M. Variations from Fig. 2 occur due to the fact that not all solids and their resulting solutions have the same density and convective velocity. For example, salicylamide has a lower solubility than I but a higher solution mass transfer velocity.

Finally, it must be stressed that a diffusional mass transfer step does not mean a diffusion-controlled mechanism for dissolution. While the dissolution of solids having solubilities below 0.001 M appears to be diffusion controlled, this does not mean that the interfacial rate is now fast compared to the diffusion rate. Certainly, the rate of interaction at the solid–liquid interface decreases as the solubility decreases, as indicated by the data in Table I showing that the effective interfacial concentration decreases with decreasing solubility. This phenomenon is not likely to change at even lower solubilities. Consequently, regardless of solubility or the approach to diffusional mass transfer, the interfacial rate will remain slower than the diffusion rate and dissolution must be considered as an interfacially controlled process.

REFERENCES

(1) R. L. Nedich and D. O. Kildsig, *J. Pharm. Sci.*, **61**, 214 (1972).

(2) C. D. Shively and D. O. Kildsig, *ibid.*, **61**, 1589 (1972).

(3) S. J. Michell, "Fluid and Particle Mechanics," Pergamon Press, London, England, 1970, pp. 289–292.

(4) C. D. Shively, Ph.D. thesis, Purdue University, West Lafayette, Ind., 1972.

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GLC–Mass Spectral Analysis of Psilocin and Psilocybin

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Abstract □ With the combined technique of GLC–mass spectrometry, psilocin and psilocybin, two hallucinogenic indoles, were analyzed as their trimethylsilyl derivatives. The method was applied to these two components in an extract of *Psilocybe cubensis* (Earle) Sing.

Keyphrases □ Psilocin—GLC–mass spectral analysis in extract of mushroom *Psilocybe cubensis* □ Psilocybin—GLC–mass spectral analysis in extract of mushroom *Psilocybe cubensis* □ GLC–mass spectrometry—analysis, psilocin and psilocybin in extract of mushroom *Psilocybe cubensis* □ Hallucinogenics—psilocin and psilocybin, GLC–mass spectral analysis in extract of mushroom *Psilocybe cubensis*

Psilocin and psilocybin, two hallucinogenic principles found in certain agarics (1), have aroused considerable interest since their isolation and characterization in the late 1950's (2, 3). The mushrooms in which they occur have been known and revered in southern Mexico for centuries. Their rediscovery in 1939 (4) and their subsequent study (5, 6) and popularization ultimately led to legislation to control their use (7).

BACKGROUND

Psilocin (3-[2-(dimethylamino)ethyl]indol-4-ol) and psilocybin, its dihydrogen phosphate ester, are unique in nature as indoles having a 4-substituent and a phosphate group. Both qualitative and quantitative methods have been utilized for their analysis. Paper chromatography (2) and TLC (8) have been used in conjunction with colorimetric reagents as well as UV spectroscopy. When pure samples are available, IR spectrometry can be used (9). Mass spectrometry (10) has been relied upon for identification of these compounds in various pharmaceutical preparations. Various fluorescence and phosphorescence techniques (11) as well as other emission spectral techniques (12) have also been used.

In an investigation of the genus *Psilocybe* and related genera, the need for a sensitive and accurate quantitative method for the analysis of psilocin and psilocybin became apparent. This method would have to be applicable to extracts of minute amounts of scarce herbarium material and vegetative mycelium obtained from liquid culture.

Previously, GLC was applied to the analysis of psilocin (13) and psilocybin (14), both as the free bases and their trimethylsilyl derivatives. GLC–mass spectrometry has been successfully applied to the bis(trimethylsilyl) derivative of bufotenine, the 5-hydroxy positional isomer of psilocin (15).

The possibility of utilizing GLC for the separation of organic molecules containing a phosphate group was suggested by work with trimethylsilyl derivatives of ribonucleotides (16).

EXPERIMENTAL[1]

Fifty milligrams of freeze-dried pileus tissue of *P. cubensis*[2] was ground to a fine powder with sand and transferred to a screw-capped vial. Methanol (5 ml) was added, and the tube was shaken at room temperature for 24 hr. The mixture was filtered, and the solid was washed with 5 ml of methanol. The filtrate was concentrated *in vacuo* to 0.5 ml, transferred to a 1.0-ml vial[3], and concentrated to dryness in a nitrogen stream. All traces of solvent were removed by evacuation on the oil pump. In an anhydrous nitrogen atmosphere, 100 μl of bis(trimethylsilyl)trifluoroacetamide[4] was added to the vial, which was then closed with a septum-lined aluminum seal[5].

