A NEW PSILOCYBIAN SPECIES OF *COPELANDIA*

R. Arnold Weeks

11840 SW 47 Street, Miami, Florida 33175

Rolf Singer

Instituto Nacional de Pesquisas da Amazonia,
Caixa Postal 478, 69000, Manaus, Amazonas, Brazil
and Field Museum of Natural History, Chicago, Illinois

and

William Lee Hearn

Department of Pharmacology, University of Miami School of Medicine,
Miami, Florida

Abstract.—*Copelandia chlorocystis sp. nov.* is described, and a key to the known species of this genus included. Psilocybin and psilocin were isolated from the new species by dry column chromatography, and the presence of baeocystin was indicated. Urea was detected by analytical tlc.

Cerebral mycetism from members of the Panaeoloideae has been recorded as early as 1916 (1) but was largely ignored until 1958 when Hofmann *et al.* isolated psilocybin and psilocin as the psychoactive principles of *Psilocybe mexicana* Heim, one of the 'sacred mushrooms' of Mexico (2–5). Psilocybin and psilocin have since been detected in numerous species of *Psilocybe, Panaeolina, Panaeolus, Copelandia, Conocybe, Pholiotina* and *Gymnopilus* (6, 7). Context blueing is a frequent indicator of psilocybin-psilocin producing species in certain of these genera (8, 9). Observations of context blueing in specimens of a previously unidentified panaeoloid collected during studies of the mycoflora of South Florida prompted a more detailed investigation of this collection. Psilocybin, psilocin, and urea were detected in dried carpophores of the material by analytical tlc against reference standards; identification of psilocybin and psilocin was confirmed by isolation through the use of dry column chromatography (dcc). Spectral data (uv, ms) were recorded for the constituents isolated.

On the basis of its characteristic colored metuloid cystidia, blueing context, and other characteristics, the collection is considered to be a new species of *Copelandia*. It differs from known species of this genus in the deep green apical color of the metuloids, in the combination of relatively smaller spores borne on two-spored basidia lacking a basal clamp, and in other characteristics. For this species we propose:

Copelandia chlorocystis Singer & Weeks *sp. nov.* Fig. 1

Pileo isabellino vel pallide cinnamomeo in centro, cinerascente marginem versus, marginem versus striatulo-ruguloso, vel nullo notato, usque ad 28 mm lato. Lamellis e sporis aquilis, albomarginatis, subdistantibus, adnexis. Stipite plerumque cremeo, basin versus brunnescente, brunneogriseo ad apicem in maturis, subtiliter albopruinato praesertim ad apicem, in siccis 30 X 0.3 mm vel maiore. Carne caerulescente tactu sicut etiam superficiebus caerulescente vel viridescente. Sporis 10.3–12–(13) X 8–9.5 μm; cystidiis metuloideis, ad apicem viridibus vel olivaceis; cheilocystidiis ampullaceis; epicute ex elementis subvesiculosis hymeniformiter dispositis efformata; dermatocystidiis stipitis vesiculosis.

Ad caespites graminum. Typus a William Lee Hearn Julio 1976 in Florida, Dade Co., lectus et in F conservatus est.

Pileus isabelline to pale cinnamon or yellowish brown in the center and white or, at least in maturity, greyish on the margin but turning buff on drying. Appearing slightly whitish, streaked over the outer third or two thirds of the radius

because of a marked unevenness of the shallowly wrinkled-pitted surfaces (whereby the elevated minute ridges remain pallid), sometimes with more elevated venation and ridges; old caps turning blue, blue-green and eventually blackish on drying; often with a ragged edge but without any sign of a veil remnant; finely striate over a few millimeters from the rim of the margin of the mature pileus; glabrous and naked; campanulate-convex, often with a small umbo; 5–28 mm broad and up to 13 mm high.

Lamellae blackish from the spores, with white edge, broad, subdistant, ascending, adnexed to adnate. Spore print black to sepia black.

