Compound[a] | Compound[a] |
|---|---|
| Indole | 3-Indolelactic acid |
| 2-Methylindole | 3-Indoleacetamide |
| 3-Methylindole | 3-Indoleacetic acid |
| 5-Methylindole | γ (Indole)-N-butyric acid |
| 5-Methoxyindole | β (Indole-3)-propionic acid |
| 5-Hydroxyindole | 3-Indolecarboxylic acid |
| 3-Indoleacetonitrile | β-Indole-3-acrylic acid |
| 3-Hydroxymethyl indole | 3-Indolealdehyde |
| 3-Indoleacetic acid ethyl ester | 3-Indoleacetaldehyde |
| 5-Hydroxy-3-indole acetic acid | 3-Hydroxyethyl indole |
| *N*-Methyltryptophan | Indoxylacetate |
| 5-Methoxy-2-carboxyindole | Indoxylbutyrate |
| 5-Benzyloxy-3-indole acetic acid | 5-Methyltryptophan |
| Tryptamine hydrochloride | L-Tryptophane |
| 5-Hydroxytryptamine creatinine sulfate | 5-Hydroxytryptophane |
| *N*,*N*-Dimethyltryptamine hydrogen-oxalate | Isatin |
| Bufotenine monooxalate | Gramine |

[a]The list indicates addition compounds, apart from well-known alkaloids

indole ($C_8H_7N$), linked to an ethylamine substituent. Structurally, psilocybin has a close resemblance with a neurotransmitter serotonin or 5-HT (**6**). Tryptamines are one of the four categories of hallucinogenic indoles in more than 20 classes of indole compounds comprising approximately 600 alkaloids.




**Tryptophan**
**5**




**Serotonin**
**6**

Psilocybin is zwitterionic in nature (containing both positive and negative charges), insoluble in most organic solvents (such as chloroform, benzene, etc.,), slightly soluble in ethanol, moderately soluble in methanol (120 parts of boiling methanol), and soluble in water (20 parts of boiling water). Aqueous solution is highly unstable and rapidly oxidizes. This is an important factor and should be taken into consideration when psilocybin is used as an analytical standard [39]. Physically, psilocybin is white, needle-like crystalline structure with melting point between 185–195 °C [14] and 190–198 °C [95]. A 1% solution of psilocybin dissolved in 50% ethanol has a pH of 5.2 [2]. Scientific work carried out in

Japan with respect to large-scale chemical synthesis of psilocybin without chromatographic purification suggests use of 4-hydroxyindole to produce psilocybin from psilocin with a yield of 85% [95].

For presumptive analysis (color test) of psilocybin, both Ehrlich and Marquis tests can be applied. Care should be taken as some other $C_2$ unsubstituted indoles (such as DMT and LSD) may give similar colors with test reagent, thus making it mandatory for a confirmatory test before extending any final concluding remarks. With Ehrlich's reagent (1 g of p-dimethylaminobenzaldehyde in 10 ml methanol + 10 ml con. orthophosphoric acid), a violet to gray-violet color indicates the possible presence of psilocybin or psilocin. The detection limit of this method is approximately 1 μg. While with Marquis' reagent (solution A: 8–10 drops of 40% formaldehyde solution to 10 ml glacial acetic acid; solution B: con. $H_2SO_4$), an orange color indicates the possible presence of psilocybin and a green-brown color the presence of psilocin. The detection limit of this method is about 10 μg. Since the positive color reaction for psilocybin with Marquis' reagent is obscured by vegetable matter and as some other natural products present in mushrooms may develop a yellow color, therefore with sulfuric acid, this color test is not suitable for the detection of psilocybin or psilocin. Mandelin tests can also be applied to detect psilocybin. Application with Mandelin reagent (1 g of ammonium vanadate in 100 ml con. $H_2SO_4$) results in the development of green color, while DMACA reagent (0.5 g of p-dimethylaminocinnamaldehyde in 50 ml methanol + 10 ml con. HCl) can be sprayed to detect after chromatographic analysis [96, 97], or few drops can directly be added over the suspected material on a spot plate. Psilocybin gives gray-violet to violet color, whereas psilocin turns blue. The DMACA reagent is more sensitive than Ehrlich reagent and also reflects better color stability. The detection limit for DMACA reagent is about 20 ng for psilocybin and 10 ng for psilocin. Other techniques used to detect and quantify psilocybin include gas chromatography coupled to mass spectrometry [98], ion mobility spectrometry [26], capillary zone electrophoresis [99], ultraviolet spectroscopy [39], infrared spectroscopy [55], high-performance liquid chromatography (HPLC) with ultraviolet [39], fluorescence [100], and electrochemical [101] or electrospray mass spectrometric methods [102]. These chromatographic techniques can also be used to detect psilocin in the body fluids.

