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Fingerprint analysis of thermolytic decarboxylation of tryptophan to tryptamine catalyzed by natural oils

Cláudia P.B. Martins[a], M. Ali Awan[b], Sally Freeman[c], Tomás Herraiz[d], John F. Alder[a], Simon D. Brandt[e,*]

[a] School of Chemical Engineering and Analytical Science, The University of Manchester, Sackville Street, P.O. Box 88, Manchester M60 1QD, UK
[b] Institute of Environmental Science and Engineering, National University of Sciences and Technology, Rawalpindi, Pakistan
[c] School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Oxford Road, Manchester M13 9PL, UK
[d] Spanish Council for Scientific Research, Instituto de Fermentaciones Industriales, CSIC, Juan de la Cierva 3, Madrid, Spain
[e] Institute for Health Research, School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK

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ABSTRACT

A number of *N*,*N*-dialkylated tryptamines show psychoactive properties in man which resulted in a renewed interest in psychopharmacological research. Attempts to manufacture these derivatives are increasing within a clandestine environment, where literature procedures are adapted and information is exchanged on the Internet. One such example is based on the thermolytic decarboxylation of tryptophan to tryptamine as the precursor to psychoactive derivatives. This procedure was proposed to make use of household solvents such as *turpentine substitute* and *white spirit* to facilitate decarboxylation. Discussions on websites also suggested the catalytic use of natural oils in order to accelerate these reactions. In this research, the analytical characterization of this preparation procedure was carried out using gas chromatography–ion trap single and tandem stage mass spectrometry in electron and chemical ionization mode that led to the identification of previously unreported 1-mono and 1,1-disubstituted tetrahydro-β-carboline (THBCs) by-products. The tryptamine product and several THBC by-products were determined quantitatively and a "fingerprint" analysis of the crude products allowed for the differentiation between the essential oil catalysts involved as indicated by the presence of tetrahydro-β-carbolines and their imine intermediates.

1. Introduction

The analytical characterization of impurities in pharmaceutical drugs and natural products is of fundamental importance in order to establish the application of proper quality control procedures [1].

The tryptamine category of psychoactive drugs is based on the presence of the 2-(1*H*-indol-3-yl)ethanamine nucleus (**1**, Fig. 1A). Biological activity derives from structural similarity to the neurotransmitter serotonin (5-hydroxytryptamine) and the substitution pattern determines the extent of biological activity. *N*,*N*-Dialkylation, for example, is found in a large number of tryptamine derivatives with psychoactive and hallucinogenic properties [2]. Naturally occurring *N*,*N*-dimethyltryptamine (DMT) can be classified as a classical representative of a hallucinogenic tryptamine [3] that is also accessible by a large number of synthetic routes [4,5]. Shulgin and Shulgin have published the preparation and psychoactive properties of a large number of tryptamine-based drugs [6]. The availability of these data on the Internet, and the fact that hallucinogens such as D-lysergic acid diethylamide (LSD) are less commonly available, has resulted in the increased popularity of tryptamine derivatives [7].

Clandestine drug synthesis [8,9] is based on the attempt to prepare such derivatives, and the fact that most of them are prohibited by legislation often places restrictions on commercial availability of starting materials and reagents. Access to a variety of Internet sources enables one to gain important information on the properties and potential dangers associated with the consumption of illegal drugs. Synthetic procedures are also often discussed and experiences are exchanged about the application of either established or newly developed or improvised synthetic procedures. Analytical profiling and fingerprinting of synthetic routes to illegal tryptamines has hitherto not been carried out in detail. Therefore, the present work aims to provide the forensic and clinical/medical community with basic information on the nature of these drugs and likely side products [4,5,10].

A two-step procedure called *The Breath of Hope* synthesis was proposed on Internet websites for the synthesis of DMT. It was suggested to employ the widely available amino acid tryptophan (Trp) as the starting material. In the presence of high boiling solvents

* Corresponding author. Tel.: +44 151 231 2184; fax: +44 151 231 2170.
E-mail address: s.brandt@ljmu.ac.uk (S.D. Brandt).

