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CASE REPORT

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Honey with *Psilocybe* mushrooms: a revival of a very old preparation on the drug market?

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Abstract In 1996 samples of suspicious honey preparations were confiscated at the Dutch-German border. The labels on the 50 ml jars indicated that the honey contained *Stropharia cubensis* (better known as *Psilocybe cubensis*). The jars were filled with honey with a ca. 1 cm layer of fine particles on the top. The particles were collected and subjected to microscopic and chemical analysis. By microscopy mushroom tissue (plectenchym) and spores typical for the genus *Psilocybe* were identified in all samples. The HPLC analysis with atmospheric pressure mass spectrometry and diode array detection revealed psilocine but psilocybine was not found. The quantitative analysis was very difficult due to the matrix problems. A search showed that the honey with *Psilocybe* can be purchased in Dutch coffee shops without any limitations although psilocine and psilocybine belong to listed substances according to Dutch law.

Key words *Psilocybe* mushrooms · Honey · Psilocybine · Psilocine

Introduction

Among about 30 000 known species of capped mushrooms at least 75 contain the hallucinogenic indole derivative psilocybine. These mushrooms belong to the genus *Psilocybe*, *Panaeolus*, *Conocybe* and *Gymnopilus*. Various mushrooms mainly belonging to the genuses *Psilocybe* and *Conocybe*, were consumed even by the Aztecs and were known as "Teonanacatl" (flesh of God) [1]. This custom survived in Mexico, where "magic mushrooms" are still used for ritual purposes. In the sixteenth century Spanish conquerors were confronted for the first time with "Teonanacatl". From this time originates the report of a Spanish monk, Fray Toribio de Benavente, who described the hallucinogenic properties of mushrooms eaten together with honey [2]. In modern times the use of psilocybine-containing mushrooms attracted public attention in 1957 after a report in Life Magazine [3]. The "magic mushrooms" were identified by Heim and Wasson [4] and Heim and Hofmann [5] as belonging to the genus *Psilocybe*. Hofmann et al. isolated, identified and consecutively synthesized the psychoactive ingredients psilocybine and psilocine [6]. The ancient cult of "magic mushrooms", suppressed by the Spanish occupiers in Mexico, was reborn in modern society not as a part of a religion, but as a ritual in the drug scene.

Nowadays, dried *Psilocybe* mushrooms and also complete kits consisting of spores, fertilizer and instructions on how to grow cultures are offered in Dutch "Coffee shops". For this reason, dried mushrooms appear fairly frequently in forensic casework and occasionally, as in this case as an unusual preparation form.

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Case history

In summer 1996 some suspicious, small glass containers were found during a routine border control of several persons travelling from the Netherlands to Germany. As the control revealed also hashish, marijuana and "Ecstasy" pills, the containers were sent to our Institute for identification. The jars were filled with a ca. 50 ml of brown, semi-liquid, honey-smelling mass, with a ca.1 cm thick grey-brown, inhomogenous upper layer, which macroscopically resembled minced plant material. The labels stated that the preparation contained acacia honey with *Stroph. cubensis* (better known as *Psilocybe cubensis*). For "best results", half to one jar per month should be taken. On two labels phone numbers of "Coffee shops" in Maastricht and Kerkrade were cited. A visit to the shop in Kerkrade revealed that honey with *Psilocybe* was sold for 25 Dfl per jar. In the same shop other drugs were also offered such as Ecstasy pills and many sorts of marijuana.

Material and methods

The containers with honey were obtained from six persons. The contents were subjected to morphological and chemical examination.

Microscopic evaluation was performed in native conditions (in a drop of water) and after treatment with Melzer's reagent.

For chemical identification, the jars with honey were heated to 70° C and the upper layer (about 10% of total volume) was collected and filtered under constant gentle heating through a nylon mesh. The filtered mass was rinsed with warm water and 1 g was extracted with 20 ml methanol, concentrated to ca.1 ml and filtered through a membrane filter (0.45 μm). The extracts were evaporated under nitrogen, reconstituted in mobile phase and 5–10 μl was injected into a chromatograph.