Stipe milk white or light cream in color, yellowish brown towards the base, at maturity brown-grey in the upper half and tending to become blackened in dried material; blueing when bruised; finely white pruinate especially at the apex, below weakly longitudinally striate; equal or subequal, hollow, about 30 X 0.3 mm but reaching up to 80 X 2 mm; basal mycelium white, tending to become blue as does the mycelium in culture.

Context white to yellowish, tending to become blue when broken (at times blueish-green), on stipe blueing within about 5 to 10 minutes. Odor farinaceous or of grain; taste mild.

Spores 10.3–12–(13) X 8–9.5 X 6–7.5–(9.5) μm, lemon shaped when seen frontally, but somewhat compressed and lentiform, in profile elliptical; a minority of spores with a bulge in the outer side near the base; with a broad truncate germ pore; without suprahiliar depression or applanation but rarely the inner side flatter or even somewhat concave; immature hyaline then fuscous; mature fuscous-fuliginous to almost black and only slightly transparent, often with a fine internal granulation; with thick (about 1 μm) complex wall; without ornamentation.

HYMENIUM.—Basidia 17–22.5 X 7.5–9.5 μm, with 2–(3) sterigmata and spores, hyaline and without basal clamp. Cheilocystidia making the edges heteromorphous, 26–39 X 10–12.2 μm, ampullaceous with equal or sometimes subclavate apex which is 3.5–5.5 μm broad and rounded-obtuse at the tip; thin walled, but not incrusted; hyaline. Metuloids 22–56 X 11–19 μm, ventricose and narrowly but prominently mucronate; wall thin but towards and on the apex abruptly or gradually thick, underneath the mucro 1–2 μm thick, in mucro even somewhat thicker, with a thin canal-like lumen or with merely a relatively thin lumen; in the upper part characteristically green or olive-green in water, ammonia and KOH mounts; the apex beset with crystals which are hyaline but tend towards greenish in ammonia (as some Psathyrellae); the tip not acute; these cystidia appearing on both the sides (among the basidia) and the edges (among the cheilocystidia) of the lamellae.

HYPHAE.—Subhymenium subcellular; hymenophoral trama at first hyaline and regular, later palest fuscidulous, thin walled and not gelatinized; inamyloid hyphae of the stipe parallel or subparallel with each other and with fuscidulous intraparietal pigment in most, in pileus often inflated to 5–23 μm diameter; clamp connections absent (in some sections some few and rare septa with questionable clamps); some hyphae eventually blue in ammonia. Hypodermium a cutis of hyphae with intraparietal melleous pigment.

CORTICAL LAYERS.—Epicutis of the pileus consisting of subvesiculose cells forming a hymeniform layer which, in some places, degenerates to an apparent epithelium; these elements interspersed with dermatocystidia in the marginal

region, the dermatocystidia like the cheilocystidia, *e.g.* 30 X 11 μm, with a neck about 5.7 μm broad, some reduced in the neck portion to cylindrical outgrowths of the pavement cells which then attain 10–30 μm diameter. Sterile cells of the surface of the stipe rather numerous but mostly not very dense; 12–24 X (6)–8.5–15 μm; subvesiculose to vesiculose and similar to the pavement cells of the epicutis of the pileus; no dermatocystidia of the ampullaceous type and no metuloids on the surface of the stipe.

HABITAT.—On sod grown in the Okeechobee region of Florida using rich black soil from the Everglades, apparently without dung of any kind added; the species growing gregariously to cespitosely or solitary, often in 'fairy rings', attached to decaying grass fragments. Fruiting from June to March.

MATERIAL STUDIED.—USA, FLORIDA. Redlands Fruit and Spice Park, Homestead, Dade Co., *July 1976, W. L. Hearn* (F. TYPE) and *September 1977, W. L. Hearn & S. Pollock* (FLAS-F #51989); M&M Sod, US 27 south of Okeelanta, Palm Beach Co., *September 1977, S. Pollock* (FLAS-F #51988); Okeechobee region, Palm Beach Co., *September 1978, R. A. Weeks* (FLAS-F #51987).

