Available online at www.sciencedirect.com
SCIENCE DIRECT®
Talanta 67 (2005) 354–359
Talanta
www.elsevier.com/locate/talanta

Determination of psilocin and psilocybin using flow injection analysis with acidic potassium permanganate and tris(2,2′-bipyridyl) ruthenium(II) chemiluminescence detection respectively

Nicole Anastos [a], Neil W. Barnett [a], Simon W. Lewis [a,*], Nicholas Gathergood [b], Peter J. Scammells [b], D. Noel Sims [c]

[a] *School of Biological and Chemical Sciences, Deakin University, Geelong, Vic. 3217, Australia*
[b] *Victorian College of Pharmacy, Monash University, Parkville, Vic. 3052, Australia*
[c] *Forensic Science SA, Adelaide, SA 5000, Australia*

Received 19 September 2004; received in revised form 16 November 2004; accepted 17 November 2004
Available online 16 February 2005

Abstract

A simple, rapid and sensitive method for the determination of psilocin and psilocybin is described. This is the first report on the determination of psilocin and psilocybin using flow injection analysis with acidic potassium permanganate and tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence. The limits of detection (signal-to-noise ratio = 3) are 9×10^{-10} M and 3×10^{-10} M for psilocin and psilocybin, respectively.

A concise synthetic route for psilocin in three steps from readily available starting materials is also described. The structures were elucidated on the basis of spectroscopic data.

Keywords: Psilocin; Psilocybin; Flow injection analysis; Chemiluminescence; Synthesis; Forensic analysis

1. Introduction

Psilocin and psilocybin are naturally occurring indoles (see Fig. 1) found in several species of mushrooms [1,2] at concentrations of up to 0.5% m/m and 2% m/m, respectively [3]. Psilocybin is rapidly dephosphorylated to psilocin in vivo [4], with the latter compound being structurally related to the neurotransmitter serotonin (see Fig. 1) which gives rise to its comparable human metabolism [5]. This molecular similarity endows psilocin with high affinity for serotonin receptors, which blocks the release of the neurotransmitter thus giving rise to hallucinogenic effects [6]. Both psilocin and psilocybin are controlled substances in many countries, as intentional intoxication from these compounds continues to be a major problem in USA and Europe [7]. The determination of psilocin and psilocybin, mostly in mushroom and body fluids, has been previously reported [8–24] utilising several techniques including thin-layer chromatography [8], gas chromatography mass spectroscopy [9–12], high-performance liquid chromatography [13–20], liquid chromatography mass spectroscopy [21], capillary electrophoresis [22,23], ion mobility spectrometry [10] and chemiluminescence [24]. The analytical figures of merit (where available) from these studies [8–24] have been summarised in Table 1.

Psilocin is present in the blood of users at low peak concentrations in the order of 5×10^{-8} M with an average half-life of about 3 h [13]. Consequently, a simple, rapid and sensitive method for quantifying both psilocin and psilocybin in various matrices would be of interest to forensic scientists. The chemiluminescence reagents acidic potassium permanganate and tris(2,2′-bipyridyl)ruthenium(III) have both been used successfully to detect a wide range of analytes including certain types of alkaloids [25,26]. The former elicited intense chemiluminescence upon reaction with serotonin [27] and it

* Corresponding author. Tel.: +61 3 5227 1365; fax: +61 3 5227 1040.
E-mail address: swlewis@deakin.edu.au (S.W. Lewis).

