Of 140 cultures tested 38 grew anaerobically (Table 1). Such growth was found particularly in members of the Mucorales, Hypocreales and Tuberculariales and sporadically in other groups. In all cases of growth, results were confirmed in tubes or shake flasks, as were a few cases where growth failed (Table 2). Dry weights up to 2·4 mg/ml were obtained. This is a wider range of fungi showing anaerobic growth than has been previously reported.

REFERENCES

BARTNICKI-GARCIA, S. & NICKERSON, W. J. (1962). Nutrition, growth and morphogenesis of *Mucor rouxii*. *J. Bact.* **84**, 841–858.

FOSTER, J. W. (1949). *Chemical activities of fungi*, p. 162. New York: Academic Press.

GUNNER, H. B. & ALEXANDER, M. (1964). Anaerobic growth of *Fusarium oxysporum*. *J. Bact.* **87**, 1309–1316.

STOTZKY, G. & GOOS, R. D. (1965). Effect of high CO_2 and O_2 tensions on the soil microbiota. *Can. J. Microbiol.* **11**, 853–868.

P. J. CURTIS, *Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield*

OCCURRENCE OF PSILOCYBIN IN THE SPOROPHORES OF PSILOCYBE SEMILANCEATA

The nature of the active principles of the sporophores of Mexican hallucinogenic Agaricales (*Psilocybe mexicana* Heim and related species) has been determined by Hofmann, Heim, Brack & Kobel (1958) and by Hofmann, Frey, Ott, Petrzilka & Troxler (1958). The principal active substance is psilocybin (4-phosphoryl-*N*,*N*-dimethyl-tryptamine) although psilocin (4-hydroxyl-*N*,*N*-dimethyltryptamine) occurs in trace amounts. *Conocybe siligineoides* Heim has also been shown to contain psilocybin (Benedict, Brady, Smith & Tyler, 1962).

The occurrence of these substances in British Agaricales has not previously been established, although there have been several instances of suspected poisoning by sporophores of *Panaeolina* and *Psilocybe* spp.

Air-dried samples of the sporophores of *Panaeolina foenisecii* (Pers. ex Fr.) Maire (1·65 g) and *Psilocybe semilanceata* (Fr. ex Secr.) Kummer (2·35 g), collected by Miss M. Holden at Rothamsted Experimental Station, Harpenden, Herts., have been investigated for the presence of psilocybin and psilocin. The finely milled tissue was extracted with methanol (2 × 20 ml) and the combined methanol extracts reduced to small volume *in vacuo*. An aliquot of each extract was chromatographed with authentic psilocybin and psilocin on silica gel G in a chloroform/methanol/ammonia (80:20:0·2) solvent system, followed by spraying with 3 % *p*-dimethylaminobenzaldehyde in concentrated HCl to detect indole derivatives by their blue-grey colour. This system separates psilocybin from psilocin although the former does not move from the origin. The *Panaeolina foenisecii* extract contained no detectable psilocybin or psilocin, and psilocin was also undetectable in the *Psilocybe semilanceata* extract.

However, the *P. semilanceata* extract contained a substance which was

inseparable from psilocybin in both the chloroform/methanol/ammonia solvent system and a methanol/acetic acid/water (75:10:15) system (Agurell, Blomkvist & Catalfomo, 1966) in which it showed an R_F of 0·25. The remaining methanol extract was partly purified by preparative thin-layer chromatography in the chloroform/methanol/ammonia solvent system, the origin then being eluted with methanol and the extract evaporated to dryness. The residue was taken up in methanol/water (1:1), and applied to a small (0·5 × 2·0 cm) column of Zeocarb 225 (H^+) ion-exchange resin. The column was washed with successive 5 ml portions of methanol/water (1:1), methanol, chloroform/methanol (1:1), methanol, and methanol/water (1:1). The column was eluted with 10 ml methanol/water (1:1) containing 5 % ammonium hydroxide (0·88 s.g.) followed by 10 ml methanol containing 1 % N-HCl. The combined eluates were evaporated to dryness and extracted with cold ethanol (0·5 ml) in which psilocybin is poorly soluble. The residue was crystallized from methanol (yield 3·3 mg) and gave a melting-point (190 °C), ultra-violet absorption spectrum ($\lambda_{max\ 267}$, 290 nm), and infra-red spectrum (in KBr) which agreed with authentic psilocybin. Mass spectrometry by direct insertion into the ion source at 250° revealed no trace of an ion at *m/e* 284 (the molecular weight of psilocybin). The highest mass peak, *m/e* 204, was accompanied by others at 160, 159, 146, 130, 84 and 58 (base peak). These peaks (with the exception of *m/e* 84) appeared in the spectrum of authentic psilocybin which likewise showed no molecular ion. The fragmentation is consistent with the structure of psilocin, and high resolution studies confirmed the composition $C_{12}H_{16}N_2O$ (found 204.1264). Presumably the substituted phosphoric acid, psilocybin, decomposes on heating to the phenol, psilocin, and metaphosphoric acid. An anology may be found in the decomposition upon distillation of monophenyl phosphate into phenol and metaphosphoric acid (Jacobsen, 1875).

