CHAPTER II

LITERATURE REVIEWS

2.1 Magic mushrooms

Magic mushrooms is the name most commonly given to mushrooms that grow naturally and have hallucinogenic (sometimes called psychedelic) properties (Bogusz et al., 1998, Seivewright and Lagundoye, 2000). The mushrooms were originally used as godly traditional medicine for centuries in the religious ceremony by the shamans in Central and South America (Kamata, et al., 2003, Stamets, 1996). Magic mushrooms are saprophytes. They can grow throughout most of the world. *Panaeolus cyanescens* was found in Hawaii, Mexico, Philippines, Eastern Australia, Thailand (Allen, 1992, Stamets, 1996). *Psilocybe cubensis* was found in Mexico, Cuba, America, Australia, India, Thailand, Vietnam, Japan (Tsujikawa et al., 2003, Allen, 1992, Stamets, 1996).

Hallucinogenic fungi can be found in many habitats such as grasslands, dung deposits, woodlands and burned lands. Most of them are small, dull-colored fungi and never attract much notice. These mushrooms belong to the genus *Panaeolus*, *Psilocybe*, *Conocybe*, *Gymnopilus*, *Inocybe* and *Pluteus*. The mushrooms mainly belong to the genus *Panaeolus* and *Psilocybe* (Stamets, 1996). Many *Psilocybe* and *Panaeolus* species will turn bluish green when they were bruised. This happens either as a normal response to growing conditions, or while they are handled as they are picked. The bluing reaction is of great interest to chemists and pharmacologists. Apparently, the blue pigmentation is a result of a phenomenon paralleling the degradation of unstable psilocin (dephosphorylated psilocybin) to presently unknown compounds by enzymes within the mushroom cells. This means is that when a *Psilocybe* or *Panaeolus* bruises bluish, the color reaction is a co-indicator that psilocin is or was present (Stamets, 1996, Levine, 1967).

Hallucinogenic mushrooms in Thailand often appear on the decomposed manure of domesticated water buffalo. The Thai people refer to mushrooms as *Hed* and formally call their buffalo *quai*. Thus, the native Thai phase for 'magic mushrooms' *Hed Keequai* can be translated as mushroom which appears after buffalo defecates. Hallucinogenic fungi in Thailand are in genus *Psilocybe* and *Panaeolus* including *Psilocybe cubensis*, *Psilocybe subcubensis*, *Panaeolus* (*Copelandia*) *cyanescens*, *Panaeolus antillarum* (Allen, 1992) and *Psilocybe samuiensis* Guzmán, Bandala and Allen (Allen, 1991, Guzmán et al.,1993, Gartz et al., 1994). Most of these mushrooms were found in the south of Thailand. Some of magic mushrooms are shown in Figure 2.1.

Psilocybe samuiensis was a new species of hallucinogenic fungi. It was first collected (from manured sandy-clay soil, in rice paddies at Koh Samui, Thailand) in 1991.

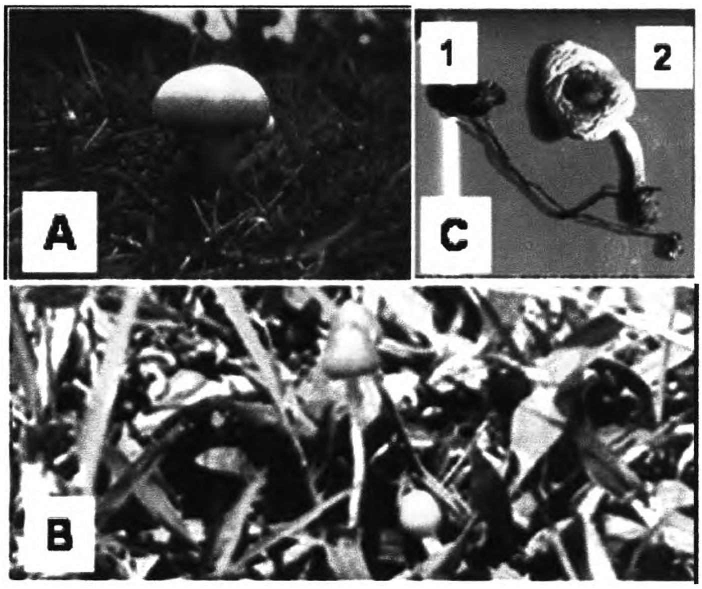


Figure 2.1 Example of hallucinogenic mushrooms in Thailand (Allen, 1992).
(A) *Psilocybe subcubensis* grew in nature
(B) *Psilocybe samuiensis* grew in nature
(C) Dry specimens of (1)*Copelandia cyanescens*, and (2) *Psilocybe cubensis*

