limited free amino acid pool in *P. cyanescens* is responsible for the failure of the fungus to accumulate psilocybin in saprophytic cultures. However, the results do suggest that the isolate of *P. cyanescens* is less responsive to phosphate nutrients and has narrower metabolic tolerance than *P. cubensis* NRRL A-9109.

P. campanulatus appeared to be the most metabolically responsive of the species tested. It grew well under the saprophytic conditions and appeared to have an adequate soluble nitrogen pool. These observations suggest the improbability of a limited supply of common amino acids as the basic factor behind the lack of serotonin production in saprophytic cultures. Some key factor controlling the formation or function of tryptophan hydroxylase is probably responsible for the different metabolic capabilities of carpophores and vegetative mycelium of this basidiomycete.

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Keyphrases

Basidiomycete cultures—phosphate effect
Phosphate effect—trichloroacetic acid-soluble, -insoluble nitrogen metabolites
Fungal free amino acid pool—hydroxytryptamine derivatives accumulation
Colorimetric analysis—spectrophotometer
TLC—separation, identity

Baeocystin and Norbaeocystin: New Analogs of Psilocybin from *Psilocybe baeocystis*

By A. Y. LEUNG* and A. G. PAUL

Two new 4-phosphoryloxytryptamine derivatives have been isolated from *Psilocybe baeocystis* grown in submerged cultures. Using ultraviolet, infrared, and mass spectral analyses the structures have been determined. Both are analogs of the psychotomimetic, psilocybin, and have been named baeocystin (monomethyl analog) and norbaeocystin (demethyl analog).

PSILOCYBIN was first isolated from a Mexican psychotomimetic mushroom, *Psilocybe mexicana* Heim, by Hofmann *et al.* (1). While the occurrence of this compound and its dephosphorylated derivative, psilocin, has been established in several species of mushrooms, analogs have not yet been described.

The formation of psilocybin in submerged cultures of *Psilocybe baeocystis* Singer and Smith has been reported previously (2). In a communication (3) the authors also have reported the isolation of baeocystin, a monomethyl analog of psilocybin, from this same fungus. The present report deals with details of the procedure for the isolation and characterization

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*Present address: Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94122

of baeocystin and of a second analog, norbaeocystin.

EXPERIMENTAL

Plant Materials—Fungal tissue was obtained from submerged cultures of the organism grown in the medium previously reported (2). Mycelial pellets were collected on a Büchner funnel, washed with distilled water, and freeze dried.

Isolation Procedure—The ground plant material (23.2 g.) was defatted with petroleum ether (30–60°) in a Soxhlet extractor (Fig. 1). After removal of solvent at room temperature, the tissue was transferred to a conical flask and extracted with successive portions of methanol at room temperature, using a magnetic stirrer, until a concentrated aliquot of an extract gave little or no color reaction with Ehrlich's reagent. The combined extracts (4 l.) were concentrated to dryness under vacuum at 40° using a Rinco rotating evaporator. The residue was mixed with 20 g. of dry powdered cellulose which had been extracted with water-saturated 1-butanol. The mixture was added to the top of a 3.5-cm. o.d. column packed to a height of 94 cm. with powdered cellulose which had been extracted with water-saturated 1-butanol for several days. The column was developed with this same solvent and 20-ml. fractions were collected at a rate of approximately 40 ml./hr. (Eluate I) using an automatic fraction collector.

Thin-layer chromatography (TLC) (2) of Eluate I indicated that Fractions 46–76 contained psilocin, 77–84 contained psilocybin and traces of psilocin, 85–149 contained a mixture of psilocybin, baeocystin, and norbaeocystin, and 150–305 contained these three compounds grossly contaminated with glycine. Fractions 85–149 were combined (Fraction 1) as were Fractions 150–305 (Fraction 2).

The solvent was removed from Fraction 1, the residue was mixed with 20 ml. of warm methanol, and the mixture filtered. The filtrate was concentrated to approximately 5 ml. at room temperature and again filtered. The filtrate was placed in a freezer at −23° for 8 days. The rosette crystals which formed were removed by filtration, washed with cold methanol, and dried in a desiccator. The mother liquid was concentrated, filtered, and returned to the freezer. Additional crystalline material was obtained giving a total yield of 140 mg. (m.p. 199–204° dec.). TLC analysis indicated it to be a mixture of psilocybin, baeocystin, and norbaeocystin.

