

doses of 6.5 g taken during an entire pregnancy have resulted in the birth of a normal healthy neonate (19). However, in an animal such as the rat in which a wide variety of compounds can affect fetal growth, acetaminophen in the doses used in this study seems to produce less of an adverse effect than does aspirin.

REFERENCES

- (1) E. M. Boyd, *J. Clin. Pharmacol.*, **10**, 222(1970).
- (2) H. N. Wright, *Toxicol. Appl. Pharmacol.*, **11**, 280(1967).
- (3) M. Goldman and C. A. Poppens, *ibid.*, **24**, 159(1973).
- (4) J. D. McColl, M. Globus, and S. Robinson, *ibid.*, **7**, 409(1965).
- (5) C. A. Kimmel, J. G. Wilson, and H. J. Schumacher, *Teratology*, **4**, 15(1971).
- (6) K. S. Larsson and H. Bostrom, *Acta Paediatr. Scand.*, **54**, 43(1965).
- (7) S. C. Smith and I. W. Monie, *Teratology*, **2**, 1(1969).
- (8) K. T. Szabo, S. M. Free, H. A. Birkhead, Y. J. Kange, E. Alston, and M. Henry, *Toxicol. Appl. Pharmacol.*, **19**, 371(1971).
- (9) J. Warkany and E. Takacs, *Am. J. Pathol.*, **35**, 315(1959).
- (10) E. Takacs and J. Warkany, *Teratology*, **1**, 109(1968).
- (11) S. Siegel, "Non Parametric Statistics," McGraw-Hill, New York, N.Y., 1956, p. 110.

- (12) R. D. Pawkins, B. J. Gould, and M. J. H. Smith, *Biochem. J.*, **99**, 702(1966).
- (13) K. Janakidevi and M. J. H. Smith, *J. Pharm. Pharmacol.*, **22**, 51(1970).
- (14) *Ibid.*, **22**, 249(1970).
- (15) J. T. Miyahara and R. Karler, *Biochem. J.*, **97**, 194(1965).
- (16) J. R. Vane, *Nature*, **231**, 232(1971).
- (17) S. Dikstein, M. Grotto, V. Zor, M. Tamari, and F. G. Sulman, *J. Endocrinol.*, **36**, 257(1966).
- (18) R. Lewis and J. Shulman, *Lancet*, **2**, 1159(1973).
- (19) L. Garrettson, J. A. Procknal, and G. Levy, *Clin. Pharmacol. Ther.*, **17**, 98(1975).

ACKNOWLEDGMENTS AND ADDRESSES

Received November 8, 1974, from the College of Pharmacy, University of Kentucky, Lexington, KY 40506

Accepted for publication February 9, 1976.

Supported in part by University of Kentucky Research Committee Grant 201-12-83100-63039.

The authors acknowledge the assistance of Mr. Michael Chestnut, Mr. Douglas Droz, Ms. Kay Gaines, and Mr. John Triplett with the manipulative procedures.

* To whom inquiries should be directed.

Baeocystin in *Psilocybe semilanceata*

DAVID B. REPKE ** and DALE THOMAS LESLIE †

Abstract □ Baeocystin and psilocybin were found in extracts of a variety of *Psilocybe semilanceata*. Psilocybin (but not baeocystin) was also detected in extracts of a related species, *Psilocybe pelliculosa*. Traces of psilocin were present in these two species. The structures of the isolated compounds were corroborated using mass spectrometry and UV spectroscopy.

Keyphrases □ Baeocystin—isolated from extracts of *Psilocybe semilanceata*, identified by mass spectrometry and UV spectroscopy
□ Psilocybin—isolated from extracts of *Psilocybe semilanceata* and *P. pelliculosa*, identified by mass spectrometry and UV spectroscopy
□ *Psilocybe semilanceata*—extracts of carpophores, baeocystin and psilocybin isolated, identified by mass spectrometry and UV spectroscopy

The genus *Psilocybe* contains many species with hallucinogenic properties (1). Their use for religious purposes in Mexico was first recorded 4 centuries ago (2). However, only in the last 35 years has serious study been devoted to this subject (3). In 1959, two active principles were isolated from several members of this genus (4): the 4-hydroxy-3-(2-dimethylaminoethyl)-indole (psilocin) and its corresponding phosphate ester (psilocybin). Since then psilocin and psilocybin have been detected in other members of *Psilocybe* and related genera (5).

Heim (6) suggested that the hallucinogenic mushroom described in a 16th century English report was *Psilocybe semilanceata* (Fr.) Kummer (Strophariaceae), considered the type species of the genus (7, 8). Investigators have verified the presence of psilocybin (but not psilocin) in several European collections of *P. semilanceata* (9–12).