The psychedelic effect of some species of genus *Psilocybe* were first described by Wasson in 1957 (Wasson, cited in Beug, 1981, Wasson and Wasson, cited in Beug, 1981). Hofman et al. (Hofmann et al., cited in Beug, 1958) isolated two important hallucinogenic compounds of the tryptamine type, psilocybin (4-phosphoryloxy-*N,N*-dimethyltryptamine), and psilocin (4-hydroxy-*N,N*-dimethyltryptamine) from *Psilocybe mexicana* (Beug and Bigwood, 1981, Keller et al., 1999, "Albert Hofmann", 1996-2000) and psilocybin was the main psychotropic compound.

Psilocin and psilocybin are the main psychoactive principle of *Psilocybe* mushrooms such as *Psilocybe semilanceata* ('liberty cap'). Psilocin and psilocybin interacts mainly with serotonin receptors such as 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptor subtypes. Serotonin receptors (also called 5-Hydroxy tryptamine receptors or 5-HT receptors) a monoamine neurotransmitter, found in cardiovascular tissue, in endothelial cells, in blood cells, and in the central nervous system (CNS), play an important role in perception and affect regulation and attention (McCall and Aghajanian, 1980, Hasler et al., 2004, Nichols, 2004, Sard et al., 2005). The hallucinogenic molecules fit into the same receptors as the neurotransmitter, and over-stimulate them, leading to fail signals being created (Ophardt, 2003). Serotonin receptor site is shown in Figure 2.2 and structure of serotonin is shown in Figure 2.3.

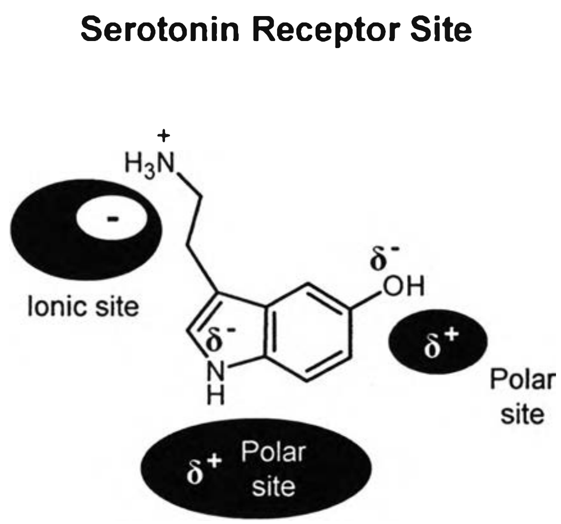
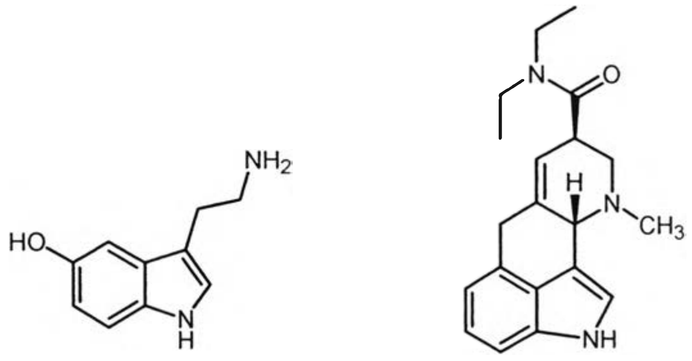


Figure 2.2 Serotonin receptor site (Ophardt, 2003)



Serotonin   Lysergic acid diethylamide (LSD)

Figure 2.3 Chemical structures of serotonin and lysergic acid diethylamide (LSD)

Psilocybin and related substances are potent hallucinogens producing pharmacological effects similar to those of lysergic acid diethylamide (LSD). After ingesting hallucinogenic mushrooms, symptoms occur within 20-60 minutes with depersonalization, hallucinations (sometimes extremely bizarre), derealization and euphoria, and symptoms such as vertigo, anxiety, agitation, headache, nausea, tachycardia, mydriasis, flushing, fever and seizures (rare). Fever and seizures have been seen in children. Symptoms are usually maximal within 2 hours and disappear within 4-6 hours, though 'flashback' may recur for weeks or months (Persson, 2003).

