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Note

Determination of psilocybin in *Psilocybe semilanceata* using high-performance liquid chromatography on a silica column

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During the last few years an increasing number of young Norwegians have used *Psilocybe semilanceata* (Fr. *ex* Secr.) Kummer as a narcotic. The first report on misuse of this mushroom was made in autumn 1977, and knowledge of its hallucinogenic properties has gradually become public through reports in the popular media. The ingestion of *P. semilanceata* has in some cases resulted in the need for treatment in hospital[1,2].

Many species of the genus *Psilocybe* are found in Norway[3], but only *P. semilanceata* is regarded as hallucinogenic. It occurs on grassy sites in most parts of the country from the middle of August to the middle of October. *P. semilanceata* is known to contain indole alkaloids, of which psilocybin is considered to be the main constituent. In order to carry out detailed studies of the potency of Norwegian *P. semilanceata* a quantitative method was required for the assay of psilocybin.

Several methods have previously been used for analysis of the hallucinogenic components of *Psilocybe* mushrooms. Both paper chromatography[4,5] and thin-layer chromatography[6,7] have been employed in conjunction with colorimetric reagents as well as with UV spectroscopy. Gas chromatography and gas chromatography–mass spectrometry[8] have been applied for the analysis of psilocin and psilocybin.

Recently two high-performance liquid chromatographic (HPLC) methods have been published[9,10]. White[9] separated the three compounds psilocin, psilocybin and baeocystin on a silica column, and Perkal *et al.*[10] described the quantitation of psilocin and psilocybin by ion-exchange chromatography. We have developed a HPLC method based on a silica column which provides a simple, rapid and accurate quantitation of the psilocybin content of Norwegian *P. semilanceata*.

EXPERIMENTAL

Chemicals

Psilocin and psilocybin were supplied by Sandoz (Basel, Switzerland). Analytical-grade methanol was obtained from E. Merck (Darmstadt, G.F.R.).

Apparatus

A reciprocating pump (Glenco Scientific, Houston, TX, U.S.A.) was used to

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deliver solvent at 1 ml min^{-1}, and a Spectra-Physics UV detector (Model 225) was used to monitor the eluent at 254 nm. A Kontron® spectrofluorometer SFM23 was used for the fluorimetric detection. Samples were introduced through a Rheodyne (Model 7120) rotary valve injector which was equipped with an external 10-μl sample loop. The column was a 25 cm × 4.6 mm I.D. stainless-steel tube, slurry-packed with small-particle silica (6-μm Partisil 5, Whatman, Maidstone, Great Britain). Separation of the components was achieved with methanol–water–1 *N* ammonium nitrate solution (220:70:10). The 1 *N* ammonium nitrate solution was buffered to pH 9.6 with 2 *N* ammonia. A VG Micromass 7070F mass spectrometer was used for the mass spectrometric investigation.

Extraction procedure

The mushrooms were dried in an oven at 50°C overnight. One accurately weighed mushroom was ground to a powder in a mortar and transferred to a glass-stoppered centrifuge-tube. A 3-ml volume of 10% 1 *N* ammonium nitrate in methanol was added and the tube was rotated for 30 min using a rotary mixer (Cenco Instruments). After centrifugation the supernatant was transferred to a 5-ml volumetric flask, and the extraction was repeated with 2 ml of the extraction solution. The two extracts were combined and diluted in 10% 1 *N* ammonium nitrate in methanol to 5.00 ml.

Quantitation

Quantitative analyses of psilocybin were based on peak height measurements. UV detection was used for routine analyses of mushroom extracts. A calibration graph was constructed for the concentration range 0.005–0.2 mg/ml. The relative standard deviation was calculated after eight assays of each of the three solutions at 0.01, 0.05 and 0.2 mg/ml. Fluorescence detection was used as a comparative method to check for interferences. A standard curve was constructed for the concentration range 0.05–0.2 mg/ml.

The calibration graphs of y (the peak height of psilocybin) against x (the concentration of psilocybin in mg/ml) were calculated according to the method of least squares.

RESULTS AND DISCUSSION

Extraction procedure

For routine HPLC analyses of the psilocybin content in dried mushrooms, a rapid and efficient extraction procedure was necessary. Methanol with 10% 1 *N* ammonium nitrate was employed as extraction solution. The mushroom extracts were stable for several weeks and had a similar composition to the mobile phase. During the drying procedure, the mushrooms lost 92% in weight.

A one-step extraction procedure for Australian *Psilocybe* species has recently been reported[10]. Homogenization for 2 min in 3 ml methanol was considered to be optimal for extraction of the active components. But Australian *P. subaeruginosa* has a low psilocybin content (0.01–0.2%) compared to the Norwegian *P. semilanceata* which we have found to be much more potent[11]. A one-step extraction procedure with 5 ml of the extraction solution was investigated, but only 91% of the psilocybin

content was extracted. The yield of psilocybin was not increased by performing the extraction at 60°C. It therefore seemed clear that extraction of Norwegian *P. semilanceata* required more than one step.

Fig. 1 shows the percentage yield of psilocybin after successive extractions with 3 ml, 2 ml, 1 ml and 1 ml of 10% 1 *N* ammonium nitrate in methanol. The proposed two-step extraction procedure guarantees that at least 98% of the total psilocybin is extracted, which is considered to be satisfactory. All the detectable mushroom components seemed to be extracted to similar extents.