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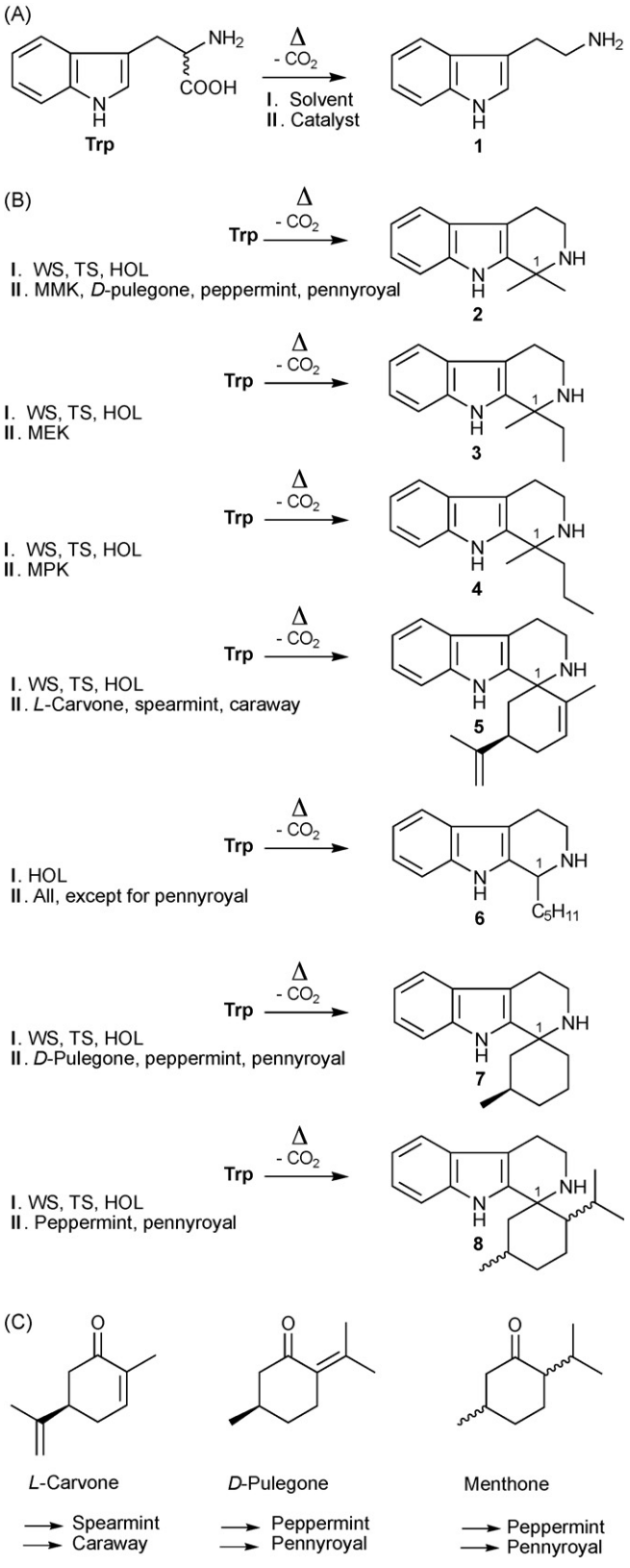

Fig. 1. (A) The synthesis of tryptamine *via* the thermolytic decarboxylation of D,L-tryptophan. (B) Different combinations of catalysts and solvents led to the detection of several tetrahydro-β-carboline (THBC) side products. (C) The main constituents of natural oils which were employed as catalysts in order to accelerate decarboxylation to tryptamine **1**. The detection of THBCs **5**, **7** and **8** confirmed their involvement in by-product formation. WS: white spirit. TS: turpentine substitute. HOL: hexan-1-ol.

and ketone catalysts, heating at reflux was proposed to generate tryptamine **1** as the intermediate, followed by the synthesis of DMT using methyl iodide and a phase transfer catalyst under alkaline conditions [11].

Previous research in this laboratory revealed that the decarboxylation of Trp indeed produced tryptamine **1** as suggested by various clandestine websites. Significant amounts of side products however, were detected and subsequently characterized as tetrahydro-β-carboline derivatives (THBCs) [12]. The overall physiological effects of these THBCs products are difficult to predict and further toxicological and biological activity studies are warranted. A potential action might be on monoamine oxidase (MAO) enzymes that metabolize hallucinogenic tryptamines. In this regard, it was assessed if the THBCs by-products (**2**–**4**) identified and isolated from tryptamine preparation reactions are inhibitors of MAO, by following a recently reported method [22]. In recent work done by the authors, these compounds were not good inhibitors of MAO, as compared to simple aromatic β-carbolines.

The presence of these THBCs impurities yielded a useful profile for the identification of the synthetic pathway because it transpired that both solvent (cyclohexanol) and ketone catalysts caused their formation, possibly due to the involvement of a Pictet-Spengler mechanism [12].

The present study explored the fact that pure solvents and reagents are often expensive and difficult to obtain within a clandestine environment, which leads to alternative methods on the Internet for the thermolytic decarboxylation of Trp. It was suggested to employ commonly available and inexpensive household products [13]. This work addressed the use of household solvents such as *turpentine substitute* (TS) and *white spirit* (WS), as well as the use of natural oils as catalysts for the decarboxylation of tryptophan. Fingerprinting and quantitative analysis were carried out using gas chromatography–ion trap tandem mass spectrometry (GC–IT-MS–MS) in electron (EI) and chemical ionization (CI) mode.