2.2 Chemical analysis of mushrooms in genus *Psilocybe*

In 1968 Leung and Pual studied chemical constituents from *Psilocybe baeocystis* grown in submerged cultures. They found psilocin, psilocybin and two new 4-phosphoryloxytryptamine derivatives, baeocystin (3-[2-(methylamino)ethyl]-1*H*-indol-4-ol dihydrogen phosphate ester) and norbaeocystin (3-aminoethyl-1*H*-indol-4-ol dihydrogen phosphate ester) (Leung and Pual, 1968).

In 1981 Koike and coworkers found psilocybin, ergosterol, ergosterol peroxide and α,α-trehalose from dried fruiting bodies of *Psilocybe argentipes*, a new specie found in Japan, 1979 (Koike et al., 1981).

In 1981 Beug and Bigwood studied quantitative analysis of psilocybin and psilocin from freeze-dried *Psilocybe baeocystis* Singer and Smith by high-performance liquid chromatography (HPLC). They found that both psilocybin and psilocin levels were highly variable: psilocybin levels ranged from 1.5 mg/g (dry weight) up to 8.5 mg/g (average 2.9 ± 1.5 mg/g), and typically 2.0 ± 0.2 mg/g. Psilocin levels ranged from zero to 5.9 mg/g (average 1.9 ± 1.5 mg/g) (Beug and Bigwood, 1981).

In 1982 Beug and Bigwood studied the analysis of eight species in genus *Psilocybe* from the Pacific Northwest, U.S.A. Among of these mushrooms, *Psilocybe semilanceata* (Fr.) Quél., the "liberty cap", a species that blues inconsistently, is most often sought. It is a consistent producer of psilocybin with levels ranging from 6.5 to 12.8 mg/g, typically in excess of 10 mg/g, making it one of the most potent species as well as one of the most content in amount. It was not found to contain psilocin. *Psilocybe cyanescens* Wakefield, a markedly bluing species, is usually even more potent than *Psilocybe semilanceata*. Psilocybin levels were found to range from 4.9 mg/g up to 16.8 mg/g dry weight with total psilocin plus psilocybin levels reaching 19.6 mg/g dry weight, nearly 2% of the dry weight. *Psilocybe stuntzii* Guzmán and Ott, sometimes contains neither psilocybin nor psilocin. They found maximum of psilocybin and psilocin were 3.6 mg/g and 0.6 mg/g, respectively. *Psilocybe baeocystis* Singer and Smith generally contains relatively low levels of both psilocybin (up to 2.8 mg/g) and of psilocin (up to 1.4 mg/g), but one collection was found containing 8.5 mg/g psilocybin and 5.9 mg/g psilocin. *Psilocybe pelliculosa* was also quite variable in psilocybin, with levels ranging from 1.2 mg/g to 7.1 mg/g dry weight. It was not found to contain psilocin. *Psilocybe inquilina* (Fr. ex Fr.) Bres., a non-bluing native species that grows on grass stems, was found to contain neither psilocybin nor psilocin. Both were also absent in *Psilocybe montana* (Pers. ex Fr.) Kummer, a non-bluing species found on dung in the spring through fall (Beug and Bigwood, 1982).

In 1994 Marcano and coworkers analyzed an amount of psilocin and psilocybin from *Psilocybe pseudobullacea* (Petch) Pegler, *Psilocybe montana* and *Psilocybe subcubensis* by thin-layer chromatographic and spectroscopic (UV) methods. Both hallucinogens were found in *Psilocybe pseudobullacea* and *Psilocybe subcubensis*, while *Psilocybe montana* was found to be exempt of these compounds. This research revealed the first occurrence of 4-hydroxygenated indole alkaloids in *Psilocybe pseudobullacea* (Marcano et al., 1994).