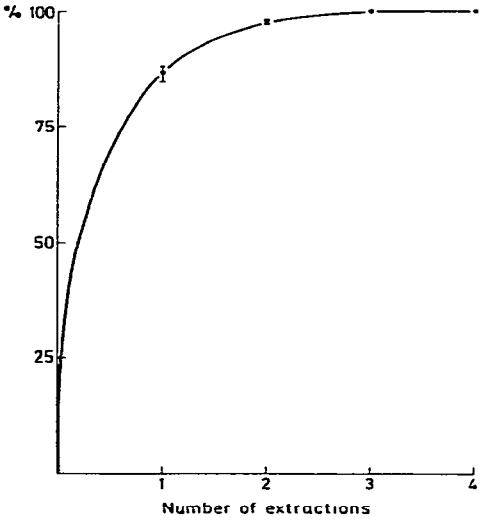

Fig. 1. Percentage yield of psilocybin (the mean value from three different mushrooms) after successive extractions with 3 ml, 2 ml, 1 ml and 1 ml of 10% 1 *N* ammonium nitrate in methanol.

Chromatography

The Australian[10] and English[9] HPLC examinations of *Psilocybe* mushrooms resulted in chromatograms having two and three peaks, respectively. The above conditions gave at least four distinct peaks, and the total analysis time was about 13 min. Typical chromatograms of Norwegian *P. semilanceata* are shown in Fig. 2. Peaks 1, 2 and 3 have not yet been identified. Baeocystin and norbaeocystin (the monomethyl and demethyl analogues of psilocybin) have previously been isolated from *P. baeocystis* grown in submerged culture[12]. The presence in English *P. semilanceata* of a compound provisionally identified as baeocystin has been reported[9]. It is possible that one or both of these substances are present. Further investigation of the chemical constituents of Norwegian *P. semilanceata* will be carried out.

From Fig. 2 it is seen that psilocin is eluted just after psilocybin. This is in contrast to the results obtained by a similar chromatographic system with Partisil 5 as stationary phase and methanol–water–1 *N* ammonium nitrate solution (240:50:10), buffered to pH 9.7 with ammonia (sp.gr. 0.88), as mobile phase[9]. Under these conditions psilocin was eluted much earlier. Because of this large variation in the retention of psilocin, the identity of our psilocin standard was verified by mass spectral analysis.

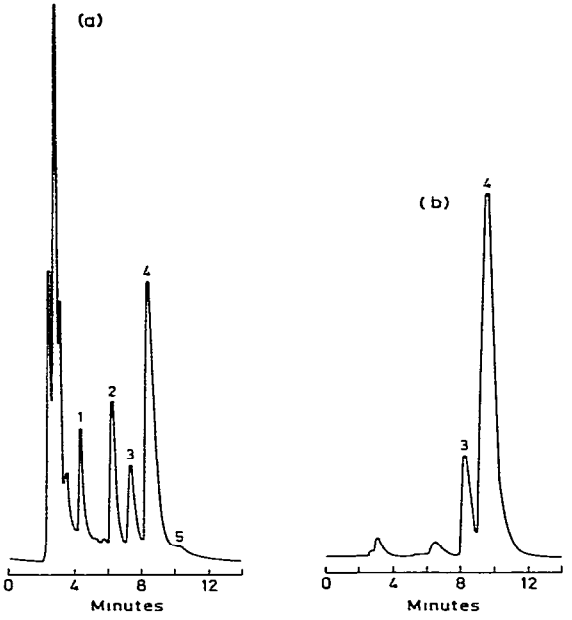

Fig. 2. Liquid chromatograms of Norwegian *P. semilanceata* extract. Detection by UV absorption at 254 nm (a) or by fluorescence at 335 nm (excitation at 267 nm) (b). Peaks: 1, 2, 3 = unknown; 4 = psilocybin; 5 = psilocin.

Separation of the components was also studied on ODS columns and the more polar Spherisorb Phenyl column, but the very polar and water soluble psilocybin did not seem to be sufficiently retarded.

Quantitative analysis

By using UV detection, a calibration graph was obtained which could be described by the equation $y = 54.73x - 0.02$ with a correlation coefficient of 0.9999. The relative standard deviations were 4.7%, 2.4% and 2.5% respectively for 0.01, 0.05 and 0.2 mg/ml solutions of psilocybin.

To check the results obtained by UV detection, a fluorescence spectrometer was used to monitor the eluent. Psilocybin has been found to fluoresce strongly at 335 nm with excitation at 267 nm[10], and the fluorescence detector was set at these wavelengths. Excellent linearity was obtained in the psilocybin fluorescence response. The standard curve could be described by the equation $y = 98.61x - 0.14$ with a correlation coefficient of 0.9993.

Mushroom extracts

Quantitative data for the psilocybin content in nine different mushrooms are given in Table I. The results obtained by UV absorption and fluorescence detection are well correlated. The fluorescence method is very specific for psilocybin, and the excellent agreement indicates that interferences can be neglected.

All the extracts analyzed contained various amounts of psilocybin and at least

TABLE I

QUANTITATIVE HPLC DATA FOR PSILOCYBIN IN DRIED MUSHROOM SAMPLES

Sample No.	*Psilocybin content (%, w/w)*	
	UV detector	*Fluorescence detector*
1	0.73	0.75
2	1.00	1.01
3	0.82	0.83
4	0.65	0.64
5	0.72	0.75
6	0.70	0.72
7	0.58	0.57
8	0.55	0.58
9	0.77	0.77

three unidentified components. Only traces of psilocin could be detected in some mushrooms.

The method described has been used in an extensive study of the potency of Norwegian *P. semilanceata*. The mushrooms were found to contain 0.2–2.0% psilocybin[11]. Norwegian *P. semilanceata* must therefore be regarded as a potent narcotic.

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