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Short Communication

Determination of phenylethylamines in hallucinogenic cactus species by high-performance liquid chromatography with photodiode-array detection

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ABSTRACT

A high-performance liquid chromatographic procedure with photodiode-array detection has been developed to create phytochemical profiles of phenylalkylamine-containing cactus species. The basic methanolic cactus extracts with methoxamine as internal standard were separated on a 3-μm ODS column with acetonitrile–water–phosphoric acid–hexylamine as the mobile phase. Peak assignation was performed by on-line UV detection (190–300 nm) and by gas chromatography–mass spectrometry of the isolated compounds. The quantitation of mescaline was done at 205 nm. The excellent sensitivity (the detection limit of mescaline was 500 pg, corresponding to 0.002% in cactus material) allowed the analysis of milligram amounts of cactus material taken from living plants. The mescaline content of the psychotropic Peyote cactus *Lophophora williamsii* (Lem. ex Salm-Dyck) Coult. ranged from 680 to 1010 mg per 100 g. The morphologically very similar *Lophophora diffusa* (Croizat) Bravo could be differentiated by the absence of mescaline and the dominant alkaloid pellotine.

INTRODUCTION

In the USA, Mexico and several countries in South America, different species of the Cactaceae family are known for their hallucinogenic properties. The most popular species are *Lophophora williamsii* (Lem. ex Salm-Dyck) Coult. and *L. diffusa* (Croizat) Bravo, both called Peyote and often confused because of their very similar morphology. *L. williamsii* grows on limestone soils of low hills and flatlands, mainly in the Chihuahua Desert of Central and Northern Mexico and in the adjacent parts of the United States, especially in the Rio Grande Valley. *L. diffusa* occurs only in a restricted area in the State of Queretaro, Mexico [1,2]. Today, Peyote is still used by several Indian tribes of northern Mexico. The use of Peyote has also spread to the United States and Canada, where members of the American Native Church still use the cactus in religious ceremonies. The main psychotropic principle of *L. williamsii* is the alkaloid mescaline, a phenylethylamine derivative. The internationally controlled mescaline can easily be synthesized by clandestine laboratories and appears from time to time on the illegal drug markets in the United States and in Europe. Minor alkaloids belong to the group of phenylethylamines (*e.g.* N-methylmescaline, hordenine) or tetrahydroisoquinolines (*e.g.* anhalamine,

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anhalonidine, anhalonine, isopellotine, pellotine). *L. diffusa* contains pellotine as the main alkaloid, with virtually no mescaline [2]. In Switzerland and other European countries *Lophophora* species are sold without legal restrictions in garden centres, flower shops and at flower markets.

Because these cactus species are morphologically very similar, and because of the potential for abuse, it was the aim of the present work to develop a selective, specific, accurate and sensitive analytical procedure using high-performance liquid chromatography with photodiode-array detection (HPLC–DAD) that would allow chemical differentiation of the two *Lophophora* species and rapid estimation of their psychotropic potency. Because wild Peyote has become endangered and is under international control [3], the sensitivity of the method should also allow the sampling of living cactus specimens without sacrificing the whole plant. To our knowledge this is the first HPLC–DAD procedure suitable for profiling *Lophophora* species. The efficiency of HPLC–DAD in the field of drug analysis, analytical toxicology, forensic chemistry and phytochemistry of psychotropic drugs has been shown previously [4–12]. Other methods for the determination of cactus alkaloids, such as normal phase HPLC [13], gas chromatography (GC) [14,15] and thin-layer chromatography (TLC) [2,15,16], use time-consuming extraction procedures or show poor resolution or low sensitivity.

EXPERIMENTAL

Instrumentation

The HPLC system consisted of a Hewlett-Packard (HP) 1090M liquid chromatograph (Hewlett-Packard, Waldbronn, Germany), a HP 1090L autosampler, a HP 1040M photodiode-array detector, a HP 79994A Chemstation (software version 1.05), a HP 7470A *x/y* plotter and a HP 2225A Thinkjet printer. The separation was performed isocratically at 25°C on a 150 × 4.6 mm I.D. column with a 20 × 4.0 mm I.D. precolumn, packed with 3-μm Spherisorb ODS-1 (Phase Separations), filled by Stagroma (Wallisellen, Switzerland). The mobile phase was acetonitrile–water (108:892) containing 5.0 ml (8.5 g) of orthophosphoric acid (85%) and 0.28 ml (0.22 g) of hexylamine per 1000 ml. The flow-rate was 1 ml/min. The eluent was filtered through a membrane filter (regenerated cellulose, 0.45 μm, Schleicher and Schuell) and degassed by sonication and during use with a constant flow of helium. Methanol was used for washing the column.