Two chromatographic methods were used to identify the compounds: high pressure liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (LC-APCI-MS) and high pressure liquid chromatography with diode array detection (HPLC-DAD).

For LC-APCI-MS a SSQ 7000 LC-API-MS spectrometer (Finnigan MAT, San Jose, USA) was used in the positive ionization mode. The samples were separated on Superspher RP 18 columns (125 × 3 mm, Merck, Darmstadt, Germany) in acetonitrile-ammonium formate buffer 50 mM, pH 3.0 (5:95) at a flow rate of 0.6 ml/min. The heated vaporizer temperature was set at 450° C and the heated capillary at 190° C. Full scan mass spectra of standard solutions of psilocybine and psilocine were taken in the range m/z 50–350 u at octapole offset voltage 10 V and 40 V. On the base of the information obtained from full scan runs, a selected ion monitoring procedure was developed which allowed to monitor for protonated molecular ions of relevant substances (psilocybine and psilocine) and the characteristic fragments. This was achieved using octapole offset voltages of 10 and 40 V alternating every 0.5 s. At an offset voltage of 10 V the protonated molecular ions (m/z 285 and 205) were measured and at 40 V the products of fragmentation (collision induced dissociation – CID) i.e. the ions m/z 205, 160, 146, 115. The scan time was 0.2 s.

HPLC-DAD was performed using a Waters model Series 900 instrument (Waters Chromatographie, Eschborn, Germany). In this method the samples were separated on Superspher RP18 column (125 × 4 mm, Merck, Darmstadt, Germany) in a mobile phase consisting of acetonitrile-triethylammonium phosphate buffer 50 mM, pH 3.0 (3:97) at a flow rate of 0.7 ml/min. UV-spectra in the range of 200–350 nm were recorded.

Results and discussion

Microscopic evaluation in water showed that the upper layer from the jars consisted of crumbled mushroom tissue with fragments of plectenchym (tissue with clamp connections) and gills (reproductive tissue) with basidia and adhering spores. Those were brown, thick- and smooth-walled with germ pores, of ellipsoid or lemon-like shape and about 8 × 16 μm dimensions (Fig. 1). With Melzer's reagent they showed negative reaction (inamyloid) with features typical for spores from the genus *Psilocybe* (Strophariaceae/Basidiomycetes) most probably *Psilocybe cubensis* [7, 8].

The LC-APCI-MS mass spectrum of psilocine taken at octapole offset voltage of 10 V showed only the protonated molecular peak (m/z 205) and acetonitrile adduct (m/z 246) (Fig. 2a). Under these conditions psilocybine showed a protonated molecular peak (m/z 285) and dephosphorylated fragment i.e. psilocine (m/z 205) (Fig. 2b). At octapole offset of 40 V both molecules underwent distinct fragmentation (Fig. 2c, d).

Psilocine was identified in all samples examined by LC-APCI-MS and HPLC-DAD but psilocybine was not found (Figs. 3–6). This was most probably caused by dephosphorylation of psilocybine to psilocine in the course of processing of the mushrooms or sample preparation. Hence, the results of the qualitative chemical analysis were fully consistent with the morphological observations. However, the quantification of psilocine in the examined samples was very difficult. The content of psilocine was determined in extracts using LC-APCI-MS and the external standardization procedure. Assuming that psilocine was present only in the isolated mushroom tissue, the content of drug ranged from 0.15 to 4 mg per 50 ml jar. It is possible, however, that psilocine or psilo-