2. Experimental

2.1. Chemicals

1-Hexanol (98%) was obtained from Aldrich (Poole, UK). *TS* and *WS* were purchased at local stores. Spearmint oil (from *Mentha spicata* L.) and D,L-tryptophan (Trp) were obtained from Fluka (Poole, UK). Peppermint, caraway and pennyroyal oils were also purchased locally. The ketones L-carvone (98%), pentan-2-one (MPK) (99.5%), D-pulegone (85%), butan-2-one (MEK) (>99%), acetone (MMK) (99.5%) were obtained from Aldrich. Silica gel for flash chromatography (particle size 40–63 μm) and silica gel aluminium TLC plates were obtained from VWR (Lutterworth, UK). All other solvents and reagents were analytical grade from Aldrich. Tryptamine **1** and 5-methoxytryptamine (I.S.) were also obtained from Aldrich. 1,1-Dimethyl-tetrahydro-β-carboline **2**, 1-methyl,1-ethyl-THBC **3** and 1-methyl,1-propyl-THBC **4** were available as standards from previous work [12].

2.2. Synthesis of tetrahydro-β-carboline by-products ***5*** and ***6***

Synthesis of THBC **5** was achieved by adapting an iodine-catalyzed Pictet-Spengler reaction [14]. Tryptamine (200 mg, 1.25 mmol) was dissolved in 10 mL ethanol, followed by the addition of L-carvone (188 mg, 1.25 mmol) and a catalytic amount of iodine. The reaction mixture was left stirring for 20 h at room temperature. The resulting product was characterized directly by GC–MS analysis.

THBC **6** was synthesised by heating at reflux a mixture of tryptamine (1.0 g, 6.25 mmol) and hexanal (1.6 g, 16.0 mmol) in 30 mL hexan-1-ol for 12 h. The resulting product was characterized directly by GC–MS analysis.

2.3. Instrumentation

The investigation employed GC–EI/CI-MS–MS analysis. Both EI and CI mass spectra were obtained on a Varian Saturn 2200 ion trap MS equipped with a Varian CP-3800 gas chromatograph (Varian, Walnut Creek, USA) and a Combi Pal autosampler (CTC Analytics, Zwingen, Switzerland). Data handling was carried out with the Workstation, Version 5.52 software. Chromatographic separation was achieved using a 5% phenyl, 95% dimethylpolysiloxane, 30 m × 0.25 mm I.D. Factor Four capillary column (VF-5 ms) with a film thickness of 0.25 μm. The carrier gas was helium at a flow rate of 1 mL/min (EFC constant flow mode). A CP-1079 injector (280 °C) was used in split mode (50:1) and a 1 μL volume injection was carried out. Transfer line, manifold and ion trap temperatures were set to 270, 90 and 200 °C, respectively. The column temperature was programmed as follows: 160 °C held for 2 min, then heat at a rate of 12 °C/min to 260 °C. This temperature was held constant for 14.67 min; total run time was 25.00 min. HPLC-grade methanol was used as the liquid CI reagent. Ionization parameters (0.5 s/scan): CI storage level 19.0 *m*/*z*; ejection amplitude 15.0 *m*/*z*; background mass 55 *m*/*z*; maximum ionization time 2000 μs; maximum reaction time 40 ms; target TIC 5000 counts. CI-MS–MS spectra were obtained by collision induced dissociation (CID) of the protonated molecule $[M+H]^+$ by application of a CID waveform excitation amplitude in the non-resonant mode. Helium was used as the damping and collision gas.

2.4. Decarboxylation of tryptophan to tryptamine *1*

The appropriate catalyst (25 mmol) was added to a suspension of D,L-tryptophan (12.25 mmol) in 30 mL of the high boiling point solvent under a stream of nitrogen. The mixture was heated at reflux until a clear solution was observed and no tryptophan detected as judged by TLC. The solvent system used was chloroform–methanol–ammonia (9:1:0.1, v/v). An acid–base extraction was performed to isolate the products from *white spirit* and *turpentine substitute*. A liquid–liquid extraction was performed by acidification using 10% (w/w) HCl. The protonated products were then extracted into 30 mL water. A 10% (w/w) sodium hydroxide solution was then added until alkaline and the free base compounds were extracted twice with 30 mL chloroform. The chloroform layer was evaporated and the sample reconstituted in methanol for analysis.

3. Results and discussion

This study explored the use of household solvents such as *turpentine substitute* (TS) and *white spirit* (WS), as well as the use of natural oils as catalysts for the thermolytic decarboxylation of the amino acid D,L-tryptophan (Trp). Discussions on websites suggested the use of several natural oils in order to accelerate formation of the desired tryptamine product **1**. This proposal was based on the occurrence of ketone constituents in these natural products and aliphatic ketones were known catalysts for this decarboxylation.

