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## Note

# Analysis of extracts from *Psilocybe semilanceata* mushrooms by high-pressure liquid chromatography

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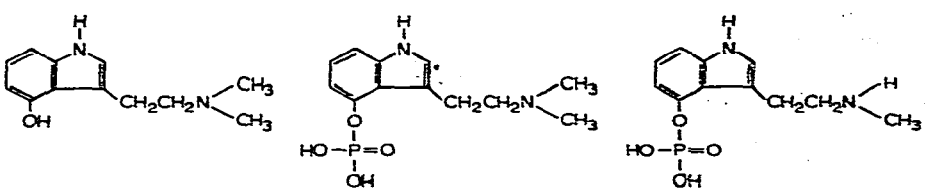
*Psilocybe semilanceata* mushrooms, commonly known as Liberty Caps, can be cultivated in this country. The mushrooms contain psilocin (1) and its phosphate ester, psilocybin (2), both of which have powerful hallucinogenic properties. Analysis of these substances has previously been achieved by thin-layer chromatography (TLC) and mass spectrometry[1], by ultraviolet (UV) and infrared spectroscopy[2,3] and by gas chromatography–mass spectrometry[4]. However, it is important to have a more simple means of analysis to confirm the presence of these drugs and this note reports a high-pressure liquid chromatographic (HPLC) method.

Examination of many extracts from the mushrooms revealed another major component in addition to psilocin and psilocybin. This was obtained in greater amounts using preparative TLC and was provisionally identified as 4-phosphoryl-N-methyltryptamine (baeocystin) (3). Recently, Repke and Leslie[5] have reported the presence of this compound in extracts of a Pacific Northwest variety of *Psilocybe semilanceata* mushrooms.



1 2 3

## EXPERIMENTAL

A reciprocating pump (Type HM, Metering Pumps, London, Great Britain) was used to deliver solvent at 2 ml/min. A Cecil instruments CE212 variable wavelength UV detector was used to monitor the eluent at 254 nm. The column was a 25 cm × 4.6 mm I.D. stainless-steel tube, slurry-packed with small particle silica (6-$\mu$m Partisil 5, Reeve Angel, Maidstone, Great Britain). A stop-flow injection system was used, with injections being made onto a piece of 8-$\mu$m stainless-steel gauze at the

top of the column. The column and injector design have been described elsewhere[6]. Separation of the components was obtained with methanol–water–1 *N* ammonium nitrate solution (240:50:10) and buffered to pH 9.7 with ammonia (sp.gr. 0.88). Extracts of the mushrooms were obtained by heating them on a steam-bath for ten minutes. The solution was decanted, evaporated under a stream of nitrogen, and the residue was redissolved in methanol–chloroform (9:1). Aliquots were then injected on to the chromatographic column.

TLC was carried out on glass plates (10 × 5 cm) coated with 0.25 mm silica gel 60 $F_{254}$ (Merck, Darmstadt, G.F.R.). The plates were developed with *n*-butanol–glacial acetic acid–water (2:1:1). The spots were located initially by UV absorption at 254 nm, and then by spraying with Van Urk reagent (dimethylaminobenzaldehyde in concentrated hydrochloric acid).

Preparative TLC was carried out on glass plates (20 × 20 cm) coated with 0.5 mm of silica gel 60 $F_{254}$. Plates were developed in the same solvent system as mentioned above, double-development being necessary to obtain adequate band separation.

A single focusing VG Micromass 12F mass spectrometer was used to obtain electron impact (EI) and chemical ionisation (CI) spectra. Samples were introduced into the mass spectrometer via a heated probe.

## RESULTS

A chromatogram, typical of that obtained from a *Psilocybe semilanceata* mushroom extract is shown in Fig. 1. Greater resolution of compound 3 from compound 2 can be be obtained by increasing the ammonia content of the solvent.

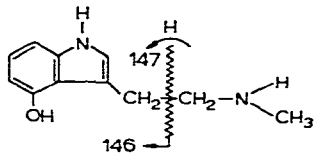
The results of the TLC analysis are given in Table I.

After spraying the plate with the reagent all the components listed in the table gave dark blue spots.

Compound 3, obtained by preparative TLC, gave a mass spectrum with a base peak of *m/e* 147 in the EI spectrum. Further ions were detected at *m/e* values of 44 (relative intensity, 71), 146 (67) and 190 (33). The ions at *m/e* 44 and 146 are fragments arising from the $\beta$-bond fission of the ethylamine side chain. The peak at *m/e* 147 results from a rearrangement which involves the transference of a proton associated with the bond fission.

In the CI spectrum the $(M+H)^+$ ion was detected by a strong peak at *m/e* 191. This corresponds with the peak seen at *m/e* 190 in the EI spectrum and confirmed that the molecular weight of the compound was 190.

mol. wt. 190




The desmethyl psilocin structure shown above can be rationalised with both spectra.

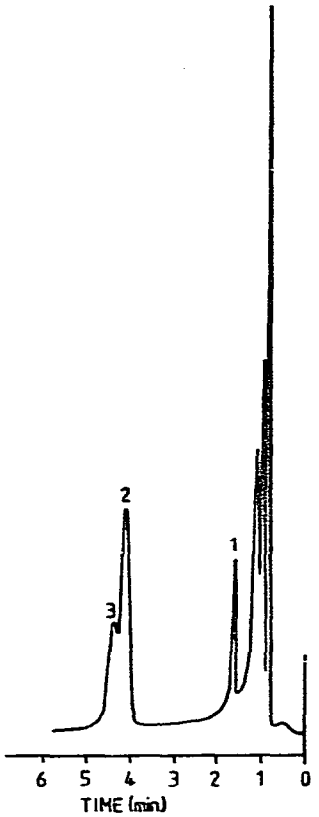



Fig. 1. Separation of a *Psilocybe semilanceata* mushroom extract. Peaks: 1 = psilocin; 2 = psilocybin; 3 = baeocystin. Conditions: Column, 25 cm × 4.6 mm I.D., filled with 6-μm Partisil 5; solvent, methanol–water–1 *N* ammonium nitrate (240:50:10) buffered to pH 9.7 with ammonia (sp. gr. 0.88); flow-rate, 2 ml/min, pressure 2500 p.s.i.; detector wavelength, 254 nm.

TABLE I

TLC RETENTION DATA

| *Compound* | $R_F$ |
|---|---|
| Psilocin (1) | 0.29 |
| Psilocybin (2) | 0.58 |
| Baeocystin (3) | 0.38 |

## DISCUSSION

HPLC system provides a convenient and speedy analysis of psilocin and psilocybin in *Psilocybe semilanceata* mushrooms.

From mass spectral results, the structure of the other major component found in the extracts, could be rationalised with that of desmethyl psilocin. However HPLC retention data would indicate that the structure is more similar to that of psilocybin than psilocin. A sample of psilocybin when analysed by a heated probe in the mass spectrometer was found to give a spectrum identical to that of psilocin, which indicates

that dephosphorylation takes place at elevated temperatures. If this was occurring with the unknown material, the parent compound would be desmethyl psilocybin (baeocystin). This compound would be expected to have a retention time very similar to that of psilocybin when analysed by HPLC.

Since Repke and Leslie[5] have reported the presence of baeocystin in Pacific Northwest *Psilocybe semilanceata* mushrooms, it would appear that it is present in mushrooms that have been cultivated in this country. Hence until a synthetically prepared sample of this material is available the compound can only be provisionally identified as baeocystin.

ACKNOWLEDGEMENTS

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