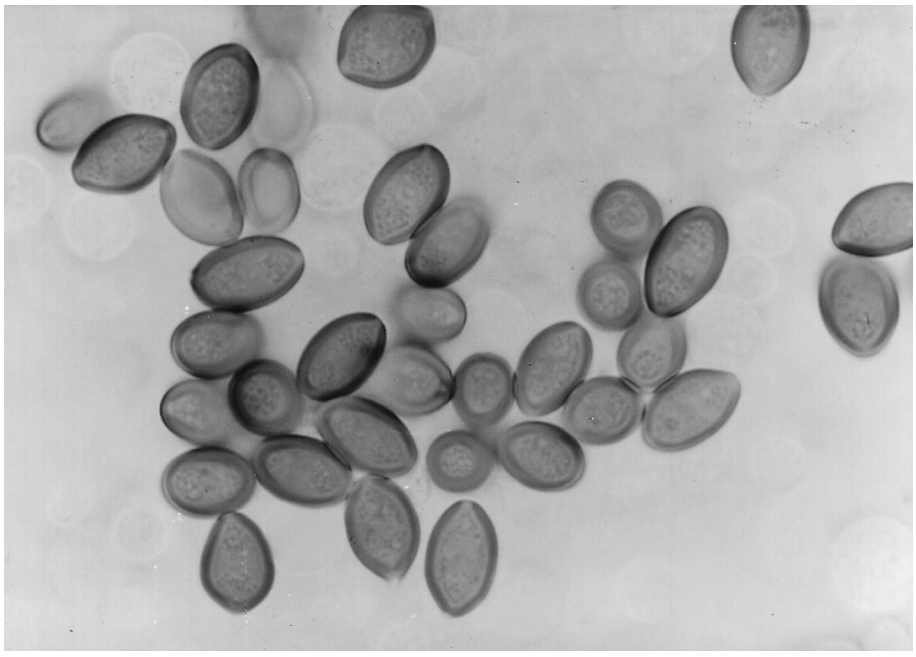

Fig. 1 Microphotography of *Psilocybe* spores isolated from the honey (1000 × magnification, oil immersion)

