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Analytical characterisation of the routes by thermolytic decarboxylation from tryptophan to tryptamine using ketone catalysts, resulting in tetrahydro-β-carboline formation

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Abstract

N-Alkylated tryptamines have complex psychoactive properties. Routes for clandestine synthesis are described on Internet websites one of which involves the thermolytic decarboxylation of tryptophan to tryptamine as a precursor to psychoactive compounds. High boiling solvents and ketone catalysts have been employed to facilitate the decarboxylation of tryptophan.

The present study has revealed that there is formation of tetrahydro-β-carboline (THBC) derivatives which may originate from reaction with both the solvent and the ketone catalyst. The application of gas chromatography electron- and chemical-ionisation ion trap tandem mass spectrometry (GC–IT-MS–MS), in combination with nuclear magnetic resonance (NMR), led to the isolation and identification of 1,1-disubstituted-tetrahydro-β-carbolines formed as major impurities in the tryptamine. Confirmation was by synthesis of the THBC derivatives from tryptamine using Pictet-Spengler cyclisation. Under EI-conditions, mass spectral characterisation of the THBCs suggests predominance of alkyl cleavage.

These impurities will yield a useful profile for identification of the synthetic pathway and likely reagents employed, particularly a "fingerprint" of the ketone catalyst and an insight into the influence of solvents and catalysts on the formation of by-products.

Keywords: Tryptamines; Hallucinogens; Decarboxylation; Forensic; Fingerprint; Tetrahydro-β-carbolines; Synthesis; Biological activities; Analytical chemistry

1. Introduction

Simple tryptamines unsubstituted on the amine, such as 2-(1H-indol-3-yl)-ethylamine (**2**), do not seem to be orally psychoactive themselves but can serve as convenient building blocks for the synthesis of psychoactive derivatives. *N*,*N*-Dialkylated tryptamines, serotonin (5-hydroxytryptamine, 5-HT) and related compounds play an integral part in the neurochemistry of the human brain. These compounds have generated growing interest in the psychiatry [1], neuroscience [2,3] and psychopharmacology [4–6] communities, as well as in recreational drug use [7]. Recent case reports [8,9] and intelligence alerts [10] reflect the increased popularity of these compounds in the recreational drugs movement and appropriate analytical procedures need to be developed [11–14].

Some of the synthetic routes to psychoactive tryptamines that are reported in the literature find their way into the clandestine community where the lack of quality control leads to low quality drugs with unpredictable biological activity and ill-defined impurity profiles [7,15].

Tryptophan (**1**) (Trp) and some derivatives are readily available and can be used as the starting material for the synthesis of the corresponding tryptamine precursor via thermal decarboxylation (Fig. 1). The chemically based conversion of Trp is by far the simplest way to the synthesis of **2** and is achieved by heating at reflux in a high boiling solvent; a variety of conditions has been adopted and used with success.

Hashimoto et al., for example, used cyclohexanol as the solvent and observed accelerated reaction times and a higher yield of amine produced, with the addition of 2-cyclohexen-1-one

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that was present also as an impurity [16]. Other workers have used diphenylmethane [17] and diphenyl ether [18]. Alternative methods [19] included a two-step catalytic decarboxylation by reacting Trp with copper acetate or zinc acetate via formation of metal chelate compounds that were then decarboxylated to produce tryptamine hydrochloride, with indole as a by-product [19].

Takano et al. [20] heated L- or DL-tryptophan at reflux in tetralin with a catalytic amount of various carbonyl compounds. A variation was reported by Eckstein et al. [21] where Trp was decarboxylated in cyclohexanol: one method employed tetralin that contained its peroxide, another used tetralone followed by tetralin. A quantitative decarboxylation of Trp in acetophenone at 130 °C, using organic peroxides as catalysts has also been described [22].

A study of various hydroxy- and methoxy-aromatic ketones as the decarboxylating media [23] concluded that the decarboxylation of Trp and other α-amino acids proceeds via the formation of stable Schiff base intermediates, i.e. imines [24] (Fig. 1). Some of these intermediates when hydrolysed by hydrochloric acid or sodium hydroxide, were found to undergo transamination to a degree depending on the ketones used [23], with yields of tryptamine between 60 and 100%.

An interesting approach that has also been discussed on Internet websites uses the natural abundance of carvone (5-*isoprenyl-2-methyl-cyclohex-2-enone)* in spearmint (*Mentha spicata*) oil as the ketone catalyst and either xylene or *white spirit* as the refluxing solvent [25]. It was also suggested that dill (*Anethum graveolens*), caraway (*Carum carvi*; *contains carvone*) or pennyroyal (*Mentha pulegium*; *contains* D-*pulegone*, (5*R*)-*methyl*-2-*isopropylidene-cyclohexanone*) essential oils could also be employed as the catalyst. Oil of Turpentine (*the steam-volatile oil from rosin*, *an exudate of pine trees*) has also been suggested as a solvent. Noteworthy in all the methods described is the range of side products that may be present as trace constituents in the final product, thus acting as indicators to the synthetic route.

The present study focused for the first time on the analytical characterisation of the synthetic route to tryptamine **2** via decarboxylation of Trp **1** in the presence of ketone catalysts,

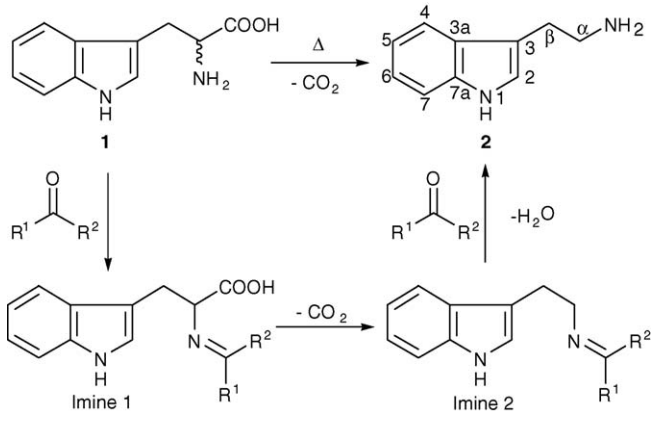

Fig. 1. Tryptophan (**1**) undergoes thermolysis and forms tryptamine (**2**). High boiling solvents and the presence of aldehyde/ketone catalysts facilitate decarboxylation. The mechanism is proposed to proceed via imine 1 and imine 2.

with an emphasis on the identification of possible by-products. It arose from a two-stage synthesis from tryptophan to tryptamine **2** and its subsequent methylation to *N*,*N*-dimethyltryptamine using methyl iodide and benzyltriethylammonium chloride/NaOH phase transfer catalyst, proposed on an internet website [26], that became known as *The Breath of Hope Synthesis*. Discussion on the internet and separately, work in the authors' laboratory repeating the proposed method revealed that it did not work well [27] but warranted further investigation for forensic purposes, due to its perceived appeal to the clandestine chemist.

2. Experimental

2.1. Materials

Cyclohexanol (99%) was from Lancaster (UK), spearmint oil (from *M. spicata* L.) from Fluka (UK) and oil of turpentine (purified) was from Riedel-de Haën (Germany). The ketones were (Aldrich, UK): L-carvone (98%), pentan-2-one (99.5%), pentan-3-one (98%), D-pulegone (85%), butan-2-one (>99%), acetone (99.5%) and (Fluka, UK) 2-cyclohexen-1-one, 98+%. Silica gel for flash chromatography (particle size 40–63 μm) and silica gel aluminium TLC plates were obtained from VWR (UK). All other solvents and reagents were analytical grade from Aldrich (UK).