0039-9140/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2004.11.038

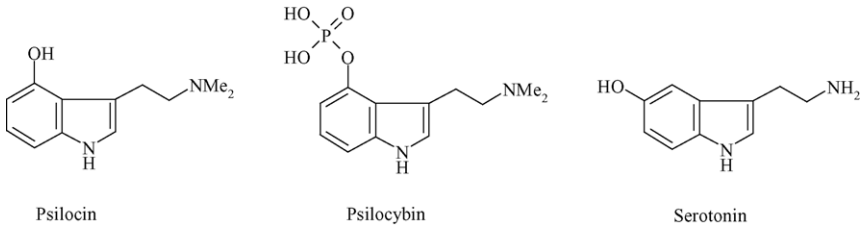


Fig. 1. Molecular structures of naturally occuring indoles.

was reasoned that this was also likely to occur with psilocin given their structural likeness (see Fig. 1). Moreover, a range of tertiary amines has exhibited good analytical detectability with tris(2,2′-bipyridyl)ruthenium(III) [28], and therefore psilocybin ought to do likewise.

This paper describes for the first time the determination of psilocin and psilocybin from *Psilocybe subaeruginosa* and *Hypholoma aurantiaca* mushrooms using flow injection analysis with acidic potassium permanganate and tris(2,2′-bipyridyl)ruthenium(II) chemiluminescence detection, respectively. Additionally, whilst psilocin was commercially available it was also prohibitively expensive and as such, small quantities were synthesised for the preparation of analytical standards [29].

2. Experimental

2.1. Instrumentation

A simple two-line flow injection analysis (FIA) manifold was used, employing a Gilson Minipuls 3 peristaltic pump (John Morris, Australia) with PVC pump tubing (1.85 mm i.d., A.I. Scientific Australia) to propel the carrier and reagent streams at a flow rate of 2.8 mL/min and 1.8 mL/min for psilocin and psilocybin determinations respectively. The manifold tubing was PTFE (0.5 mm i.d., Chromalytic Technology, Australia) and standard solutions were injected manually (70 μL) using a six-port valve (Upchurch V.240, Activon, Australia). The carrier and reagent streams were merged at a T-piece positioned 30 mm from a flat-coiled PTFE (0.5 mm i.d.) flow cell mounted flush against the photomultiplier tube window (THORN-EMI 9924BS, ETP, Australia) which was operated at 900 V, using a stable power supply (THORN-EMI Model PM28BN). The flow cell and photomultiplier tube were encased in a light tight housing and the output was monitored using a chart recorder (Yokogawa Electric Works Ltd, Tokyo, Japan). Chemiluminescence responses were measured manually using peak height. All pH measurements were made using a Jenko pH meter (CHK Engineering, Australia). ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz using a Varian Unity Plus NMR (Varian, Palo Alto, California, USA). Electrospray ionization mass spectra were determined in a positive ion mode using a Micromass Platform II Mass Spectrometer (Micromass, Altrincham, Cheshire, UK).

Table 1
Detection limits reported in the literature for the determination of psilocin and psilocybin

Technique	Psilocin (mol/L)	Psilocybin (mol/L)	Reference
Ion mobility spectrometry		1.4×10^{-4}	[10]
GC–MS	2.4×10^{-8}		[11]
HPLC			
(i) UV absorption	3.9×10^{-6}	3.5×10^{-6}	[16]
(ii) Fluorescence	9.7×10^{-6}	1.7×10^{-6}	
(iii) Electrochemical	3.7×10^{-8}	1.7×10^{-6}	
HPLC UV absorption	3.9×10^{-6}	3.5×10^{-6}	[18]
Continuous addition of reagent chemiluminescence	4.8×10^{-5}		[24]