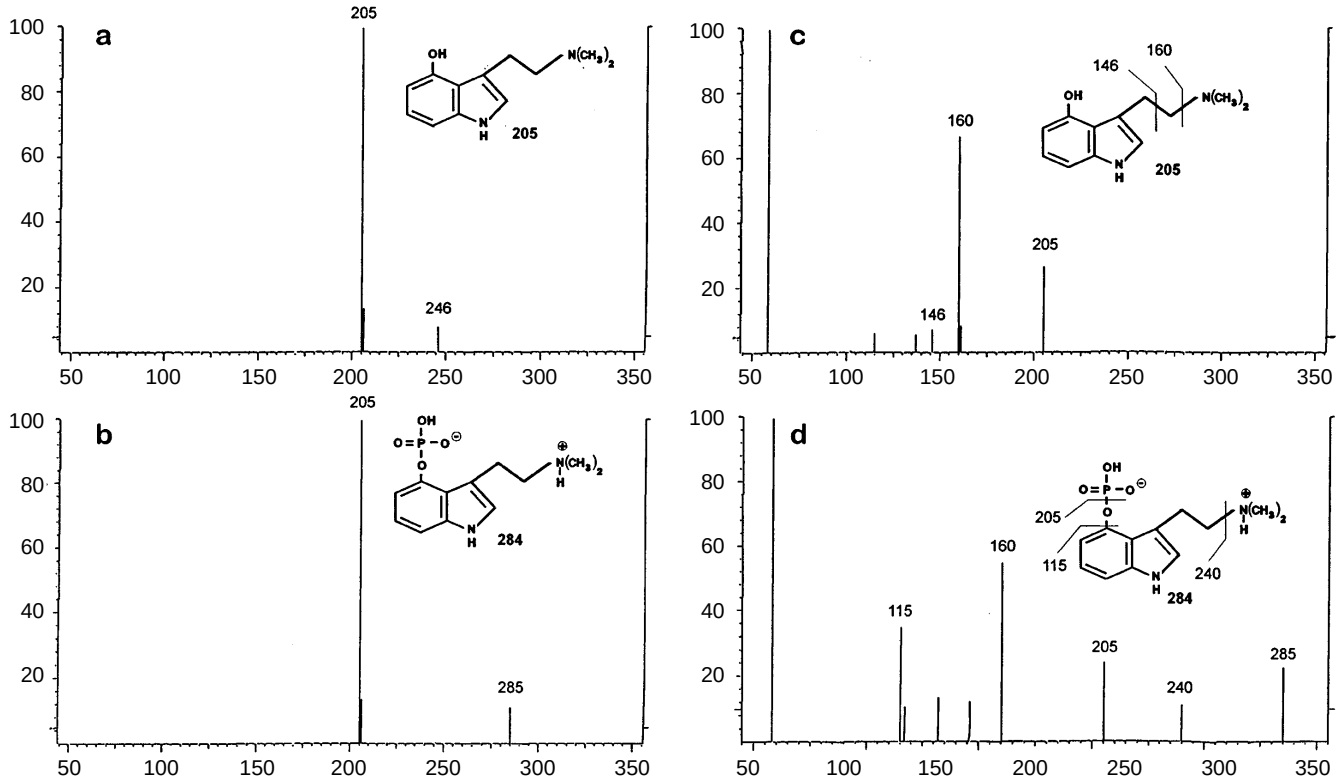

Fig. 2 a–d Mass spectra of psilocybine and psilocine. At octapole offset values of 10 V psilocine shows only protonated molecular ion and acetonitrile addict (4a), in the case of psilocybine protonated molecular ion and psilocine as dephpsphorylated fragment is seen (4b). At octapole offset of 40 V psilocine (4c) and psilocybine (4d) underwent distinct fragmentation

cybine were also present in the honey. Therefore the total content of drug could be substantially higher. Unfortunately, the isolation of psilocybine or psilocine from honey does not seem to be feasible.

It is strongly assumed that the intake of examined mushroom preparation will precipitate the symptoms characteristic for psilocybine. The first description of the hallucinogenic action of honey with "magic mushrooms" given by Fray Toribio de Benavente [2] is as follows: "The first thing which they ate at the gathering was small, black mushrooms which they called nanacatl (teonanacatl). These are intoxicating and cause visions and even provoke sensuousness.... They ate these little mushrooms with honey, and when they began to be excited by them, they began to dance, some singing, some weeping, for they were already intoxicated by the mushrooms.... When the intoxication from the little mushrooms had passed, they talked over among themselves the visions which they had seen". Self-experiments demonstrated that the ingestion of *Psilocybe* mushrooms in doses corresponding to 12 mg of drug precipitated hallucinogenic action lasting 3–5 h [9]. Several species of *Psilocybe* fungi may contain 0.25–0.98% of psilocybin [10].

Other unconventional preparations of *Psilocybe* mushrooms, are "blue mead" (honey wine with blue *Psilocybe*

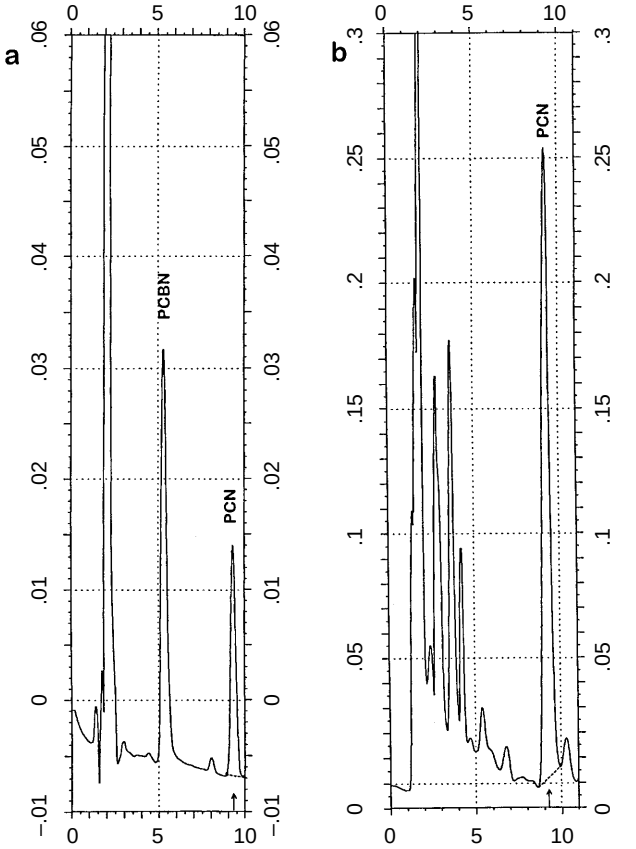

Fig. 3 a, b HPLC-DAD chromatogram of standard mixture of psilocybine (PCBN) and psilocine (PCN) (100 ng of each substance injected) (4a) and of extract from minced mushroom mass from "Herbal Honey" (4b). Pilot signal at 220 nm

