Baeocystin in *Psilocybe*, *Conocybe* and *Panaeolus*

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ABSTRACT.—Sixty collections of ten species referred to three families of the Agaricales have been analyzed for the presence of baeocystin by thin-layer chromatography. Baeocystin was detected in collections of *Psilocybe*, *Conocybe*, and *Panaeolus* from the U.S.A., Canada, Mexico, and Peru. Laboratory cultivated fruitbodies of *Psilocybe cubensis*, *P. semilanceata*, and *P. cyanescens* were also studied.

Intra-species variation in the presence and decay rate of baeocystin, psilocybin, and psilocin are discussed in terms of age and storage factors. In addition, evidence is presented to support the presence of 4-hydroxytryptamine in collections of *P. baeocystis* and *P. cyanescens*. The possible significance of baeocystin and 4-hydroxytryptamine in the biosynthesis of psilocybin in these organisms is discussed.

A recent report (1) described the isolation of baeocystin [4-phosphoryloxy-3-(2-methylaminoethyl)indole] from collections of *Psilocybe semilanceata* (Fr.) Kummer. Previously, baeocystin had been detected only in *Psilocybe baeocystis* Singer and Smith (2, 3). This report now describes some further observations regarding the occurrence of baeocystin in species referred to three families of Agaricales.

Stein, Closs, and Gabel (4) isolated a compound from an agaric that they described as *Panaeolus venenosus* Murr., a species which is now considered synonomous with *Panaeolus subbalteatus* (Berk. and Br.) Sacc. (5, 6). This compound was similar to psilocybin [4-phosphoryloxy-3-(2-dimethylaminoethyl)-indole] but exhibited a higher melting point. No structural elucidation was offered. In a study of the indole alkaloids of the Strophariaceae, Leung (2) and Leung and Paul (3) detected, isolated, and determined the structures of two psilocybin analogs which they named baeocystin and norbaeocystin. These compounds differed from psilocybin only by the lack of one and two methyl groups on the terminal nitrogen, respectively. This finding led Leung and Paul (3) to suggest that the compound isolated from *Panaeolus venenosus* (4) was baeocystin. The finding of the two analogs of psilocybin in *P. baeocystis* prompted Leung and Paul (3) to suggest a major role for these compounds in the biosynthesis of psilocybin in this species. Agurell and Nilsson (7) also proposed a minor pathway to psilocybin in *P. cubensis* (Earle) Singer (=*Stropharia cubensis* Earle) involving O-phosphorylation prior to N-methylation. However, they suggested that the major pathway was *via* hydroxylation of N,N-dimethyltryptamine followed by phosphorylation (7, 8).

Psilocybin and its dephosphorylated counterpart, psilocin, have been detected in various species of *Psilocybe* (Fr.) Kummer (family Strophariaceae Singer and Smith), *Panaeolus* (Fr.) Quel. (family Coprinaceae Roze), and *Conocybe* Fayod (family Bolbitaceae Singer). Species of *Stropharia* (Fr.) Quel., *Copelandia* Bres., *Panaeolina* Maire, and *Pholiotina* Fayod, which have been reported to contain psilocybin and/or psilocin or to be psychoactive, are now usually referred to *Psilocybe* (9), *Panaeolus* (6, 10), and *Conocybe* (11). Psychoactive species of these genera have been reported to occur in North (12, 13)

and South America (9), Europe (14), Asia (14, 15), Australia (16), and Africa (6, 9).

Although pharmacological or clinical studies of baeocystin and norbaeocystin are lacking, McCawley, Brummett, and Dana (17) reported several toxic (one fatal) reactions due to accidental ingestion of agarics thought to be *P. baeocystis*. However, the actual specimens involved in the intoxications were not identified.

EXPERIMENTAL

MATERIAL STUDIED.—Collections of the following species were examined in this study: *P. baeocystis* Singer & Smith, *P. cubensis* (Earle) Singer, *P. cyanescens* Wakefield, *P. pelliculosa* (Smith) Singer & Smith, *P. semilanceata* (Fr.) Kummer, *P. silvatica* (Peck) Singer & Smith, *P. stuntzii* Guzmán & Ott, *Conocybe smithii* Watling (=*Galera cyanopes* Kauffman), *Conocybe cyanopus* (Atk.) Kühner *sensu* Kühner, and *Panaeolus subbalteatus* (Berk. & Br.) Sacc.

