THE OCCURRENCE OF THE PSYCHOTOMIMETIC AGENT PSILOCYBIN IN AN AUSTRALIAN AGARIC, *PSILOCYBE SUBAERUGINOSA*

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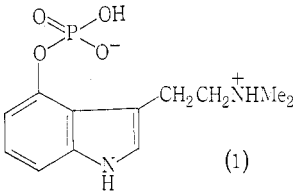

(1)

The psychotomimetic agents psilocybin[1,2] (1) and its dephosphorylated derivative psilocin[2,3] were first isolated[2,4] from the hallucinogenic Mexican mushroom *Psilocybe mexicana* Heim. The presence of psilocybin, sometimes accompanied by psilocin, has subsequently been demonstrated in a number of species of the genus *Psilocybe* from Mexico,[2,5,6] North America,[7–9] and Europe,[10,11] in *Stropharia cubensis* from Mexico,[2,5] Thailand, and Cambodia,[2] in *Conocybe cyanopus*[9,11] and *smithii*[11] from North America, in a *Copelandia* from France,[12] and in *Panaeolus sphinctrinus* from Mexico[13a] (although this last occurrence has not been confirmed[10,14]). The mono- and bis-demethyl analogues are found together with psilocybin in submerged cultures of *Ps. baeocystis*.[8,15] The occurrence of psilocybin or derivatives is not universal throughout the genus *Psilocybe*, however, and *Ps. yungensis*[13b] and *atrobrunnea*[8] lack such metabolites.

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No evidence exists of the tribal use of mushrooms by Australian aborigines, in the manner of the collective hysteria of some New Guinea natives or the magico-religious ceremonies of the Aztecs.[2,16] However, recent publications[17] on hallucinogenic effects attributed to *Ps. cubensis* growing in southern Queensland and northern New South Wales prompted us to examine *Ps. subaeruginosa*, an indigenous species found in New South Wales, Victoria, and South Australia.[18] The stipe and pileus of this species at maturity exhibit[18] the blue-green staining frequently associated[9,11] with the presence of psilocybin or psilocin, or both, which is probably due to enzymic oxidation of psilocin.[19] The toxicity of the species is unknown.

Ps. subaeruginosa was collected in the Australian Capital Territory in late autumn. Preliminary extraction of the dried carpophores with ether and then ethyl acetate removed lipid materials, which included ergosterol. Methanol afforded extracts showing the ultraviolet chromophore of psilocybin, which were chromatographed on cellulose columns. Psilocybin, isolated in 0·45% yield, was identified by chromatographic and spectroscopic comparison with authentic material. Psilocin, which separates from psilocybin in the chromatographic systems employed, could not be detected even spectroscopically and if present its concentration must be less than 1% that of psilocybin. The absence of free psilocin may be related to the apparent oxidase activity of the mature carpophores, mentioned earlier. Examination of the other metabolites of *Ps. subaeruginosa* is in progress.

Experimental

Ultraviolet spectra were measured on a Unicam SP800 spectrometer in ethanol unless otherwise stated. Mass spectra were recorded on an AEI MS902 instrument using the direct insertion probe at 70 eV. Cellulose powder was Whatman chromatographic grade CC31. Paper chromatograms were run ascending on Whatman No. 1 paper in water-saturated n-butanol,[9] thin-layer chromatograms on Merck Kieselgel G in n-butanol–acetic acid–water (24 : 10 : 10). Chromatograms were examined for fluorescence in ultraviolet light before spraying with Ehrlich's reagent (2% *p*-dimethylaminobenzaldehyde in 1N hydrochloric acid). Psilocybin gives a violet fluorescence in ultraviolet light, and a pink colour reaction turning violet with Ehrlich's reagent.

Isolation of Psilocybin

Air-dried carpophores of *Ps. subaeruginosa* (2·6 g) were powdered and extracted with ether in a Soxhlet apparatus for 12 hr. The semi-solid extract (73 mg) showed ultraviolet absorption, λ_{max} 262, 271, 282, and 293 mμ in EtOH, characteristic of a steroidal 5,7-diene.[20] T.l.c. on Kieselgel G in benzene–ether (1 : 1) resolved a number of components and confirmed the presence of ergosterol on comparison with authentic material. Further Soxhlet extraction of the carpophores with ethyl acetate gave a brown gum (10 mg) which was not examined further.

The carpophores were extracted for several hours with six portions of cold methanol, until the extracts show no further psilocybin-type ultraviolet absorption. Paper and thin-layer chromatography of the extracts showed a number of components, including psilocybin (R_F in the two systems 0·09 and 0·19 respectively, identical with authentic material). Neither psilocin nor tryptophan could be detected in the extracts.

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The first three methanol extracts were combined, evaporated, redissolved in methanol (7 ml) containing water (1 ml), and absorbed onto cellulose powder (4 g). After air-drying, this powder was slurried in water-saturated n-butanol and added to the top of a cellulose powder column (30 g, 30 by 1·5 cm) which had previously been washed with the same solvent until the eluate showed no ultraviolet absorption. Development of the chromatogram with water-saturated n-butanol afforded eluent fractions (150, 4 ml each) which were monitored by ultraviolet spectroscopy. Fractions 24–40 showed absorption corresponding to pure psilocybin, and were combined. Evaporation gave psilocybin (11·3 mg), m.p. 185–195° from methanol (lit.[2,8] records m.p. varying from 185–195° to 205–210° from methanol), identical in t.l.c. behaviour (R_F 0·19) and ultraviolet spectrum λ_{max} 269, λ_{infl} 278, and λ_{max} 290 nm (cf.[2]) with authentic material. The mass spectrum (*m/e* 204, 160, 159, 146, 130, 117, 115, and 58) was identical with that of authentic psilocybin, but in contrast to the literature[15] showed no molecular ion.

Similar chromatography of methanol extracts 3–6 gave additional psilocybin (0·5 mg).

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