Solvent was removed from Fraction 2, the residue dissolved in 25 ml. of warm methanol, and the solution concentrated to approximately 6 ml. The concentrate was filtered and the filtrate placed in the freezer for several hours. The heavy precipitate which formed was removed by filtration. TLC analysis indicated that it was glycine. The filtrate was further concentrated to 2 ml. and placed in the freezer for 3 days. No crystalline material was obtained.

The filtrate was combined with the mother liquid from Fraction 1 and this combined solution was mixed with 5 g. of extracted powdered cellulose. The mixture was dried and transferred to a powdered cellulose column (3.5 × 97 cm.) which had previously been extracted with water-saturated 1-butanol. The column was developed with this solvent and fractions richest in psilocybin, baeocystin, and norbaeocystin, but free of glycine, were combined (Fraction 3).

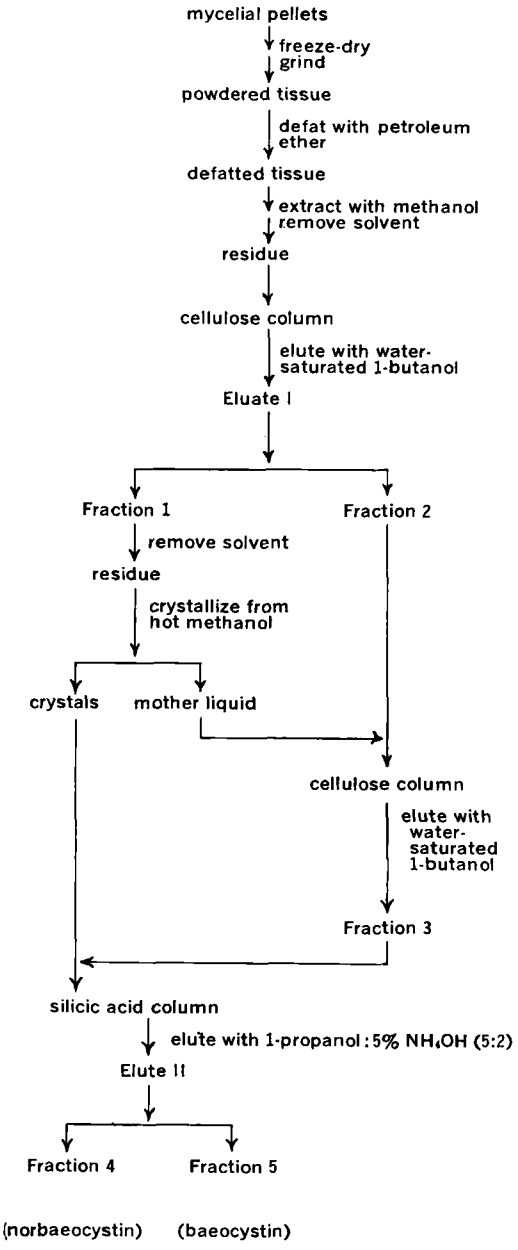


Fig. 1—Isolation procedure.

Solvent was removed from Fraction 3 and the residue was dissolved in a small volume of hot methanol. One hundred milligrams of crystalline material isolated from Fraction 1 was added to the solution and additional hot methanol was added to completely dissolve the material. This solution was transferred to a column of activated silicic acid (3 × 45 cm.). The column was developed with 1-propanol–5% ammonium hydroxide (5:2), using pressure, at a rate of approximately 9 ml./hr.

Eluate II was collected in 3-ml. fractions. TLC analysis of Eluate II indicated that Fractions 25–66 contained norbaeocystin, 70–175 contained baeocystin, and 180–270 contained psilocybin. Fractions 25–66 were combined (Fraction 4), as were Fractions 70–175 (Fraction 5).

The solvent was removed from Fraction 4 and the residue was dissolved in approximately 7 ml. of hot methanol. The solution was concentrated to approximately 1 ml., filtered, further concentrated to approximately 0.5 ml., and placed in the freezer. Crystals which formed at the end of 40 days were removed by filtration, washed with cold methanol–water (1:1), and dried in a desiccator. The yield of norbaeocystin (m.p. 188–192° dec.) was 2 mg.

The solvent was removed from Fraction 5 and the residue was dissolved in approximately 9 ml. of hot methanol. This solution was concentrated to approximately 1.5 ml., filtered, and the filtrate placed in the freezer. After 3 days, crystals which formed were removed by filtration and dried in a desiccator. The yield of baeocystin (m.p. 254–258° dec.) was 6.5 mg.