The absorption coefficients (ε) of mescaline (2–250 μg/ml mobile phase) were measured on a HP 8452A UV–VIS-diode-array spectrophotometer with a HP Vectra ES/12 PC (HP 89530 MS/DOS UV–VIS software).

The preparative work was done on a Waters HPLC system, consisting of an M 6000-A pump, a U6K injector, a 440 absorbance detector and a Shimadzu Chromatopac C-R1A data processor. Peak isolation was performed on a 250 × 10 mm I.D. column, packed with 5-μm Spherisorb ODS-1 using a slurry technique [17]. The mobile phase was acetonitrile–water (125:875), containing 5.0 ml (8.5 g) of orthophosphoric acid (85%) and 0.28 ml (0.22 g) of hexylamine per 1000 ml at a flow-rate of 4 ml/min. The detection wavelength was set at 254 nm.

The GC–MS system used for peak identification was a Hewlett-Packard HP 5990 gas chromatograph with a HP 5970 mass selective detector, a HP Chemstation (Pascal Rev. 3.1), a HP 2225A Thinkjet printer and a HP 7470A *x/y* plotter. A J&W DB-5 bonded-phase capillary column (20 m × 0.18 mm I.D. and 0.40-μm coating) was inserted directly into the ion source. The injector and transfer line temperatures were 250 and 260°C, respectively. The oven temperature was programmed from 70 to 250°C at 10°C/min. The scan range was *m/z* 33–400, and the scan-rate was set at 1.17 scans/s. Helium was used as carrier gas at a flow-rate of 0.7 ml/min (49 cm/s).

Chemicals and reagents

Hexylamine 99% (purum) was provided by Fluka (Buchs, Switzerland). Water used for HPLC was bidistilled. All other chemicals and solvents were of analytical or HPLC grade, purchased from Merck (Darmstadt, Germany). Mescaline hydrochloride was supplied by Laboratoires Plan (Geneva, Switzerland), and N-methylmescaline hydrochloride and methoxamine hydrochloride were provided by Sigma (St. Louis, MO, USA). Anhalamine, anhalonidine, anhalonine, isopellotine and pellotine were gifts from Hoffmann-LaRoche (Basle, Switzerland/Nutley, USA).

Cactus samples

The specimens of *Lophophora williamsii* (Lem. ex Salm-Dyck) Coult. and *L. diffusa* (Croizat) Bravo (Cactaceae) were bought at flower shops and shopping centres in Switzerland or obtained from private collections.

Methods

For the differentiation of living cactus specimens, cubic pieces with a fresh weight of *ca.* 0.8 g were lyophilized and stored in a desiccator under vacuum. After pulverization with a grinder, an accurately weighed amount of the powdered sample (*ca.* 10 mg) was washed with four 1-ml volumes of diethyl ether by sonication for 5 min and filtration through a 0.2-μm regenerated cellulose filter (Spartan 13/30, Schleicher and Schuell). The defatted sample was extracted with four 0.5-ml volumes of methanol–ammonia 33% (99:1), containing 150.0 mg/l methoxamine hydrochloride as the internal standard (I.S.) by sonication for 5 min and filtration through a 0.2-μm regenerated cellulose filter. Aliquots of 5 μl were injected into the HPLC–DAD system.

Mescaline was quantitated by measuring the peak areas of mescaline and the I.S. at 205 nm. The calibration graph was obtained by measuring five standard solutions in the concentration range 3.8–15.3 μg/ml mescaline with the addition of 25.6 μg/ml I.S. (aqueous solution, calculated as base).

The inter-day precision of the method was determined by analysing a dried and pulverized cactus specimen. The analyses were repeated three times on two different days at an interval of 1 week using the procedure described above.

The recovery of mescaline from cactus material was determined by analysing a dried and pulverized mescaline-free *L. diffusa* specimen spiked with a methanolic solution of 1 mg/ml mescaline, corresponding to 10 mg/g dried material. The solvent was evaporated before analysis. The analyses were repeated four times using the procedure described above.