Solvent system does play very important role in the isolation and extraction of psilocybin. It is quite evident from the early phase of research activities that very polar solvent, such as methanol or mixtures of ethanol and water, is most suitable for the isolation of psilocybin. The polar properties of the phosphate group make psilocybin soluble in water and methanol (polar solvent), and as a result solubility decreases with the decrease in polarity, i.e., less soluble in polar solvent. In comparison, psilocin is less polar and thus readily soluble in polar solvent, such as 1-chlorobutane. For thin layer chromatography, solvent systems comprising of n-butanol + acetic acid + water (20:10:10) or methanol + con. ammonia solution (100:1.5) may be used to develop the plates, and visualization of the blue spots on fluorescent background can be made using ultraviolet (UV) light at 254 and 365 nm. The absorption characteristics of both

psilocybin and psilocin in the UV region are quite prominent. Both exhibit native fluorescence and are electrochemically active.

Mushrooms of the genus *Psilocybe* generally have a brownish cap (pileus) when moist in fresh fruiting bodies but fade gradually in color during the process of drying. In addition, some species (typically the hallucinogenic species) have the tendency to change the color (a condition called blue staining reaction) when fruiting body is damaged. This is also described as blueing phenomenon. Some authors do conclude that the blueing phenomenon ultimately reflects and provides a reasonable correlation between psilocybin and other indole derivatives content present in that particular species [43, 103]. However, at the same time, others suggest not to use this phenomenon as a definite method for identification or determining the potency of a mushroom [6, 17]. The phenomenon that psilocybin has the tendency to form blue color in solution can further be linked to its conversion into psilocin (which is relatively unstable in solution due to its phenolic hydroxyl –OH group) by dephosphorylation in presence of enzyme phosphatase followed by oxidation of psilocin by cytochrome oxidase, or copper oxidase or $Fe^{2+}$ [104]. Animal module also supported and suggested a similar mechanism for the development of blue color by these mushrooms when psilocybin was incubated with the homogenates of rat kidney or other mammalian tissues. A rapid liberation of psilocin was observed through the action of alkaline phosphate which further undergoes oxidative degradation and forms a blue-colored product [105]. A mitochondrial enzyme (cytochrome oxidase) was found responsible in the oxidation of psilocin to a dark blue color by the tissue homogenates [106].

## 4 Biosynthesis

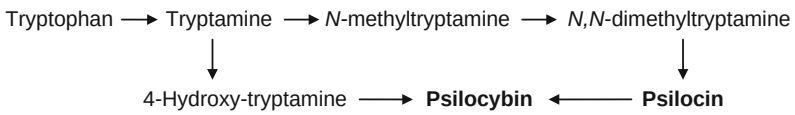
Studies on biosynthesis mechanism and the routes including enzymes involved in different steps are the best known toll in understanding the conversion or production of various biochemicals in living system. In case of psilocybin biosynthesis, though the experimental evidences are limited, but the overall mechanism can safely be discussed starting from chorismic acid, which is produced through shikimic acid pathway, a well-known pathway present in fungi which regulates the production of various biochemicals, including psilocybin and some other tryptamine-based psychedelic drugs, such as DMT and LSD.

The shikimic acid pathway leading to the production of chorismic acid is regulated in the cytosol of the fungal cells. Cytosol or intracellular fluid (cytoplasmic matrix) is a complex mixture of substances dissolved in water. These include ions (such as calcium, sodium, and potassium), macromolecules, and large complexes of enzymes that act together to carry out metabolic pathways. Production of chorismic acid in the cytosol is ultimately utilized in the synthesis of folate, ubiquinone, and amino acids, the most important of which is tryptophan which plays a major role in the biosynthesis of psilocybin.