The extraction was repeated using 58.9 g. of fnngal tissue. The procedures followed were identical with those described except that it was nccessary to chromatograph Fraction 2 on a cellulose column prior to combining this fraction with the mother liquid of Fraction 1, in order to free it of glycine. Fraction 4 yielded 4 mg. of norbaeocystin and Fraction 5 yielded 8 mg. of baeocystin.

Characterization—Both norbaeocystin and baeocystin exhibited identical color reactions on TLC with Ehrlich's reagent and with a modified phosphate reagent (4) as did psilocybin. On two-dimensional TLC, using solvent systems *A* and *B*, these compounds chromatographed close to psilocybin but far from all the other indole compounds tested (2). The R_f values of norbaeocystin were 0.17 ± 0.02 (solvent *A*) and 0.58 ± 0.02 (solvent *B*), while those of baeocystin were 0.16 ± 0.01 (solvent *A*) and 0.46 ± 0.02 (solvent *B*).

The UV spectra[1] of baeocystin and norbaeocystin were superimposable and were identical with that of psilocybin, showing absorption maxima at 221, 268, 280, and 290 mμ (Fig. 2), indicating that they all have the same chromophore, a 4-phosphoryloxytryptamine nucleus. Slightly different chromophores such as those present in psilocin or bufotenine have distinctly different absorption maxima (1, 5).

The IR spectrum[2] of norbaeocystin showed sharp absorption peaks at 3,376 cm.$^{-1}$ and 1,640 cm.$^{-1}$ (Fig. 3) and that of baeocystin showed sharp absorption peaks at 3,275 cm.$^{-1}$ and 1,640 cm.$^{-1}$ (Fig. 4) which are absent in the IR spectrum of psilocybin (1). These absorption bands are indicative of primary or secondary amines.

The low resolution mass spectrum[3] of norbaeocystin showed a weak molecular ion peak at m/e = 256, corresponding to I. Both low- and high-resolution mass spectra[4] showed a strong peak at

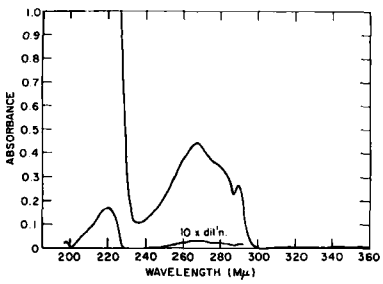
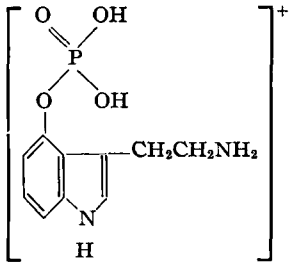


Fig. 2—Ultraviolet absorption spectrum of baeocystin and norbaeocystin (20 mcg./ml. methanol).

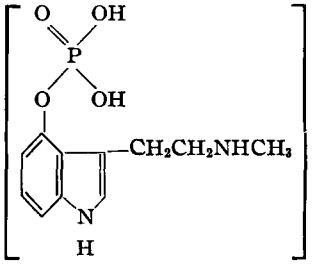
m/e = 176 due to the dephosphorylated species. The empirical formula of this species was shown by high-resolution mass spectral data to be $C_{10}H_{12}N_2O$ with an accurate mass of 176.0957 (calcd., 176.0950).



I

The low-resolution mass spectrum of baeocystin showed a weak molecular ion peak at m/e = 270, corresponding to II. Both low- and high-resolution mass spectra showed a strong peak at m/e = 190 due to the dephosphorylated species. The empirical formula of this species was shown by high-resolution mass spectral data to be $C_{11}H_{14}N_2O$ with an accurate mass of 190.1102 (calcd., 190.1106).

Similarly, psilocybin gave a weak molecular ion peak at m/e = 284 and a strong peak of the dephosphorylated species at m/e = 204.



II

In addition, baeocystin and psilocybin showed strong peaks at m/e = 44 [$CH_2{=}\overset{+}{N}HCH_3$] and m/e = 58 [$CH_2{=}\overset{+}{N}(CH_3)_2$], respectively. However, the corresponding peak of norbaeocystin at m/e = 30 [$CH_2{=}\overset{+}{N}H_2$] lacked prominence. This may be attributed to the lack of resonance of this ion as contrasted with the other two.