All collections are deposited in the herbaria of either the Escuela Nacional Ciencias Biológicas, Instituto Politecnico Nacional, Mexico, D.F. (ENCB); the University of Michigan, Ann Arbor, Michigan (MICH); the San Francisco State University, San Francisco, California (SFSU); the Royal Botanical Garden, Edinburgh (E); the Centraalbureau voor Schimmelcultures, Baarn (CBS); or the Institute for Fermentation, Osaka (IFO). Some exceptionally large collections were deposited in more than one herbarium.

When fresh material of some species was unavailable, laboratory cultivation for the production of fruiting bodies was attempted. Cultures were isolated from the internal tissue of carpophores of certain collections (except for *P. cyanescens* LESLIE 1870 which was isolated from a spore deposit) and were maintained in pure culture on sterilized MEA (Difco malt extract, 20 g; Difco agar, 15 g; distilled water, 1 liter). Cotton-plugged 500 ml Erlenmeyer flasks and jar assemblies (as described by San Antonio (19)) were filled with composted natural materials (listed in Table 1), sterilized by autoclaving, cooled, innoculated from stock cultures, and incubated at 23°. Illumination was provided for 10 hrs/day by 400–700 foot-candles of fluorescent light. Fruitbodies were produced by cultures of *P. cubensis* in 3 to 8 wks while the strain of *P. semilanceata* produced carpophores after 84 wks, and the culture of *P. cyanescens* produced primordia after 24 to 35 wks. Subcultures of these strains are on deposit at the CBS and IFO.

ANALYTICAL METHODS AND EXTRACTION PROCEDURES.—Thin-layer chromatography was carried out on 20 x 20 cm glass plates coated with 0.25 mm layers of silica gel GF (Analtech, Newark, Del.). The solvent systems investigated were A) 6% aqueous ammonia/*n*-propanol (2:5), B) *n*-butanol/glacial acetic acid/water (2:1:1), and C) 1.5% ammonia in methanol (20). The standard of baeocystin was isolated and characterized earlier (1).

Weighed amounts (5–50 mg) of dried fungal material were ground to a powder and extracted by shaking with methanol (2.0 ml) at room temperature for 20 hrs. This time period was found to be sufficient for complete extraction of the alkaloids. The samples were centrifuged and 1.0 ml of the supernatant drawn off and concentrated in a stream of dry nitrogen at 30°. The dry extracts were reconstituted by addition of 100 μl of methanol. An aliquot (20 μl) of each solution was applied to a thin-layer plate. Solvent system A was found to give the best results in terms of resolution of the multi-component extract. After development the plates were dried in a stream of warm air and examined under short wave uv light. Baeocystin and other indoles were visualized by spraying the plates with 1% 4-dimethylaminobenzaldehyde in ethanol containing 5% hydrochloric acid followed by exposure to hydrogen chloride vapors. Baeocystin exhibited a pink to purple to blue color reaction. A semi-quantitative estimation was made by comparison with varying concentrations of the standard chromatographed under the same conditions. Quantitative values obtained by tlc methods are not considered more accurate than ±20% (23, 24).

SYNTHESIS.—The standard of 3-(2-aminoethyl)-indol-4-ol (4-hydroxytryptamine) was synthesized as follows:

4-ACETOXYINDOLE-3-GLYOXYLAMIDE.—A solution of 4-acetoxyindole (500 mg, 2.86 mmol) in diethyl ether (2.0 ml) was added dropwise to a stirred solution of oxalyl chloride (0.5 ml, 5.86 mmol) in anhydrous diethyl ether (2.0 ml) at 0° (21). After the addition, the reaction was stirred at 0° for 5 hrs. The heterogeneous reaction mixture was then added to a cold, saturated solution of anhydrous ammonia in ether (150 ml). Water (50 ml) was added, and the white suspended solid was collected by filtration and dried *in vacuo*. After recrystallization from hot water the product had mp 198–200°, 445 mg (63%).