2.2. Instrumentation

The investigation employed gas chromatography combined with electron- and chemical-ionisation ion trap (single and double stage) mass spectrometry (GC–IT-EI/CI-MS–MS) and nuclear magnetic resonance (NMR).

EI and CI mass spectra were obtained on a Varian Saturn 2200 ion trap MS equipped with a Varian CP-3800 gas chromatograph (Varian, USA) and a Combi Pal autosampler (CTC Analytics, Switzerland). Data handling was completed with Saturn GC/MS Workstation, Version 5.52 software. Chromatographic separation was achieved using a 5% phenyl, 30 m × 0.25 mm CP-Sil 8 CB Low Bleed/MS column with a film thickness of 0.25 μm. The carrier gas was helium at a flow rate of 1 ml min^{-1} (EFC constant flow mode). A CP-1177 injector (280 °C) was used in split mode (50:1). The transfer line, manifold and ion trap temperatures were set to 270, 95 and 200 °C, respectively. The column temperature was programmed as follows: 40 °C and held for 1 min, then heat at a rate of 50 °C min^{-1} to 260 °C and held at this temperature for 14.6 min; total run time was 20 min.

HPLC grade methanol was used as the liquid CI reagent. Ionisation parameters (0.5 s/scan)—CI storage level: 19.0 *m/z*; ejection amplitude: 15.0 *m/z*; background mass: 55 *m/z*; maximum ionisation 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 $[M+H]^+$ within the ion trap, using helium, by application of a CID waveform excitation amplitude in the non-resonant mode. Excitation storage level was set to 48.0 *m/z*. The excitation amplitude was

set to 30 V unless stated otherwise. The number of ions in the trap was controlled by an automatic gain control function.

NMR spectra were recorded using a Bruker DPX 300 at 300.1 MHz (^{1}H NMR) or 75.5 MHz (^{13}C NMR) at 300 K and the solvent used was $CDCl_3$, unless stated otherwise; chemical shifts were reported relative to TMS at $\delta = 0$ ppm. NMR spectra were obtained by ^{1}H NMR, proton decoupled ^{13}C, Distortionless Enhancement Polarisation Transfer DEPT-135 (pulse angle 135°) and ^{1}H–^{13}C COSY (Heteronuclear Multiple Quantum Coherence, HMQC) experiments.

The identities of the synthesised compounds were confirmed by ESI-TOF-MS exact mass measurements (experimental error ≤5 ppm) and NMR spectroscopy.

2.3. Decarboxylation of tryptophan

The appropriate catalyst was added to a suspension of tryptophan in a high boiling-point solvent under a nitrogen blanket. The mixture was heated at reflux and stirred vigorously until a clear reaction mixture was observed. TLC analysis of the product mixture indicated that tryptophan was no longer present. Quantitative estimation of the final product mixture was performed using a standard addition technique and the calculated yields were found to be in broad agreement with those isolated by flash chromatography using chloroform:methanol:aq. ammonia (0.88 s.g.), 9:1:0.1 as eluent. The general procedure outlined above was performed using the solvents and catalysts described in Table 1.

2.4. General synthetic procedure for 1,1-disubstituted 1,2,3,4-tetrahydro-β-carbolines

Reference materials to confirm identification of the THBC by-products were prepared by a modified Pictet-Spengler procedure [28]. Tryptamine (300 mg, 1.87 mmol) was added to a solution of 30 ml toluene and 2 ml trifluoroacetic acid. The appropriate ketone (28 mmol) was added and the mixture stirred at 60 °C overnight. The reaction mixture was concentrated under reduced pressure and the crude residue made alkaline with 10% (w/w) aq. sodium hydroxide. The free base compounds were extracted three times with 40 ml chloroform and washed twice with water. The chloroform layer was evaporated under reduced pressure and subjected to flash chromatography (*solvent system as above*). The corresponding THBCs were isolated as oils and dried under vacuum over P_2O_5 where some of the products solidified.

THBC derivatives **6** and **7** were synthesised simultaneously using pulegone as the ketone catalyst, with heating at 60 °C for 3 days.

Data for 1-ethyl-1-methyl-THBC **3** (204 mg, 0.95 mmol, 51%)—^{1}H NMR: 7.70 (1H, br s, N-9H), 7.48 (1H, dd, H-5, *J* 7.5, 0.8 Hz), 7.32 (1H, dd, H-8, *J* 6.9, 1.1 Hz), 7.16 (1H, td, H-7, *J* 7.2, 1.5 Hz), 7.09 (1H, td, H-6, *J* 7.3, 1.1 Hz), 3.31–3.13 (2H, m, CH_2-3), 2.74 (2H, t, CH_2-4, *J* 5.9 Hz), 2.18 (1H, br s, N-2H), 1.87 (1H, dq, 1′-CHAHB, J_{gem} 11.5 Hz, *J* 7.5 Hz), 1.80 (1H, dq, 1′-CHAHB, J_{gem} 11.5 Hz, *J* 7.5 Hz), 1.45 (3H, s, 3′-CH_3), 0.91 (3H, t, 2′-CH_3, *J* 7.5 Hz). ^{13}C NMR: 139.9 (C-9a), 136.0 (C-8a), 127.8 (C-4b), 121.8 (C-7), 119.7 (C-6), 118.5 (C-5), 111.1 (C-8), 109.0 (C-4a), 53.9 (C-1), 40.1 (CH_2-3), 34.4 (1′-CH_2), 27.2 (3′-CH_3), 23.4 (CH_2-4), 8.7 (2′-CH_3). HREIMS—theory: 214.1465; observed: 214.1468 (delta: 1.6 ppm).

Data for 1,1-diethyl-THBC **4** (247 mg, 1.08 mmol, 58%)—^{1}H NMR: 7.65 (1H, br s, N-9H), 7.49 (1H, br d, H-5, *J* 8.0 Hz), 7.32 (1H, br d, H-8, *J* 8.0 Hz), 7.15 (1H, td, H-7, *J* 7.4, 1.3 Hz), 7.09 (1H, td, H-6, *J* 7.3, 1.0 Hz), 3.19 (2H, t, CH_2-3, *J* 5.8 Hz), 2.71 (2H, t, CH_2-4, *J* 5.5 Hz), 1.79 (4H, q, 1′/4′-CH_2, *J* 7.4 Hz), 1.63 (1H, br s, N-2 H), 0.87 (6H, t, 2′/3′-CH_3, *J* 7.5 Hz). ^{13}C NMR: 138.6 (C-9a), 135.6 (C-8a), 127.4 (C-4b), 121.4 (C-7), 119.2 (C-6), 118.1 (C-5), 110.6 (C-8), 109.8 (C-4a), 56.4 (C-1), 39.8 (CH_2-3), 32.1 (1′/2′-CH_2), 23.0 (CH_2-4), 8.4 (3′/4′-CH_3). HREIMS—theory: 228.1621; observed: 228.1624 (delta: 1.3 ppm).

