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Note

High-performance liquid chromatographic determination of some psychotropic indole derivatives

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The indole derivatives psilocybin and psilocin were identified as active compounds of hallucinogenic mushrooms of the genus *Psilocybe*[1,2]. These compounds and their demethylated analogue baeocystin were later also found in many species of fungi[3–7]. A reliable determination of these compounds is very important in forensic analysis, toxicology and mushroom research. As the biosynthesis of psilocybin has not yet been satisfactorily clarified[8], it was of interest to monitor potential precursors and intermediates.

Thin-layer chromatography and/or high-performance liquid chromatography (HPLC) on silica or reversed-phase HPLC have often been used for the analysis of psychotropic indole derivatives[9–17]. In addition to conventional UV-photometric measurements, fluorimetric[10,12,15] and electrochemical[12,17] detection were used.

A simple method for determination of five hallucinogenic indole derivatives and serotonin is proposed in this study. The use of UV-photometric and electrochemical detection for "non-chromatographic separation" of non-readily separable compounds is described. The method was applied to the analysis of mycelium and fruit bodies of the fungus *Psilocybe bohemica* Šebek.

EXPERIMENTAL

Chemicals

Psilocybin and psilocin were from Sandoz (Basle, Switzerland), the other standard substances from Sigma (St. Louis, MO, U.S.A.). All other chemicals were of analytical grade from Lachema (Brno, Czechoslovakia).

Instrumentation

The liquid chromatograph comprised a 3 B high-pressure pump, injection valve Model 7105, LC-75 spectrophotometric detector and Chromatographics 2 data system (all from Perkin-Elmer, Norwalk, CT, U.S.A.). A voltammetric 641 VA-Detector (Metrohm, Herisau, Switzerland) was connected in series with the UV detector. The separation was performed on an analytical column (250 mm × 4 mm I.D.) packed with Separon SGX C_{18}, 7 μm (Tessek, Prague, Czechoslovakia). The mobile phase was a mixture of buffer pH 3.1 (5.1 g KH_2PO_4, 2.1 g KOH and 13.1 g citric acid monohydrate) with ethanol, flow-rate 1.0 ml/min.

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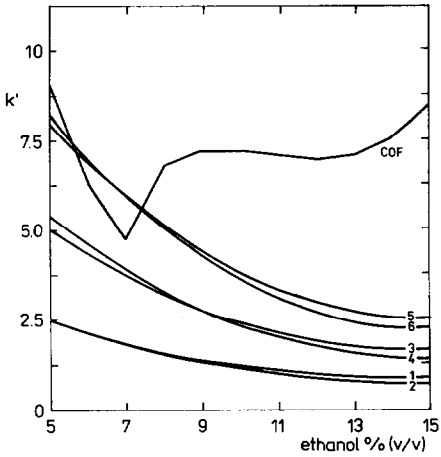

Fig. 1. Dependence of the capacity factor and the chromatographic optimization function (COF) on the ethanol content in the mobile phase. 1, Psilocybin; 2, serotonin; 3, tryptophan; 4, bufotenine; 5, tryptamine; 6, psilocin.

RESULTS AND DISCUSSION

For the optimization of the ethanol content in the mobile phase, we used a procedure based on computer processing of retention and detection data of the compounds studied[18]. Fig. 1 shows that, under the given conditions, it is not possible to separate chromatographically the pairs psilocybin–serotonin, tryptophan–bufotenine and tryptamine–psilocin. Separation of these pairs of compounds is optimal at 10% (v/v) ethanol in the mobile phase. A chromatogram of a mixture of standard substances in this mobile phase is shown in Fig. 2. All pairs of compounds are perfectly baseline separated.