Apart from these differences, the mass spectra of all three compounds showed prominent peaks at

[1] Beckman DK-2A ratio recording spectrophotometer.
[2] Perkin-Elmer 337 grating infrared spectrophotometer.
[3] The authors are indebted to Dr. M. Grostic, The Upjohn Co., Kalamazoo, Mich., for the low-resolution mass spectral data.
[4] The authors are indebted to Dr. F. Vane, Hoffmann-LaRoche, Inc., Nutley, N. J., for the high-resolution mass spectral data.

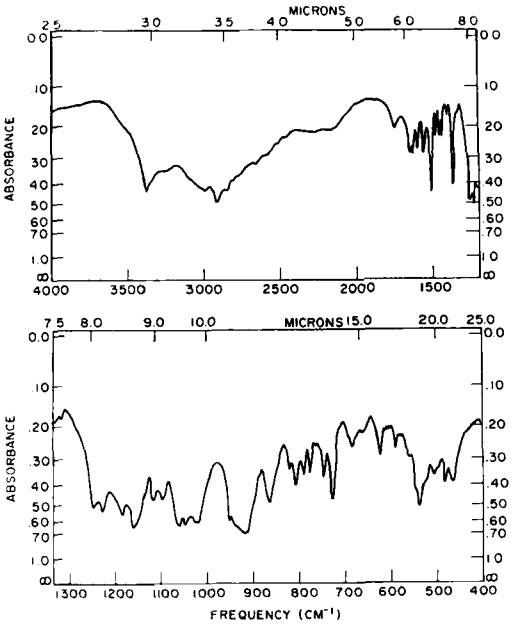


Fig. 3—Infrared spectrum of norbaeocystin (0.9% in KBr).

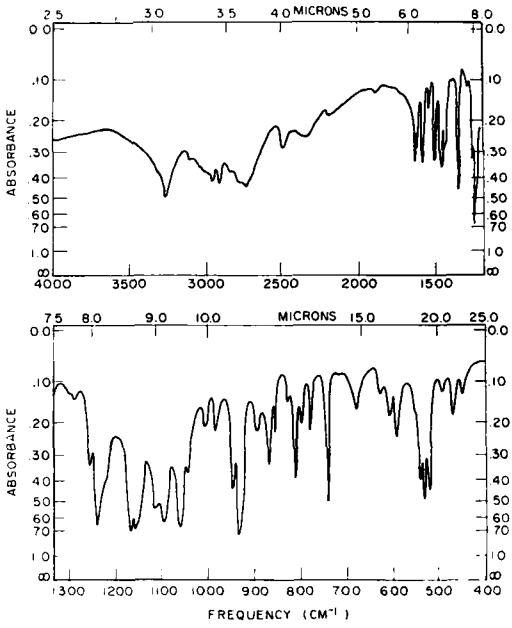


Fig. 4—Infrared spectrum of baeocystin (1.0% in KBr).

m/e = 160, 159, 146, 130, and 117. The proposed fragmentation patterns of these compounds are similar (Fig. 5).

These data indicate that baeocystin is the monomethyl analog (II) and that norbaeocystin is the demethyl analog (I) of psilocybin.

DISCUSSION AND CONCLUSIONS

Despite the simplicity of the structure of psilocybin, there have been no convincing reports on the occurrence of its analogs in nature. The only reports which suggest their presence in fungi have been made by Eugster (6) and by Stein *et al.* (7). From