TS (b.p. 140–300 °C), WS (b.p. 155–200 °C) and hexan-1-ol (b.p. 156 °C) were used in combination with a variety of ketone catalysts. The range of ketones included aliphatic representatives such as acetone (MMK), butan-2-one (MEK) or pentan-2-one (MPK), as well as natural product components, i.e. L-carvone, D-pulegone, oils of spearmint, caraway, peppermint or pennyroyal, respectively.

Table 1
Yields of tryptamine (**1**) and tetrahydro-β-carbolines by-products (THBCs) after decarboxylation of tryptophan in a variety of high boiling point solvents

Solvent	Catalyst	**1** (% yield)	THBC no. (% yield)
	MMK[a]	10	**2** (3)
	MEK[b]	20	**3** (12)
	MPK[c]	17	**4** (2)
	L-Carvone	26	**5**
TS[d]	Spearmint oil	20	**5**
	Caraway	45	**5**
	D-Pulegone	60	**2** (11), **7**
	Peppermint	30	**2** (1), **7**, **8**
	Pennyroyal	21	**2** (12), **7**, **8**
	MMK[a]	23	**2** (3)
	MEK[b]	25	**3** (13)
	MPK[c]	10	**4** (5)
	L-Carvone	46	**5**
WS[e]	Spearmint oil	70	**5**
	Caraway	19	**5**
	D-Pulegone	71	**2** (15), **7**
	Peppermint	75	**2** (9), **7**, **8**
	Pennyroyal	24	**2** (7), **7**, **8**
	MMK[a]	24	**2** (13), **6**
	MEK[b]	75	**3** (4), **6**
	MPK[c]	58	**4** (5), **6**
	L-Carvone	95	**5**, **6**
HOL[f]	Spearmint oil	90	**5**, **6**
	Caraway	43	**5**, **6**
	D-Pulegone	62	**2** (10), **6**, **7**
	Peppermint	37	**2** (1), **6**, **7**, **8**
	Pennyroyal	61	**2** (17), **7**, **8**

Tryptamine (**1**) yields appeared to show solvent-dependence. TS group 28 ± 16%; WS group 40 ± 26%; hexan-1-ol group 61 ± 24%.
[a] Acetone.
[b] Butan-2-one.
[c] Pentan-2-one.
[d] Turpentine substitute.
[e] White spirit.
[f] Hexan-1-ol.

Table 1 provides an overview of the employed solvent–ketone catalyst combinations.

3.1. Calibration and method validation for the determination of tryptamine and THBCs by GC–MS

A GC–MS method was developed for the quantitative determination of tryptamine **1** and THBC by-products **2**–**4** by direct injection and without the need of chemical derivatization that is commonly used for THBC analysis [15]. Calibration curves for tryptamine **1**, and THBCs **2**–**4** were generated based on their on-column masses (OCM), ranging from 0.35 to 7.5, 0.037 to 35, 0.15 to 8 and 0.13 to 15 ng, respectively, using 5-methoxytryptamine as an internal standard (I.S.). The concentration of internal standard was kept constant in each of the samples and all GC–MS determinations (TIC) were carried out in triplicate as summarized in Table 2. In the case of THBCs **2**–**4** a linear regression approach was used to fit the appropriate trendline to the data. For tryptamine **1** however, the response was observed to be non-linear. A second-order polynomial was used to fit the appropriate calibration data with greater accuracy (Microsoft Excel) achieving a correlation coefficients (r^2) of 0.998. All other responses demonstrated good linearity over the on-column mass ranges studied with correlation coefficients $r^2 = 0.998$, 0.994 and 0.999 for **2**, **3** and **4** respectively.

Accuracy was determined as recovery percentage ($[C_o/C_s] \times 100\%$) between found (C_o) and known (C_s) amounts of the above mentioned compounds. Standards were run in triplicate at OCM values of 1.8 and 5.2 ng **1**, 0.37 ng **2**, 0.75 ng **3** and 0.67 ng **4**, respectively (Table 2). System precision (GC response)

Table 2
Determination of linearity, accuracy, precision, limit of detection (LOD) and limit of quantification (LOQ) for analytes **1**–**4**

Analyte[a]	Line of best fit (r^2)	C_s [ng][b]	C_o [ng] (±SD)[c]	Recovery [%][d]	RSD [%]	LOD (pg)[e]	LOQ (pg)[e]
1	$y = 0.0716x^2 + 0.1829x + 0.0961$ (0.998)	1.80 5.20	1.89 (0.036) 4.91 (0.05)	105.0 94.4	1.90 1.02	35.8	340.0
2	$y = 0.3878x + 0.0146$ (0.998)	0.37	0.34 (0.006)	92.0	1.78	10.0	80.0
3	$y = 2.4072x + 0.4416$ (0.994)	0.75	0.76 (0.018)	102.0	2.43	7.6	60.0
4	$y = 2.2171x - 0.387$ (0.999)	0.67	0.68 (0.018)	101.5	2.73	2.7	30.0

y represents peak area ratio (T/I.S.) and x represents OCM (on-column mass) of analyte (ng).
[a] Structures of analytes are shown in Fig. 1.
[b] C_s: mass of analyte in standard.
[c] C_o: observed mean mass of analyte ($n = 3$).
[d] Percent recoveries were calculated by $(C_o/C_s) \times 100\%$.
[e] On-column mass.