Peaks were assigned by spiking cactus extracts with solutions of reference compounds. For the GC–MS confirmation of the correct peak identification, 100 mg of dried cactus material (*L. williamsii*) were extracted as described above; ten 100-μl volumes of this extract were injected in the semi-preparative HPLC system, and the main constituents were isolated by peak collection. The combined fractions were then reduced to about half volume under a stream of nitrogen, alkalized with ammonia 33% to pH 10–11, and extracted with four 5-ml volumes of chloroform. After evaporation under a stream of nitrogen, the residue was dissolved in 1 ml of methanol and 1 μl was injected splitless into the GC–MS system.

RESULTS AND DISCUSSION

With alkalized methanol (solvent/sample, 200:1, v/w) and sonication, mescaline is almost quantitatively extracted from the finely powdered and defatted cactus matrix. The efficiency of the extraction step has been demonstrated previously [11]. The recovery of mescaline was greater than 99%, showing that the loss of mescaline during the defatting process, which is necessary to remove interfering lipids and waxes, is negligible.

Among the reversed-phase materials tested, only the 3-μm spherical C_{18} phase with a minimum plate number of 120 000/m (calculated for the mescaline peak) showed the efficiency necessary to obtain the HPLC profiles of a complex cactus extract differentiating major and minor alkaloids (Figs. 1 and 2). It is well known that basic compounds may show a pronounced tailing effect on certain reversed-phase columns owing to interactions with the residual polar silanol groups of the stationary phase [18,19]. The addition of an amine modifier to the mobile phase as a masking agent for the silanol groups improves the peak shape and changes the capacity factor (k') of basic substances [20,21]. It has to be noted that the selectivity of the chromatographic system can be widely influenced by changing not only the ratio of acetonitrile to water but also the concentration of hexylamine. With the addition of orthophosphoric acid to the mobile phase, an acidic eluent with a pH of *ca.* 2 is obtained, so that the components of interest, such as mescaline and other basic phenylalkylamine derivatives, are protonated and eluted as associates with phosphate ions. The described chromatographic system, with slight modifications in the ratio of the four components, is routinely used in our laboratory for the analysis of a wide range of basic substances of toxicological and forensic interest. For example, cocaine and cocaine metabolites [9], ring-substituted ampheta-

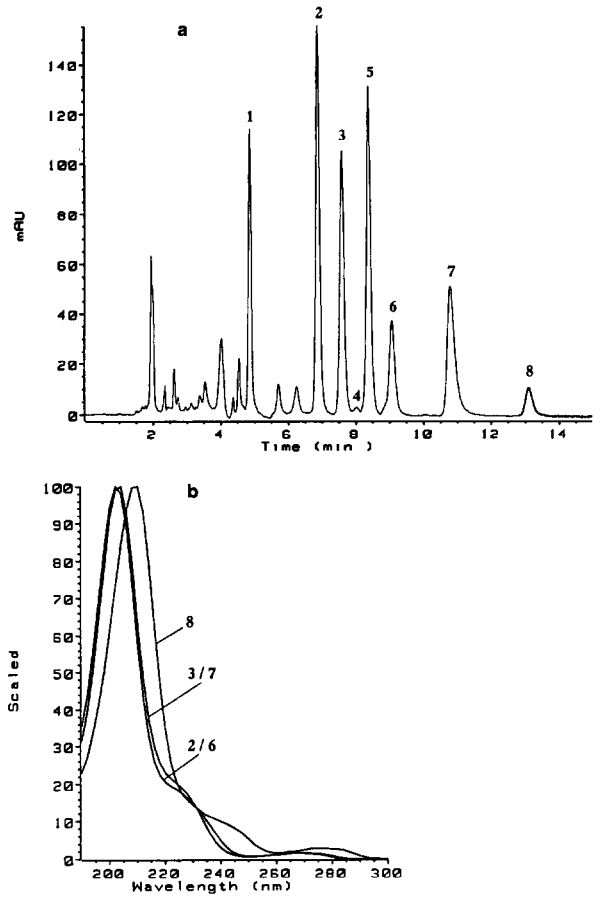

Fig. 1. (a) Chromatogram and (b) on-line UV spectra (DAD) of an extract of *L. williamsii* recorded at 205 nm. Peaks: 1 = anhalamine; 2 = mescaline; 3 = anhalonidine; 4 = isopellotine; 5 = methoxamine; 6 = N-methylmescaline; 7 = pellotine; 8 = anhalonine. Chromatographic conditions as described in Experimental.