Data for 1-methyl-1-propyl-THBC **5** (281 mg, 1.23 mmol, 66%)—^{1}H NMR: 7.66 (1H, br s, N-9H), 7.48 (1H, br d, H-5, *J* 8.0 Hz), 7.31 (1H, br d, H-8, *J* 8.0 Hz), 7.15 (1H, td, H-7, *J* 7.8, 1.3 Hz), 7.09 (1H, td, H-6, *J* 7.3, 1.1 Hz), 3.24 (1H, dt, CH_2-3, *J* 13.2, 5.1 Hz), 3.16 (1H, dt, CH_2-3, *J* 13.2, 5.6 Hz), 2.71–2.70 (2H, m, CH_2-4), 1.82 (1H, br s, N-2H), 1.79–1.70 (2H, m, 1′-CH_2), 1.44 (3H, s, 4′-CH_3), 1.47–1.33 (1H, m, 2′-CHAHB), 1.32–1.18 (1H, m, 2′-CHAHB), 0.90 (3H, t, 3′-CH_3, *J* 7.3 Hz). ^{13}C NMR: 140.1 (C-9a), 135.9 (C-8a), 127.8 (C-4b), 121.9 (C-7), 119.7 (C-6), 118.6 (C-5), 111.0 (C-8), 108.8 (C-4a), 53.7 (C-1), 44.5 (1′-CH_2), 40.2 (CH_2-3), 27.7 (4′-CH_3), 23.4 (CH_2-4), 17.1 (2′-CH_2), 14.9 (3′-CH_3). HREIMS—theory: 228.1621; observed: 228.1619 (delta: 0.9 ppm).

Data for 1,1-dimethyl-THBC **6** (143 mg, 0.71 mmol, 38%)—^{1}H NMR: 7.74 (1H, br s, N-9H), 7.48 (1H, dd, H-5, *J* 7.5, 0.75 Hz), 7.31 (1H, dd, H-8, *J* 8.1, 1.2 Hz), 7.15 (1H, td, H-7, *J* 7.5, 1.2 Hz), 7.09 (1H, td, H-6, *J* 7.4, 1.3 Hz), 3.22 (2H, t, CH_2-3, *J* 5.8 Hz), 2.73 (2H, t, CH_2-4, *J* 5.8 Hz), 1.98 (1H, br s, N-2H), 1.49 (6H, s, 1′/2′-CH_3). ^{13}C NMR: 140.5 (C-9a), 136.0 (C-8a), 127.7 (C-4b), 122.0 (C-7), 119.8 (C-6), 118.6 (C-5),

Table 1
Adapted literature procedures for the decarboxylation of tryptophan (**1**)

Trp (**1**) (mmol)	Solvent (ml)	Catalyst	Reference
12.25	Cyclohexanol (30)	MMK, MEK, MPK, EEK, 2-cyclohexen-1-one (4.3 mmol),[a] D-pulegone (0.3 ml)	[25]
12.25	Tetralin (30)	MMK, MEK, MPK, EEK, 2-cyclohexen-1-one (4.3 mmol),[a] D-pulegone, L-carvone (0.3 ml)	[20]
17.16	Turpentine (30)	D-Pulegone, L-carvone, spearmint oil (0.3 ml)	[25]
4.90	Diphenyl ether (50)	Not employed	[18]
1.22	Diphenylmethane (10g)	Not employed	[17]
1.23	Quinoline (30)	Not employed	[29]

[a] See Table 2 for abbreviations.

111.1 (C-8), 107.7 (C-4a), 51.0 (C-1), 40.2 (CH_2-3), 29.3 (1′/2′-CH_3), 23.2 (CH_2-4). HREIMS—theory: 200.1308; observed: 200.1306 (delta: 1.0 ppm).

Data for spiro-3-methylcyclohexane-THBC **7** (130 mg, 0.51 mmol, 27%)—^{1}H NMR (CD_3OD): 7.35 (1H, dd, H-5, *J* 7.2, 0.75 Hz), 7.25 (1H, br d, H-8, *J* 7.9 Hz), 7.01 (1H, td, H-7, *J* 7.5, 1.1 Hz), 6.93 (1H, td, H-6, *J* 7.3, 1.0 Hz), 3.08 (2H, t, CH_2-3, *J* 5.8 Hz), 2.70 (2H, t, CH_2-4, *J* 5.8 Hz), 1.69–1.92 (7H, m), 1.51 (1H, t, *J* 13.4 Hz), 1.10–0.95 (1H, m), 0.94 (3H, d, CH-$\underline{CH_3}$, *J* 6.0 Hz). ^{13}C NMR: 141.6 (C-9a), 137.6 (C-8a), 128.5 (C-4b), 121.8 (C-7), 119.5 (C-6), 118.6 (C-5), 111.7 (C-8), 107.6 (C-4a), 54.9 (C-1), 45.6 (CH_2), 40.0 (CH_2-3), 36.0 (CH_2), 35.4 (CH_2), 28.4 (CH), 23.2 (CH-$\underline{CH_3}$), 23.1 (CH_2-4), 22.3 (CH_2). HREIMS—theory: 254.1778; observed: 254.1786 (delta: 3.3 ppm).

Data for 1-spirocyclohexane-THBC **8** (179 mg, 0.74 mmol, 40%)—^{1}H NMR: 7.92 (1H, br s, N-9H), 7.48 (1H, dd, H-5, *J* 7.5, 0.75 Hz), 7.30 (1H, dd, H-8, *J* 7.2, 1.1 Hz), 7.13 (1H, td, H-7, *J* 7.2, 1.5 Hz), 7.08 (1H, td, H-6, *J* 7.2, 1.1 Hz), 3.14 (2 H, t, CH_2-3, *J* 5.8 Hz), 2.70 (2H, t, CH_2-4, *J* 5.6 Hz), 1.98–1.22 (10H, m, H-1′–H-5′). ^{13}C NMR: 141.6 (C-9a), 135.8 (C-8a), 127.9 (C-4b), 121.8 (C-7), 119.7 (C-6), 118.6 (C-5), 111.1 (C-8), 108.4 (C-4a), 52.7 (C-1), 39.6 (CH_2-3), 37.0 (CH_2), 26.1 (CH_2), 23.5 (CH_2-4), 21.8 (CH_2). HREIMS—theory: 240.1621; observed: 240.1639 (delta: 7.7 ppm).

Data for *N*-benzylidene-tryptamine **9**; synthesis was carried out as above but without the addition of acid and with only one equivalent of benzaldehyde. Evaporation of toluene was followed by recrystallisation with petroleum spirit (60–80 °C)/$CHCl_3$ and yielded a beige solid (334 mg, 1.35 mmol, 72%)—^{1}H NMR 8.15 (1H, s, N=CH), 8.08 (1H, br s, NH), 7.72–7.68 (2H, H-2′/6′, m), 7.66 (1H, d, H-4, *J* 8.3 Hz), 7.40–7.38 (3H, H-3′/4′/5′, m), 7.32 (1H, d, H-7, *J* 7.9 Hz), 7.18 (1H, t, H-6, *J* 7.6 Hz), 7.11 (1H, t, H-5, *J* 7.1 Hz), 6.97 (1H, s, H-2), 3.93 (2H, t, CH_2-α, *J* 7.2 Hz), 3.16 (2H, t, CH_2-β, *J* 7.2 Hz). ^{13}C NMR 161.9 (N=CH), 136.6 (C-7a and C-1′ overlap), 131.0 (C-4′), 129.0 (2x C-3′/5′), 128.5 (2x C-2′/6′), 127.9 (C-3a), 122.6 (C-2), 122.3 (C-6), 119.6 (C-5), 119.4 (C-4), 114.4 (C-3), 111.5 (C-7), 62.4 (CH_2-4), 27.3 (CH_2-3). HREIMS—theory: 248.1308; observed: 248.1309 (delta: 0.4 ppm).