was determined from relative standard deviation (RSD) after triplicate injections of standard solutions used for the construction of calibration curves. The method precision was estimated by injecting standards **1**–**4** in triplicate. Recoveries were obtained by using the calibration equations at lower and/or upper parts of the calibration ranges. Table 2 indicates that recoveries ranged from 92 to 105% indicating a satisfactory level of accuracy.

The system precision was determined from the peak area ratios of analytes and I.S., based on triplicate injections, and showed acceptable reproducibility with RSD values between 0.6 and 3.7% **1**, 0.6 and 8.6% **2**, 2.8 and 7.3% **3** and 2.3 and 11.3% **4**, respectively. The method precision was assessed after triplicate measurements of **1**–**4** standards using calibration equations in Table 2. It can also be seen in Table 2 that the obtained RSD values for compounds **1**–**4** were all below 3%. Limit of detection (LOD) and limit of quantification (LOQ) values were determined by serial dilution of **1**–**4** until a signal-to-noise ratio of 3 and 10 were obtained.

3.2. Decarboxylation of tryptophan to tryptamine *1*

Table 1 shows that thermolytic decarboxylation of Trp provided the desired product **1** but yields varied greatly between 10 and 95%. The use of natural oils was found to accelerate the reactions as judged by their reaction times (data not shown). A well-defined trend was not apparent when considering the nature of the employed ketone catalysts and their impact on tryptamine yields. L-Carvone is known to be a major constituent found in spearmint (*M. spicata*) and caraway (*Carum carvi*), whereas pennyroyal (*Mentha pulegium*) and peppermint oil (*Mentha piperita*) contain significant amounts of D-pulegone. Inspection of tryptamine yields and comparison between the main ketone constituent with the corresponding essential oils indicated that the influence of the particular solvent was important as well (Table 1).

WS and TS are inexpensive and commonly available products. The use of these household solvents led to longer reaction times and lower tryptamine yields when compared with the pure hexan-1-ol (HOL) alternative (Table 1). A general trend indicated that tryptamine yields increased in the order of TS, WS and HOL, respectively. Thermolytic decarboxylation was previously found to be temperature-dependent. This was indicated by reduced reaction times when reaction temperatures increased by the choice of an appropriate solvent with increasing boiling points [12]. In the present study, a similar relation between temperature and tryptamine yields was not observed for both household solvents. This was in contrast with the fact that their boiling points were higher in comparison with hexan-1-ol. TS and WS represent complex mixtures of hydrocarbons which are indicated by their large boiling point range as mentioned above. TS is a complex mixture of refined hydrocarbon distillates, mainly in the C_9–C_{16} range and WS is a mixture of saturated aliphatic and alicyclic C_7 to C_{12} hydrocarbons [16]. The exact composition of both household products is not documented and it currently remains unknown whether the presence of uncharacterized constituents caused unpredictable side reactions which interfered with the formation of tryptamine and reduced yields. A solvent delay of 10 min was used during GC–MS analysis in order to protect the filament. Complex chromatographic patterns were observed which showed some resemblance to fuel-based sample materials when these two household solvents were analysed without filament delay. Fig. 2A displays a characteristic hydrocarbon GC–MS trace where TS was used as a solvent without catalytic addition of an essential oil. Tryptamine was detected as the main compound and the absence of THBC by-products can also be noted in this chromatogram. Both features illustrated their impact on product purity and by-product formation for forensic fingerprint analysis.

3.3. Fingerprinting the formation of tetrahydro-β-carboline by-products *2*–*8*

The presence of several 1,1-disubstituted THBC side products was previously observed after decarboxylation of tryptophan to tryptamine as a consequence of interactions with aliphatic ketone catalysts [12]. As indicated in Table 1, similar THBC derivatives were identified under the applied conditions. This provided support for the ability to fingerprint the employed ketone catalyst even when household solvents were used. In addition to the quantitative identification of previously reported THBCs **2**–**4** (Table 1), novel and previously unreported THBCs side products **5**, **6** and **8** were detected in the current study when essential oils were employed as catalysts (Fig. 1B). Structural representations of the major constituents commonly found in the employed natural oils, namely carvone (found in spearmint and caraway), D-pulegone (found in peppermint and pennyroyal) and menthone (found in peppermint and pennyroyal) are shown in Fig. 1C. Based on the dominating presence of the particular ketone within the essential oil it was therefore possible to infer the employed natural product used for the ketone-accelerated thermolysis of Trp as judged by the detection of the corresponding THBC by-product, irrespective of the employed solvent, Fig. 1B.