In 1994 Gartz, Allen and Merlin analyzed the alkaloids content in cultured fruit bodies of *Psilocybe samuiensis* and naturally occurring mushrooms of this species. HPLC analyzed of both naturally occurring and in vitro cultivated fruit bodies of *Psilocybe samuiensis* revealed high concentrations of psilocybin and psilocin. Psilocybin levels varied from 0.23% up to 0.90% dry weight, whilst psilocin levels varied from 0.05% up to 0.81% dry weight. A small amount of baeocystin were also detected and varied from 0.01% up to 0.5% dry weight. The psilocybin content was highest in caps. Psilocybin was also found in the cultured non-bluing mycelia of *Psilocybe samuiensis* and varied from 0.24% up to 0.32% dry weight (Gartz, Allen and Merlin, 1994).

In 1996 Gartz analyzed indole derivatives in dried fruit bodies of *Psilocybe bohemica* from the garden culture and from the cultivation on rice grain. The investigation showed the occurrence of psilocybin, small amount of psilocin and baeocystin in *Psilocybe bohemica* from garden culture. The concentration of these indole derivatives were similar to the levels found in naturally grown fruit bodies or in mushrooms from the cultivation on wet rice grain (Gartz, 1996).

In 1999 Keller and coworkers determined psilocin and psilocybin content in cap and stem of *Psilocybe subcubensis*. Psilocybin and psilocin were found in an amount of 0.86% and 0.02% dry weight in the caps, respectively. In the stems they found 0.8% dry weight of psilocybin and 0.03% dry weight of psilocin (Keller et al., 1999).

In 2000 Musshoff, Madea and Beike determined psilocybin and psilocin in *Psilocybe* mushrooms, *Psilocybe cubensis*, *Psilocybe semilanceata* and *Psilocybe tampanensis*. The alkaloids were ranged zero up to 1.07% dry weight of psilocybin and 0.01% up to 0.23% dry weight of psilocin in *Psilocybe cubensis*, 0.01% up to 0.91% dry weight of psilocybin and 0.01% up to 0.90% dry weight of psilocin in

Psilocybe semilanceata, zero up to 0.19% dry weight of psilocybin and 0.01% up to 0.03% of psilocin in *Psilocybe tampanensis* (Musshoff, Madea and Beike, 2000).

In 2003 Tsujikawa et al. (2003) determined psilocybin and psilocin in *Psilocybe cubensis* from Japan. The psilocin and psilocybin content in *Psilocybe cubensis* were in the range of 0.17% up to 0.78% dry weight and 0.44% up to 1.35% dry weight respectively in the caps, 0.09% up to 0.30% dry weight and 0.05% up to 1.27% dry weight respectively in the stems. Hallucinogenic alkaloids (psilocin and psilocybin) had a tendency to be contained in the cap more than the stem (Tsujikawa et al., 2003).