3. Results and discussion

Thermal decarboxylation of Trp requires the use of high-boiling solvents and ketone catalysts for accelerated conversion. Based on the literature, the most commonly used solvents were chosen: cyclohexanol (b.p. 160–161 °C) and tetralin (1,2,3,4-tetrahydronaphthalene, b.p. 207 °C). Diphenyl ether (b.p. 259 °C) and diphenylmethane (b.p. 264 °C) were used in order to investigate the impact of higher temperatures. Oils of turpentine (b.p. 153–175 °C) and quinoline (b.p. 237 °C) [29] were also employed.

A range of ketone catalysts was used that included simple, symmetrically substituted acetone (MMK) and pentan-3-one (EEK). Asymmetrically substituted ketones butan-2-one (MEK) and pentan-2-one (MPK) were also employed. In addition, decarboxylation with 2-cyclohexen-1-one, an activated α,β-unsaturated ketone, spearmint oil, L-carvone and D-pulegone were employed. All reactions are summarised in Table 2.

Table 2
Yield of tryptamine **2**, tetrahydro-β-carboline by-products **3**–**8**, *N*-benzylidene **9** formed during the decarboxylation of tryptophan (**1**)[a]

Solvent	Catalyst	t_R[b] (h)	**2** (% yield)	THBC no. (% yield)
Cyclohexanol	MMK[c]	51	46.2	**6** (14.5) **8** (25.0)
Cyclohexanol	MEK[d]	56	54.3	**3** (4.2) **8** (21.0)
Cyclohexanol	MPK[e]	18	65.0	**5** (4.4) **8** (25.5)
Cyclohexanol	EEK[f]	17	56.0	**4** (9.4) **8** (27.2)
Cyclohexanol	D-Pulegone[g]	20	50.2	**6** (4.9) **7** (12.5) **8** (31.0)
Cyclohexanol	2-Cy[h]	1	68.7	**8** (9.3)
Cyclohexanol	–	100	34.4	**8** (8.4)
Tetralin	MMK[c]	1	78.3	**6** (0.2)
Tetralin	MEK[d]	1	82.5	**3** (0.4)
Tetralin	MPK[e]	1	87.9	**5** (6.0)
Tetralin	EEK[f]	1	86.9	**4** (3.9)
Tetralin	D-Pulegone[g]	1	93.3	**6** (0.5) **7** (0.7)
Tetralin	2-Cy[h]	0.5	89.8	–
Tetralin	–	10	81.2	–
Tetralin	L-Carvone[i]	1	87.9	–
Turpentine	D-Pulegone[g]	30	64.1	**6** (5.0) **7** (4.8)
Turpentine	Carvone[i]	24	60.0	–
Turpentine	Spearmint oil	28	67.1	–
Diphenyl ether	–	1	56.0	–
Diphenylmethane	–	0.5	65.6	**9** (2.0)
Quinoline	–	0.5	73.2	–

[a] Yields of tryptamine 45–93% were obtained in all of these methods. The use of ketone catalysts accelerated the decarboxylation procedure in all cases which, however, also resulted in significant by-product formation. This was particularly the case when cyclohexanol was used as the solvent.
[b] Reaction time.
[c] Acetone.
[d] Butan-2-one.
[e] Pentan-2-one.
[f] Pentan-3-one.
[g] (*R*)-5-methyl-2-(1-methylethylidene)-cyclohexanone.
[h] 2-Cyclohexen-1-one.
[i] (*R*)-5-isoprenyl-2-methyl-cyclohex-2-enone.

The addition of ketones led to shorter reaction times compared with heating at reflux in solvents alone. For example, time to complete decarboxylation of tryptophan in cyclohexanol was 100 h, whereas addition of a ketone catalyst caused a rate acceleration between two- and five-fold. 2-Cyclohexen-1-one enabled decarboxylation in 1 h, in broad agreement with Hashimoto et al. who reported 1.5 h [16]. In tetralin alone, the procedure was completed in 10 h, whereas the addition of ketones led to completion in 0.5–1 h. Higher temperatures did not require the use of catalysts: for example, decarboxylation in diphenylmethane was

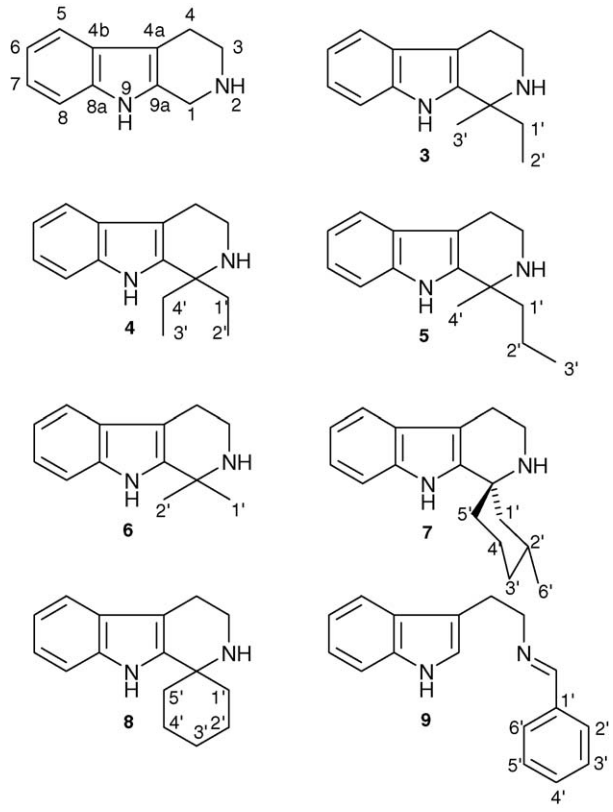

Fig. 2. 1,1-Disubstituted-tetrahydro-β-carbolines **3–8** have been identified as the major by-products during decarboxylations, particularly when cyclohexanol was used as the solvent. *N*-Benzylidene-tryptamine **9** was found during decarboxylation in diphenylmethane, possibly in the presence of benzaldehyde contamination of the solvent. Note the different numbering system when compared with tryptamines.

achieved in 20 min. Spearmint oil, L-carvone and D-pulegone effected a rate acceleration comparable with the addition of simple ketones. The ketone catalysis of the reaction involved the formation of an imine, as shown in Fig. 1.

GC–IT-MS analysis of products from reactions carried out using cyclohexanol as the solvent, showed they were contaminated with 1,1-disubstituted-THBCs [*up to* 48%, Table 2] that resulted from reaction with some of the ketone catalysts. THBC impurities were isolated by flash chromatography, characterised by 1D- and 2D NMR and subjected to mass spectrometric analysis. The inferred identities were confirmed by synthesis of the compounds (Section 2.4). These showed identical characteristics in all respects to the identified THBC impurities (Fig. 2 for structures).

Fig. 3(A) shows a representative GC–IT-MS chromatogram of the product, after thermolysis of Trp (**1**) in cyclohexanol with pentan-2-one as the catalyst. Three significant peaks were observed that indicated the presence of two by-products at 10.05 and 13.99 min. These were identified as THBC-derivatives **5** and **8**, respectively. The tryptamine product **2** eluted at 8.3 min. Tryptamine produced its characteristic EI-induced mass spectrum with a hydroquinolinium peak at *m/z* 131 and a quinolinium base peak at *m/z* 130 [30,31] (Fig. 4(A1)). Under CI-IT-MS-MS conditions (Fig. 4(A2)) base peak formation at *m/z* 144 was presumably effected via loss of ammonia from $[M+H]^+$ at *m/z* 161. Three additional compounds of minor abundance were detected (inset Fig. 3(B)). Based on their EI- and CI-MS–MS spectra (Fig. 4) they were tentatively identified as monoalkylated tryptamine derivatives **5a** and **8a** and as the imine **5b**. With the exception of the spirocyclohexane-THBC **8** [32] (Fig. 4(F1)), mass spectrometric data on 1,1-disubstituted-THBCs are sparsely available in the literature. It was therefore of interest to carry out a more detailed inspection of the fragmentation behaviour.