The two starting materials of the shikimic acid pathway, phosphoenolpyruvate (a product of glycolysis) and erythrose 4-phosphate (a product of pentose

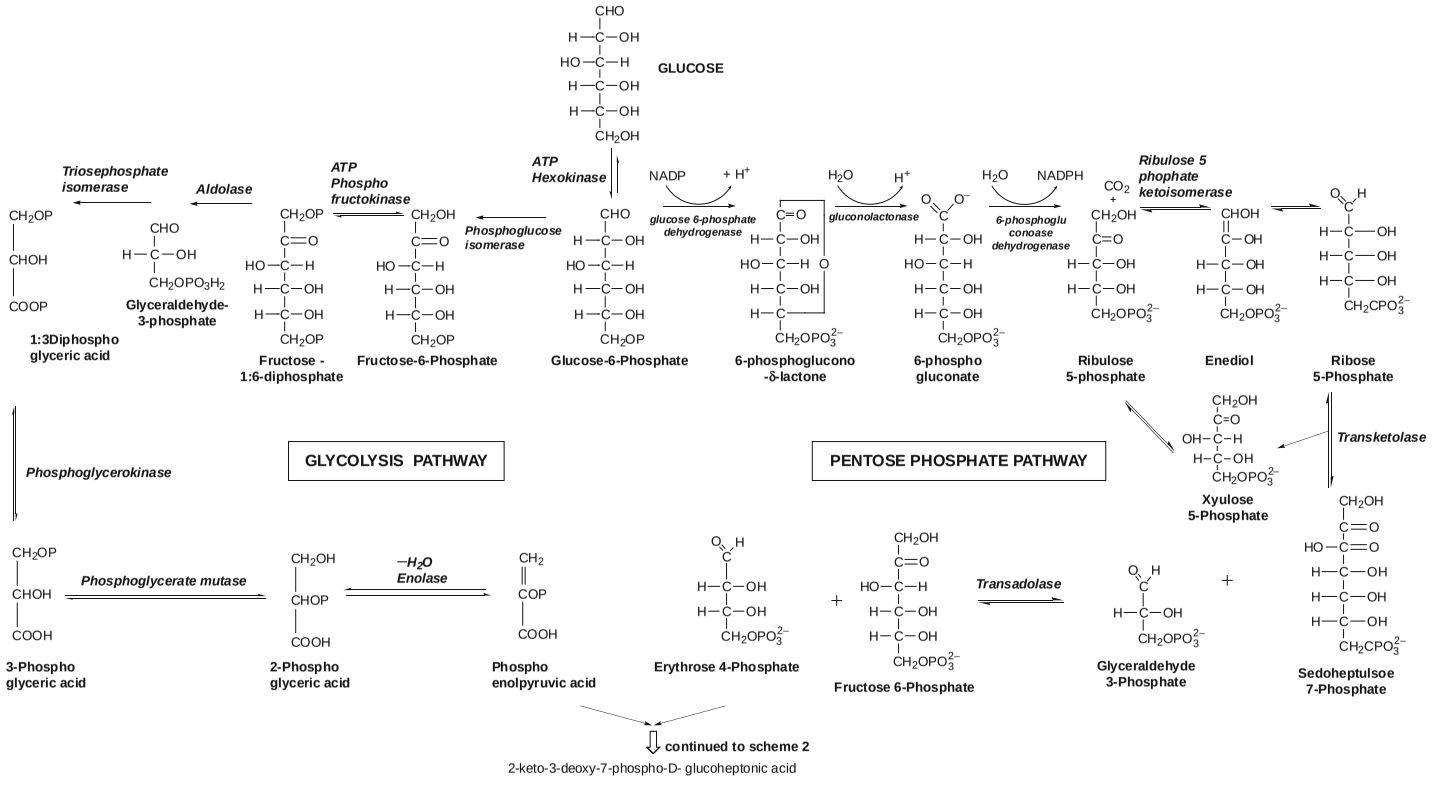
phosphate pathway), act as phosphate donors. Glucose is phosphorylated by ATP, which loses a phosphoryl group to become ADP. The glucose 6-phosphate thus produced, enter into two different pathways, glycolysis and pentose phosphate pathway, to produce both the starting materials required to regulate shikimic acid pathway with the production of chorismic acid, a substrate to give anthranilic acid which is a precursor of tryptophan (Scheme 18.1). With a few minor exceptions, tryptophan and its decarboxylation product, tryptamine, give rise to the large class of indole alkaloids, including psilocybin [107, 108] (Scheme 18.2).