The presence of the psilocybin analogs 4-phosphor-

xyloxy-3-(2-methylaminoethyl)indole and its demethyl counterpart (baeocystin and norbaeocystin, respectively) have been reported only in *P. baeocystis* Singer and Smith (13). These compounds were not found in *P. atrobrunnea* (Lasch) Gillet, *P. pelliculosa* (Smith) Singer and Smith, *P. caerulipes* (Peck) Sacc., or *P. strictipes* Singer and Smith (14).

During investigations of the indole alkaloids of the genus *Psilocybe*, a compound with a mobility slightly slower than psilocybin was consistently detected on thin-layer chromatograms of extracts of a Pacific Northwest variety of *P. semilanceata*. This compound was observed in all collections of this species examined over several years.

EXPERIMENTAL¹

Freeze-dried carpophores of *P. semilanceata*², 824 mg, were ground to a powder and extracted by shaking with methanol at room tem-

¹ TLC was carried out using 0.25- and 1.0-mm layers of silica gel GF on glass plates. The solvent system used was 1-propanol-5% ammonium hydroxide (5:2) (13). Mass spectra were obtained with an Atlas CH-4 spectrometer via direct inlet. The probe temperature was 300°, and the ion source potential was 70 eV. A Cary model 15 spectrophotometer was used to determine UV spectra.

² The collections are in the herbarium of the Escuela Nacional de Ciencias Biológicas, I. P. N., Mexico, D. F., Mexico, and some are also on deposit at the herbarium of the University of Michigan, Ann Arbor, Mich. Carpophores of *P. semilanceata* were collected in pastures at the following locations: Grays Harbor County, Wash., 1972–1975 [LESLIE 1351 (also at MICH), 1807 (also at MICH), 2426 (also at MICH), and 2647]; Jefferson County, Wash., 1973–1975 [LESLIE 1843 (also at MICH), 2409 (also at MICH), and 2664]; Randle, Wash., 1975 (LESLIE 2710); and Sixes, Ore., 1975 (LESLIE 2781). Collections of *P. pelliculosa* were found in forests on wood debris and sawdust in Grays Harbor County, Wash., 1973 (LESLIE 1822) and Maytown, Wash., 1975 (LESLIE 2758). The taxon, designated here as *P. semilanceata*, conforms to published descriptions of the European *P. semilanceata*, but some doubt remains concerning this specific placement which further study may clarify.

perature for 20 hr. The mixture was filtered, and the solids were washed with methanol. The combined filtrate and washings were concentrated *in vacuo*. Analytical TLC of the extract (two developments) showed psilocybin (R_f 0.27) and the slower compound (R_f 0.22). Both spots exhibited the same dark-blue coloration when sprayed with 2% 4-dimethylaminobenzaldehyde in ethanol and exposed to hydrogen chloride vapors. Trace amounts of psilocin were also detected.

The concentrated residue was preparatively chromatographed on a 1.0-mm layer of silica gel in the described solvent system. A band containing the two components was removed from the plate, and the compounds were eluted with methanol containing 5% aqueous ammonia. The eluate was concentrated under reduced pressure, and the residue was rechromatographed on a 0.25-mm layer of silica gel to resolve the two components. Two developments in the propanol-ammonia system followed by elution of the bands afforded psilocybin (3 mg, 0.36%), mp 175–180° [lit. (15) mp 185–195°] and its slower-moving counterpart (1 mg, 0.12%), mp 245–248° [lit. (14) mp 254–258°]. Carpophores of *P. pelliculosa* were extracted in a similar manner to give 0.08% psilocybin. Traces of psilocin were also detected.

RESULTS AND DISCUSSION

The UV spectra of the two compounds were superimposable, with λ_{\max} (methanol) 290 (ϵ 4000), 280 (sh) (5000), 268 (6300), and 222 (40,000) nm, typical of psilocybin (4). The mass spectra were consistent with published data for psilocybin (11) and baecocystin (13). Psilocybin showed peaks at *m/e* 204 (relative abundance, 19%), 160 (4), 159 (3), 146 (6), 130 (3), 117 (2), and 58 (100). Baecocystin showed peaks at 190 (8), 160 (4), 159 (4), 147 (16), 146 (14), 130 (4), 117 (5), and 44 (100).

Each compound exhibited typical fragmentations of 3-indolethylamines (16), *e.g.*, a base peak arising from β -bond fission of the ethylamine side chain (psilocybin, *m/e* 58; and baecocystin, *m/e* 44). While neither compound showed a parent peak, both showed signals for the dephosphorylated species (psilocybin, *m/e* 204; and baecocystin, *m/e* 190). The concentration of psilocybin in *P. semilanceata* was consistent with isolated yields reported from European collections (9, 11).

