

## Blueing in *Conocybe*, *Psilocybe*, and a *Stropharia* Species and the Detection of Psilocybin

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### TAXONOMY<sup>3</sup>

#### PSILOCYBE AND STROPHARIA

It is now a familiar observation that stropharioid fungi which in fresh specimens stain naturally blue or blue-green at the base of the stipe and often completely blue to the stipe apex when handled may contain the hallucinogenic drugs psilocybin and/or psilocin or closely related compounds. This generalization has resulted from the now well-documented work on the *Psilocybe* spp. used by Mexican Indians (18) in religious rituals and from subsequent studies on related species.

The correlation between staining and the occurrence of active constituents was of particular interest since one of us (R. W.) had successfully cultured *Stropharia fimetaria* Orton, a fungus described fairly recently from Scotland, and noticed that some of the carpophores developed a very noticeable bluish green stain. Indeed Orton (10) himself mentions this fact in the original description. Materials of both the type and of carpophores grown in sterile culture from basidiospores of the type were analysed for the presence of hallucinogenic principles; results as will be shown below were positive.

Orton pointed out that *S. fimetaria* was described in *Stropharia* in order to fall into line with the *New Check List of British Agarics and Boleti* (3), but some characteristics would place it in *Psilocybe*. The absence of chrysocystidia, the presence of long cucurbitiform to lageniform cheilocystidia, and now the presence of psilocybin are three factors which favour the transference of this fungus to the genus *Psilocybe*.

The presence of a veil which is sometimes annuliform is not considered sufficient to retain the taxon in *Stropharia* even when coupled with a viscid pellicle on the pileus, for several *Psilocybe* spp possess this latter character under humid conditions. *S. fimetaria* differs from *S. squamosa*, to which it was compared, in the structure of the subcutis; it is not subcellular. Singer (12) has transferred *Agaricus squamosus* Pers. ex Fr. to *Naematoloma* (= *Hypholoma* of the *Check List* authors) on the basis of anatomical features.

The following new combination is proposed:

***Psilocybe fimetaria*** (Orton) Watling comb. nov.

*Stropharia fimetaria* Orton, 1964 Notes R. Bot. Gdn. Ed. 26 (1), 49.

In Northern England and Scotland *Psilocybe semilanceata* (Fr. ex Secr.) Kummer consistently has a blueing stipe. This has been called var. *caerulescens* Cooke but appears to differ from the type variety in no other way than the flesh of the stipe changing colour. During the last three collecting seasons over half of the specimens found have at least some blueing on the stipe particularly towards the base. I think that the variety *caerulescens* represents nothing but a carpophore with an extreme form of blueing, just as in a single colony of *P. fimetaria* different carpophores exhibit different amounts of blueing. However, the blueing of *P.*

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*semilanceata* is of great interest as Tyler (personal communication) found psilocybin in extracts of a *Psilocybe* sp identical with those ingested by some University of British Columbia students for psychotomimetic effects. Specimens of this mushroom were later identified by A. H. Smith as *P. semilanceata*. In the present study, psilocybin was detected, as will be shown below, from both the typical form of *P. semilanceata* and its var. *caerulescens*.

The above observations suggest that *P. fimetaria* and *P. semilanceata* under the present taxonomic arrangements of Singer (15) should be included as members of *Psilocybe* Section *Caerulescentes* as outlined in greater detail by Singer and Smith (16) and Singer (13). *P. semilanceata* is usually taken as type of the genus, and therefore Section *Caerulescentes* falls into synonymy with Section *Psilocybe*. There is really very little after consulting Singer (15) which is significant other than blueing to separate Section *Psilocybe* from *Caerulescentes*, and from the above observations blueing is not a completely reliable character. Pileus shape is not sufficient to retain the sections separate as can be seen simply by comparing Singer and Smith's drawings of *P. pelliculosa*, *P. mexicana* (Section *Caerulescentes*), and *P. semilanceata* (Section *Psilocybe*).

The *Psilocybe merdaria* group has very great affinities with *Deconica* as is suggested by Orton's key (9) to that genus and by Huijsman's (6) work; it is now recognized that *Deconica* may be a superfluous genus. If *Deconica* is included in *Psilocybe*, as is done by Singer (15), then it would be useful to separate the different *Psilocybe* spp on a combination of rather different characters than at present used. Such a treatment is to be discussed elsewhere, when British collections of *Psilocybe cyanescens* are studied.