The vial was heated at 140° for 15 min. Prolonged reaction periods (up to 2.5 hr) showed that this length of time was sufficient for quantitative

[1] GLC was carried out using a Hewlett-Packard model 402 gas chromatograph equipped with hydrogen flame-ionization detectors. The column used was a 1.6-m × 2.8-mm i.d. glass U-tube with 1.5% SE-30 on 100–120-mesh Chromosorb W. Chromatography conditions were: injection block, 220°; detector, 280°; oven, temperature programmed from 150 to 250° at 7.5°/min; chart speed, 0.64 cm/min; and helium carrier gas, 50 ml/min. TLC utilized glass plates with 0.25-mm layers of silica gel GF using 1-propanol–5% ammonium hydroxide (5:2) (17).

GLC–mass spectrometry was carried out with a Finnigan model 9500 gas chromatograph coupled to a Finnigan model 3100 D quadrupole mass spectrometer through a single-stage glass jet separator. A System/250 data system (Systems Industries) was used to control the mass spectrometer and acquire data. The mass spectrometer parameters were: interface temperature, 200–225°; transfer line, 150–175°; manifold temperature, 100°; and ion source potential, 70 ev. Two sets of chromatography conditions were employed: (*a*) essentially the same as those listed for GLC, and (*b*) a 0.75-m × 2-mm i.d. glass U-tube with 3% OV-101 on 100–120-mesh Gas Chrom Q temperature programmed from 200 to 275° at 10°/min with a helium flow rate of 20 ml/min.

[2] Carpophores of *Psilocybe cubensis* (Earle) Singer (= *Stropharia cubensis* Earle) (Strophariaceae) were obtained by aseptic cultivation of a strain of this fungus on sterile horse manure. The strain was isolated from pileus tissue of a fresh carpophore of *P. cubensis* collected Aug. 31, 1974, in Huautla de Jimenez, Oaxaca, Mexico, and was maintained on malt extract agar. The origin and maintenance of the culture and the production of fruiting bodies were similar to those reported previously (5, 6, 18). Carpophores were authoritatively identified by Dr. Gastón Guzmán, Escuela Nacional de Ciencias Biológicas (ENCB), I.P.N., Mexico, D.F., Mexico. Herbarium material is on deposit at the ENCB herbarium and the University of Michigan herbarium as LESLIE 1902 and at the Institute for Fermentation herbarium, Osaka, Japan, as IFO-H 11703. Subcultures are on deposit at the Institute for Fermentation (IFO 30176) and the Centraalbureau vorr Schimmelcultures, Baarn, The Netherlands (CBS 134.76).

[3] Hewlett-Packard 5080-8712.

[4] Regisil, Regis Chemical Co.

[5] Hewlett-Packard 5080-8713.

derivatization. The resulting solution was cooled to room temperature, and 1.0-μl aliquots were used for subsequent analyses. Reference standards of psilocin and psilocybin were derivatized in the same manner, and quantitation was performed using an external standard of bis(trimethylsilyl)psilocin following the general method described by Gudzinowicz (19). The silanized solutions were stable for several weeks when stored at 4° under anhydrous conditions.

RESULTS AND DISCUSSION

Under the temperature-programmed chromatography conditions described (SE-30), the retention times for bis(trimethylsilyl)psilocin (I) and tris(trimethylsilyl)psilocybin (II) were 8.45 and 13.10 min, respectively. These two compounds were clearly separated from other components in the natural extract. Comparison with the external standard showed the concentrations to be 0.420% psilocin and 0.168% psilocybin. These values are not inconsistent with the total yields obtained by Heim and Hofmann (20) from various cultivated strains of *P. cubensis*. However, in the present analysis, the ratio of psilocin to psilocybin consistently exceeded 1, in contrast to the isolated yields of each compound reported by Heim and Hofmann (20). The present results were confirmed by TLC. The difference in these results might be attributed to the decomposition of psilocin during isolation procedures.

While the mass spectrum of I both as the standard and from the natural extract could be recorded easily, the mass spectrum of II could not be obtained with the longer SE-30 column. However, the conditions utilized with the OV-101 column proved rewarding, and a satisfactory spectrum was recorded. Under these conditions, II was eluted in 3.6 min.