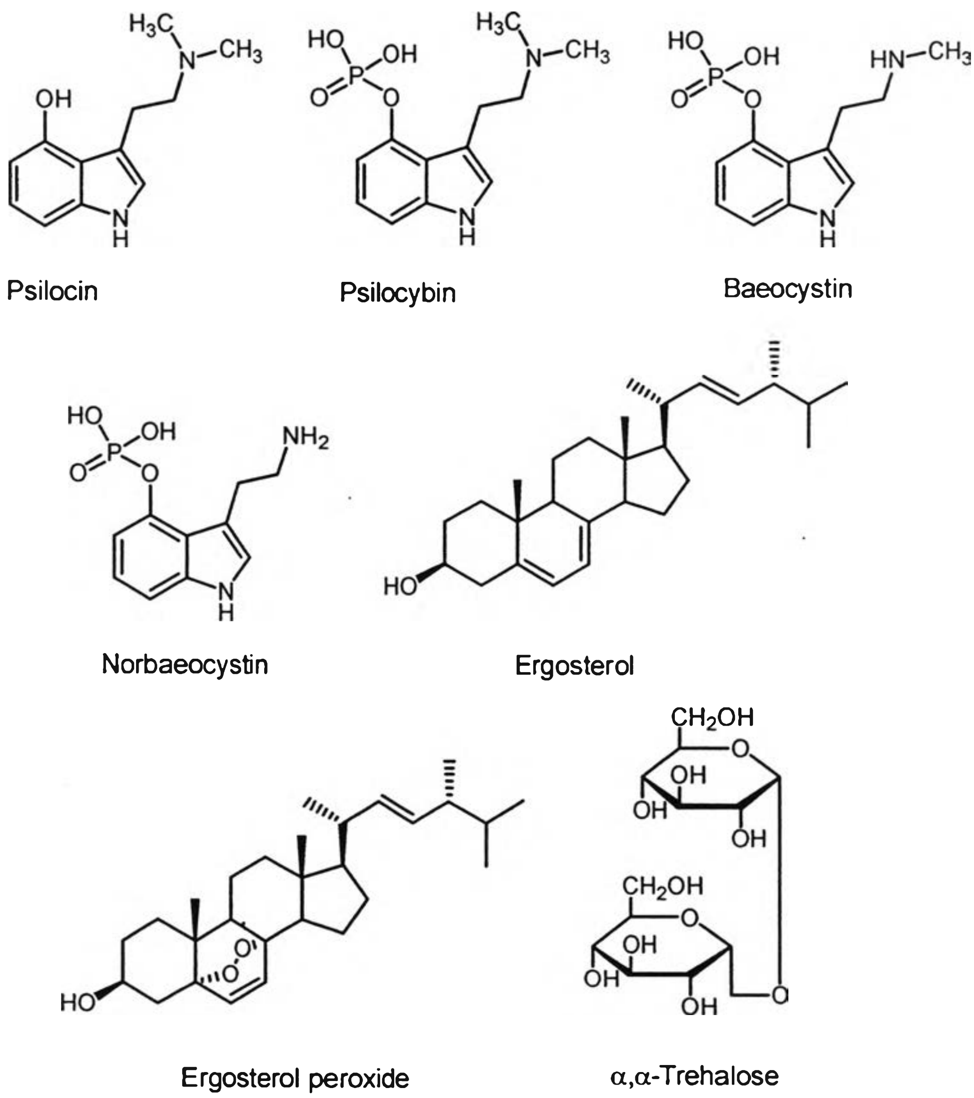
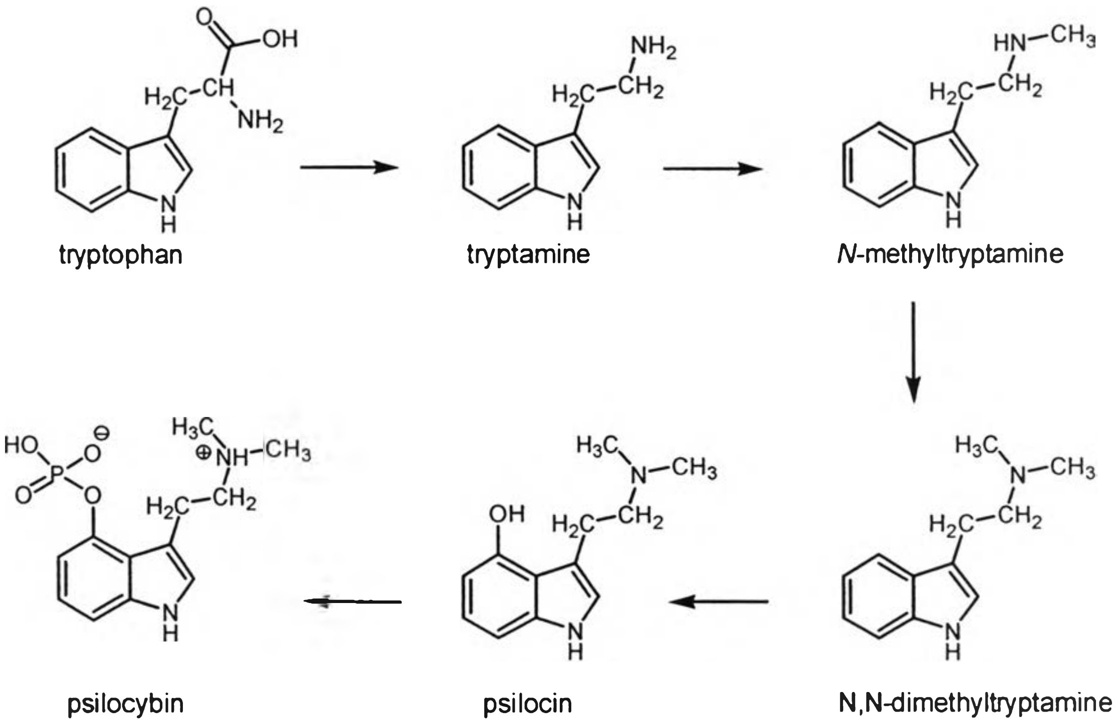