#### CONOCYBE<sup>4</sup>

Heim (4, 5) has recorded that *Conocybe siligineoides* is probably used in the religious ceremonies of certain tribes of Mexican Indians, and although this has been queried, it became more likely when Benedict and co-workers (1) detected psilocybin in another species of *Conocybe*. Guzman (personal communication), however, after extensive work in Mexico on these hallucinogenic fungi, has failed to collect the fungus and has found no further evidence of its being used by the Indians.

Examination of the type of *Galerula cyanopus* Atk. shows that it is a true member of the genus *Conocybe*; Kühner (7) correctly placed the fungus, as *Conocybe cyanopoda*, in the *Bolbitiaceae*. In parallel to *Psilocybe fimetaria*, *Conocybe cyanopus*, as its epithet implies, has a blueing base to the stipe and this could suggest a further connection, this time in the genus *Conocybe*, between blueing and the presence in the carpophore tissue of psilocybin as had been found in *Psilocybe*. Kühner had not seen *C. cyanopus* and published the notes on collections made in Europe by J. Schaeffer and in North America by A. H. Smith. In his accompanying diagrams the two collections are figured side by side, and differences immediately spring to mind. Similar differences are found in fig. 42 of the same book, and in this case it is now considered that one-half of the figure illustrates *C. pygmaeoaffinis*, the other half *C. striaepes*.

Schaeffer's collections are obviously the same as the material from Washington chemically analysed by Benedict *et al.* (1) and made available for examination. On the other hand Smith's notes refer to an agaric widespread in Michigan and named by Kauffman *Galera cyanopes*, only a few weeks after Atkinson's fungus had been published. Kauffman's type material is badly preserved but careful analysis shows that a hymenoderm is present and all other characters are in keeping with fresh material collected near the type locality. A very thorough examination of the type material was made, as will be shown below, particularly to assess

<sup>4</sup>This part of the paper constitutes "Observations on the Bolbitiaceae III."

the presence or absence of pileocystidia. Thus an investigation was instigated to determine whether there was one or two species of *Conocybe* which turned blue and if so whether both contained psilocybin. The result of taxonomic studies indicated that two species exist, and chemical analysis showed that both contain psilocybin.

Full descriptions are given below to assist in the separation of these two very closely related taxa, both of which turn blue-green at the base of the stipe. They are close in some macroscopic and some microscopic characters as well as in name, and it is proposed here to replace *Galera cyanopes* by the name *Conocybe smithii*.<sup>5</sup> This will eliminate confusion and possible ambiguity which would most certainly occur with two taxa both in the same subgenus and, although of different habitat, found within a similar geographical area.

CONOCYBE CYANOPUS (Atk.) Kühner, 1935 in *Le Genre Galera*. Fig. 1.

*Galera cyanopus* Atk. in *Proc. Am. Phil. Soc.* 57: 367, 1918.

Pileus (7-) 8-12 (-25) mm convex, almost semiglobate, slightly expanding, hardly or not umbonate, slightly wrinkled about disc, rusty fulvous to snuff or dark brown to almost umber, particularly towards the disc when fresh, margin hardly striate and with faint filamentous fragments of veil. Stipe 20-40 x 1-1.5 mm white, silky striate, becoming greyish then brownish streaky at apex on handling or at maturity, deep blue-green at base. Gills dull rust brown, not crowded, with white flocculose margin. Flesh whitish to hyaline, slightly darker at the base of the stipe, becoming brown; taste and odour not distinctive. Basidia 4-spored, ca. 20 x 7-8.5  $\mu$ , hyaline in KOH; basidiospores 6.5-7.5 (-8.5) x 4.5-5  $\mu$  inequilateral in side view, broadly ellipsoid, fairly thick-walled, dull rust brown in KOH, smooth with small but distinct germ-pore and apiculus. Cheilocystidia swollen at base and with a cylindrical tapered neck and obtuse apex, rarely subcapitate, 7.5-10 x 20-25  $\mu$ , neck up to 15  $\mu$  long and apex 3-5  $\mu$  in diameter; pleurocystidia lacking. Caulocystidia not parallel in shape to cheilocystidia except those at very apex of stipe, but then with more cylindric-lageniform neck, up to 90  $\mu$  long, apex 4  $\mu$  wide, and becoming adpressed to the stipe, below apex flexuous, irregular. Hymenoderm present, typically with numerous pileocystidia similar in shape to the cheilocystidia but sometimes more frequently subcapitate 18-25 x 7-8  $\mu$ , apex 4-5 (-6.5)  $\mu$  in diameter. Habitat: on lawns.