Formation of THBC by-products was previously suggested to occur *via* Pictet-Spengler cyclization following interaction between the amine and ketone components. This procedure was also used to confirm their identity by synthesis [12]. In the present study, a similar approach was undertaken for the preparation of the novel THBC derivatives **5**, **6** and **8** and although isolation of the products was unsuccessful, GC–MS characterization allowed for their unambiguous identification based on identical retention times and mass spectra.

The Pictet-Spengler reaction requires the presence of an imine intermediate as part of the condensation between carbonyl source (ketone catalyst) and either Trp or tryptamine. Fig. 2 shows a

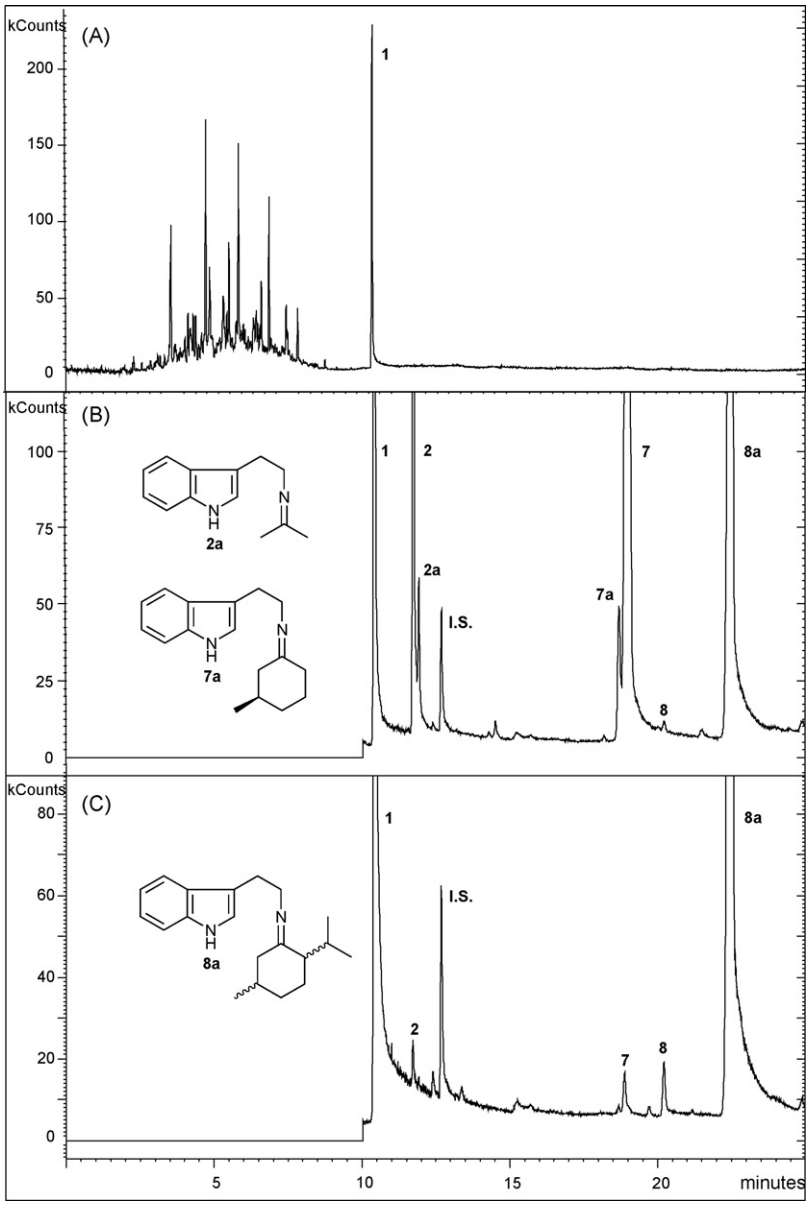

Fig. 2. Representative GC–IT-MS chromatograms after thermolytic decarboxylation of D,L-tryptophan to tryptamine **1** using turpentine substitute (TS) as the solvent. (A) No catalyst was used. The crude tryptamine product was significantly contaminated with a very complex mixture of hydrocarbons which form the basis of the solvent. (B) TS in combination with pennyroyal oil used as catalyst. (C) TS in combination with peppermint oil as catalyst. Both essential oil catalysts led to formation of tetrahydro-β-carboline by-products. Internal standard (I.S.): 5-methoxytryptamine.

representative example of a GC–MS total ion chromatogram of the product after thermolytic decarboxylation of tryptophan to tryptamine using TS with oils of pennyroyal oil (Fig. 2B) and peppermint (Fig. 2C). Both imine intermediates and cyclized THBC products were detectable.

