Occurence of Psilocybin and Psilocin in Certain *Conocybe* and *Psilocybe* Species

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The 4-substituted tryptamine derivatives psilocybin and psilocin are the only principles isolated from mushrooms which are capable of inducing psychotomimetic effects in the human being following ingestion. Bufotenine has been found in certain *Amanita* species, but it does not produce such effects when administered orally.

With one exception, all of the species previously shown to contain psilocybin and/or psilocin are members of the genus *Psilocybe*, section Caerulescentes *sensu* Singer & Smith (9), although some other authors place one of these species in the genus *Stropharia*. The exception is *Panaeolus sphinctrinus* (Fr.) Quél. from which Heim and Wasson (4) reported the isolation of 0.19 percent of crystalline psilocybin.

Apparently not all species of the section Caerulescentes contain psilocybin or psilocin. Chromatographic studies failed to establish the presence of either of these compounds in 3–5 g of *Psilocybe yungensis* Singer & Smith (4), even though the species was shown to possess a distinct psychotropic action. Conversely, a number of species outside the genera *Psilocybe* and *Panaeolus* have been reported to induce psychosis, but the nature of their active principles remains unknown. Included in this group is *Conocybe siligineoides* Heim, a lignicolous species of Mexican origin which has been designated as one of the hallucinogenic mushrooms employed by the Mazatec Indians in their magico-religious ceremonies (4). No physiological or chemical evidence has been obtained to substantiate this report, and, in view of the uncertainties which still abound in this difficult area of study, such evidence was required to establish *Conocybe* as a genus containing hallucinogenic species.

During an extensive screening program devoted to the identification of active principles contained in mushrooms found in the Pacific Northwest, two previously uninvestigated species, *Conocybe cyanopus* (Atk.) Kühner and *Psilocybe cyanescens* Wakefield, were found to contain indole derivatives and were subsequently subjected to a detailed investigation. An additional collection of *Psilocybe baeocystis* Singer & Smith was also obtained, and, in view of the previous report of the presence of psilocin but the absence of psilocybin in this species (1), it was subjected to a re-examination.

EXPERIMENTAL

Source of Specimens.—*Conocybe cyanopus* and *Psilocybe cyanescens* were collected during the fall of 1961 from a yard in the Seattle, Washington area. The latter species had previously been reported only from Kew Gardens, Surrey, England (9). Attention was directed to both of these species by the peculiar bluish stains on the fruiting bodies which became more pronounced when they were handled or dried (figures 1 and 2). *Psilocybe baeocystis* was collected in December, 1961 from a yard near Milwaukie, Oregon. Representative specimens of these collections have been deposited in the University of Michigan Herbarium.

Analytical Procedures.—Carpophores were dried in a circulating-air oven at 50° C, milled to a fine powder, and extracted (100 mg) with cold methanol (5 ml). Quantities (50–100 μl) of this extract and standard solutions of psilocybin and psilocin were spotted, singly and in admixture, on sheets of Whatman No. 1 filter paper. These chromatograms were formed for approximately two hours in 150–

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mm Petri dishes by the circular procedure of Rutter (7). Three solvent systems were employed for each sample. These were water-saturated *n*–butanol, *n*–butanol–acetic acid–water (4:1:5), and *n*-propanol–1 N ammonium hydroxide solution (5:1). The chromatograms were air-dried and sprayed with *p*–dimethylaminobenzaldehyde reagent (PDAB) (12) 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.

For spectrophotometric studies larger quantities (250–300 mg) of carpophores were extracted, the total extract streaked on sheets of Whatman No. 3 filter paper, and the chromatogram formed ascendingly for approximately twenty hours in the *n*–butanol:acetic acid:water solvent system. After thorough drying, the