Material examined: Type, Ithaca, New York, found amongst grass, Atkinson 23302 (CORNELL). Dried material: Seattle, Washington, 4 September 1961, V. E. Tyler (MICH); on lawn, Plainsville, Colorado, August 1956, Smith 51596 (MICH); same locality, 8 August 1956, Smith 51588 (MICH).

Description and Figure: As *C. cyanopoda* in *Le Genre Galera*, 1935: p. 130, 2° Description de J. Schaeffer, on lawns, Germany (Berlin, Potsdam and Harz).<sup>6</sup>

*Conocybe cyanopus* differs from *Conocybe smithii* in the gills lacking cinnamon flush, wider spores, larger size and sturdier stature of the carpophores, greater number of pileocystidia, narrower cheilocystidia which are of a slightly different shape (rarely subcapitate), white silky stipe as is seen in *Conocybe coprophila*, and darker colour of pileus which is hardly if at all striate.

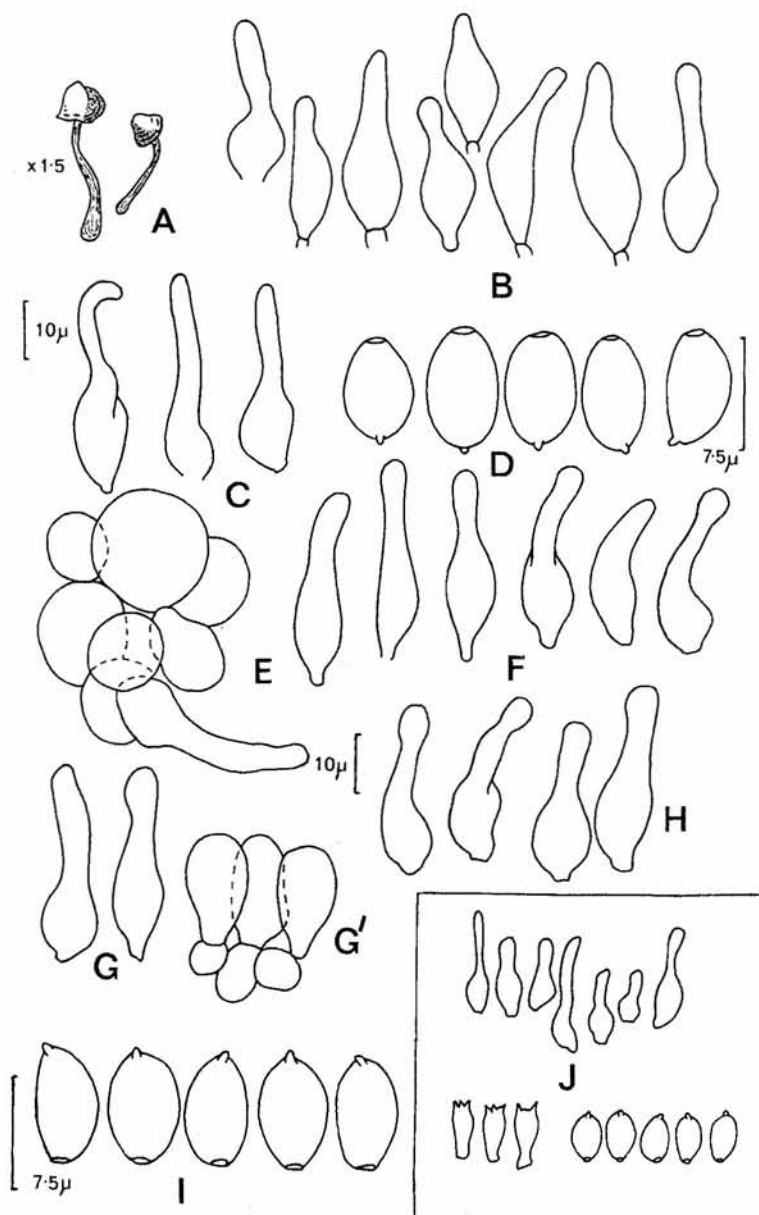
CONOCYBE SMITHII Watling Fig. 2

*Galera cyanopes* Kauffman, 1918

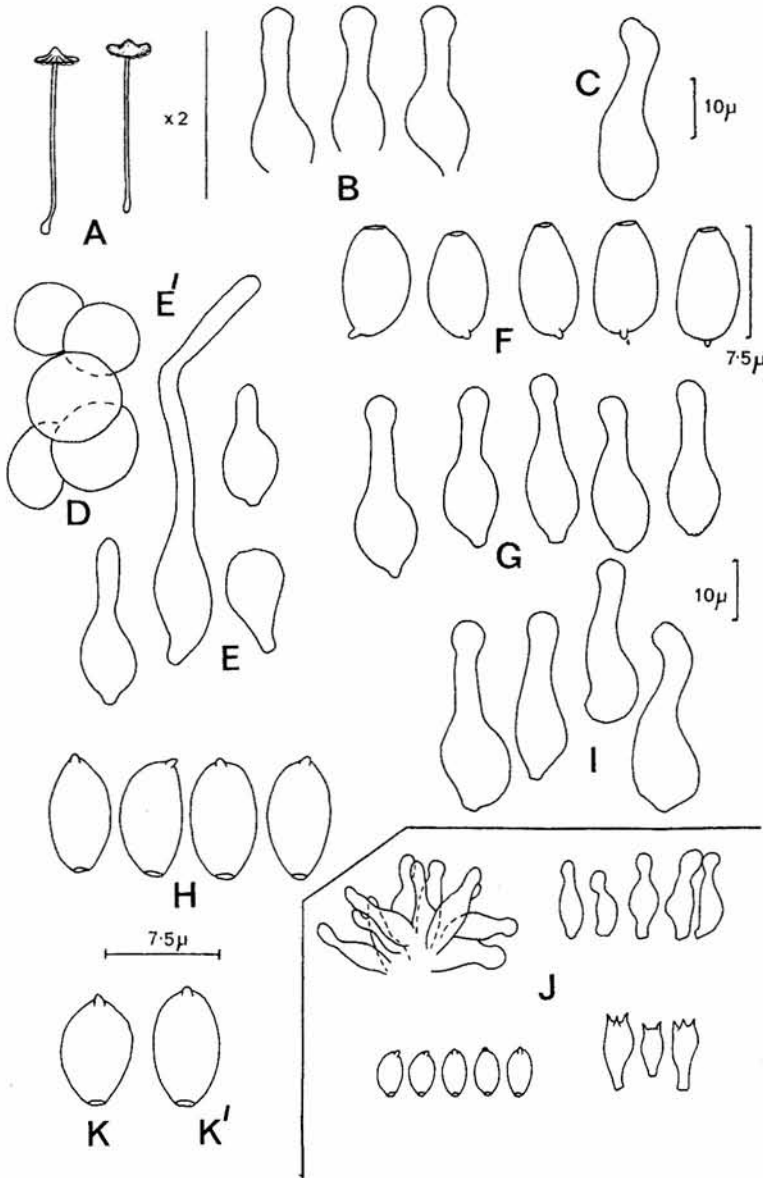
Pileus 3-10 (-13) mm, obtusely conic soon expanding at the margin to be-