As mentioned above, both essential oils are known to contain D-pulegone and menthone (Fig. 1C) and correspondingly, it was expected to detect a similar contribution to THBC by-product formation. Inspection of the GC–MS traces revealed a significantly different fingerprint as indicated in Fig. 2B and C. The major difference between the two traces manifested in the dramatically increased presence of THBC impurities **2** and **7** and their imine intermediates **2a** and **7a**, when pennyroyal oil was used as the catalyst. Previous work indicated that the presence of D-pulegone resulted in the formation of both THBC **2** and **7**, possibly *via* a retro-aldol mechanism [12]. This phenomenon may have indicated that the D-pulegone content in pennyroyal may have been higher than in peppermint as judged by the decreased occurrence of these two THBCs displayed in Fig. 2C. Under the conditions used similar signal responses have been obtained for the detection of **8a** that is the imine intermediate after reaction with the menthone constituent of both essential oils. It appeared that the extent of cyclization of the imine **8a** to afford the THBC **8** was comparatively low which may be rationalized by steric factors or by the presence of water after condensation.

3.4. Characterization of products by mass spectrometry

In cases where isolation of identified by-products proved impossible, MS detection enabled this to be carried out based on previous work on mass spectral characterization of THBC derivatives [12]. EI-MS spectra of the main products detected during

Table 3
EI-MS spectra of the main products detected during the decarboxylation of D,L-tryptophan

Analyte	R.T. (min)	M_w	Base peak	Other ions *m/z* (%)
1	10.49	160	130	77 (10), 103 (12), 131 (36), 144 (10)
2	11.70	200	185	144 (14), 170 (9), 186 (15), 200 (5)
3	12.59	214	185	68 (5), 144 (14), 199 (15)
4	13.30	228	185	68 (7), 144 (20), 168 (5), 213 (12)
5	21.49	292	292	183 (24), 208 (58), 209 (59), 238 (21)
6	16.85	242	171	115 (6), 144 (10), 154 (14), 186 (5)
7	18.92	254	211	154 (21), 184 (27), 196 (31), 197 (54), 230 (18)
8	20.38	296	211	41 (23), 183 (23), 254 (15), 282 (35), 296 (23)

the decarboxylation of Trp are shown in Table 3. Tryptamine **1** showed characteristic hydroquinolinium $[C_9H_9N]^+$ and quinolinium $[C_9H_8N]^+$ ion peaks at *m/z* 131 and *m/z* 130, respectively, which facilitated differentiation from cyclized THBC counterparts. A typical ion series at *m/z* 103 (loss of HCN) and *m/z* 77 (loss of C_2H_2) was also observed [17–19]. Imine intermediates could also be distinguished from THBCs based on their retention time differences and the fact that they also displayed tryptamine-like fragmentation patterns.

1,1-Disubstituted THBCs **2**–**4**, carrying an open chain substitution pattern presented a characteristic alkyl radical loss that was consistent with previous work [12] that exhibited base peak formation at *m/z* 185. Asymmetrically 1,1-disubstituted derivatives showed preferential loss of the larger alkyl group in agreement with Stevenson's rule.

The 1-monoalkylated THBC **6** also displayed cleavage of the C_5H_{11} alkyl radical to form a dihydro-β-carbolinium base peak at *m/z* 171 [18,20]. The mass spectrum for compound **7** was in agreement with the data reported previously and may have occurred after ring opening *via* α-cleavage. Subsequent alkyl losses may have been responsible for the formation of ions at *m/z* 197 and *m/z* 211 [12,21]. Accordingly, a corresponding fragmentation pattern was observed for compounds **5** and **8** based on their structural similarities. Therefore, the *m/z* 211 species was the base peak for THBC **8** and *m/z* 209 was detected for the spirocyclohexane derivative **5**. It was also interesting to observe that a strong molecular ion was present which indicated stability of these species under EI conditions. In the case of THBC **5** the molecular ion formed the base peak.

Chemical ionization is a soft ionization method useful for the determination of the protonated molecule $[M+H]^+$. A loss of 17 amu, presumably *via* cleavage of ammonia, was a characteristic feature for THBC derivatives. This $[M+H-17]^+$ ion was detected during CI-MS–MS analysis of most THBC compounds. It appeared that loss of ammonia was indicative of unsubstituted nitrogen at position 2 since N-alkylation precluded its detection. However, an exception was observed under tandem MS conditions for **5** and **8** where loss of 17 amu was not detected (Table 4).