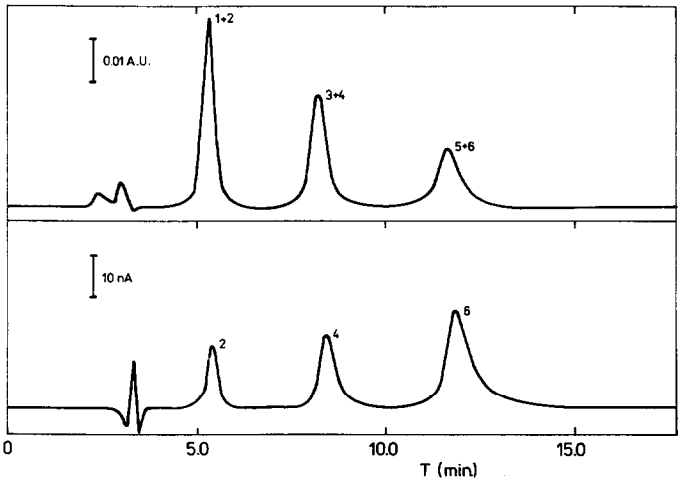

Fig. 2. Chromatogam of a mixture of hallucinogens with electrochemical (lower trace) and UV-photometric (upper trace) detection. Designation of substances as in Fig. 1.

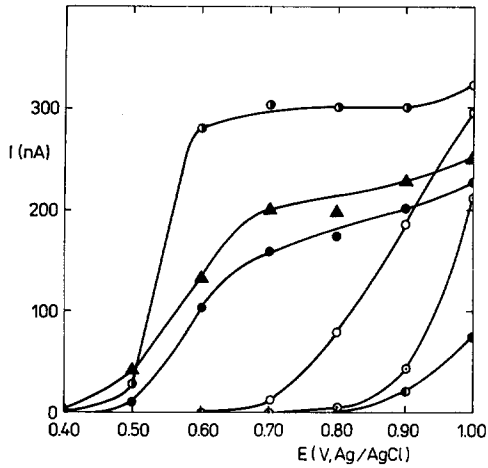

Fig. 3. Hydrodynamic voltammograms of psilocybin (○), serotonin (●), tryptophan (◐), bufotenine (◑), tryptamine (⊙) and psilocin (▲). Mobile phase: citrate–phosphate buffer pH 3.1 with 10% (v/v) ethanol. Flow-rate: 1.0 ml/min.

It is possible to separate chromatographically non-separable pairs by electrochemical selective detection. From the hydrodynamic voltammograms (Fig. 3) it follows that 4- and 5-hydroxylated derivatives are selectively detected at a working electrode potential of approximately +0.60 V (Ag/AgCl), whereas the half-wave potential of the other substances is higher than +0.80 V.

The external standard method was used for quantitation. The calibration must be performed with two standard mixtures of different compositions. The first mixture (M1) consisted of all compounds to be determined, the second mixture (M2) contained serotonin, bufotenine and psilocin. The signal of the electrochemical detector was evaluated directly; the concentration of the compounds detected only by the

TABLE I

CALIBRATION DATA FOR THE UV PHOTOMETRIC AND ELECTROCHEMICAL DETECTORS

Substance	*Detector type*	*Slope*[a]	*Correlation coefficient*	*Detection limit (ng)*	*Relative standard deviation*[b] *(%)*
Psilocybin	UV	0.3767	0.9963	40	6.3
Tryptophan		0.4344	0.9856	35	3.4
Tryptamine		0.1924	0.9851	79	3.8
Serotonin	ED	56.956	0.9470	4.3	9.1
Bufotenine		15.648	0.9581	15	7.4
Psilocin		26.432	0.9740	9.4	7.2

[a] For UV photometric detector at 267 nm in a.u. · 10^4/ng and for electrochemical detector at +0.60 V (Ag/AgCl) in pA/ng.

[b] Five parallel determinations.

UV-photometric detector were calculated according to

$$C = \frac{H(\text{UV, S}) - H(\text{EC, S})H(\text{UV, M2})/H(\text{EC, M2})}{H(\text{UV, M1}) - H(\text{EC, M1})H(\text{UV, M2})/H(\text{EC, M2})} \cdot \frac{V(\text{M1})}{V(\text{S})} \cdot C(\text{M1})$$

where C is the concentration, H the peak height, UV and EC refer to the detector type, M1 and M2 to the calibration mixture used, S to the sample and V is the volume injected.

Table I presents the calibration data obtained by the method described. This method of chromatogram evaluation is less accurate than conventional methods. However, it is very advantageous in cases when a satisfactory chromatographic separation is impossible.

We used the method for the analysis of methanolic extracts of the fungus *Psilocybe bohemica*. Values of 0.93% of psilocybin, 0.01% of psilocin, 0.01% of tryptophan and 0.02% of tryptamine per dry mass of fruit bodies were detected. The concentration in the mycelium is lower by about an order of magnitude.

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