In agreement with Leung and Paul (14), baecocystin was not detected in *P. pelliculosa*. Psilocybin (but not psilocin) has been reported previously in this species (17).

Preliminary investigations suggest the presence of baecocystin in other species and genera. In fact, Leung and Paul (13) and Benedict (18) suggested that baecocystin may have been the unidentified compound observed by Stein *et al.* (19) in a species of *Panaeolus*.

Pharmacological effects of the analogs of psilocybin have not been studied.

REFERENCES

- (1) R. Heim and R. G. Wasson, "Les Champignons Hallucinogènes du Mexique," Museum National D'Histoire Naturelle, Paris, France, 1959.
- (2) B. de Sahagún, "Florentine Codex, General History of New Spain," Books III and XI, A. J. O. Anderson and C. E. Dibble, Translators, University of Utah Press, Salt Lake City, Utah, 1970.
- (3) R. G. Wasson, *Bot. Mus. Leaflet Harv. Univ.*, **20**, 25(1963).
- (4) A. Hofmann, R. Heim, A. Brack, H. Kobel, A. Frey, H. Ott, T. Petrzilka, and F. Troxler, *Helv. Chim. Acta*, **42**, 1557(1959).
- (5) R. E. Schultes and A. Hofmann, "The Botany and Chemistry of Hallucinogens," Charles C Thomas, Springfield, Ill., 1973, pp. 36–52.
- (6) R. Heim, *Natur. Can.*, **98**, 415 (1971).
- (7) R. Singer, "The Agaricales in Modern Taxonomy," 2nd ed., J. Cramer, Weinheim, Germany, 1962, p. 542.
- (8) P. Konrad and A. Maublanc, "Révision des Hyménomycètes de France," Paul LeChevalier, Paris, France, 1937, p. 84.
- (9) A. Hofmann, R. Heim, and H. Tschertter, *C. R. Acad. Sci.*, **257**, 10(1963).
- (10) R. G. Benedict, V. E. Tyler, and R. Watling, *Lloydia*, **30**, 150(1967).
- (11) P. G. Mantle and E. S. Waight, *Trans. Br. Mycol. Soc.*, **53**, 302(1969).
- (12) M. Semerdzieva and F. Nerud, *Ceska Mykol.*, **27**, 42(1973).
- (13) A. Y. Leung and A. G. Paul, *J. Pharm. Sci.*, **57**, 1667(1968).
- (14) *Ibid.*, **56**, 146(1967).
- (15) A. Hofmann, R. Heim, A. Brack, and H. Kobel, *Experientia*, **14**, 107(1958).
- (16) H. Budzikiewicz, C. Djerassi, and D. H. Williams, "Structural Elucidation of Natural Products by Mass Spectrometry," vol. 1, Holden-Day, San Francisco, Calif., 1964, pp. 42–45.
- (17) V. E. Tyler, *Lloydia*, **24**, 71(1961).
- (18) R. G. Benedict, in "Microbial Toxins," S. Kadis, A. Ciegler, and S. J. Ajl, Eds., Academic, New York, N.Y., 1972, chap. 11.
- (19) S. I. Stein, G. L. Closs, and N. W. Gabel, *Mycopathol. Mycol. App.*, **11**, 205(1959).

ACKNOWLEDGMENTS AND ADDRESSES

Received December 29, 1975, from *148-B West Dana Street, Mountain View, CA 94041, and †104 Whitney Avenue, Los Gatos, CA 95030

Accepted for publication May 28, 1976.

The authors thank Lornie Leslie and Jean Leslie for their help with this study and also Jerry Boydstein for assistance in collecting *P. pelliculosa* near Maytown, Wash.

* To whom inquiries should be directed.

Simple Device for GLC Separations of Cannabinoids Using a Surface-Coated Open Tube Column without Stream Splitting

N. K. McCALLUM* and E. R. CAIRNS

Abstract □ A simple device that allows the GLC analysis of greater than 10 μ l of sample solution on capillary columns is described. The conditions necessary for application of this device to the quantitative analysis of cannabinoids are elaborated.

Keyphrases □ Cannabinoids—GLC analysis, device using a surface-coated open tube column □ GLC—analysis, cannabinoids, device using a surface-coated open tube column □ Marijuana—GLC analysis of constituents, device using a surface-coated open tube column

GLC is the method of choice for rapid qualitative and quantitative analysis of marijuana constituents. Much literature (1) is devoted to the use of this analytical tech-

nique with packed columns. Several investigators, however, reported problems with packed GLC columns for these analyses. Some long chain alkanes have similar re-