Calculated for $C_{12}H_{10}N_2O_4$ (246.23): C 58.53 H 4.09 N 11.38
Found: C 58.89 H 4.09 N 11.55

4-HYDROXYTRYPTAMINE.—A solution of 1N diborane-tetrahydrofuran complex in tetrahydrofuran (3.0 ml, 3.0 mmol) was added to a solution of 4-acetoxyindole-3-glyoxylamide (100 mg, 0.41

TABLE 1. *Occurrence of baeocystin in* Psilocybe, Conocybe, *and* Panaeolus.

Species analyzed	Collection number and herbaria	Origin of collection	Habit and habitat	Method[a] of drying	Age[b, c] when analyzed	Concentration[d] of baeocystin
Psilocybe baeocystis	LESLIE 2731 ENCB	Thurston Co., Washington October 30, 1975	Scattered to gregarious on wood and bark chips	AD	3	0.02 –.03%
Psilocybe baeocystis	"	"	"	"[e]	66	0
Psilocybe baeocystis	LESLIE 3833 ENCB	Thurston Co., Washington November 11, 1976	Scattered to gregarious on wood and bark chips	FD	4	0.02 –.04%
Psilocybe baeocystis	"	"	"	"	20	0
Psilocybe baeocystis	LESLIE 3995 ENCB	Washington Co., Oregon November 20, 1976	Scattered to gregarious on wood and bark chips	FD	4	0.01 –.02%
Psilocybe baeocystis	LESLIE 3971 ENCB	Lane Co., Oregon (County of type location) November 3, 1976	Scattered to gregarious in lawn, on wood and bark chips	FD	4	0.03 –.06%
Psilocybe baeocystis	LESLIE 3649 ENCB	Vancouver, British Columbia, Canada November 3, 1976	Scattered to gregarious on wood and bark chips	FD	4	0.08 –.10%
Psilocybe baeocystis	HDT 24723 SFSU	San Francisco Co., California January 17, 1970	Gregarious on ground	AD	260	0
Psilocybe cyanescens	LESLIE 2732 ENCB	Thurston Co., Washington October 30, 1975	Gregarious to caespitose on wood and bark chips	AD	3	0.02 –.03%
Psilocybe cyanescens	"	"	"	"	66	0
Psilocybe cyanescens	LESLIE 3692 ENCB	Thurston Co., Washington November 3, 1976	Gregarious to caespitose on wood and bark chips	FD	4	0.02 –.04%
Psilocybe cyanescens	"	"	"	"	20	0.01 –.03%
Psilocybe cyanescens	LESLIE 3972 ENCB	Lane Co., Oregon November 20, 1976	Gregarious to caespitose on rotten log	AD	4	0.004–.01%
Psilocybe cyanescens	LESLIE 1870 ENCB	San Francisco Co., California January 1973	Solitary in leaves, twigs, and humus in wooded area	AD	163	0
Psilocybe cyanescens	Culture from	Laboratory culture	Cultivated on composted wood chips and leaves	FD	1	0.004–.007%

	LESLIE 1870 IFO CBS					
Psilocybe cyanescens.....	"	" "	Cultivated on composted horse manure	FD	1	0.004–.007%
Psilocybe cyanescens.....	HDT 32138 SFSU	San Francisco Co., California January 6, 1974	Gregarious on ground	AD	148	0.005–.008%
Psilocybe cyanescens.....	HDT 26886 SFSU	San Francisco Co., California November 9, 1970	Gregarious on ground	AD	260	0
Psilocybe cubensis.....	LESLIE 4185 (Schroeder) [f] ENCB	Harris Co., Texas April 1976	Scattered on cow manure in pasture	AD	188	0
Psilocybe cubensis.....	LESLIE 1902 ENCB, MICH	Huautla de Jimenez, Estado de Oaxaca, Mexico August 31, 1974	Solitary on cow dung, on forest path	AD	53	0
Psilocybe cubensis.....	Culture from LESLIE 1902 IFO CBS	Laboratory culture	Cultivated on sterilized horse dung	FD	2	0.002–.003%
Psilocybe cubensis.....	"	"	"	"	52	0
Psilocybe cubensis.....	"	"	Stored at −5° under anhydrous conditions	"	52	0.001–.002%
Psilocybe cubensis.....	"	"	Cultivated on MEA	AD	2	0.001–.002%
Psilocybe cubensis.....	"	"	Cultivated on sterilized wheat bran and sand	AD	1	0.007–.01%
Psilocybe cubensis.....	"	"	Stored at −5° under anhydrous conditions	"	52	0.004–.009%
Psilocybe cubensis.....	"	"	Cultivated on sterilized composted grass	AD	1	0
Psilocybe cubensis.....	"	"	Cultivated on sterilized foliage of *Camellia thea* Link	AD	1	0
Psilocybe cubensis.....	"	"	Cultivated on sterilized composted foliage of *Pseudotsuga menziesii* (Mirb.) Franco	AD	4	0