Earlier findings published in 1961 [107] suggested that psilocybin is finally been synthesize from tryptophan. The hypothesis was based on the results obtained from still cultures of *Psilocybe semperviva*. Subsequent studies on the subject carried out using *Psilocybe cubensis* under submerged cultural condition also supported this hypothesis that psilocybin may biosynthetically derive from tryptophan and tryptamine [108]. More details were then published to clarify the sequence of events which leads from tryptophan to psilocybin by incorporating $H^3$– and $C^{14}$- labeled hypothetical intermediates (such as DL-Tryptophan-$^3$H, Tryptamine-$^{14}$C, *N*-methyltryptamine-$C^{14}$-$^3$H, *N,N*-Dimethyltryptamine-$^{14}$ C-$^3$H, 4-hydroxytryptamine-$^{14}$ C-$^3$H, Psilocin-$^3$H, DL-4-OH-Tryptophan-$^3$H) into psilocybin in submerged cultures of *Psilocybe cubensis* [109, 110]. Other reports [111, 112] indicated no effect or influence of tryptophan on the biosynthesis of psilocybin when added in the culture medium during submerged fermentation of *Psilocybe cubensis* and *Psilocybe baeocystis*. It can be concluded from the results of most findings that tryptophan has to be modified by decarboxylation, methylation of the amino group, hydroxylation of the 4-position of the indole moiety, and phosphorylation of the 4-hydroxyindole moiety for the production of psilocybin with below sequence [108–110, 113].