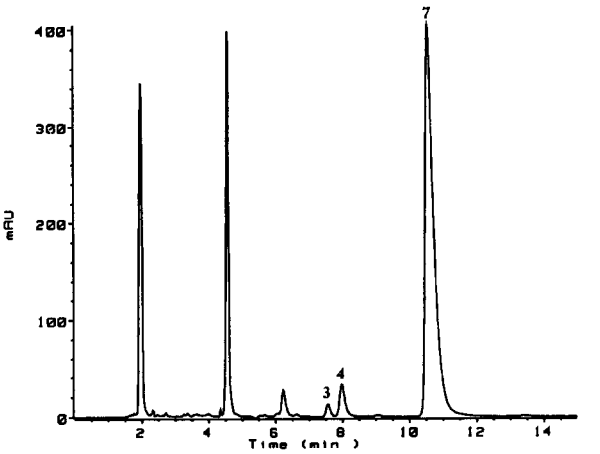

Fig. 2. Chromatogram of an extract of *L. diffusa*.

mines (*e.g.* methylenedioxymethylamphetamine, MDMA; methylenedioxyamphetamine, MDA) [11,22], cathinone [10], norpseudoephedrine [10] and methadone [8] can be analysed in different matrices, *e.g.* biological fluids and pharmaceutical formulations. Another advantage of this mobile phase is the low UV cut-off, which allows the detection of mescaline at its major UV absorption maximum (see Fig. 1: 205 nm, log ε 4.625) and thus to obtain a sixty times higher sensitivity compared to the maximum at 268 nm (log ε 2.833) mentioned in the literature [23]. The detection limit for mescaline at 205 nm and a signal-to-noise ratio of 5:1 was 500 pg, corresponding to 0.002% mescaline in cactus material. The excellent sensitivity makes it possible to analyse milligram amounts of the tissue of living cactus specimens, for example when studying the distribution of alkaloids or the influence of the vegetation period on the alkaloid pattern of one single plant. The linearity range for mescaline was 3.8–15.3 μg/ml with a correlation coefficient (r) of 0.999. The coefficient of variation of the inter-day precision was 3.3%.

Peak detection and identification of the alkaloids was performed by comparing retention times and UV spectra against those of standards, and by peak purity check (matching up-slope, apex and down-slope spectra) using a photodiode-array detector. Fig. 1a and b show the HPLC profile of an extract of *L. williamsii* and the on-line UV spectra of the main phenylethylamine and tetrahydroisoquinoline alkaloids, respectively. The UV spectra of mescaline and N-methylmescaline are identical and can be characterized by the major absorption maximum at 205 nm, a shoulder at 226 nm and a minor maximum at 270 nm. Pellotine and anhalonidine also have identical spectra, but can be differentiated from mescaline and N-methylmescaline by a slight bathochromic shift in the region of 190–230 nm. Anhalonine shows a major UV maximum at 208 nm. The peak assignation of mescaline, N-methylmescaline and pellotine was confirmed by isolation with preparative HPLC followed by GC–MS analy-

sis. The mass spectra were compared with those of reference compounds and showed the characteristic ions: for mescaline, m/z 211 (M^+), 182 (base peak), 181, 167, 151 and 148; for N-methylmescaline, m/z 225 (M^+), 182, 167, 151 and 44 (base peak); for pellotine, m/z 222 (M^+-15, base peak), 207, 206, 189, 178 and 161.

As demonstrated in Figs. 1a and 2, the typical HPLC profile of *L. williamsii* is generally more complex than that of *L. diffusa*. The alkaloid pattern of *L. williamsii* is dominated by mescaline, the psychotropic principle. The mescaline content of six specimens varied between 680 and 1010 mg per 100 g (calculated per dry weight). Other major alkaloids are the tetrahydroisoquinoline derivatives anhalamine and anhalonidine. Minor alkaloids are N-methylmescaline, isopellotine, pellotine and anhalonine. The very different alkaloid profile of *L. diffusa* is characterized by the dominant presence of pellotine and the absence of mescaline, anhalamine and anhalonine. The peak appearing at 4.5 min could not be identified. The UV spectrum indicates an alkaloid of the tetrahydroisoquinoline type.

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