TABLE 1. *Continued.*

Species analyzed	Collection number and herbaria	Origin of collection	Habit and habitat	Method[a] of drying	Age[d, e] when analyzed	Concentration[d] of baeocystin
Psilocybe cubensis	LESLIE 4171 ENCB (Bigwood, T. & D. McKenna) IFO, CBS	Pucallpa, Peru	Cultivated on sterilized horse manure	FD	1	0.02%
Psilocybe cubensis	"	"	Cultivated on MEA	FD	1	0.006%
Psilocybe cubensis[g]	LESLIE 3976	Origin unknown	Cultivated on sterilized horse manure	FD	1	0.009%
Psilocybe cubensis	ENCB, "	"	Cultivated on MEA	FD	1	0.006%
Psilocybe pelliculosa	LESLIE 2758 ENCB	Thurston Co., Washington October 30, 1975	Gregarious to caespitose on rotted sawdust in forest	AD	6	0
Psilocybe pelliculosa	REPKE & LESLIE 76–62 ENCB	Thurston Co., Washington October 31, 1976	Gregarious to caespitose on rotted sawdust in forest	FD	1	0.02 –.05%
Psilocybe pelliculosa	LESLIE 3942 ENCB	Lane Co., Oregon (15 km from type location) November 16, 1976	Gregarious to caespitose on twigs and humus in forest	FD	4	0
Psilocybe pelliculosa	LESLIE 3877 ENCB	Whatcom Co., Washington November 1, 1976	Gregarious to caespitose on twigs and humus in forest	AD	5	0.008–.01%
Psilocybe pelliculosa	"	"	"	"	20	0.007–.01%
Psilocybe pelliculosa	LESLIE 4299 (Glowa & Berry)	Vancouver, British Columbia, Canada November 1976	Gregarious on twigs and humus in forest	AD	2	0.02 –.04%
Psilocybe semilanceata	LESLIE 1351 ENCB, MICH	Grays Harbor Co., Washington October 23, 1972	Scattered to gregarious in pasture	AD	15	0.10 –.12%
Psilocybe semilanceata	"	"	"	"	52	0.07 –.09%
Psilocybe semilanceata	"	"	"	"	208	0.02 –.03%

Psilocybe semilanceata....	LESLIE - 2409 ENCB, MICH	Jefferson Co., Washington November 30, 1974	Scattered to gregarious in pasture	AD	6	0.09 –.13%
Psilocybe semilanceata....	"	"	"	"	104	0.05 –.07%
Psilocybe semilanceata....	Culture from LESLIE 2409 IFO, CBS	Laboratory culture	Cultivated on sterilized rotted branches of *Quercus* sp.	FD	1	0.005%
Psilocybe semilanceata....	LESLIE 2710 ENCB, MICH	Lewis Co., Washington October 26, 1975	Scattered to gregarious in pasture	FD	4	0.09– .14%
Psilocybe semilanceata....	"	"	"	"	64	0
Psilocybe semilanceata....	LESLIE 3832 ENCB	Thurston Co., Washington November 11, 1976	Scattered to gregarious in pasture	FD	3	0.13 –.17%
Psilocybe semilanceata....	LESLIE 2781 ENCB	Curry Co., Oregon October 25, 1975	Scattered to gregarious in pasture	AD	3	0.07–.12%
Psilocybe semilanceata....	LESLIE 3928 ENCB	Lane Co., Oregon November 16, 1976	Scattered to gregarious in pasture	AD	4	0.04 –.06%
Psilocybe semilanceata....	LESLIE 3651 ENCB	Vancouver, British Columbia, Canada November 3, 1976	Scattered to gregarious in lawn	AD	4	0.06 –.11%
Psilocybe silvatica.....	LESLIE 1822 ENCB	Grays Harbor Co., Washington October 7, 1973	Gregarious to caespitose on twigs and humus in forest	AD	8	0
Psilocybe silvatica.....	LESLIE 3827 ENCB	Thurston Co., Washington November 11, 1976	Gregarious to caespitose on rotted sawdust in forest	FD	4	0–.007%
Psilocybe silvatica.....	"	"	"	"	20	0–.004%
Psilocybe silvatica.....	LESLIE 3842 ENCB	Thurston Co., Washington November 11, 1976	Gregarious to caespitose on rotted sawdust in forest	FD	4	0.01 –.02%
Psilocybe silvatica.....	"	"	"	"	20	0.005–.01%
Psilocybe stuntzii[h].....	LESLIE 2709 ENCB	Lewis Co., Washington October 26, 1975	Scattered to gregarious in pasture	FD	1	0.006–.02%
Psilocybe stuntzii.....	LESLIE 3908 ENCB	Thurston Co., Washington November 13, 1976	Gregarious to caespitose in lawn on wood and bark chips	AD	4	0.004–.008%
Psilocybe stuntzii.....	"	"	"	"	20	0.004–.006%