The phosphorus composition was determined by micro-analysis, a value of 6·8 % (calculated 9·8 % for psilocybin) being obtained in spite of the small quantity (1·4 mg) of sample analysed.

Thus this conclusively identifies the presence of psilocybin (approximately 0·15 %) for the first time in the sporophores of *P. semilanceata* and is also the first record of the natural occurrence of this substance in this country. Further, on the basis of the effective hallucinogenic dose for pure psilocybin (Hofmann, Heim, Brack & Kobel, 1958), ingestion of more than about 3 g of air-dry sporophores of *P. semilanceata* might be expected to be hallucinogenic.

The supply of reference samples of psilocybin and psilocin by Sandoz Ltd., Basle, Switzerland, is gratefully acknowledged.

REFERENCES

AGURELL, S., BLOMKVIST, S. & CATALFOMO, P. (1966). Biosynthesis of psilocybin in submerged culture of *Psilocybe cubensis*. *Acta pharm. Suecica* **3**, 37–44.

BENEDICT, R. G., BRADY, L. R., SMITH, A. H. & TYLER, V. E. (1962). Occurrence of psilocybin and psilocin in certain *Conocybe* and *Psilocybe* species. *Lloydia* **25**, 156–159.

JACOBSEN, G. (1875). Ueber phenylphorphorsauren und chloride derselben. *Ber. dt. chem. Ges.* **8**, 1519–1523.

HOFMANN, A., FREY, A., OTT, H., PETRZILKA, T. & TROXLER, F. (1958). Konstitutionsaufklarung und synthese von psilocybin. *Experientia* **14**, 397–399.

HOFMANN, A., HEIM, R., BRACK, A. & KOBEL, H. (1958). Psilocybin, ein psychotroper Wirkstoff aus dem mexikanischen Rauschpilz *Psilocybe mexicana* Heim. *Experientia* **14**, 107–109.

P. G. MANTLE and E. S. WAIGHT, *Departments of Biochemistry and Chemistry, Imperial College, London, S.W. 7*

DISTRIBUTION AND PROPERTIES OF BASIC PROTEINS IN THE PHIALIDE OF PENICILLIUM NOTATUM DURING CONIDIATION

In a study of the cytochemical changes in the phialide of *Penicillium notatum* Westling during conidial development intense cytoplasmic staining in alkaline fast green was observed in the early stages of phialide differentiation which declined on maturation of the penicillus. Since it was also observed that cytoplasmic ribonucleic acid (RNA) distribution closely paralleled that of the basic proteins, the possible relationship between the two was followed throughout development.

Penicillium notatum was grown on malt extract agar disks on coverslips for 3–5 days at 20 °C which gave a range of coverslip cultures consisting primarily of young phialides after 3 days' growth to predominantly mature or senescent penicilli after 4 and 5 days' growth respectively. Coverslips were removed from the cultures and the adhering colonies were fixed in either 95 % alcohol, or acetic:alcohol 3:1, or 10 % neutral buffered formalin.

Since the methyl green-pyronin and Feulgen techniques are not generally suitable for fungal material and gave inconsistent results in *P. notatum*, nucleic acids were stained with toluidine blue or trypan blue or by the acridine orange fluorescence method of Bertalanffy & Bertalanffy (1960). General protein staining was achieved by the mercury-bromophenol blue method at low pH and the coupled tetrazolium reaction (Danielli, 1947). Basic proteins were selectively stained by alkaline fast green FCF (pH 8·1) by the method of Alfert & Geschwind (1953), but since fading in permanent mounts was rapid, temporary preparations were made in glycerine jelly. Nucleic acids were removed, as applicable, with 5 % trichloroacetic acid (TCA) for 15 min at 90°, with ribonuclease (RNase) or cold perchloric acid, or with deoxyribonuclease (DNase).

Deamination was carried out by treatment with a mixture of equal volumes of 5 % TCA and 5 % sodium nitrite solutions at 20° for 30 min, a treatment known to destroy the ε-amino groups of lysine but not the guanidino groups of arginine. Amino groups were blocked by fixation or post-fixation in 10 % neutral buffered formalin for 3 h at 20°. Arginine was detected by the Sakaguchi reaction.

RNA and total protein methods revealed high concentrations of these substances in the young phialides with decreased amounts in the older structures. Removal of nucleic acids with TCA followed by alkaline fast