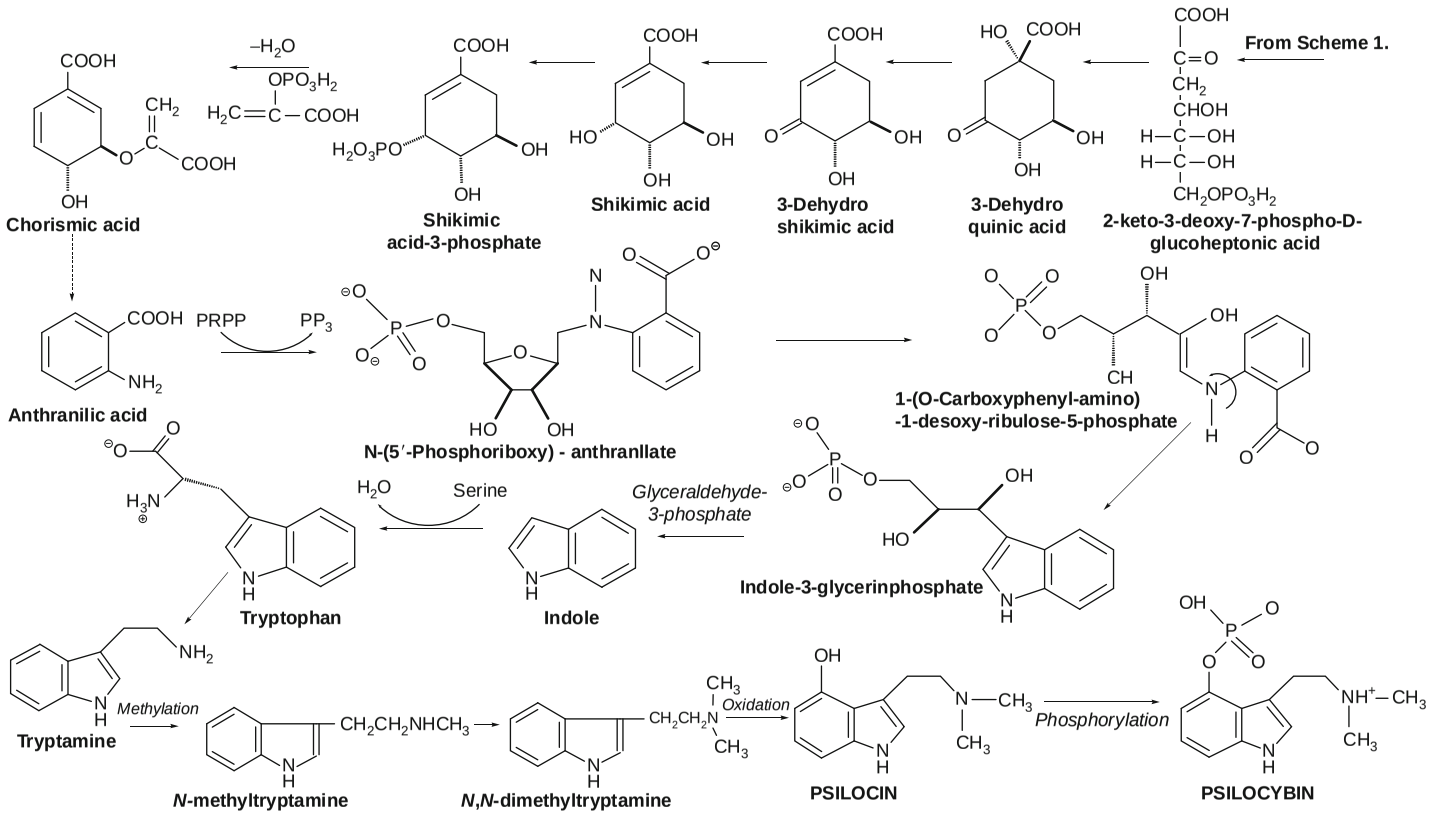



However, this may not be the only route. The fungus still may have an alternate route to convert 4-hydroxytryptamine to psilocybin. This probably because phosphorylated intermediate baeocystin and norbaeocystin are also produced, in addition to psilocybin, which is most likely produced via one phosphorylase enzyme from psilocin.

The radioactive tryptamine is observed to function as a better precursor than the tryptophan and 4-hydroxytryptophan in submerged culture of *Psilocybe cubensis* [113]; it is reasonable to conclude that the decarboxylation of tryptophan to tryptamine is the most crucial step in the biosynthetic pathway. This step is highly sensitive to feedback inhibition due to allosteric enzymes. It is believed that as the concentration of psilocin increases in the culture media, it starts inhibiting further conversion of tryptophan to tryptamine, and as a result tryptophan will not be converted to tryptamine, and thus no psilocin or psilocybin is




**Scheme 18.1** Glycolysis and pentose phosphate pathway




**Scheme 18.2** A proposed biosynthesis pathway of psilocybin biological activities

formed. Further addition of tryptophan will have no impact on the formation of psilocybin. While conversion of tryptamine to N-methyltryptamine is not as sensitive to feedback inhibition; therefore, addition of tryptamine will increase the production of psilocin. Apart from this, all other steps are also not as sensitive to feedback inhibition either. There still exists some controversy among authors regarding biosynthetic route from tryptamine to psilocybin [114]. When *Psilocybe cubensis* (mini-cultures) grows under deuterium-labeled precursor solution, a wide range of tryptamine can readily be absorbed by mycelia and translocated into developing mushrooms. The deuterated tryptamine can be incorporated more efficiently into psilocin and psilocybin than were monomethyltryptamine and dimethyltryptamine, suggesting that though hydroxylation enzyme operates normally on tryptamine, but may be flexible enough to oxidize dimethyltryptamine or other natural compounds forced on it at a high concentration. As the hydroxylation of dimethyltryptamine to produce psilocin was noted to occur with NIH shift, therefore an intermediate compound (tryptamine-4,5-epoxide) is most probably present between tryptamine and psilocin.

It is really difficult to interpret and match the data obtained through submerged cultural condition with that of fruit bodies grown in wild to ascertain the biosynthesis mechanism. Some observations indicate that surface cultures of *Psilocybe semilanceata* have a high hydroxylation and phosphorylation capacity, although the ability to methylate tryptamine derivatives is low. These findings are in accordance with the earlier mentioned hypothesis that the fungus may have alternate route to convert 4-hydroxytryptamine to psilocybin.