TABLE 1. *Continued.*

Species analyzed	Collection number and herbaria	Origin of collection	Habit and habitat	Method[a] of drying	Age[b, c] when analyzed	Concentration[d] of baeocystin
Psilocybe stuntzii	LESLIE 4000 ENCB	Hood River Co., Oregon November 20, 1976	Gregarious to caespitose on wood and bark chips	AD	12	0
Psilocybe stuntzii	LESLIE 3996 ENCB, MICH, E	Washington Co., Oregon November 22, 1976	Gregarious to caespitose on wood and bark chips	AD	4	0.006–.02%
Psilocybe stuntzii	"	"	"	"	12	0.002–.004%
Psilocybe stuntzii	LESLIE 4293 (Kroeger) ENCB	Vancouver, British Columbia, Canada October 1976	Gregarious to caespitose on wood and bark chips	AD	22	0.004–.009%
Conocybe smithii	LESLIE 1840 E	Jefferson Co., Washington November 1973	Solitary in grass and moss	AD	183	0
Conocybe smithii	LESLIE 3726 E	Thurston Co., Washington November 7, 1976	Solitary in grass and moss in pasture	AD	4	0.04 –.08%
Conocybe cyanopus sensu Kühner	LESLIE 2767 (Boydston) E	Thurston Co., Washington August 1975	Scattered in grass and moss in lawn	AD	13	0.05%
Conocybe cyanopus sensu Kühner	LESLIE 3981, 4026 4290 (all Kroeger), LESLIE 4300, 4301 (both Glowa & Berry) LESLIE 3650 E	Vancouver, British Columbia, Canada August–November 1976	Scattered in grass and moss in lawn	AD	4–20	0.03 –0.10%
Panaeolus subbalteatus	LESLIE 2753 ENCB	Thurston Co., Washington November 1975	Scattered to subcaespitose in well manured garden	AD	4	0.002–.003%

Panaeolus subbalteatus....	"	"	"	"	52	0.002–.003%
Panaeolus subbalteatus....	LESLIE 3764 ENCB	Lane Co., Oregon November 1976	Scattered to subcaespitose on horse manure pile	FD	4	0.002–.005%
Paneaolus subbalteatus....	"	"	"	"	25	0.002–.005%
Panaeolus subbalteatus....	LESLIE 3773 ENCB	Lane Co., Oregon November 1976	Scattered to subcaespitose in well manured garden	FD	4	0.001–.002%
Panaeolus subbalteatus....	LESLIE 4735 (Warth) ENCB	Carroll Co., Maryland November 1974	Scattered to gregarious on horse manure pile	AD	32	0

[a]AD = Air dried at 40°; FD = Freeze-dried.
[b]Specimens were stored at 22° and 5–10% relative humidity unless otherwise noted.
[c]Time (in weeks) between collection and analysis.
[d]Based on dry weight. Range of percent indicates more than one carpophore analyzed per collection.
[e]Ditto marks refer to repeated analysis of the same material after further storage.
[f]Actual Collectors name appears in parenthesis following collection number.
[g]This collection is a small spored form of *P. cubensis* with basidiospores measuring 11.5–12.5 (–13.5) x 7.5–9 x 7.5–8 μ
[h]This collection differs from the type description of *P. stuntzii* in the smaller and more fragile carpophores, the thinner stipe which is rarely, and then only faintly caerulescent, the non-caerulescent context, and the habitat not on buried or exposed wood debris or chips.