3.1. Electron ionisation ion trap mass spectrometry

The identified 1,1-disubstituted-THBC side products can be grouped into compounds with an open chain substitution pattern at C1 (**3–6**) and a closed ring, spirocyclohexane motif (**7** and **8**). EI-IT-MS of the open chain analogues (summarised in Table 3) resulted predominantly in fragments that can be rationalised by loss of an alkyl radical via α-cleavage (radical-site-initiation). Loss of a methyl radical for example, would then be responsible for the base peak at *m/z* 185 for the 1,1-dimethyl-THBC **6** as exemplified in Fig. 4(G1).

Correspondingly, asymmetrically substituted derivatives at C1 showed a preferential loss of the larger group that formed the base peak. For example, both 1-Me-1-Et-THBC **3** and 1-Me-1-Pr-THBC **5** (Fig. 4(B1)) showed a base peak at *m/z* 185 due to the loss of C_2H_5 and C_3H_7 radicals, respectively. A cleavage of a methyl radical however, was observed to a minor extent also, leading to the formation of *m/z* 199 (**3**, 12%) and *m/z* 213 (**5**, 17%), respectively. The observation of alkyl-cleavages was consistent with Coutts et al. [33] and Gynther [31] who reported on the EI mass spectra of several 1-monosubstituted-THBCs. 1-Ethyl-monosubstituted-THBCs have also been reported to show a dominating C_2H_5 radical cleavage [34].

EI mass spectra of both spirocyclohexane-THBCs **7** and **8** are shown in Fig. 4(H1 and F1) and summarised in Table 3. A suggested fragmentation for key-ions of derivative **7** is based on ring opening via α-cleavage. Subsequent alkyl losses (43 Da and 29 Da) may then be responsible for the base peak formation at *m/z* 197 and a species at *m/z* 211, respectively. A prominent fragment at *m/z* 184 was also found, which was in agreement with the observations of Rodríguez and Gil-Lopetegui [32].

The methylated spirocyclohexane-THBC **7** (Fig. 4(H1), Table 3) that was formed during the decarboxylation of Trp in the presence of D-pulegone showed an EI-IT-MS with common key-fragments that showed identical exact masses (*not shown*). The base peak was observed at *m/z* 211. The ion at *m/z* 239 may be rationalised by a loss of a methyl group.

It is noteworthy that when diphenylmethane was used as the solvent an additional peak at 16.4 min (*not shown*) was observed in the GC–IT-MS chromatogram. CI-IT-MS–MS revealed a protonated molecule at 249 Da that was originally thought to be 1-phenyl-THBC. Synthesis of that standard, employing tryptamine and benzaldehyde, showed similar retention time and identical CI-IT-MS-MS, i.e. two major fragments at 220 and 144 Da, respectively. Inspection of the EI-IT-MS however, gave a totally different spectrum and therefore a different compound.

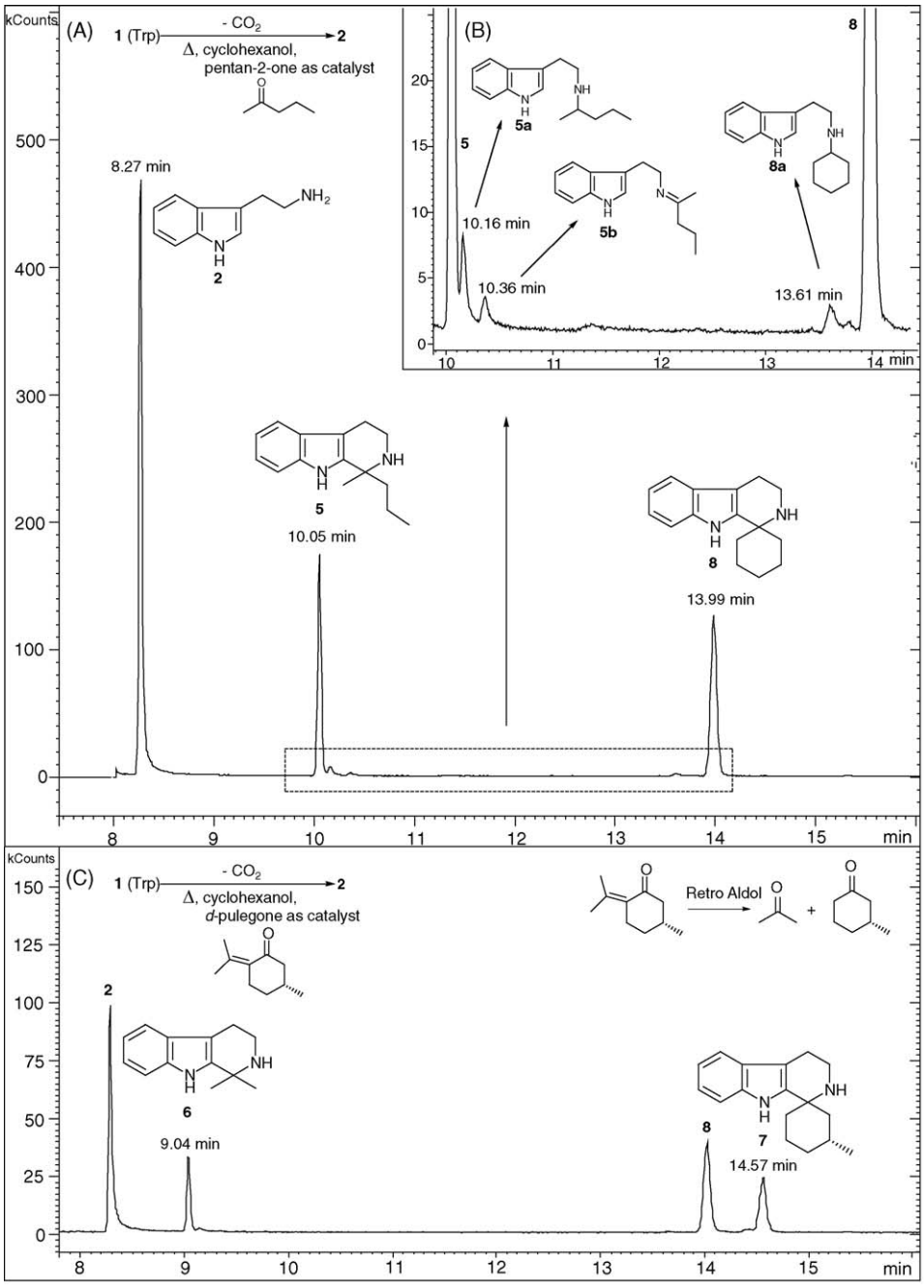

Fig. 3. Representatative example of thermal decarboxylation of tryptophan (Trp) (**1**) in cyclohexanol and its conversion to tryptamine (**2**). (A) A GC–IT-MS chromatogram of the product after catalytic conversion with pentan-2-one; two impurities of major significance were identified as 1,1-disubstituted-THBCs **5** and **8** that indicated the participation of the catalyst and the solvent. (B) Three chromatographic peaks were assigned to tryptamine derivatives **5a**, **5b** and **8a**. (C) When catalytic amounts of D-pulegone were used, in addition to the presence of **2**, the detection of THBCs **6** and **7** indicated the influence of the catalyst. In all cases, THBC formation was thought to occur by Pictet-Spengler cyclisation via involvement of the ketone catalysts. Compounds **6** and **7** may have been formed under similar conditions after degradation of D-pulegone by a retro-aldol mechanism; see text for details. The corresponding EI-IT-MS and CI-IT-MS-MS mass spectra are shown in Fig. 4.