## 5 Biological Acitivies

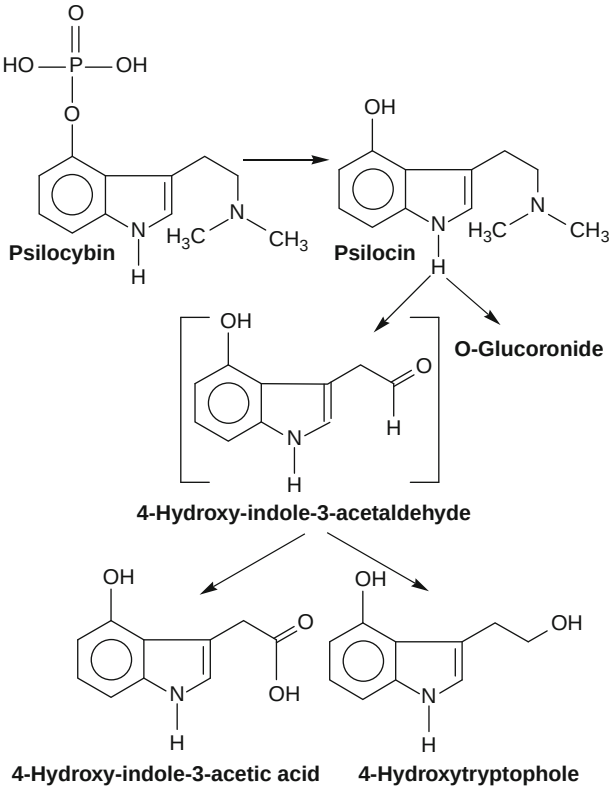
Despite the introduction of psilocybin more than five decades back by Sandoz, under the brand name of Indocybin, limited data are available for discussion. The original Indocybin tablets manufactured during 1958–1965 are still available in its original packaging (glass bottle with metal screw on lid, labeled: Sandoz Pharmaceuticals, 50 tablets, Psilocybin, each tablet contains 10 mg) in Bureau of Forensic Services Laboratory, Eureka, California. Traces of psilocin were still detectable by GC/MS in these tablets, but most peaks reflect tablet excipients. During the early mid-1960s (until it is classified as Schedule I in 1970 in the USA), psilocybin was used in psychiatric and psychological research as well as psychotherapy. Psilocybin mushrooms have had numerous medicinal and religious uses in dozens of cultures throughout history and have a drastically lower potential for abuse than other Schedule I drugs. Also, the UN's International Narcotics Control Board (INCB) has made it clear that *Psilocybe* mushrooms are not controlled by the UN [115, 116]. Research into the biological activity of psilocybin resumed in the mid-1990s, and currently a number of research institutes are working on biological activities of psilocybin to ascertain its clinical role

in different disorders and syndromes. In view of highly scattered biological activities of psilocybin, it will be appropriate to discuss this topic under subheadings, such as Pharmacology (Pharmacodynamics and Pharmacokinetics); Physiological and Psychological Effects; Therapeutic Role (Advanced Stage Cancer, Cluster Headaches, Obsessive-Compulsive Disorders); and Toxicity (Side Effects and Risks).

## 5.1 Pharmacology (Pharmacodynamics and Pharmacokinetics)

After oral ingestion, psilocybin is rapidly dephosphorylated into psilocin which is structurally related to serotonin (5-HT) and lysergide (LSD-25). Psilocin has high affinity (Ki = 6nM) for 5-HT receptors, especially the 5-$HT_{2A}$ in the brain (cerebral cortex) where it mimics the effects of serotonin and thus capable of altering perception, mood, consciousness, and cognition. It also has affinity (but binds less tightly, Ki = 190 nM) with other serotonergic receptors, such as 5-$HT_{1A}$ (hippocampus), 5-$HT_{1D}$ (cranial blood vessels), and 5-$HT_{2C}$ (choroid plexus) and thus can also contribute to the subjective and behavioral effects. Psilocybin and psilocin have no affinity for dopamine $D_2$ receptors [117]. The mode of action can precisely be explained in a way that psilocybin acts by altering the concentration of indoles, including serotonin, in the central nervous system and thus interfering with the transmission and processing of external stimuli. The psychotomimetic effects of psilocybin can be blocked in a dose-dependent manner by the 5-$HT_{2A}$ antagonist drugs, such as ketanserin and risperidone [118].