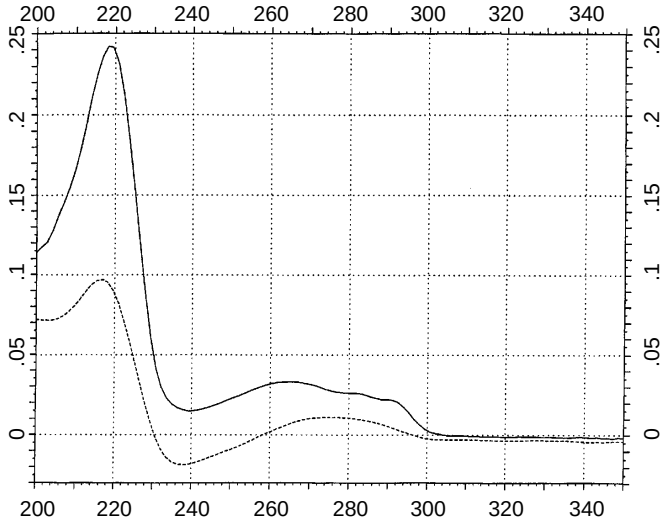

Fig. 4 UV spectra taken by diode array detector from the extract peak as on Fig. 4b (broken line) and from the psilocine standard (solid line)

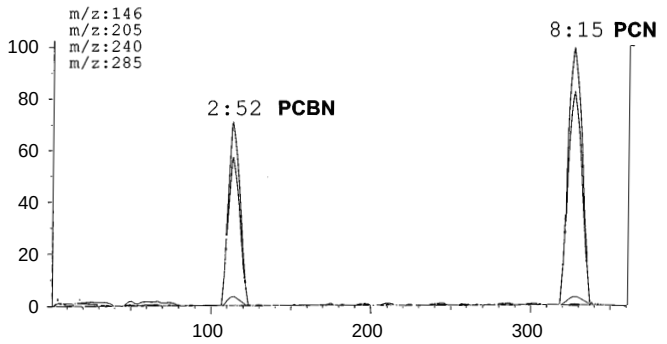

Fig. 5 LC-APCI-MS chromatogram of standard mixture of psilocybine (PCBN) and psilocine (PCN), 50 ng of each substance injected

mexicana mushrooms) and pizza with *Psilocybe* mushrooms (Internet sources).

Both in Germany and in The Netherlands psilocybine and psilocine are classified as scheduled substances (§1, Abs.1, Anl.1 des BTM-Gesetzes in Germany, Art.2, Abs.1, Anl.C Opiumwet in the Netherlands). The cultivation or posession of whole *Psilocybe* mushroooms and its spores are not restricted by German law.

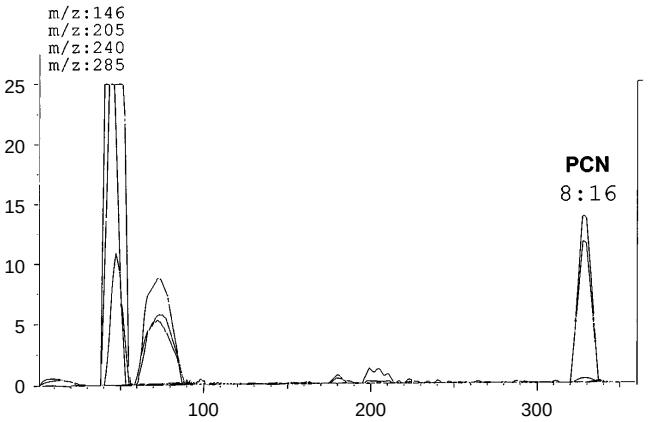

Fig. 6 LC-APCI-MS chromatogram of the extract of "Herbal Honey". Psilocine concentration was 60 μg/g of mushroom tissue

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