The mass spectrum of I exhibited peaks at *m/e* 348 (M^+) (relative intensity 4%), 333 (1), 291 (6), 290 (21), 75 (3), 73 (39), and 58 (100). The mass spectrum of II showed peaks at *m/e* 500 (M^+) (1%), 485 (1), 442 (6), 77 (9), 75 (15), 73 (47), and 58 (100). While these two spectra are relatively simple in appearance, they are consistent with known fragmentation patterns of this type of compound. The parent peak at *m/e* 348 in I indicates the presence of two trimethylsilyl groups. As expected for 3-indolylethylamines, the significant fragments at *m/e* 290 and *m/e* 58 arise by fission of the bond beta to the nitrogen in the dimethylaminoethyl side chain (21). The M − 15 peak at *m/e* 333 and the peaks at *m/e* 75 and 73 are consistent with known fragmentations of trimethylsilyl ethers (22). The parent peak in the spectrum of II at *m/e* 500, the β-bond fission fragment at *m/e* 442 (M − 58), and the M − 15 peak at *m/e* 485 are evidence for the presence of three trimethylsilyl groups. Again, the peak at *m/e* 58 due to β-bond fission is the most prominent. The mass spectrum of the eluted psilocybin derivative confirms the stability of the silanized molecule under the instrumental conditions used.

Analyses by this method are routinely performed using 5–20 mg of dried fungal tissue.

REFERENCES

(1) R. E. Schultes and A. Hofmann, "The Botany and Chemistry of Hallucinogens," Charles C Thomas, Springfield, Ill., 1973, pp. 36–52.

(2) A. Hofmann, R. Heim, A. Brack, H. Kobel, A. Frey, H. Ott, T. Petrzilka, and F. Troxler, *Helv. Chim. Acta*, **42**, 1557 (1959).

(3) A. Hofmann, *Bull. Narc.*, **23**, 3 (1971).

(4) R. E. Schultes, *Bot. Mus. Leaft., Harv. Univ.*, **7**, 37 (1939).

(5) R. Heim and R. G. Wasson, "Les Champignons Hallucinogènes du Mexique," Museum National D'Histoire Naturelle, Paris, France, 1959, pp. 224–243.

(6) R. Singer, *Mycologia*, **50**, 239 (1958).

(7) 91st Congress of the United States, "Comprehensive Drug Abuse and Control Act of 1970," Public Law 91-513, Oct. 27, 1970.

(8) R. Heim, K. Genest, D. W. Hughes, and G. Belec, *J. Forensic Sci. Soc.*, **6**, 192 (1966).

(9) R. J. Mesley and W. H. Evans, *J. Pharm. Pharmacol.*, **22**, 321 (1970).

(10) S. W. Bellman, *J. Assoc. Offic. Anal. Chem.*, **51**, 164 (1968).

(11) J. J. Aaron, L. B. Sanders, and J. D. Winefordner, *Clin. Chim. Acta*, **45**, 375 (1973).

(12) N. Fiussello, *Mycopathol. Mycol. Appl.*, **39**, 329 (1969).

(13) B. Holmstedt, W. J. A. VandenHeuvel, W. L. Gardiner, and E. C. Horning, *Anal. Biochem.*, **8**, 151 (1964).

(14) M. Lerner and M. D. Katsiaficas, *Bull. Narc.*, **21**, 47 (1969).

(15) N. Narasimhachari, J. Spaide, and B. Heller, *J. Chromatogr. Sci.*, **9**, 502 (1971).

(16) T. Hashizume and Y. Sasaki, *Anal. Biochem.*, **15**, 199 (1966).

(17) A. Y. Leung, A. H. Smith, and A. G. Paul, *J. Pharm. Sci.*, **54**, 1576 (1965).

(18) L. R. Kneebone, in "Developments in Industrial Microbiology," vol. 1, Plenum, New York, N.Y., 1960, p. 109.

(19) B. J. Gudzinowicz, "Gas Chromatographic Analysis of Drugs and Pesticides," Dekker, New York, N.Y., 1967, pp. 164–174.

(20) R. Heim and A. Hofmann, *Rev. Mycol.*, **23**, 347 (1958).

(21) 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.

(22) A. E. Pierce, "Silylation of Organic Compounds," Pierce Chemical Co., Rockford, Ill., 1968, pp. 33–39.

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High-Pressure Liquid Chromatographic Separation of 5,6-*cis*- and *trans*-Prostaglandin A_2

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Abstract □ A high-pressure liquid chromatographic procedure capable of separating the *cis*- and *trans*-isomers of prostaglandin A_2 is described.

Keyphrases □ Prostaglandin A_2—high-pressure liquid chromatographic analysis, *cis*- and *trans*-isomers □ High-pressure liquid chromatography—analysis, *cis*- and *trans*-prostaglandin A_2

Prostaglandin A_2 (I) normally has the *cis*-configuration at the 5,6-double bond. However, extracts from the gorgonian *Plexaura homomalla*, a soft coral found in the Caribbean area, contained significant amounts of 5,6-*trans*-prostaglandin A_2 (II) (1). Therefore, a procedure capable of separating the *cis*- and *trans*-isomers in prostaglandin samples is needed.

A recent paper (2) described the high-pressure liquid