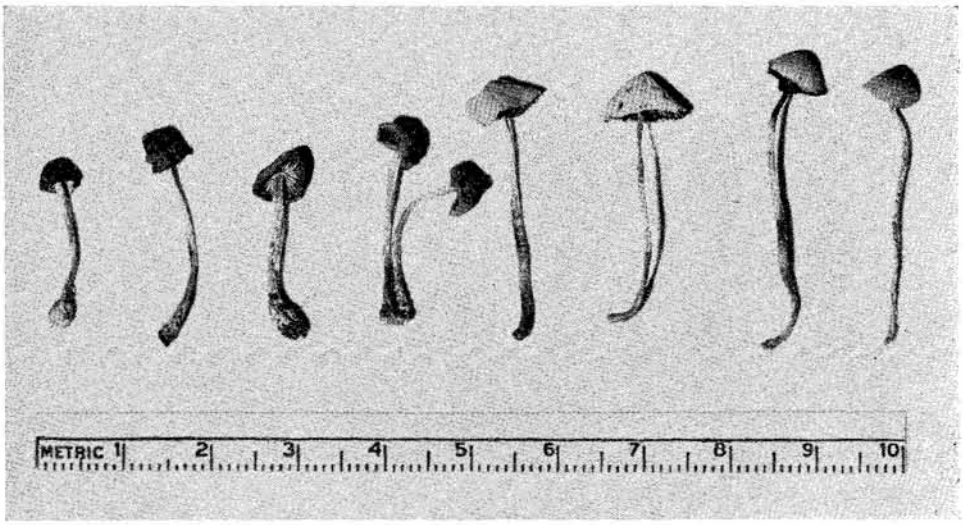

FIG. 1. *Dried carpophores of* ***Conocybe cyanopus.***

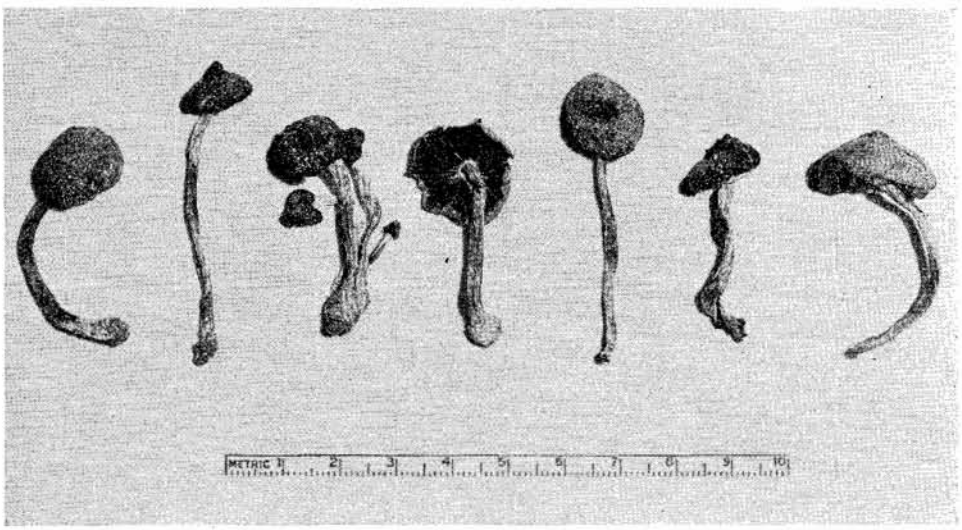

FIG. 2. *Dried carpophores of* ***Psilocybe cyanescens.***

areas corresponding to psilocybin were cut from the sheets, eluted with methanol, and the absorbance of the solutions measured in a Beckman spectrophotometer, model DU.

Results.—Chromatograms of extracts of carpophores of *Conocybe cyanopus* exhibited, after spraying with PDAB, a reddish violet zone which failed to separate from reference psilocybin in all three solvent systems. Chromatographic examination of relatively large quantities (ca. 100 μl) of the extract did not permit detection of psilocin. The ultraviolet absorption spectrum of the methanol eluate of the compound corresponding to psilocybin exhibited maxima at 268 and 290 mμ and minima at 240 and 288 mμ. These values are in agreement with those previously reported for psilocybin (5).