<sup>5</sup>*Conocybe smithii* Watling nom. nov. in honour of an outstanding agaricologist Professor A. H. Smith. Basionym *Galera cyanopes* Kauffman in *Agaricaceae of Michigan*, p. 500, 1918. Ad *Conocybem cyanopodum* (Atk.) Kühner 1935 proxime accedit, sed a sporis leviter longioribus; Carpophoribus coloribus, formibus et cheilocystidiis subcapitatis vel capitatis differt. Statura graciliore et habitatione insignis.

<sup>6</sup>F. Parker-Rhodes (personal communication) has found what he called *C. cyanopus* once in rather soggy grass on Skokholm Island, Carmathenshire, U. K.; no material is available for examination.

FIG. 1. *Conocybe cyanopus* (Atk.) Kühner

(A) Sketch of dried material, Smith 51588; (B) Cheilocystidia, type of *Galerula cyanopus* Atkinson; (C) Caulocystidia, type Atkinson; (D) Basidiospores, type Atkinson; (E) Pileus 'scalp' showing presence of pileocystidium, Smith 51588; (F) Cheilocystidia, Smith 51588; (G) Pileocystidia, Smith 51588; (G') Hymenoderm elements, Smith 51588; (H) Caulocystidia, Smith 51588; (I) Basidiospores, coll. Tyler, Washington; (J) Sketch of Schaeffer's material figured in Kühner (1935).