2.2. Reagents

All reagents were of analytical grade unless otherwise specified and solutions were prepared with high-quality purified water from a MilliQ system (Millipore, North Ryde, NSW, Australia). Sodium hexametaphosphate and orthophosphoric acid were purchased from BDH (Poole, UK). Potassium permanganate and lead dioxide were from Ajax Chemicals (Liverpool, NSW, Australia). Tris(2,2′-bipyridyl)ruthenium(II) chloride hexahydrate was supplied by Strem Chemicals (Newburyport, MA, USA). 3-Butyn-1-ol, tosyl chloride, dimethylamine, trimethylsilyl chloride, *N*-butyl lithium, iodine, palladium(II) acetate, triphenylphosphine, tetraethylammonium chloride, diisopropylethylamine, and trifluoroacetic acid were purchased from Aldrich (Castle Hill, NSW, Australia). *Tert*-butyl lithium and boron tribromide were purchased from APS Chemicals (Seven Hills, NSW, Australia). Dichloromethane (Aldrich) was freshly distilled from CaH_2 prior to use. Diethyl ether and tetrahydrofuran (Aldrich) were freshly distilled from Na/benzophenone before use. Triethylamine (Aldrich) was dried over KOH. Psilocybin was supplied from the Victoria Police Forensic Services Centre. Dried mushroom samples of *Psilocybe subaeruginosa* and *Hypholoma aurantiaca* were supplied from the Royal Botanical Gardens Melbourne, Australia.

Potassium permanganate (0.5 mM) was made up in the carrier solution, sodium hexametaphosphate (0.05% m/v), and both streams were adjusted to pH 2.0 with orthophosphoric acid (0.1 M). Tris(2,2′-bipyridyl)ruthenium(II) chloride

(2 mM) was dissolved in sulfuric acid (0.05 M) prior to oxidation with approximately 0.1 g of lead dioxide Stock solutions of psilocin (9×10^{-5} M) and psilocybin (3.5×10^{-5} M) were prepared in methanol and working standards were obtained by serial dilution with sodium polyphosphate solution (0.05% m/v). Mushroom samples were soaked in methanol overnight and diluted (1:100) with sodium polyphosphate solution (0.05% m/v).

3. Results and discussion

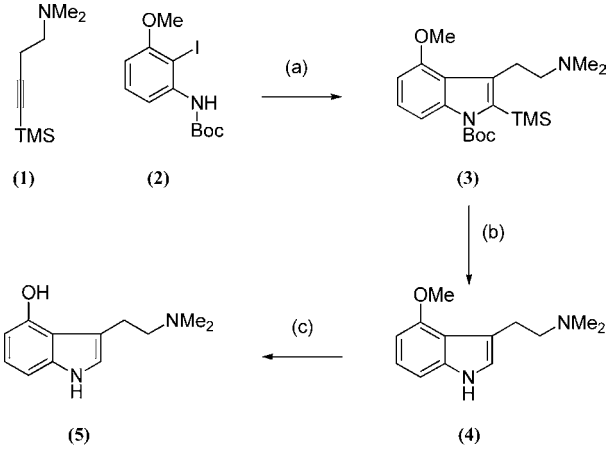
3.1. Synthesis of psilocin

Several routes to psilocin have been reported [30–34]; however, these required either costly starting materials or extremely toxic reagents. Accordingly, we developed a three-step synthesis from readily available starting materials (see Scheme 1), which avoids these limitations [30–34].

3.1.1. Step 1

N,*N*-Dimethyl-4-(trimethylsilyl)-3-butyn-1-amine (1): 3-Butyn-1-ol (25.0 g, 357 mmol, 1 eq.) and tosyl chloride (73.9 g, 392 mmol, 1.1 eq.) were dissolved in dry dichloromethane (1 L) and dry triethylamine (73.9 mL, 53.9 g, 540 mmol, 1.5 eq.) was added at room temperature under a nitrogen flush. The reaction mixture was stirred overnight and washed with water (200 mL). The organic phase was separated and solvents were removed by rotary evaporation using a 25 °C water bath. The flask containing the crude tosylate was charged with an aqueous solution of dimethylamine (300 mL of 40% m/m, 2.7 moles, 7.5 eq.) and the cloudy suspension stirred at room temperature overnight. The clear reaction solution was extracted with diethyl ether (6×150 mL), and then sodium chloride was added to the aqueous phase, which was re-extracted with diethyl ether (3×150 mL). Fractional distillation of the diethyl ether at 1 atm gave a crude product containing residual tosyl impurities, which was purified by vacuum transfer to afford 3-butyn-1-dimethylamine (24.4 g, 251 mmol). The 3-butyn-1-dimethylamine (9.72 g, 100 mmol, 1.0 eq.) was dissolved in diethyl ether (700 mL) under a nitrogen flush and *n*-butyllithium (86.7 mL of 1.5 M in hexanes, 130 mmol, 1.3 eq.) was added dropwise over 2 h at −10 °C to 0 °C and then stirred at 0 °C for a further 2 h. Trimethylsilyl chloride (20 mL, 150 mmol, 1.5 eq.) was added at 0 °C–10 °C and then stirred for 2 h. After addition of water (100 mL), the organic phase was washed with brine (50 mL) and dried over anhydrous sodium sulfate, filtered and solvents were removed by rotary evaporation (20 mmHg, 25 °C). The title compound was obtained in an 88% yield (14.9 g, 88 mmol) by vacuum transfer (0.1 mmHg).