Psilocybin and psilocin are stoichiometrically equivalent in potency. After dephosphorylation to psilocin, presumably via a first pass effect by hepatic metabolism, it is easily taken up by tissues. More than 50% of the psilocybin is absorbed through the lining of mouth, stomach, and intestine and is detectable as psilocin in the blood within 20–40 min. Distribution throughout the body is quite uniform, including the brain [119, 120]. The absolute bioavailability of psilocin after oral administration of psilocybin is recorded as 52.7 ± 20%. The average peak concentration of 8.2 ± 2.8 ng $ml^{-1}$ ($C_{max}$ ranging from 4 to 21 ng $ml^{-1}$) is usually achieved within 2 h (105 ± 37 min.) after oral administration of 10–20 mg psilocybin (0.224 ± 0.02 mg $kg^{-1}$ body weight) and decline over the next 3–4 h. While after intravenous injection of 1 mg psilocybin, peak plasma concentration of 12.9 ± 5.6 ng $ml^{-1}$ reaches within 1.9 ± 1.0 min. The average half-life ranges between 2.5 and 3 h [121–123], while quantification of psilocin can be made within 6–7 h after oral administration [121, 124]. Psilocin is excreted mainly as glucuronide and to some extent as unchanged psilocin as well in urine. According to one report, 65% of the absorbed psilocybin is excreted into urine and 15–20% into bile and feces within 24 h [125]. Other metabolites detected in quantities include 4-hydroxyindole-3-yl-acetaldehyde, 4-hydroxyindole-3-yl-acetic acid, and 4-hydroxytryptophol [121, 126–129]. A proposed metabolic route for psilocybin is suggested in Scheme 18.3.




**Scheme 18.3** Metabolism of psilocybin

## 5.2 Physiological and Psychological Effects

The effects of psilocybin are quite variable and usually dependent upon dose and duration of intake. This may also be linked with the individual brain chemistry and physiology which may have significant role in determining the response to psilocybin. Though the recreational dose ranges from 10 to 50 mg, yet only 4–10 mg is enough to induce hallucination [5, 6]. Some people may require relatively high dose to gain a low-dose effects of psilocybin. The mental and physical tolerance to psilocybin may build but dissipates quickly. So dose adjustment (especially for frequent users) may be required to avoid side effects. Cross-tolerance is also possible between psilocybin and LSD, as well as between psilocybin and phenylethylamine hallucinogens, such as mescaline and 2,5-dimethoxy-4-methylamphetamine [130–132].

The general features which are characteristic of the psychedelic reaction are same for psilocybin as well. The most common features include changes in perception (perceptual changes such as illusion, pseudohallucination, and hallucination) and changes in vision (objects, pictures, or patterns seem to come alive, shift, ripple, or

become wavy). The psychological symptoms are greatly affected after psilocybin ingestions. Various trial reports published elsewhere [133–135] highlight these effects when psilocybin was given to healthy volunteers in decreasing order. The emotional alterations noted in 100% of the volunteers include disorders/alterations of consciousness in 91%, depersonalization in 84%, perceptual alterations in 75%, disorders/alteration of volition and psychomotor behavior in 34%, body image distortions in 25%, disorders/alteration of attention in 22%, disturbances in thought process in 22%, and disorders of memory in 19% of the volunteers.

## 5.3 Therapeutic Role (Advanced Stage Cancer, Cluster Headaches, Obsessive-Compulsive Disorders)

Results of various double-blind, placebo-controlled pilot studies carried out with psilocybin in the management of these conditions or reducing the severity of the various associated symptoms are highly encouraging. To reduce anxiety and improved quality of life in people with advanced stage of cancer, psilocybin has been observed as good alternate [136, 137]. In case of cluster headaches which has been recognized as one of the worst pain syndromes and difficult to control through presently available drugs, psilocybin has been reported to have promising effect in reducing the severity of the attack as well as to increase the attack-free periods in such patients [138–140]. A substantial reduction in symptoms in patients associated with obsessive-compulsive disorders (OCD) has also been noted with psilocybin therapy. The proposed mechanism and reason for this is the reduction in serotonin levels at 5-$HT_{2A}$ receptors due to psilocybin which ultimately suppresses the responsiveness to serotonin [141, 142]. In most cases, only sub-hallucinogenic dose of psilocybin is required to achieve the desired therapeutic effect without any major side effects.

## 5.4 Toxicity (Side Effects and Risks)

The therapeutic index of psilocybin as given by "The Registry of Toxic Effects of Chemical Substances" is quite high (therapeutic index = 641), in comparison to aspirin and nicotine for which the values are quite low (therapeutic index = 199 and 21, respectively), representing better a better safety profile [143]. Therapeutic index is the ratio of the dose that produces toxicity ($TD_{50}$) to the dose that produces a clinically desired or effective response ($ED_{50}$) in a population. The therapeutic index is a measure of a drug's safety, because a larger value indicates a wide margin between doses that are effective and doses that are toxic. $LD_{50}$ in rats by oral route is 280 mg $kg^{-1}$ while that of intravenous route in rabbit is 12.50 mg $kg^{-1}$ [144]. The common side effects are very similar to those reported for other hallucinogens, such as ergoline and LSD. These include perception of time and space, derealization, depersonalization, dilated pupils, dizziness, fatigue, impaired concentration, unusual body sensations and thoughts, transient increase in blood pressure or heart rate, transient psychosis, change in mood, nausea, etc. These effects are mostly noted as

acute which usually last for about 4–6 h [117, 145–148]. Oral intake of psilocybin appears to be at the lowest risk out of all other psychoactive drugs, while long-term toxicity studies in mice indicated no teratogenicity or mutagenicity [149, 150].