mmol) in 5.0 ml anydrous tetrahydrofuran under a nitrogen atmosphere. The orange solution was kept at room temperature for 16 hrs, then methanol was added dropwise to destroy excess diborane. The clear solution was concentrated *in vacuo*. The dark brown oil was distilled *in vacuo* (Kugelrohr apparatus) at 0.05 mm collecting material boiling at 150–175°. The clear oily product (15 mg, 21%), while homogeneous by tlc, proved to be exceedingly unstable, as predicted by earlier work (21) with this type of compound. It could not be induced to crystallize and rapidly darkened upon standing or in solution in a variety of solvents. Attempted further purification by chromatography on silica resulted in decomposition. The compound was characterized by mass spectrometry of its *bis*-trimethylsilyl derivative using the method described earlier (22). The following peaks were observed: m/e 320 (M^+) (relative intensity 15%), 305 (3%), 293 (4%), 292 (15%), 291 (42%), 290 (84%), 288 (3%), 218 (3%), 102 (1%), 75 (5%), 74 (12%), 73 (100%), 59 (3%), 45 (17%), and 30 (5%). This spectrum was analogous to that of the trimethylsilyl derivative of psilocin (22).

RESULTS AND DISCUSSION

Table 1 illustrates the results of the analysis for the detection of baeocystin in 60 collections of 10 species of agarics. Previous analyses of baeocystin utilized a two-dimensional thin-layer chromatography technique (2, 3). We have found that uni-directional tlc using solvent system A results in the clear resolution of baeocystin ($R_F = 0.12$), psilocybin ($R_F = 0.16$), and other components.[1] These two substances were not separated by systems B and C. Several experimental factors are important for successful analysis: 1) The solvent must be prepared just prior to the chromatographic run and equilibrated for just 10 min in the solvent tank (unlined). 2) The solvent front should migrate at least 15 cm from the origin (running time, 3 hrs.). Shorter distances or aged solvent mixtures result in tailing and overlap of the baeocystin spot with that of psilocybin. Any overlap is critical, since in all collections in which baeocystin was detected the ratio of psilocybin to baeocystin was 4:1 to 20:1. 3) For maximum color development the exposure of the plates to hydrogen chloride vapors should be for a minimum of 6 hrs. The limits of detection of baeocystin under these conditions are 0.05 μg/spot. 4) Specimens should be analyzed as soon as possible after collection. Following these procedures, psilocin ($R_F = 0.71$, system A) was also well resolved. Baeocystin was never detected in the absence of psilocybin. In agreement with previous reports (1, 6, 11, 13, 18, 25) psilocin and psilocybin were detected in all collections when first examined except that psilocin was not detected in collections of *Conocybe* and *Panaeolus*, or in *P. cyanescens* (H.D. Thiers 26886).

Psilocin has not been previously reported in *P. stuntzii*. Neither this species nor *P. cyanescens* has been reported from Oregon. The detection of psilocybin and/or psilocin in *P. cubensis* or *Panaeolus subbalteatus* from the U.S.A. and *P. cubensis* from South America has not been previously reported, nor have these compounds been reported from any collections of any species from California. *P. silvatica* has not been previously analyzed, nor has this species been reported from the western U.S.A. Baeocystin, psilocybin, and psilocin are reported for the first time from Canadian collections of *P. baeocystis, P. pelliculosa, P. stuntzii,* and several collections of *Conocybe cyanopus* sensu Kühner.