FIG. 2. *Conocybe smithii* Watling

(A) Sketch of fresh and dried material (right), Watling A1700/G686; (B) Cheilocystidia, type of *Galera cyanopus* Kauffman; (C) Caulocystidium, type Kauffman; (D) Pileus 'scalp'; note no hint of development of prolongations on constituent cells, Watling A1700/G686; (E) Poorly developed pileocystidia, Watling A1700/G686; (E') Abnormally long, although narrow, pileocystidium as found rarely in Watling A1700/G686; (F) Basidiospores, type Kauffman; (G) Cheilocystidia, Watling A87/G627; (H) Basidiospores, Watling A1700/G686; (I) Caulocystidia, Watling A1700/G686; (J) Sketch of A. H. Smith's material figured in Kühner (1935) i.e. Smith 29; (K & K') illustrate the difference between a basidiospore of *C. cyanopus* and one of *C. smithii*.

come distinctly umbonate, ochraceous tawny, tawny cinnamon, darker at the disc which is usually more tawny, striate for  $\frac{2}{3}$  or more, hygrophanous, fading to pinkish buff or dingy tan on drying, glistening. Stipe 10-50 (-70) x .75-1.0 mm (1.5 mm at base), slender slightly swollen at the very base, fragile, pure white then watery white and finally flushed with ochraceous pallid but only faintly so, base particularly tinged greenish grey or greyish blue but sometimes tinged above as well, finally quite ochraceous, fibrillose to glabrescent at base, smooth or faintly pubescent at very apex. Gills adnate soon receding, subdistant to crowded, narrow to moderately broad, ochraceous buff at first becoming cinnamon rust, margin = concolorous with face, even or becoming minutely crenulate or flocculose. Flesh watery white; odour none; taste not distinctive. Basidia 4-spored, 8-10 x 18-20  $\mu$ , hyaline in KOH; basidiospores (6.5-) 7-9 x 4-4.5 (-5)  $\mu$  ellipsoid inequilateral in side view, faintly truncate at apex, rust cinnamon in KOH, smooth with a small although distinct germ-pore. Cheilocystidia 20-40 x 9-15  $\mu$  fusoid ventricose, hyaline, thin-walled, smooth, apex 4-7.5  $\mu$  in diameter; pleurocystidia absent. Caulocystidia similar to cheilocystidia but more variable, more flexuous and with obtuse to subcapitate head. Hymenoderm present, typical, composed of pyriform cells 10-25  $\mu$  broad, pileocystidia rare; clamp-connections present. Habitat: scattered amongst mosses in boggy areas and wet ditches.

Material examined: Type amongst *Polytrichum* in swamp, under *Populus* spp Michigan, July 26, 1909 (MICH). Fresh material amongst *Hypnum cupressiforme* and *Drapanocladus* sp in mixed *Thuja* bog under dense shade, Malony Lake, Emmet Co., Michigan, 27 July 1965, Homola and Watling, Watling A 1700/9676 (E); amongst *Sphagnum* in *Thuja* bog under dense shade, Rees' Bog, Cheboygan Co., Michigan, 12 July 1965 Guzman and Watling, Watling A 87/4627 (E) Substrate pH 8.0 and pH 7.0 respectively.

Herbarium material: amongst wet moss, George Res., Pickney, Washtenaw Co., Michigan, U.S.A., 19 July 1934, Smith 29 (MICH);<sup>7</sup> scattered on moss, Hoh River, Washington, 6 June 1939, Smith 14113 (MICH); on mossy log, Rees' Bog, Cheboygan Co., Michigan, 25 June 1946, Smith and Brooks, Smith 21469 (MICH); on wet soil, Maple River, Cheboygan Co., Michigan, 13 July 1947, Smith 25677 (MICH); same locality, 14 July 1947; same locality, Smith 26060 (MICH); in *Sphagnum* Bog, Mud Lake, Michigan, 20 June 1949, Harding 60 (MICH); power-line clearing, under dense shade, Emmet Co., Michigan, 8 September 1953, Smith 42702 (MICH); same locality, 16 September 1953, Smith 43206 (MICH); Rifle River, Lipton Co., Michigan, 30 May 1951, Smith 3633 (MICH); in swamp, Upper Maple River, Emmet Co., Michigan, 19 June 1955, Smith and Sharp, Smith 49557 (MICH); in mossy area, Cheboygan Co., Michigan, 3 July 1961, Smith and Reid (K).

Description and Figure: As *Conocybe cyanopoda* in *Le Genre Galera*, 1935, p. 128, 1<sup>o</sup> Description de A. H. Smith.