^{1}H NMR (300 MHz, $CDCl_3$) 2.58–2.45 (m, 2H), 2.42–2.34 (m, 2H), 2.23 (s, 6H), 0.12 (s, 9H). ^{13}C (75 MHz) δ 105.48, 85.24, 58.46, 45.27, 18.83, 0.24. HRMS: calculated for $C_9H_{19}NSi$ $[M+H]^+$ 170.1365. Found 170.1374.



(a) $Pd(OAc)_2$ (0.2 equiv), PPh_3 (0.4 equiv), NEt_4Cl (1 equiv), *i*-Pr_2EtN, DMF, 80°C; (b) neat TFA, 25°C, 3h; (c) BBr_3, CH_2Cl_2, -78 to 25°C

Scheme 1. Synthesis of psilocin.

3.1.2. Step 2

N-*tert*-Butoxycarbonyl-2-iodo-3-methoxyaniline (2): Dry diethyl ether (800 mL) was added to *N*-*tert*-butoxycarbonyl-3-methoxyaniline (14.8 g, 66.7 mmol, 1 eq.). The suspension was stirred at room temperature until all solids had dissolved. The solution was cooled to −75 °C using a dry ice/acetone bath and *tert*-butyllithium was added (139 mL of 1.2 M, 167 mmol, 2.5 eq.) maintaining the temperature below −70 °C. The reaction mixture was stirred at −75 °C for 3 h and allowed to warm to −20 °C. The solution was again cooled to −75 °C and quenched by the addition of a solution of iodine (42.2 g in 600 mL of dry diethyl ether, 167 mmol, 2.5 eq.) via a cannula, ensuring the reaction temperature did not rise above −65 °C. The reaction mixture was warmed to room temperature slowly overnight. Aqueous sodium sulfite (150 mL of 10% m/v) was added and the mixture transferred to a separating funnel. The organic phase was separated and washed with sodium hydrogen carbonate (150 mL of 5% m/v), brine (150 mL) and dried over anhydrous magnesium sulfate. After filtration and evaporation of the solvent, the crude product was purified by column chromatography [SiO_2, diethyl ether and hexane (5:95) to (10:90)] to give the title compound in 61% yield (14.2 g, 40.7 mmol). The iodide was further purified by recrystallisation from hexane (40 mL) and the ^{1}H and ^{13}C NMR data were in agreement with the literature [34].

3.1.3. Step 3

4-*N*,*N*-Dimethyl-1-*tert*-butoxycarbonyl-2-trimethylsilyl-4-methoxytryptamine (3): A dry flask was charged with 1-*tert*-butoxycarbonyl-2-iodo-3-methoxyaniline (3.21 g, 9.16 mmol, 1 eq.), 4-(trimethylsilyl)-3-butyn-1-dimethylamine (3.10 g, 18.3 mmol, 2 eq.), palladium(II) acetate (420 mg, 1.84 mmol, 0.2 eq.), triphenylphosphine (960 mg, 3.68 mmol,