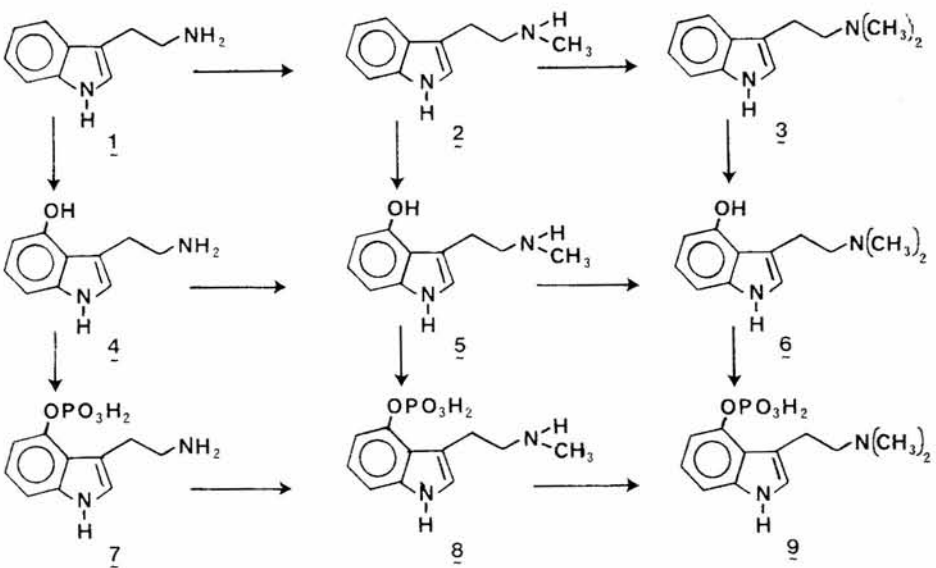
We also wish to present preliminary evidence to support the presence of 4-hydroxytryptamine (**4**, Scheme 1) in varying concentrations in all collections of *P. baeocystis* and *P. cyanescens*. This compound co-chromatographs ($R_F = 0.55$, system A; $R_F = 0.77$, system B) in admixture with a synthetic standard and exhibits the same color reactions as the standard, e.g., rapid darkening from light brown to black on tlc plates on exposure to air and an immediate purple color on spraying with 4-dimethylaminobenzaldehyde reagent. Psilocin also shows these color reactions. In the collection of *C. smithii* (LESLIE 3726) and the laboratory cultivated *P. semilanceata*, a compound ($R_F = 0.08$, system

[1]Leung's extensive study of 98 solvent systems (2) revealed that system A was most effective for the resolution of these compounds.

A) moving slightly slower than baeocystin and having identical color reactions was observed on tlc. This compound is present in only trace amounts and very likely is norbaeocystin. Isolation studies concerning these compounds are now underway.

The widespread occurrence of baeocystin suggests that the biosynthesis of psilocybin involving hydroxylation prior to *N*-methylation (3, 7) may be the major route. The occurrence of 4-hydroxytryptamine in wild carpophores of some species would also lend support to this pathway. *N*-methyltryptamine is readily incorporated into the synthesis of psilocybin when added to laboratory cultures of *P. cubensis* (7), and these results have been used as convincing evidence to support the proposed pathway involving *N*-methylation prior to hydroxylation (7, 8). However, neither *N*-methyl- nor *N,N*-dimethyltryptamine have been detected in any of the psilocybin-containing agarics. A third reaction sequence was proposed in which tryptophan is 4-hydroxylated followed by decarboxylation, *N*-methylation, and *O*-phosphorylation (26). This apparently conflicting data serves to illustrate some of the difficulties encountered in assigning specific biosynthetic routes for secondary metabolites such as psilocybin. A number of factors must be considered in any discussion of this problem. Many non-specific enzyme systems exist in fungi which will catalyze reactions of more than one substrate. This is the case with the mixed-function oxygenases (27) which have the ability to oxidize exogenously added compounds, as well as normal, obligatory intermediates. As Turner has aptly stated:

> ". . . This lack of specificity of some oxidases has led to confusion in the study of biosynthetic pathways since the ability to transform a supposed intermediate into a natural product has sometimes been taken to imply a natural role for the precursor . . ." (27, p. 5)



SCHEME 1

The lack of specificity of enzymes has led Bu'Lock (28) to propose the idea of a metabolic grid, instead of a simple linear reaction sequence, in explaining biosynthetic phenomena. Scheme 1 illustrates this concept as applied to the compounds under discussion here. This shows that four enzyme systems can each

react with three different substrates resulting in six possible pathways from tryptamine, **1** (derived from tryptophan) to psilocybin, **9**. The detection of a precursor in the organism may not be sufficient to define it as an intermediate, since it may only be in equilibrium with a true intermediate (29). Conversely, some intermediates may not be detectable if they undergo reaction when bound to proteins. The phenolic amines (such as psilocin, **6**) could also arise, in part, by hydrolytic cleavage of the O-phosphate group during handling and work-up of the specimens. Therefore, the detection (or the lack of detection) of any of the compounds in scheme 1 must be interpreted with caution when assigning biosynthetic pathways.