The type of *Galera cyanopes* consists of one good carpophore and several collapsed specimens (Spores 8-8.5 x 4-4.5  $\mu$ , not 6.5-7  $\mu$  wide as stated by Kauffman, elongate ellipsoid, smooth with distinct, although small, germ-pore and apiculus. Basidia 4-spored, 7.5-9.5  $\mu$  broad; cheilocystidia with obtuse to subcapitate apices; pleurocystidia lacking; cells of hymenoderm 12-14  $\mu$  wide).

#### DETECTION OF PSILOCYBIN<sup>8</sup>

Collections which are referable to each of the species described in this paper i.e. *Conocybe cyanopus*, *C. smithii*, *Psilocybe semilanceata* (and var. *caerulescens*) and *P. fimetaria* have all been subjected to careful analysis in order to detect the presence of psilocybin and/or psilocin. One, *C. cyanopus*, has been the subject of an earlier investigation by Benedict *et al.* (1).

<sup>7</sup>Fig. 40 M in *Le Genre Galera* is from this collection.

<sup>8</sup>by R. G. B. and V. E. T.

**Method.**—Quantities of the finely powdered dried carpophores (usually 30–50 mg) were placed in small vials and extracted with cold methanol (2.5 ml/100 mg) by agitating overnight on a rotary shaking machine. The vials and their contents were centrifuged and the clear extract analysed chromatographically. Two such methods were employed.

For paper chromatography, 50–100  $\mu$ l of the extracts and standard solutions of psilocybin and psilocin were spotted, singly and in admixture, on sheets of Whatman no. 1 filter paper. These chromatograms were developed for approximately two hours in 150-mm Petri dishes by the circular procedure of Rutter (11). Three solvent systems were employed for each sample; these were water-saturated *n*-butanol, *n*-butanol-acetic acid-water (4:1:1), and *n*-propanol-1 *N* ammonium hydroxide (5:1). The chromatograms were air-dried and sprayed with *p*-dimethylaminobenzaldehyde reagent (17) which produced a reddish violet zone with psilocybin and a bluish violet zone with psilocin. Pauly's reagent (2), which formed a reddish orange zone with psilocin, was also employed.

The thin-layer chromatographic procedure outlined by Leung and co-workers (8) was also utilized. This involved application of quantities of the extracts (<30  $\mu$ l) and reference standards to plates spread with a mixture of Silica Gel G and Kieselguhr G (2:1) and development with a wash-liquid composed of either (i) *n*-propanol-5% ammonium hydroxide (5:2) or (ii) *n*-butanol-acetic acid-water (2:1:1). Psilocybin and psilocin were detected as previously described.

TABLE 1. Occurrence of 4-substituted tryptamines in *Conocybe* and *Psilocybe* species.

| Species examined  | Collection    | Locality   | 4-Substituted tryptamine derivative identified |          |
|---|---------------|------------|--|----------|
|   |               |            | Psilocybin                                     | Psilocin |
| <i>Conocybe smithii</i> .....                                   | July 3, 1961  | Michigan   | +  | —        |
| <i>Conocybe cyanopus</i> .....                                  | Sept. 4, 1961 | Washington | +  | —        |
| <i>Psilocybe fimetaria</i> .....                                | Oct. 11, 1960 | Scotland   | +  | —        |
| <i>Psilocybe fimetaria</i> .....                                | Oct. 16, 1961 | Scotland   | +  | —        |
| <i>Psilocybe semilanceata</i> .....                             | Aug. 23, 1961 | Scotland   | +  | —        |
| <i>Psilocybe semilanceata</i> .....                             | Aug. 19, 1964 | Scotland   | +  | —        |
| <i>Psilocybe semilanceata</i> var.<br><i>caerulescens</i> ..... | Oct. 13, 1965 | Scotland   | +  | —        |

**Results.**—The results of the examination of various species for 4-substituted tryptamine derivatives are recorded in table 1.

Psilocybin was readily detected in all species examined, even in a collection of *C. smithii* of which less than a 10-mg quantity of dried carpophores was available for examination. Failure to observe the presence of psilocin may be attributed to the relatively small quantities of mushroom tissue tested rather than to its absolute absence from these species. Thus further support is given to the suggestion that certain types of blueing in agarics indicate the presence of tryptamine derivatives.

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personal collections of Bolbitiaceae and those in the Herbarium of the University of Michigan at the disposal of one of us (R. W.) during the course of this study.

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