Chromatograms of *Psilocybe cyanescens* extract formed in two of the solvent systems were somewhat difficult to interpret due to the presence of an additional compound which gave a stable greenish blue color with PDAB. In the water-saturated *n*–butanol system this compound had an R_F of approximately 0.37 which is identical with a similarly reacting compound in *Psilocybe pelliculosa* A. H. Smith (13). It separated clearly from psilocybin (R_F 0.27) and psilocin (R_F 0.51) in this system, but in the *n*-butanol–acetic acid–water system it tended to obscure the psilocin (R_F 0.76), and in the *n*-propanol–1 N ammonium hydroxide system it partially overlaid the psilocybin (R_F 0.12). The identity of this compound has not been established.

Both psilocybin and psilocin were identified chromatographically in the *Psilocybe cyanescens* extracts; the former compound was present in greater amount. The zone corresponding to psilocybin was eluted with methanol, and its ultraviolet absorption spectrum found to coincide with that of known psilocybin (maxima 269 and 290 mμ, minima 240 and 288 mμ).

In agreement with our previous study (1), extracts of *Psilocybe baeocystis* carpophores showed a prominent zone corresponding to psilocin in each of the three chromatographic systems. In addition, a faint zone corresponding to psilocybin was also noted when reasonably large quantities (ca. 100 μl) of the extract were chromatographed.

DISCUSSION

Identification of psilocybin in *Conocybe cyanopus* extends the reported occurrence of this compound to three genera, *Conocybe*, *Panaeolus*, and *Psilocybe*, of the Agaricaceae. It may be viewed as additional evidence to support the unverified claim that another species of *Conocybe* has been employed as a hallucinogenic mushroom by certain Mexican Indians.

The relatively uncomplicated chemical structures of psilocybin and psilocin render a restricted occurrence extremely unlikely, and it is very probable that they may be responsible for the reported psychotropic activities of such species as *Psathyrella sepulchralis* Singer, Smith & Guzmán (10), *Russula nondorbingi* Singer (8), and even *Amanita muscaria* (Fr.) S. F. Gray and *Amanita pantherina* (Fr.) Quél. (11). In fact, Eugster (3) has reported the presence in *Amanita muscaria* of a 4–hydroxyindole derivative resembling psilocybin, but experimental details and proof are lacking.

Psilocybe cyanescens appears to be a typical member of the section Caerulescentes with respect to its content of these compounds, since it contains both psilocybin and a lesser amount of psilocin. *Psilocybe baeocystis* differs from the other members of the section in that both samples which have been investigated contain relatively large amounts of psilocin but little, or no, psilocybin. This distinctive difference in the relative abundance of the two constituents would appear to be a chemical characteristic of some taxonomic utility for this species.

The fact that the characteristic bluish stains which form on the stipe and pileus of *Psilocybe* species belonging to this section are also present in *Conocybe*

cyanopus suggests that this characteristic is related in some way to the identical active components. Horita and Weber (6) have shown that incubation of psilocybin with homogenates of rat kidney and other mammalian tissues caused a rapid liberation of psilocin through the action of alkaline phosphatase. The psilocin thus formed underwent further oxidative degradation to form a blue-colored product, possibly an *o*-quinone derivative of psilocin. It is conceivable that the bluish stains found on these mushrooms result from an identical reaction, but no experimental evidence is available. If this postulation is correct, bluish stains of this type may serve as a valuable guide to species containing psilocybin and/or psilocin. This characteristic reaction must be carefully differentiated from others which yield a similarly colored end product, such as the conversion of boletol to boletoquinone in certain species of *Boletus*.

Although Wasson (14) has recently enumerated the species of hallucinogenic mushrooms employed in Mexico for divinatory purposes, no listing has been made of species in which the occurrence of psilocybin and/or psilocin has actually been demonstrated. These now include: *Conocybe cyanopus*, *Panaeolus sphinctrinus*, *Psilocybe aztecorum* Heim, *Psilocybe baeocystis*, *Psilocybe caerulescens* Murr. var. *maztecorum* Heim, *Psilocybe cubensis* (Earle) Singer, *Psilocybe cyanescens*, *Psilocybe mexicana* Heim, *Psilocybe pelliculosa*, *Psilocybe semperviva* Heim & Cailleux, *Psilocybe wassonii* Heim, and *Psilocybe zapotecorum* Heim (1, 4, 13).

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