The 1-phenyl-THBC spectrum displayed a molecular ion base peak at *m/z* 248 and three major fragments at 219 (47%), 218 (63%) and 171 Da (30%), respectively. The by-product instead showed a *m/z* 130 base peak with its molecular ion at 248 (15%). Three key-ions were observed at *m/z* 103 (11%, loss of HCN from *m/z* 130), *m/z* 91 (31%) and *m/z* 77 (17%), respectively. The fact that the base peak appeared at 130 Da gave reason to believe that no cyclisation had occurred in that compound, i.e. showing a feature that is typical for tryptamines (Fig. 4(A1) and Table 3 for the mass spectrum of tryptamine **2**).

The presence of an identical mass (248 Da) provided further indication for the presence of an imine **9**, *N*-benzylidene-tryptamine (Fig. 2). From a forensic viewpoint (e.g. *possible lack of high vacuum pumps for solvent evaporation under reduced*

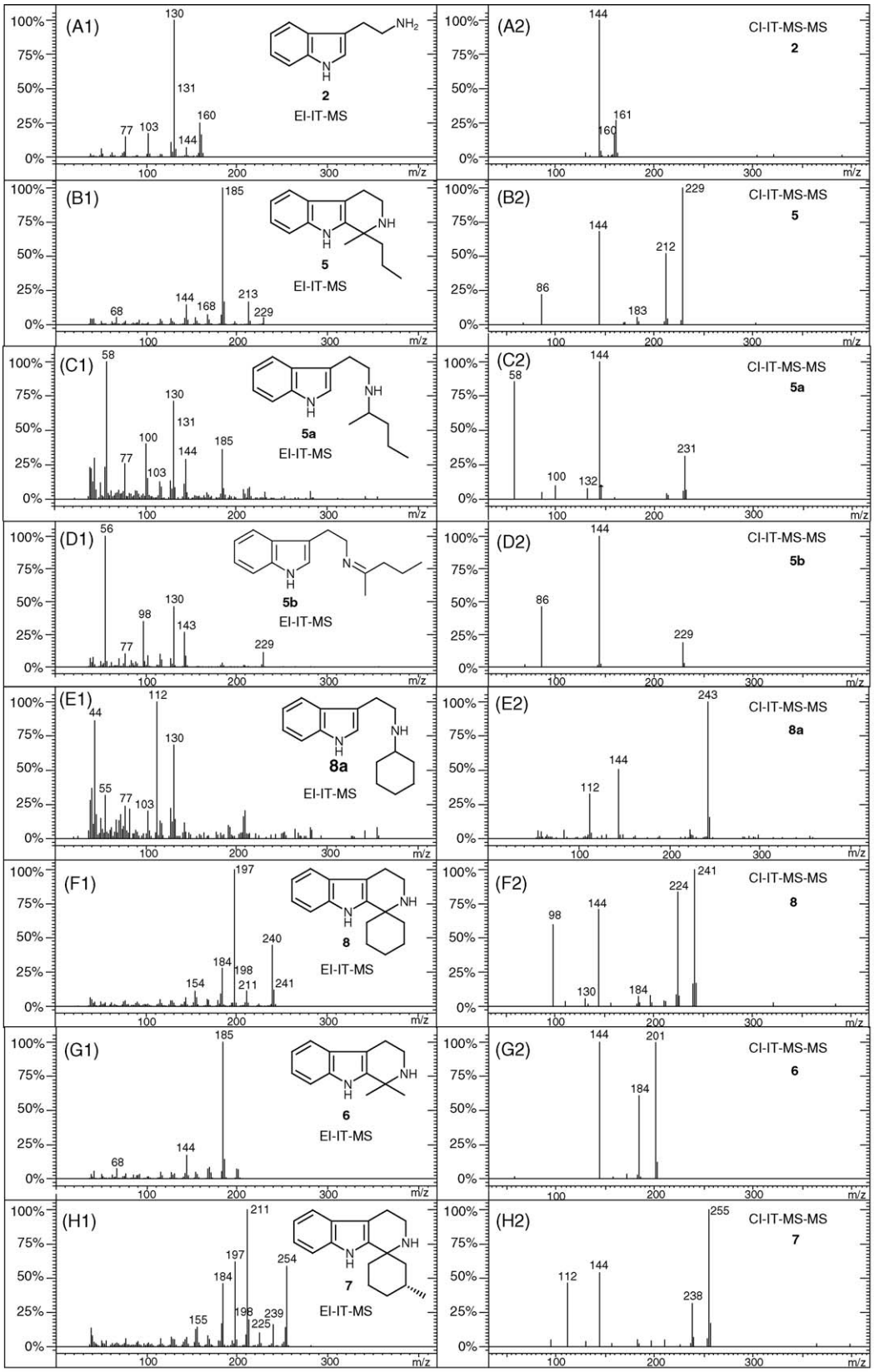

Fig. 4. Representative mass spectra that correspond to the GC–IT-MS chromatogram in Fig. 3. Left column (A1–H1): EI-IT-MS mass spectra. Right column (A2–H2): CI-IT-MS-MS mass spectra. Open-chain 1,1-disubstituted-THBCs such as **5** and **6** showed primarily alkyl loss under EI conditions. Ring-substituted THBCs **7** and **8** were also thought to show alkyl loss after ring-opening. Monosubstituted tryptamine derivatives such as **5a**, **5b** and **8a** were identified based on the similarity to tryptamine **2**. Of particular importance under EI conditions was the observation of *m*/*z* 131 and *m*/*z* 130. Compounds of the same molecular weight, e.g. **5** and **5b**, showed different fragmentation patterns under EI (B1 and D1) and CI (B2 and D2) conditions, respectively. See text for details.

Table 3
EI-IT-MS spectra of identified THBCs, by-products **3–8** and *N*-benzylidene tryptamine **9** during decarboxylation

No.	Relative intensity (%) of generated key ions			
	t_R[a] (min)	M[b]	Base peak	Others *m/z*
2	8.27	160 (25)	130	144 (7), 131 (53), 103 (17), 77 (15)
3	9.55	215 (11)	185	199 (12), 168 (7), 144 (16), 68 (6)
4	10.01	229 (6)	199	144 (14)
5	10.05	229 (5)	185	213 (17), 168 (7), 144 (15), 68 (6)
6	9.04	201 (7)	185	200 (7), 168 (7), 144 (17)
7	14.57	254 (59)	211	239 (16), 198 (26), 197 (62), 184 (46), 155 (14), 154 (14)
8	13.99	240 (48)	197	211 (11), 184 (29), 154 (12), 39 (8)
9	16.48	248 (15)	130	118 (9), 103 (9), 91 (31), 77 (17)

[a] GC–MS retention times.
[b] Open-chain THBCs **3–6** produced $M+1$ ions, presumably due to ion–molecule reactions in the ion trap.

pressure in a clandestine synthesis scenario), a work-up procedure could involve an acid-base extraction as indeed is often discussed on web sites. It seems therefore possible that especially under acidic conditions, Pictet-Spengler cyclisation of compound **9** could occur in order to form 1-phenyl-THBC that would be an impurity in the tryptamine product **2**.