We have found that the age of specimens, i.e., storage time between collection and analysis, is of importance when evaluating chemical data. Unfortunately, most previous investigators have given little consideration to this experimental factor. This age factor is demonstrated by the results of the analyses of *P. baeocystis* (LESLIE 2731) and *P. cyanescens* (LESLIE 2732). Baeocystin was originally detected in both collections; however, after 66 wks of storage at 22° and 5–10% relative humidity, this compound could not be detected in either collection. Psilocybin and psilocin similarly disappeared in *P. baeocystis* (LESLIE 2731). The rate of decomposition of baeocystin was irregular in these two species and in some collections of *P. baeocystis* and *P. cyanescens* this compound was detected even after a considerably longer storage time. Analysis of cultivated *P. cubensis* (derived from LESLIE 1902) grown on horse dung shows that baeocystin, psilocybin, and psilocin could not be detected in dried material stored at 22° for 52 wks. However, fragments of the same carpophore stored under anhydrous conditions at −5° for 52 wks and the freshly dried material (14 days) both contained these compounds. Similar decreases in baeocystin content related to storage were observed for collections of *P. semilanceata, P. silvatica,* and *P. stuntzii.* By contrast, the amount of baeocystin (and psilocybin) found in one collection of *Panaeolus subbalteatus* (LESLIE 2753) after 52 wks storage was the same as that detected in freshly dried specimens from the same collection.

In previous reports, Leung and Paul (2, 3) and Repke and Leslie (1) were unable to detect baeocystin in collections of *P. pelliculosa*. The failure to detect this compound may have been due to the age of the samples. However, this storage factor alone cannot adequately explain the variation in the detection of baeocystin in this species, since, in the course of this study, analyses of specimens of similar age produced different results. A similar, seemingly sporadic, appearance of psilocybin has been observed in *Psilocybe* (30) and *Panaeolus* (6, 30). Reports of negative data must be interpreted cautiously, especially when only limited amounts of material have been examined. In a separate report (30) we have shown that the disappearance of psilocybin in carpophores of several species can be very rapid. Variation in these metabolites has been well demonstrated by investigations utilizing vegetative mycelia of *Psilocybe* species cultivated under controlled conditions on liquid media (31–34). Changes in media formulation and time of harvest showed considerable variation in production and disappearance of some of these metabolites. It is possible that similar biochemical activities may occur in the hyphae of the fruit body.

There is no evidence to suggest that baeocystin was the causative agent in the observed toxic reactions supposedly involving *P. baeocystis* (17) in view of the common intentional ingestion (35) of many of the species reported here to contain baeocystin. As illustrated in scheme 1, psilocin **6**, differs from psilocybin **9**, by the absence of a phosphate ester group, as 4-hydroxy-*N*-methyltryptamine **5** differs from baeocystin **8**. Since the pharmacologic effects of psilocybin and psilocin are essentially the same (36), it is reasonable to suggest that the same relatiosnip holds true for baeocystin and 4-hydroxy-*N*-methyl-

tryptamine. In a study of various hydroxyindole alkylamines, Cerletti, Taeschler, and Weidmann (37) found qualitatively similar effects produced by 4-hydroxytryptamine, 4-hydroxy-*N*-methyltryptamine, and psilocin in a variety of animal and *in vitro* tests. These compounds elicited the same pressor response in the cat at comparable dose levels. Psilocin was found to have a more pronounced activation of spinal reflexes than its demethylated counterparts. Using the ability to inhibit pre- *versus* postsynaptic serotonin receptors as a measure of hallucinogenic potency, Haigler and Aghajanian (38) recently demonstrated that 4-hydroxytryptamine is much less active than psilocin. In view of the foregoing discussion, it is possible that baeocystin and norbaeocystin have no more central activity than psilocybin.

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