0.4 eq.), tetraethylammonium chloride (1.52 g, 9.12 mmol, 1 eq.), diisopropylethylamine (3.54 g, 4.8 mL, 27.4 mmol, 3 eq.) and DMF (65 mL) under a nitrogen flush and heated to 80 °C for 48 h. After cooling, DMF was removed by rotary evaporation and ethyl acetate (50 mL) and water (50 mL) were added to the residue. The aqueous phase was extracted with ethyl acetate (3 × 25 mL) and the organic phase washed with sodium hydrogen carbonate (25 mL of 5% m/v), brine (25 mL) and solvents were removed by rotary evaporation. The crude was purified by column chromatography [SiO_2, chloroform, methanol and ammonia (90:10:1)] to give the title compound as a light brown oil in 69% yield (2.45 g, 6.30 mmol). ^{1}H NMR (300 MHz, $CDCl_3$) 7.57 (d, J = 8.0 Hz, 1H), 7.17 (dd, J = 8.0 and 8.0 Hz, 1H), 6.61 (d, J = 8.0 Hz, 1H), 3.92 (s, 3H), 3.23–3.17 (m, 2H), 2.61–2.55 (m, 2H), 2.40 (s, 6H), 1.68 (s, 9H), 0.41 (s, 9H). ^{13}C (75 MHz) δ 154.19, 151.55, 139.27, 135.37, 130.50, 125.40, 120.89, 108.28, 102.84, 83.70, 61.73, 55.15, 45.35, 28.32, 24.75, 2.71. HRMS: calculated for $C_{21}H_{35}N_2O_3Si$ $[M + H]^+$ 391.2417. Found 391.2415.

3.1.4. Step 4

N,*N*-Dimethyl-4-methoxytryptamine (4): Trifluoroacetic acid (15 mL) was added to 4-*N*,*N*-dimethyl-1-*tert*-butoxycarbonyl-2-trimethylsilyl-4-methoxytryptamine (1.24 g, 3.10 mmol) at room temperature and stirred for 3 h. Volatiles were removed by rotary evaporation and dichloromethane (50 mL) was added to the residue. The organic phase was washed with saturated sodium hydrogen carbonate solution (50 mL), and the aqueous phase was extracted with dichloromethane (3 × 25 mL). Solvents were removed by rotary evaporation and the crude product was purified by column chromatography [SiO_2, chloroform, methanol and ammonia (80:20:1)] to give the title compound as a light brown solid in 58% yield (0.40 g, 1.8 mmol). This product was recrystallised from toluene/hexane to give white crystals. (m.p. 92 °C–93 °C, literature m.p. 93 °C, [34]. The NMR data were in agreement with the literature [34].

3.1.5. Step 5

Psilocin (5): A dry flask was charged with *N*,*N*-dimethyl-4-methoxytryptamine (12.0 mg, 55 μmol), dichloromethane (5 mL), cooled to −78 °C, and boron tribromide (0.25 mL of 1 M in dichloromethane, 0. 25 mmol) added drop wise. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. Volatiles were removed in vacuo and the residue was dissolved in dichloromethane (5 mL). Sodium hydrogen carbonate (25 mg) and methanol (5 mL) were added to the solution and stirred at room temperature for 45 min. The solvent was removed in vacuo and the crude product purified by column chromatography [SiO_2, methanol, ethyl acetate and ammonia (20:80:2)] to give the title compound as a white solid in 61% yield (6.8 mg, 33 μmol). ^{1}H NMR (300 MHz, CD_3OD) 7.06 (s, 1H), 6.92–6.84 (m, 2H), 6.38 (dd, J = 6.5 and 1.5 Hz, 1H), 3.53 (t, J = 7.5 Hz, 2H), 3.30 (m, 2H), 2.92 (s, 6H). ^{13}C (75 MHz) δ 152.57 (C), 140.89 (C), 123.94 (CH), 123.36 (CH), 117.69 (C), 109.85 (C), 104.77 (CH), 104.40 (CH), 61.09 (CH_2), 43.89 (CH_3), 23.62 (CH_2). MS (ESI): *m/z*, 205 $[M + H]^+$ (100%), 59 (90%). HRMS: calculated for $C_{12}H_{17}N_2O$ [M + H] 205.1341. Found 205.1332. The ^{1}H NMR data were in agreement with the literature [34].