The structural assignment of impurities **5a**, **5b** and **8a** shown in the inset of Fig. 3(B) were based on the appearance of two characteristic fragments in their EI-IT-MS. In the mass spectra of compounds **5a** (Fig. 4(C1)) and **8a** (Fig. 4(E1)), that were identified as *N*-monoalkylated tryptamines, these characteristic ions were exemplified by the combined presence of the hydroquinolinium/quinolinium peaks at *m/z* 131 and *m/z* 130 [31] with the presence of iminium ions (CH_2=N^+HR) at *m/z* 100 (**5a**, Fig. 4(C1)) and *m/z* 112 (**8a**, Fig. 4(E1)) that were also typically observed for *N*,*N*-dialkylated tryptamines via β-cleavage [35].

Secondary fragmentations of the iminium ion at *m/z* 100 (**5a**, Fig. 4(C1)) may correspondingly account for the base peak at *m/z* 58 in that EI-induced mass spectrum. The imine derivative **5b** (Fig. 3(D1)) did show the aromatic *m/z* 130 ion as well. There is some indication in the literature that these imines may show a corresponding iminium ion structure, in this case at *m/z* 98, that may be represented by CH_2=N^+=Me(Pr) [32]. Both imine **5b** and its THBC counterpart **5** have the same nominal mass (228 Da) but can be conveniently distinguished by their mass spectra (Fig. 3(D1) versus Fig. 3(B1)).

3.2. Chemical ionisation ion trap mass spectrometry

Chemical ionisation is particularly useful for the determination of the protonated molecule $[M+H]^+$ at the expense of reduced fragmentation [36]. In order to increase the information content, a tandem experiment was required. This was achieved by subjecting $[M+H]^+$ to CID within an ion trap mass spectrometer using methanol as a liquid CI reagent [37]. The application of a moderate, non-resonant excitation amplitude (30 V) generated a sufficient number of product ions while preserving a significant signal intensity of the protonated molecule.

As expected, CI tandem mass spectra of compounds **2–9**, Fig. 4(A2–H2), were found to be much simpler when compared to their EI spectra. Table 4, gives a summary of the intensity of these key ions. Loss of ammonia $[M+H\text{-}17]^+$ was prominent in all THBCs, presumably effected by elimination via α-cleavage. Two further ions common to all the THBCs, under study, were also observed at *m/z* 144 and a compound-specific species that depended on the 1,1-substitution pattern.

3.3. Mechanism of impurity formation

The role of the ketone catalyst is proposed to involve the formation of an imine (*imine* 1, Fig. 1). This then induces loss of carbon dioxide from the carboxylic acid group as the first formed anion can be resonance stabilised by conjugation with the C=N double bond. Protonation gives *imine* 2 (Fig. 1), which upon hydrolysis gives tryptamine **2** and the ketone catalyst (Fig. 1). The formation of THBC derivatives **3–8** detected during the decarboxylation of Trp can be due to a Pictet-Spengler reaction utilising *imine* 2 (Figs. 1 and 5A). Although acid catalysts and protic solvents have been used routinely for Pictet-Spengler reactions, they are also known to occur in non-acidic aprotic media. Under these conditions the electrophilic nature of the imine double bond has been observed to be the driving force for cyclisation (*for a review*, *see* [38]). The possibility of *imine* 1 (Fig. 1) to form the THBC-3-carboxylic acid derivative was of interest but this was not detected under the conditions used. One might furthermore have expected transamination side reactions

Table 4
CI-IT-MS–MS spectra of tryptamine product **2**, identified THBC by-products **3–8** and *N*-benzylidene tryptamine **9** during decarboxylation of tryptophan

No.	Relative intensity (%) of generated key ions		
	$[M+H]^+$[a]	Base peak	Others *m/z*
2	161 (38)	144	131 (9), 94 (6)
3	215 (100)	215	198 (52), 144 (47), 72 (6)
4	229 (100)	229	212 (49), 183 (7), 144 (31), 86 (16)
5	229 (100)	229	212 (52), 183 (6), 144 (68), 86 (22)
6	201 (99)	144	184 (61)
7	255 (100)	255	238 (31), 144 (54), 112 (46)224 (59)
8	241 (100)	241	197 (7), 144 (56), 98 (45)
9	249 (43)	144	220 (34), 232 (3), 206 (5), 106 (4)

[a] Protonated molecule $[M+H]^+$ using methanol as the liquid CI reagent. The excitation amplitude was set to 30 V.

[23], with the formation of indole-3-acetaldehyde, but neither was observed under these conditions.

The use of cyclohexanol as the high boiling solvent with an unactivated ketone catalyst (e.g. *acetone and methyl ethyl ketone*) led to substantial THBC formation, in particular THBC **8** which was found with all unactivated ketone catalysts; as a result lower yields of tryptamine were observed (Table 2). A significantly lower yield of **8** and a higher yield of tryptamine was observed however, when the activated ketone 2-cyclohexen-1-one was employed as the catalyst. With no catalyst, formation of **8** and the yield of tryptamine were both reduced significantly. The formation of **8** in the presence of an aliphatic ketone suggested interaction of the catalyst with the alcohol solvent. That cyclohexanol may have been responsible for the significant formation of THBCs appeared conceivable, as when tetralin was used as solvent, significantly lower amounts of these impurities were formed (Table 3).

A Pictet-Spengler mechanism would require a carbonyl source such as a cyclohexanone to produce **8**. GC–MS analysis of the cyclohexanol revealed no ketone impurity, implying therefore formation of cyclohexanone during the reaction. Fig. 5(A) provides a possible mechanism for the release of cyclohexanone, based on a hemiaminal intermediate that could be formed by reaction of imine **5b** (Fig. 5A) and cyclohexanol. Support for this putative mechanism was the presence of monoalkylated tryptamine **5a**. Mass spectral analysis gave strong indications that **5a** was present, Fig. 3(B), and further analysis is underway.

3.4. NMR spectroscopy

For decarboxylation of Trp **1** with D-pulegone as the catalyst, Fig. 3(C), thermolysis in cyclohexanol gave three major THBC impurities **6**, **7** and **8**. Detection of 1,1-dimethyl-THBC **6** and the methylated spirocyclohexane-THBC **7**, indicated the degradation of D-pulegone. A mechanism is shown in Fig. 5(B), comprising a Michael-addition of water to D-pulegone followed by a retro-Aldol reaction. The products are acetone and 3-methylcyclohexanone (3MC), which would then react with tryptamine **2** to give the imines for the Pictet-Spengler cyclisation. Authentic samples of THBC **6** and **7** were prepared under Pictet-Spengler conditions using tryptamine, trifluoroacetic acid and D-pulegone (Section 2.4).

The isolated compounds from the decarboxylation of tryptophan and authentic standards prepared by the alternative route were identical by both MS and NMR. The NMR spectra of compounds **3**, **6**, **8** [39] and **9** [40] have been reported in the literature and our data are in complete agreement. The spectra of the novel compounds **4**, **5** and **7** all contain distinctive features indicative of the substitution pattern at the C1 position. 1,1-Diethyl THBC **4** has a quartet at δ 1.79 (4H, J 7.4 Hz) and a triplet at δ 0.87 (6H, J 7.5 Hz) for the ethyl substituents.