Figure 2.4 Chemical structures from hallucinogenic mushrooms

2.3 Biosynthesis of psilocin and psilocybin

Psilocin and psilocybin are closely related to 4-hydroxylated indoles. Psilocybin may biosynthetically derive from tryptophan and tryptamine. A possible pathway of psilocin and psilocybin biosynthesis is shown in Scheme 2.1 (Agurell and Nilsson, 1968a, Agurell and Nilsson, 1968b).



Scheme 2.1 A possible pathway of psilocin and psilocybin biosynthesis as described by Agurell and Nilsson (Agurell and Nilsson, 1968, Agurell and Nilsson, 1968b).

2.4 The ethnomycology of *Psilocybe samuiensis*

2.4.1 Cap

The cap is 7-15 mm in diameter, subconvex to conic-convex, conic umbonate or campanulate-umbonate, frequently with a small papilla. It is viscid with a separable pellicle, even and striate to sulcate at the margin, and hygrophanous. It is chestnut or reddish brown to straw yellow when young, fading in drying to straw or brownish (Guzmán, et al., 1993, Gartz, et al., 1994, Stamets, 1996).

2.4.2 Gills

The gills are adnate to adnexed, clay color, becoming violaceous brown or chocolate brown-violet when dry, with white edges (Guzmán, et al., 1993, Gartz, et al., 1994, Stamets, 1996).

2.4.3 Stipe

The stipe is 40-65 x 1.52 mm, equal or slightly subbulbous. It is hollow, white or whitish to pale straw color and covered with white fibrils. It is context concolorous with pileus, bluing with slightly farinaceous taste and odour (Guzmán, et al., 1993, Gartz, et al., 1994, Stamets, 1996).

2.4.4 Microscopic features

Microscopic features of *Psilocybe samuiensis* were described in Guzmán, et al., 1993, Gartz, et al., 1994 and Stamets, 1996.

Spores: 10.4-12.8 x 6.4-8 µm, rhomboid or subrhomboid in face-view, broadly ellipsoid or subellipsoid in side-view, with yellow brown, thick (0.4-0.8 µm) wall, and with broad flattened germ pore.

Basidia: 20-28 x 7.2-8.8 µm, 4 spored, hyaline subcylindric-clavate.

Pleurocystidia: 16-20 x 4.8-6.4 µm scattered, hyaline,ventricose sublageniform.

Cheilocystidia: 18.4-28(-30) x (4.8-)5-7.2(-8) µm, abundant, hyaline, ventricose-lageniform or sometimes submoniliform, frequently with an irregular branched neck.

Subhymenium: with narrow hyphae to globose or subglobose cells, 2.4-8 µm diameter, hyaline but with incrusted yellow pigment on the walls.

Hymenial trama regular: with cylindric hyphae, 24-16 µm wide, hyaline to yellowish.

Epicutis: formed of a galantinized layer, with more or less parallel, hyaline hyphae, 0.8-4 µm diameter.

Hypodermium: with cylindric hyphae, 4-12 µm diameter, hyaline, but with yellowish incrusting pigment.

Clamp connections: special septal structures are usually formed in *Psilocybe* mushrooms

2.4.5 Habitat

Psilocybe samuiensis was first collected in soil of mixed sand and clay, among fan palms in rice paddies situated 2 km west of the village of Ban Hua Thanon, Koh Samui, Thailand. It dose not fruit directly in manure but appears scattered or gregarious in the manured soil of rice paddies. This fungus fruits from early July through late August (Guzmán, et al., 1993, Gartz, et al., 1994, Stamets, 1996).