The last step was repeated several times to obtain suitable quantities.

3.2. Analytical reaction conditions

A series of univariate searches were performed to ascertain the chemical and hydrodynamic conditions that afforded the maximum signal response, corrected for any blank contributions. The parameters investigated were reagent concentration, pH, flow rate and injection volume while the psilocin and psilocybin concentrations were maintained at 9×10^{-7} M and 3.5×10^{-6} M, respectively.

For the detection of psilocin, the concentration of potassium permanganate was varied from 0.1 mM to 1 mM with 0.5 mM giving the greatest emission; above or below this value the intensity was significantly diminished. Changing the sodium hexametaphosphate concentrations in the range between 0.0% m/v and 1.0% m/v produced an initial steep rise in response, which reached a maximum at 0.05% m/v and declined slowly thereafter. Increasing the reaction pH from 1.5 to 6.0 resulted in the highest chemiluminescence intensity being recorded at 2.0 and above this pH the signal rapidly decreased to a plateau at 4.0. The chemical conditions above combined with a total flow rate (through the detector) of 2.8 mL/min and a sample injection volume of 70 μL gave the best signals and these parameters were maintained throughout the current investigation. Not surprisingly, the chemical environment and FIA parameters employed for the detection of psilocin were very similar to those reported previously [27] for serotonin with the exception of the sodium hexametaphosphate concentration that was approximately an order of magnitude lower in the present study. Given the essential nature of this reagent for the efficient production of chemiluminescence from acidic potassium permanganate oxidations of various analytes [26] and the structural similarities between psilocin and serotonin [27], it is difficult to rationalise the significantly reduced concentration of sodium hexametaphosphate. Conversely, it should be noted that using the same chemistry for the determination of the drug salbutamol [35] a similarly low level of sodium hexametaphosphate (0.1% m/v) yielded the best signal-to-noise ratio. Moreover, as the mechanism by which chemiluminescence is produced from acidic potassium permanganate reactions in the presence of sodium hexametaphosphate is unknown, attempts to account for these observations would be mere conjecture.

With respect to psilocybin detection, varying the tris(2,2′-bipyridyl)ruthenium(III) concentration from 0.5 mM up to 5.0 mM resulted in an initial steep increase in intensity to a maximum at 2.5 mM after which a plateau in the response was observed. A substantial increase in the blank was also monitored as the tris(2,2′-bipyridyl)ruthenium(III) concentration

increased over the above range with best signal to background ratio occurring at 2.0 mM. The emission intensity was also affected by the reaction pH. The pH range investigated was from 1.0 to 5.0 with the highest response recorded at 1.5. Above pH 1.5, the chemiluminescence intensity decreased swiftly until at pH 5.0 where there was no measurable signal. The chemical conditions above combined with a total flow rate (through the detector) of 1.8 mL/min and a sample injection volume of 70 μL gave the best signals and these parameters were maintained throughout the current investigation. While the tris(2,2′-bipyridyl) ruthenium(III) concentration was similar to that employed previously [28] for an array of analytes the reaction pH was considerably lower than we have previously employed with either codeine [36] or sodium oxalate [37] but comparable to that used for the determination of ranitidine [35]. This observation most probably reflects differences in the respective chemiluminescence mechanisms that are both analyte and environment dependent [28].

3.3. Analytical performance

Six aqueous standards were prepared over each of the concentration ranges from 9×10^{-11} M to 4.5×10^{-6} M for psilocin and from 1.75×10^{-11} M to 1.75×10^{-6} M for psilocybin. The resultant calibration functions and analytical figures of merit have been summarised in Table 2. The non-linear nature of these types of calibrations has been noted before [27,35,36]. This behaviour most likely stems from changes in the kinetics of the light production, brought about by the significant variation in the analyte to reagent ratio across the relatively large calibration range. Not unexpectedly, linearity was approximated within narrower concentration ranges, specifically 9×10^{-6} M to 4.5×10^{-7} M for psilocin and 3.5×10^{-8} M to 3.5×10^{-10} M for psilocybin.