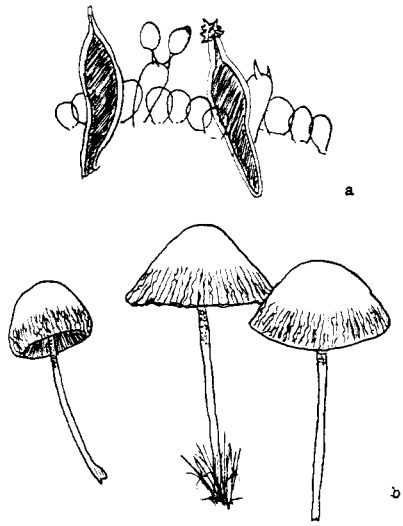

FIG. 1. *Copelandia chlorocystis*; a) portion of the hymenium, (X900); b) carpophores (X3–4); from the type.

Field notes made from a collection at the University of Miami Marine Lab, Miami, Dade Co., Florida by W. L. Hearn in August of 1971 were included in portions of the description. Unfortunately, voucher specimens from this "unusual" collection were not retained, and the material remained unidentified until our examination of the 1976 recollection.

Copelandia has been reduced to an infragenetic taxon by some authors who prefer to transfer this genus to *Panaeolus* (Fr.) Quel. (10–12). This treatment seemed to have some merit as long as only one species was believed to belong in *Copelandia* and a broader genus concept was thought to be desirable. However, counting the species described herein, we have now at least six binomials, each,

as we believe, specifically different from one another and all showing the characteristic colored metuloid cystidia and blueing context, without any forms known intermediate between these and *Panaeolus*. We maintain, therefore, in accordance with Singer (13), Horak (14), and Malençon & Bertault (15) that a narrower generic concept in the Panaeoloideae is warranted.

The new species differs from known species of this genus in the characteristically deep green apex of the metuloids and in the combination of relatively smaller spores borne on two-spored basidia lacking a basal clamp. This latter characteristic is remarkable since the bisporous 'variety' of *C. papilionacea* (*sensu* Bres.), *C. papilionacea* var. *bispora* Malençon & Bertault (15), has abundant clamp connections. Other wild forms of *Copelandia* have consistently four-spored basidia, even those without clamp connections. Only Olah (12) reports two-spored forms of his *Panaeolus cambodginiensis* (*C. cambodginiensis sensu stricto*), produced in cultures originating from the four-spored wild form. Our species is certainly not a two-spored form of *C. cambodginiensis*, because of the green metuloid cystidia and other obvious differences. Nor can it be a bisporous form of one of the larger spored species, since experience shows that bisporous forms of four-spored species have either larger or, more rarely, equally as large spores as the four-spored type, never smaller spores. Our species is therefore an autonomus species.

Reexamination of the type of *C. westii* (Murr.) Sing. in comparison with species now known supports its distinction from *C. cyanescens* (Berk. & Br.) Sing. We add a key to the known species of *Copelandia* (excluding *C. anomalus* (Murr.) Saccardo & Trott., which is now under examination); all types excepting that of *C. bispora* have been studied by us.