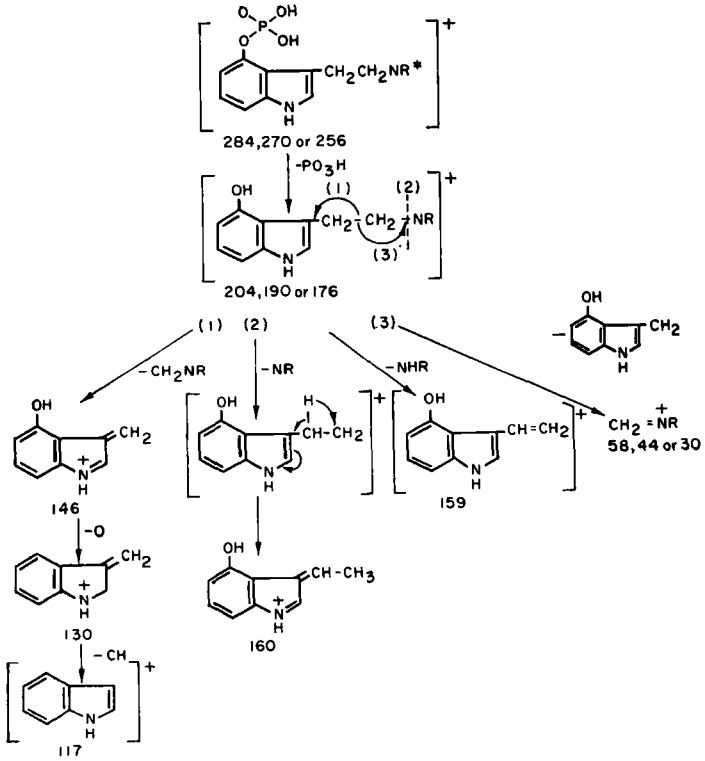


Fig. 5—Proposed fragmentation patterns of psilocybin, baeocystin, and norbaeocystin. R = $(CH_3)_2$, psilocybin; R = HCH_3, baeocystin; R = H_2, norbaeocystin.

Amanita muscaria (Fr.) Hook, Eugster isolated an extremely unstable 4-substituted indole derivative having UV spectrum practically identical with that of psilocybin. Stein *et al.* also isolated an unknown compound from *Panaeolus venenosus* Murr. This compound also had an almost identical UV spectrum as psilocybin, with absorption maxima at 220, 265, and 288 mμ. Its high melting point (>250° dec.) suggests its identity with baeocystin.

The occurrence of baeocystin and norbaeocystin in *P. baeocystis* indicates that this fungus can serve as an appropriate organism for studying the biosynthesis and the metabolism of psilocybin. Earlier studies by Agurell *et al.* (8, 9) indicated that *Psilocybe cubensis* uses the following pathway in the biosynthesis of psilocybin: tryptophan → tryptamine → *N*-methyltryptamine → *N,N*-dimethyltryptamine → psilocin → psilocybin. Recent studies by Agurell and Nilsson (10) suggest that this fungus can also use an alternative route wherein phosphorylation precedes methylation. The high concentration of 4-hydroxytryptamine incorporated into psilocybin and the detection of psilocybin-like compounds were cited as evidence for this alternative pathway. The presence of psilocybin analogs in *P. baeocystis* suggests that the alternative pathway is probably the major route utilized by this fungus. Hence, it should be an ideal organism for further study of the alternative route.

The isolation of baeocystin and norbaeocystin offers a possibility of testing the serotonin hypothesis of mental illness (11). Norbaeocystin and baeocystin are the closest known serotonin analogs which have one of their enzyme-susceptible groups protected. Whether or not this may have any biochemical or pharmacological significance remains to be studied.

SUMMARY

The detection of unknown tryptamine derivatives in *P. baeocystis* has led to the isolation of two new compounds from submerged cultures of this fungus. The isolation procedure involved column chromatography of a methanol extract of the fungal tissue on powdered cellulose and on activated silicic acid. The structures of these compounds have been determined to be the monomethyl and demethyl analogs of psilocybin by TLC characteristics, color reactions, UV, IR, and mass spectral analyses. These compounds have been named baeocystin (monomethyl) and norbaeocystin (demethyl).

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Keyphrases

Psilocybin analogs—*Psilocybe baeocystis*
Baeocystin—isolation, identification
Norbaeocystin—isolation, identification
TLC—separation, identity
UV spectrophotometry—identity
IR spectrophotometry—identity
Mass spectroscopy—structure

N-Acyl Analogs of *N*-(2-Cyanoethyl)cyclohexylamine with CNS Activity

By W. D. ROLL

A series of substituted aryl amides of *N*-(2-cyanoethyl)cyclohexylamine was synthesized and screened for their effect on the central nervous system. Members of this series exhibit a pronounced effect on the spontaneous motor activity of mice, usually accompanied by varying degrees of activity on blood pressure in rats.

SUBSTITUTED AMIDE analogs of *N*-(2-cyanoethyl)cyclohexylamine (Abbott Laboratories) appear to act on the central nervous system either by stimulation or depression in small animals depending on the dosage. The compounds reported herein exert a depressant action on the spontaneous motor activity of mice at a dosage of 4

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