A comparison of the information presented in Tables 1 and 2 reveals that detection limits attained in the present investigation are more than competitive with those achieved with various other techniques. Indeed these figures approach the detectability previously realised for codeine [36], morphine and serotonin [27] using FIA with tris(2,2′-bipyridyl)ruthenium(II) and acidic potassium permanganate chemiluminescence detection, respectively.

Table 2
Analytical figures of merit for psilocin and psilocybin utilising FIA with chemiluminescence detection

Compound	L.O.D.[a] (M)	Calibration equation	r^{2}[b]	R.S.D. (%)
Psilocin	9×10^{-10}	$y = 3E + 12x^2 + 6E + 0.7x + 41.38$	0.9996	2.5[c]
Psilocybin	3.5×10^{-10}	$y = -4E + 16x^2 + 3E + 0.9x + 51.58$	0.9988	2.9[d]

[a] Limit of detection (signal-to-noise ratio = 3).
[b] $n = 10$.
[c] Relative standard deviation ($n = 10$, 9.0×10^{-6} M).
[d] Relative standard deviation ($n = 10$, 3.5×10^{-6} M).

Table 3
Psilocin and psilocybin content of dried mushroom samples using chemiluminescence detection

	% Psilocin (mg/g)	% Psilocybin (mg/g)
Psilocybe subaeruginosa	0.038	1.41
Hypholoma aurantiaca	0.021	0.93

3.4. Analysis of mushroom samples

The methodologies developed herein may be directly applicable to the determination of these alkaloids in either mushrooms or extracts thereof. The diluted mushroom extracts were injected five times and the measured chemiluminescence signals were compared to the calibration function and the average concentration was calculated (Table 3). The concentration and detectibility of psilocin and psilocybin are dependent on several variables. (i) Complete loss of psilocin and psilocybin will occur if mushrooms are left at room temperature for an extended period of time. (ii) Psilocin is also prone to oxidation. These preliminary findings will be applied to a dual chemiluminescent detection system for HPLC [38], thus facilitating the determination of both psilocin and psilocybin in body fluids, and the results from this study will be published in due course.

4. Conclusion

Chemiluminescence can be considered a complementary method to conventional chromatographic techniques used for the analysis of psilocin and psilocybin. This present work has demonstrated a sensitive method based on flow injection analysis coupled to chemiluminescence detection for the determination of these naturally occurring indoles. A major feature of this work is the selective determination of psilocin and psilocybin, which can be applied to the analysis of real samples.

Acknowledgements

The authors wish to thank Dr Jim Pearson from the Victoria Police Forensic Services Centre for providing them with a psilocybin reference standard, and Dr Tom May from the Royal Botanical Gardens Melbourne for providing mushroom samples.

References

[1] A. Horita, L.J. Weber, Biochem. Pharmacol. 7 (1961) 47.
[2] A. Horita, L.J. Weber, Toxicol. Appl. Pharmacol. 4 (1962) 730.
[3] S. Pedersen-Bjergaard, E. Sannes, K.E. Rasmussen, F. Tonnesen, J. Chromatogr. B 694 (1997) 375.
[4] A. Campbell, The Australina Illicit Drug Guide, Black Inc., Melbourne, Australia, 2001.