1. Large-spored species; spores (10.8)–12.3–14.7–(16–19) μm long; metuloids yellowish, melleous, cinnamon rarely with an umber or olive apex; clamp connections present in carpophores with two-spored basidia.
   2. Hyphae of the carpophore with numerous clamp connections.
      3. Basidia two-spored; trauma often scarcely blueing; North African species:
         C. bispora (Malencon & Bernault) Singer & Weeks *comb. nov.*
         (*Copelandia papilionacea* var *bispora* M & B (15))
      3. Basidia four-spored; trauma distinctly blueing; widespread in the tropics and subtropics:
         C. cyanescens (Berk. & Br.) Sing.[1]
   2. Hyphae of the carpophore without clamp connections; basidia four-spored; Floridian species:
      C. westii (Murr.) Sing.
1. Medium-spored species; spores (8.5)–9–12–(13) μm long; metuloids often green; clamp connections absent in carpophores with two spored basidia.
   4. Metuloids at apex consistently green to olive-green; clamp connections absent or extremely rare; basidia 2–(3) spored; cheilocystidia large, 26–39 X 10–12.2 μm; Floridian species:
      C. chlorocystis Singer & Weeks
   4. Metuloids yellowish, melleous, cinnamon or chestnut at the apex; cheilocystidia smaller, 12–20–(30) X 2.5–6–(10) μm; Asiatic:
      5. Wall in the middle of the metuloids, in the ventral portion, 0.3–0.5 μm thick and yellowish in alkali; metuloids 10–19 μm broad; spores 10–11.5–(12) X 7.7–8.5 X 6.2–7 μm; metuloids usually with a chestnut zone in the lower part of the apex:
         C. cambodginiensis (Olah & Heim) Singer & Weeks *comb. nov.* (*Panaeolus cambodginiensis* Olah & Heim (11))
      5. Wall in the middle of the metuloids 0.5–1.5 μm thick and yellow in alkali; metuloids 9–16 μm broad; spores 8.5–12 X 6.5–7.7–(9) X 5–5.5–(7) μm; metuloids with less deeply colored walls, apical region uniformly ochraceous to chestnut:
         C. tropicalis (Olah) Singer & Weeks *comb. nov.* (*Panaeolus tropicalis* Olah (11))

[1]The spores of the type were indicated slightly smaller than those found by modern authors, but the original description does not fit any of the smaller-spored group.

EXPERIMENTAL[2]

EXTRACTION AND ANALYTICAL TLC.—Dried carpophores of *C. chlorocystis* (90 mg; half of the total collection of Hearn 1976) were finely powdered and extracted with 3 X 10 ml methanol. The combined, filtered extracts were evaporated *in vacuo*, and the residue (24.8 mg) was redissolved in 3 ml methanol for analysis.

The extract and standard solutions of psilocybin, psilocin, and urea were spotted, both singly and in admixture, on precoated sheets of Merck silica gel 60 F–254 and Merck cellulose. Silica plates were developed in methanol-NH_4OH (100:1.5) (A), n-propanol-5% aq. NH_4OH (5:2) (B), and n-butanol-acetic acid-water (2:1:1) (C). Cellulose plates were developed in n-butanol-acetic acid-water (12:3:5) (D) and n-propanol-1N NH_4OH (5:1) (E). All plates were developed to a height of at least 10 cm and allowed to air dry.

Visualization was affected by spraying duplicate plates with a 2% solution of *p*-dimethylaminobenzaldehyde in hydrochloric acid-ethanol (1:1) (PDAB) and with diazotised sulphanilic acid prepared according to Krebs *et al.* (16) (Pauly reagent).

Components of the extract co-chromatographed with their respective standards in all systems and did not separate from reference solutions during chromatography in admixture. Observed R_f values were in agreement with previously published data (9, 17–19). Trace components of the extract failed to co-chromatograph in all systems with other available standards. Each spot gave color reactions with the visualization sprays correspondent with those of the respective standard. Psilocybin turned an immediate reddish-purple with PDAB, fading to a violet hue; psilocin gave an immediate strong blue, fading to violet; urea yielded a slowly developing bright yellow. Psilocin reacted with Pauly reagent with a deep red-orange, turning to red-brown; psilocybin and urea failed to react with this reagent. Systems A & B gave superior resolution of the components of the extract and were used to monitor the subsequent isolation procedures.

ISOLATION AND IDENTIFICATION.—Availability of a much larger collection of carpophores in September of 1977 (Hearn & Pollock 1977) provided sufficient material to attempt isolation of the components tentatively identified as psilocybin and psilocin. Dry column chromatography was selected as the preferred method due to its speed and yield. Solvent system A was selected for development in order to minimize development time and sample decomposition. Dried carpophores (850 mg) were powdered and extracted as before with 100 ml portions of methanol in darkness. The combined, filtered extracts were evaporated *in vacuo*, at 30°, in darkness and stored dry, at 0°, under nitrogen and in darkness.