1-Methyl-1-propyl THBC **5** gives a singlet at δ 1.44 (3H) for the 1-methyl substituent. Due to the unsymmetrical substitution, **5** has a chiral centre, which makes the protons of each of the two methylene groups non-equivalent resulting in geminal coupling, however the complex patterns could not be completely assigned for the 1-propyl group: δ 0.90 (3H, t, J 7.3 Hz, Me-CH_2), 1.32–1.18 (1H, m, Me-CHAHB), 1.47–1.33 (1H, m, Me-CHAHB) and 1.79–1.70 (2H, m, Me-CH_2-CH_2). A complex pattern was also observed for the ethyl group of 1-ethyl-1-methyl THBC **3**, due to prochirality of the methylene protons, and here the pattern for the methylene group was assigned: δ

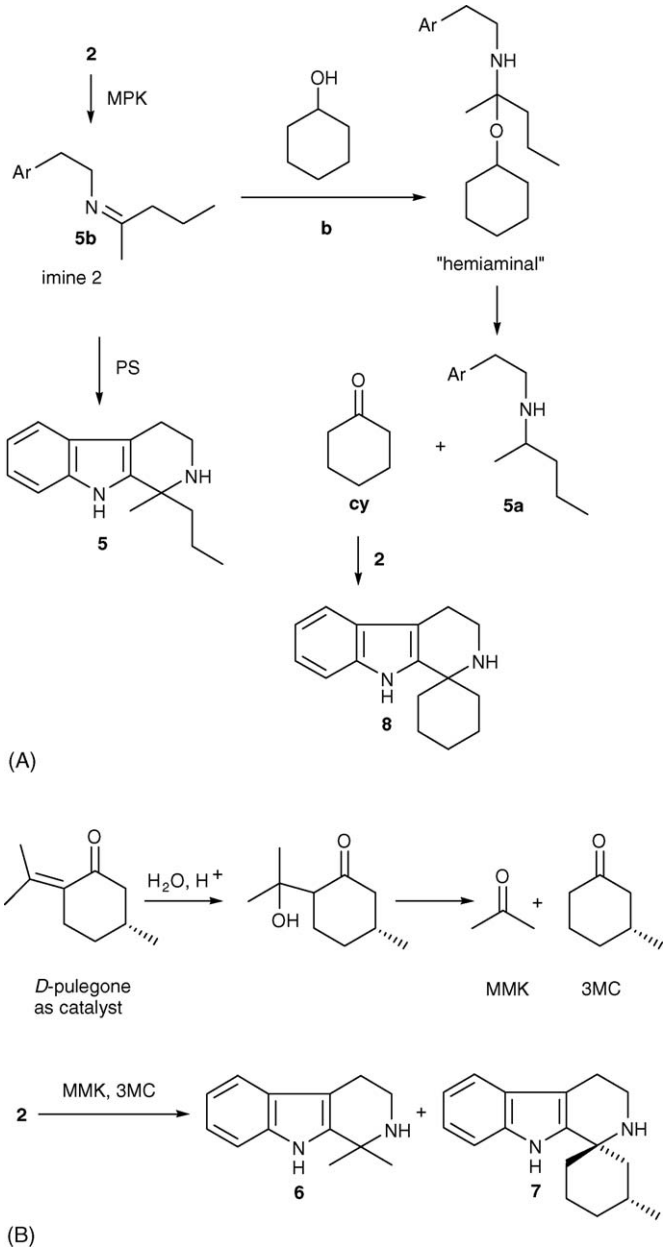

Fig. 5. (A) Imine formation (**5b**) from tryptamine **2** in cyclohexanol, catalysed by methyl-propyl-ketone (in this example) led to the detection of THBC **8** and a catalyst-derived THBC (**5** in this example). A possible mechanism is shown in pathway **b** via hemiaminal formation and subsequent generation of cyclohexanone (cy), which could then participate in a Pictet-Spengler cyclisation. The detection of concomitant product **5a** is supportive of this mechanism (see also Fig. 3(A and B) for chromatogram). (B) A retro-aldol mechanism may have been responsible for the detection of THBCs **6** and **7** when D-pulegone was used as the catalyst for thermolytic decarboxylation of tryptophan. Here, the release of acetone (MMK) and 3-methylcyclohexanone (3MC) may similarly give rise to a Pictet-Spengler reaction (refer also to Fig. 3(C) for chromatogram).

1.80 (dq, 1H, J_{gem} 11.5, J 7.5) and 1.87 (dq, 1H, J_{gem} 11.5, J 7.5).

The spectrum recorded for spiro-3-methylcyclohexane THBC **7** contained a similar coupling pattern to that observed for the unsubstituted spirocyclohexane THBC **8**. The distinguishing feature of the 3-methyl analogue **7** is the presence of a clean doublet at δ 0.94 (3H, J 6.0 Hz) for the 3-methyl substituent, supporting the formation of only 1 diastereoisomer. The chirality at C-3 is fixed by pulegone, therefore the most stable chair conformation of the preferred diastereoisomer of product **7**, with both the methyl and indole rings adopting equatorial positions, is shown in Fig. 2.

3.5. Possible implications of the THBC impurities

The presence of THBC impurities may lead to complex and unpredictable psychopharmacological interactions, either alone or in combination with the main products and precursors. 1-Monosubstituted tetrahydroharmine (7-methoxy-1-Me-THBC, THH) for example, is known for its interactions with monoamine oxidase and the 5-HT reuptake transporter and was found to be involved in the pharmacology of hallucinogenic plant mixtures such as *Ayahuasca*, via increased availability of 5-HT (*for a review*, *see* [41]). Although THH itself is not hallucinogenic [42], a number of THBC derivatives were observed to show significant affinities at a range of binding sites, such as imidazoline I_2 [43,44] and 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors [45,46]. Particularly 5-$HT_{2A/2C}$ receptors are thought to be implicated in the psychopharmacological profiles of the classical hallucinogenic compounds like lysergic acid diethylamide (LSD), 2,5-dimethoxy-4-iodoamphetamine (DOI) or *N*,*N*-dimethyltryptamine (DMT, that also includes 5-HT_{1A} agonism) [5].

Several THBC derivatives and their aromatised counterparts are present in plants, foodstuffs and mammals, and are known to have a wide range of biological activity [47–50]. The spirocyclohexane-THBC **8** *komavine* has been isolated from plants of the *Nitraria* genus and its pharmacology is unknown [51]. Some of these plants have been reported to show vasoactive, serotonin-like activity, which however was attributed to other alkaloids than **8** [52]. Any biological activity of the novel 1,1-disubstituted compounds identified in this study remains to be investigated.

4. Conclusions

The study has revealed two major analytical features of the thermolytic decarboxylation of tryptophan. There is formation of THBC derivatives that may originate from reaction with both the solvent, e.g. cyclohexanol, and with the ketone catalyst. These impurities, often at significant levels (Table 2), will yield a useful profile for identification of synthetic pathway and likely reagents employed, particularly a "fingerprint" of the ketone catalyst.

Detailed GC–MS examination has revealed possible mechanisms for the decarboxylation and THBC formation. Under EI conditions, MS characterisation of the THBCs indicates the predominance of alkyl cleavage. Authentic samples of the THBC derivatives have been prepared from tryptamine via Pictet-Spengler cyclisation.

This seemingly simple thermolytic decarboxylation reaction ironically underlines the problems associated with illicitly manufactured drugs and precursors that may contain significant levels of impurities about which little or nothing of their toxicity is known. The possible interaction of the contaminants and the principal product in the human body, further clouds the effects of the drug composition, and may put the user at mortal risk.

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