[5] S. Helsley, D. Fiorella, R. Rabin, J. Winter, Prog. Neuropsychopharmacol. Biol. Psychiatry 22 (1998) 649.
[6] F.X. Vollenweider, M.A. Geyer, Brain Res. Bull. 56 (2001) 495.
[7] K.S. Borowiak, K. Ciechanowski, P. Waloszczyk, J. Toxicol. Clin. Toxicol. 36 (1998) 47.
[8] M.W. Beug, J. Bigwood, J. Chromatogr. 207 (1981) 379.
[9] D.B. Repke, D.T. Leslie, D.M. Mandell, N.G. Kish, J. Pharmaceutical Sci. 66 (1977) 743.
[10] T. Keller, A. Schneider, P. Regenscheit, R. Dirnhofer, T. Rucker, J. Jaspers, W. Kisser, Forensic Sci. Int. 99 (1999) 93.
[11] G. Sticht, H. Kaferstein, Forensic Sci. Int. 113 (2000) 403.
[12] A.F. Grieshaber, K.A. Moore, B. Levine, J. Forensic Sci. 46 (2001) 627.
[13] B.M. Thomson, J. Forensic Sci. 25 (1980) 779.
[14] A.L. Christiansen, K.E. Rasmussen, F. Tonnesen, J. Chromatogr. 210 (1981) 163.
[15] A.L. Christiansen, K.E. Rasmussen, J. Chromatogr. 244 (1982) 357.
[16] A.L. Christiansen, K.E. Rasmussen, J. Chromatogr. 270 (1983) 293.
[17] R. Vanhaelen-Fastre, M. Vanhaelen, J. Chromatogr. 312 (1984) 467.
[18] S. Borner, R. Brenneisen, J. Chromatogr. 408 (1987) 402.
[19] R. Kysilka, M. Wurst, J. Chromatogr. 464 (1989) 434.
[20] H. Lindenblatt, E. Kramer, P. Holzmann-Erens, E. Gouzoulis-Mayfrank, K.A. Kovar, J. Chromatogr. B 709 (1998) 255.
[21] T. Kamata, M. Nishikawa, M. Katagi, H. Tsuchihashi, J. Chromatogr. B 796 (2003) 421.
[22] M. Frost, H. Kohler, Forensic Sci. Int. 92 (1998) 213.
[23] I.S. Lurie, P.A. Hays, K. Parker, Electrophoresis 25 (2004) 1580.
[24] J. Cepas, M. Silva, D. Perez-Bendito, Analyst 121 (1996) 49.
[25] R.D. Gerardi, N.W. Barnett, P. Jones, Anal. Chim. Acta 388 (1999) 1.
[26] B.J. Hindson, N.W. Barnett, Anal. Chim. Acta 445 (2001) 1.
[27] N.W. Barnett, B.J. Hindson, S.W. Lewis, Anal. Chim. Acta 362 (1998) 131.
[28] R.D. Gerardi, N.W. Barnett, S.W. Lewis, Anal. Chim. Acta 378 (1999) 1.
[29] N. Gathergood, P.J. Scammells, Org. Lett. 5 (2003) 921.
[30] F. Troxler, F. Seemann, A. Hofmann, Helvet. Chim. Acta 42 (1959) 2073.
[31] F. Yamada, M. Tamura, M. Somei, Heterocycles 49 (1998) 451.
[32] S. Ametamey, F.X. Vollenweider, J. Patt, D. Bourquin, F. Hasler, H.F. Beer, P.A. Schubiger, J. Label. Compd. Radiopharm. 41 (1998) 585.
[33] D.E. Nichols, S. Frescas, Synthesis 6 (1999) 935.
[34] H. Sakagami, K. Ogasawara, Heterocycles 51 (5) (1999) 1131.
[35] N.W. Barnett, B.J. Hindson, S.W. Lewis, Anal. Chim. Acta 384 (1999) 151.
[36] N.W. Barnett, T.A. Bowser, R.D. Gerardi, B. Smith, Anal. Chim. Acta 318 (1996) 309.
[37] N.W. Barnett, S.W. Lewis, S.D. Purcell, P. Jones, Anal. Chim. Acta 458 (2002) 291.
[38] C.E. Lenehan, Ph.D. Thesis, Deakin University, Geelong, Australia, 2002.