## 6 Biotechnological Approaches

The biotechnological approach has now occupied a prominent place while discussing topics in applied biology. However, the features and objects may vary based on scope of the study. In search of new drug molecules or to evaluate the existing therapeutically active molecules, especially from higher fungi, is increasing day by day. The higher fungi have tremendous capability to synthesize biologically active compounds, especially the alkaloids with diversified chemistry and pharmacological activity. However, unfortunately less attention has so far been given by the researchers to utilize biotechnological approach to produce tryptamines (e.g., psilocybin and psilocin), perhaps because of the legal status and their classification under Schedule I drug category in the USA which restricted such studies and funding as well. The Schedule I drugs deemed to have a high potential for abuse and are not recognized for medical use. In Canada, both psilocybin and psilocin are classified as restricted drugs and are listed in Schedule III of Canada's Food and Drugs Act [151]. However, with the change in scenario and after having some prominent results of psilocybin in the management of various disorders, the situation is thus demanding substantial quantity of such molecules for pilot study and commercialization. So the improvement in strains capability, media, and culture conditions as well as regulation of enzyme activity involved in different steps of the biosynthetic pathway of psilocybin require more attention. The function of enzymes is to effect the integration of overall metabolic pathway. The formation alkaloid relates to endogenous metabolism. Thus, the energy released during the process is utilized to maintain the viability and integrity of the cells. In the biosynthesis of psilocybin, tryptophan plays important role, both as a regulatory and a precursor. It will be worth to utilize biotechnological approach to study the enzyme tryptophan decarboxylase (responsible for the decarboxylation of tryptophan to form tryptamine) and its regulatory mechanism. This is the last major step in the pathway that is significantly inhibited by a self-feedback mechanism. Due to this, tryptophan decarboxylase stops converting tryptophan to tryptamine, and as a result less psilocybin is produced. No report available showing role of biotechnology in this direction or subject. The only reference available describes molecular cloning of enzymes (enzyme coded by cDNA) from the psilocybin biosynthesis pathway in *Psilocybe tampanensis* to determine enzymatic activities [152].

DNA-based approach (phylogenetic approach) has been reported by Canadian investigators for various species of psilocybin mushrooms which are quite helpful in the identification of species that contain the psychoactive compounds [153]. The method includes DNA amplification and sequencing of the internal transcribed spacer region of the rDNA (ITS-1) and a 5′ portion of the nuclear large ribosomal

subunit of rRNA (nLSU rRNA or 28 S). The method is quite useful in the identification of unknown fungal species with regard to presence or absence of psilocybin and psilocin. Genetic techniques based on polymerase chain reactions (PCR) of specific regions of the genomes can also be used to identify and differentiate between psilocybin and non-psilocybin-producing species and strains of various mushrooms. The submerged culture technique for the production psilocybin is still believed to be the best biotechnological approach. Though improvement can also be done through field cultivation of mushrooms, yet it will be quite laborious, expensive, time consuming, and will also depend upon several environmental factors not easily manageable.

## 7 Conclusion/Prospects

With the increasing number of psychotic patients worldwide, there is a definite need to explore and find new pathways of treatment by both discovering new molecules as well as utilizing known drugs by modifying the dimension of the research activities and protocols. The introduction of biotechnology will have significant impact to this approach. Both psilocybin and psilocin are excellent examples such actives to initiate research in a broader way to explore these two bioactive indole alkaloids not only to see the possibility of their use in psychiatry but to widen their application for the treatment of other disorders as well. Thus, further research studies on these psychedelic to examine their overall effect on cerebral nervous system are proposed. Apart from this, the clinical role of these novel alkaloids can further be explored by modifying the dosage form from immediate to modified (extended) release dosage form to establish more convenience in terms of dosage and to reduce side effects. Prospects are quite open but seem to be challenging.

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