The activity grade of the analytical silica plates was determined and a nylon tube column (80 cm X 3 cm id), packed with 70–140 mesh silica gel adjusted to the activity of the tlc plates, was prepared as described by Loev & Goodman (20). The extract was taken up in a minimum volume of methanol, mixed with a small amount of silica, dried under a stream of nitrogen, and placed on top of the column. The column was then developed until the solvent reached the bottom of the silica, at which time the remaining solvent was removed from the reservoir and the column placed on its side to stop development.

Immediately after development, the column was sampled at 5 mm intervals by piercing the nylon with capillary tubes. Samples were grouped into zones according to tlc patterns. The column was then sectioned by slicing along the zones, and the sections were eluted with solvent A until the silica no longer gave a positive spot test with PDAB. Each elute was filtered through a fine sintered glass funnel and held under nitrogen until evaporation at 30° *in vacuo* and in darkness. Processing of the column fractions was carried out as rapidly as possible in order to minimize sample decomposition. All fractions were weighed and stored dry at 0° under nitrogen and in darkness.

Purity of the psilocybin and psilocin fractions thus obtained was determined by analytical tlc to be sufficient for spectroscopy. Traces of a slower moving contaminant in the psilocybin fraction were noted, but further purification was not attempted due to the small amount of material isolated. This procedure yielded 3.9 mg of psilocybin (0.46% dry weight) and 2.5 mg of psilocin (0.29% dry weight). Fractions containing other indolic components underwent rapid decomposition during workup of the column sections, making further purification impossible. Isolation of these trace components by alternate methods is now under investigation.

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[2]Carpophores of *C. chlorocystis* used in the analysis were air dried on collection and stored dessicated over silica until used. Voucher locations are listed in the taxonomic section. Precoated tlc plates on aluminum backing were obtained from Brinkman Instruments, Chicago. Macherey, Nagel & Co. MN Silica Gel 70–140 mesh ASTM from Brinkman and nylon tubing from ICN Pharmaceuticals, Cleveland, Ohio, were used in dry column chromatography. Uv spectra were obtained on a Beckman DB-G grating spectrophotometer, and mass spectra were determined with a Hitachi Perkin-Elmer RMU-6D instrument operating at 70 ev and 360°. Standards of psilocybin and psilocin were supplied by Dr. R. W. Morris of the Department of Pharmacognosy and Pharmacology, College of Pharmacy, University of Illinois at the Medical Center (UIMC), Chicago.

The following spectral data were obtained: psilocybin sample—uv, λ max (MeOH) 222, 226, 280(sh) and 290 nm; ms, *m/e* 204 (21%), 160 (3%), 159 (4%), 146 (5%), 130 (3%), 117 (4%) and 58 (100%); psilocin sample—uv, λ max (MeOH) 222, 260(sh), 268, 285 and 294 nm; ms, *m/e* 204 (21%), 160 (2%), 159 (5%), 146 (8%), 130 (4%), 117 (4%) and 58 (100%). These data are consistent with spectral data published for psilocybin and psilocin (5, 18, 19). Two additional significant peaks were observed in the ms of the psilocyin sample, at *m/e* 190 (8%) and 44 (100%). The *m/e* 44 peak was 9% of the height of the *m/e* 58 base peak. These data suggest that the slower moving contaminant in the psilocybin sample may be baeocystin.

The psilocybin and psilocin occur in *C. chlorocystis* is not surprising. Both compounds have been detected in *C. cyanescens* (21), in *C. cambodginiensis* (12) and in *C. tropicalis* (12). Urea is also common to members of the Panaeoloideae (22). Material isolated by Closs & Gabel from *Panaeolus venenosus* Murr. (*Panaeolus subbalteatus* Berk. & Br.) (23) was most probably urea (Compound I) and baeocystin (Compound II). Baeocystin has recently been isolated from *Panaeolus subbalteatus* (19) but is otherwise unknown in the Panaeoloideae. This is the first reported indication of its occurrence in *Copelandia*.

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