Table 4
CI-IT-MS–MS spectra of the main products detected during the decarboxylation of tryptophan

Analyte	$[M+H]^+$(%)	Base peak *m/z*	Other ions *m/z* (%)
1	161 (25)	144	131 (10)
2	201 (90)	144	184 (15)
3	215 (100)	215	72 (8), 144 (46), 198 (58)
4	229 (100)	229	86 (3), 144 (40), 183 (5), 212 (25)
5	293 (100)	293	144 (68), 150 (47)
6	243 (80)	226	144 (67), 214 (13)
7	255 (100)	255	112 (40), 144 (60), 224 (51), 238 (34)
8	297 (100)	297	56 (4), 144 (56)

The excitation amplitude was set to 40 V, with exception of compounds **5** (90 V), **7** (60 V) and **8** (80 V).

The application of moderate, non-resonant excitation amplitude (40 V) generated a sufficient number of product ions while preserving a significant signal intensity of the protonated molecule except for **5** and **8**. Higher excitation amplitude values were used for THBCs **5**, **7** and **8** in order to increase their fragmentation which may be rationalized by the presence of an additional ring (Table 4).

4. Conclusions

Previously unreported THBC derivatives have been identified, characterized and the respective structures proposed as side products during an Internet-based synthetic procedure for the preparation of tryptamine utilising the decarboxylation of typtophan. Solvents are not easily obtainable by clandestine chemists, which have triggered the use of household products. Tryptamine can be synthesised using readily available domestic solvents and natural essential oils. A GC–IT-MS method for determination of tryptamine and by-products was developed and showed good linearity, sensitivity, accuracy and precision. The reactions produce characteristic traces of THBC and the imine intermediates. The detection of by-products in the tryptamine provided sufficient data to point towards the synthetic route and the catalyst employed. The detection of these novel THBC derivatives also proved that it was possible to identify the natural oils used during the synthetic procedure. An analytical characterization of the routes proposed on the Internet is useful for forensic and clinical purposes where the lack of quality control procedures is inherently problematic. The overall physiological effects of these poorly purified products are difficult to predict and further toxicological studies are warranted.

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References

[1] S. Görög (Ed.), Identification and Determination of Impurities in Drugs, Elsevier, Amsterdam, 2000.
[2] A.T. Shulgin, in: R.R. Laing (Ed.), Hallucinogens. A Forensic Drug Handbook, Elsevier Science, London, 2003, p. 67.
[3] D.E. Nichols, Pharmacol. Ther. 101 (2004) 131.
[4] S. Freeman, J.F. Alder, Eur. J. Med. Chem. 37 (2002) 527.
[5] S.D. Brandt, S. Freeman, P. McGagh, N. Abdul-Halim, J.F. Alder, J. Pharm. Biomed. Anal. 36 (2004) 675.
[6] A.T. Shulgin, A. Shulgin, TIHKAL: The Continuation, Transform Press, Berkeley, CA, 1997.
[7] K. Babu, E.W. Boyer, C. Hernon, D.E. Brush, Clin. Pediatr. Emerg. Med. 6 (2005) 81.
[8] W.H. Soine, Med. Res. Rev. 6 (1986) 41.
[9] R.R. Laing (Ed.), Hallucinogens: A Forensic Drug Handbook, Elsevier Science, London, 2003.
[10] S.D. Brandt, S. Freeman, I.A. Fleet, P. McGagh, J.F. Alder, Analyst 129 (2004) 1047.
[11] Anon., Drone #342, published on the now defunct Hive website (September 1998).
[12] S.D. Brandt, D. Mansell, S. Freeman, I.A. Fleet, J.F. Alder, J. Pharm. Biomed. Anal. 41 (2006) 872.
[13] Synthesis of Tryptamine from Tryptophan by Rhodium, Chemistry Archive, 2005, http://designer-drugs.com/pte/12.162.180.114/dcd/chemistry/tryptophan.html.
[14] Y. Lingam, D.M. Rao, D.R. Bhowmik, P.S. Santu, K.R. Rao, A. Islam, Tetrahedron Lett. 48 (2007) 7243.
[15] F. Musshof, T. Daldrup, W. Bonte, A. Leitner, O.M. Lesch, J. Chromatogr. B 683 (1996) 163.
[16] R. Phillips, in: P. Wexler (Ed.), Encyclopedia of Toxicology, Elsevier, Oxford, 2005, p. 100.
[17] M.W. Couch, C.M. Williams, Anal. Biochem. 50 (1972) 612.
[18] J. Gynther, Acta Chem. Scand. B 42 (1988) 433.
[19] T. Herraiz, Rapid Commun. Mass Spectrom. 11 (1997) 762.
[20] R.T. Coutts, R.A. Locock, G.W.A. Slywka, Org. Mass Spectrom. 3 (1970) 879.
[21] J.G. Rodríguez, P. Gil-Lopetegui, J. Heterocyclic Chem. 30 (1993) 373.
[22] T. Herraiz, C. Chaparro, J. Chromatogr. A 1120 (2006) 237.