Psilocybe samuiensis is microscopically similar to *Psilocybe mexicana*, but the form and size of spores, as well as the presence of pleurocystidia, its macroscopic feature, and habitat are very similar to *Psilocybe semilanceata*. Guzmán et al. (Guzmán et al., 1993) placed this species in the section Mexicanae because of the big rhomboid or subrhomboid spores which separate this species from *Psilocybe mexicana* and other species in the *Psilocybe* section Mexicanae. Although *Psilocybe samuiensis* is microscopically similar to *Psilocybe mexicana*, it macroscopically resembles *Psilocybe semilanceata*. However, the latter species differs macroscopically from *Psilocybe samuiensis* by the height or length of their respective stipes and the color of the fruit bodies. *Psilocybe samuiensis* and *Psilocybe semilanceata* attain heights of 40-65 x 1.52 mm and 70-110 x 1.52 mm respectively (Gartz et al., 1994). Macroscopic feature and microscopic feature of *Psilocybe samuiensis* is show in Figure 2.5 and picture of *Psilocybe semilanceata* is show in Figure 2.6.

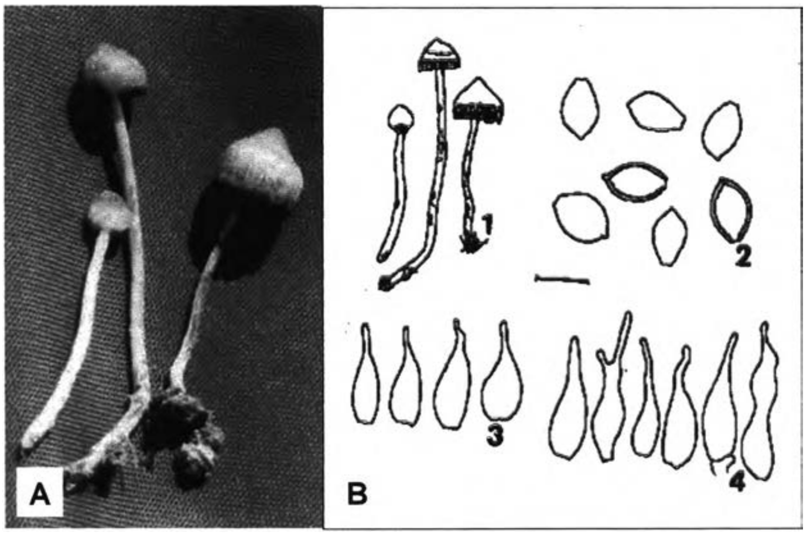


Figure 2.5 (A) Photograph of *Psilocybe samuiensis* (Gartz et al.,1994)

(B) Drawing picture of 1: basidiomes, 2: basidiospores, 3: pleurocystidia, 4: cheilocystidia (all from the type)(scale bare = 10 µm in 2-4, and 15 cm in 1) (Guzmán et al., 1993).

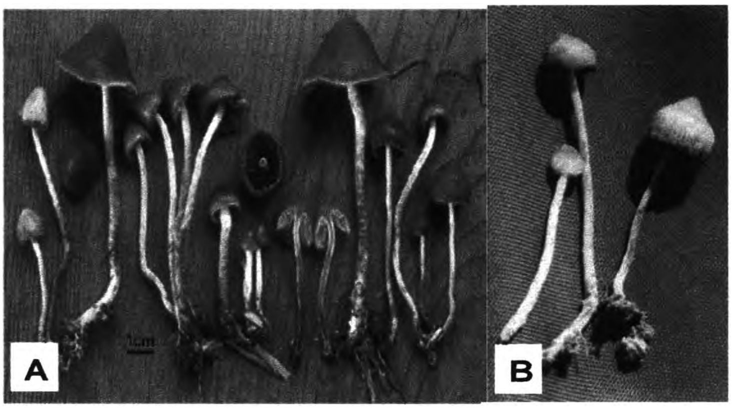


Figure 2.6 (A) *Psilocybe semilanceata* (Persson, 2003)

(B) *Psilocybe samuiensis* (Gartz, et al., 1994)