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Forensic Science International 178 (2008) 162–170
Forensic Science International
www.elsevier.com/locate/forsciint

Halogenated solvent interactions with *N*,*N*-dimethyltryptamine: Formation of quaternary ammonium salts and their artificially induced rearrangements during analysis

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Received 20 December 2007; received in revised form 16 March 2008; accepted 18 March 2008
Available online 1 May 2008

Abstract

The psychoactive properties of *N*,*N*-dimethyltryptamine (DMT) **1a** are known to induce altered states of consciousness in humans. This particular attribute attracts great interest from a variety of scientific and also clandestine communities. Our recent research has confirmed that DMT reacts with dichloromethane (DCM), either as a result of work-up or storage to give a quaternary *N*-chloromethyl ammonium salt **2a**. Furthermore, this was observed to undergo rearrangement during analysis using gas chromatography–mass spectrometry (GC–MS) with products including 3-(2-chloroethyl)indole **3** and 2-methyltetrahydro-β-carboline **4** (2-Me-THBC). This study further investigates this so far unexplored area of solvent interactions by the exposure of DMT to other halogenated solvents including dibromomethane and 1,2-dichloroethane (DCE). The *N*-bromomethyl- and *N*-chloroethyl quaternary ammonium derivatives were subsequently characterised by ion trap GC–MS in electron and chemical ionisation tandem MS mode and by NMR spectroscopy. The DCE-derived derivative formed at least six rearrangement products in the total ion chromatogram. Identification of mass spectrometry generated by-products was verified by conventional or microwave-accelerated synthesis. The use of deuterated DCM and deuterated DMT **1b** provided insights into the mechanism of the rearrangements. The presence of potentially characteristic marker molecules may allow the identification of solvents used during the manufacture of controlled substances, which is often neglected since these are considered inert.

Keywords: Tryptamines; Hallucinogens; Forensic; Analytical chemistry; Mass spectrometry

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1. Introduction

The neuroactive properties of *N*,*N*-dimethyltryptamine (DMT) **1a** (Fig. 1) can lead to the manifestation of altered states of consciousness in humans which is currently believed, at least in part, to involve serotonergic neurotransmission [1,2]. The increasing interest in tryptamine-based hallucinogens and their impact on the human mind and body arises from the search for a variety of medical applications [3]. The DMT core structure serves also as a template for a variety of derivatives used for the treatment of several clinical conditions. One such example is the 5-HT$_{1B/1D}$ agonist Sumatriptan, a methylsulfonamide derivative of DMT used for the management of migraine attacks [4]. 5-Methoxy-2-phenyl-DMT (BGC20-761) has recently been probed as a potential enhancer for long-term memory in mature adult rats and in young rats that have been exposed to scopolamine, possibly *via* 5-HT$_6$ receptor antagonism [5]. N_1-Arylsulfonyl-substituted derivatives of DMT have also been found to interact strongly with 5-HT$_6$ receptors and are investigated for their potential to treat obesity and neuropsychiatric disorders [6,7].

On the other end of the spectrum is the attempt to supply these derivatives, either within a clandestine environment or *via*

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doi:10.1016/j.forsciint.2008.03.013

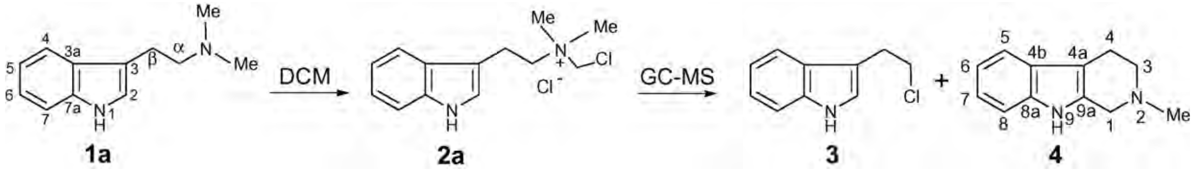


Fig. 1. A previous investigation revealed that exposure of *N*,*N*-dimethyltryptamine **1a** (DMT) to dichloromethane (DCM) led to the precipitation of quaternary *N*-chloromethyl-DMT chloride **2** which was analytically accessible by HPLC analysis. This derivative was not detectable under GC–MS conditions, but instead rearrangement was observed during analysis to form 3-(2-chloroethyl)indole **3** and 2-methyltetrahydro-β-carboline **4** [13].

the purchase of structurally modified analogues from internet websites. A large number of synthetic routes to tryptamines are documented in the literature and the identification of potentially toxic contaminants present in manufactured preparations is therefore required to assist clinical and forensic investigations [8–11].

Recent reports [12,13] indicated that DMT **1a** was found to be sensitive to contact with dichloromethane (DCM), either during storage or short-term exposure during work-up which led to the identification of *N*-chloromethyl DMT chloride **2a** (Fig. 1). When **2a** was subjected to gas chromatography–mass spectrometry (GC–MS) analysis however, rearrangements were observed instead which resulted in the absence of **2a** and detection of 3-(2-chloroethyl)indole **3** and 2-methyltetrahydro-β-carboline (2-Me-THBC) **4** in the total ion chromatograms [13] (Fig. 1). Although it underscored the complementary value of LC–UV/MS analysis, where **2a** was detectable without rearrangements being observed, it was also deemed necessary to probe this artificially induced analyte–solvent interaction. The rationale behind this study was based on the fact that GC–MS is often used as the major tool for the identification of impurities in illegally produced compounds, for example, in an attempt to identify a synthetic route of an illegally manufactured drug. The presence of artificially produced **3** and **4** during GC–MS analysis would introduce some potential for misinterpretation since these entities would erroneously be assumed to be synthesis-related.

This study probes the impact of alternative halogenated solvents such as 1,2-dichloroethane (DCE) and dibromomethane (DBM) on by-product formation and rearrangement under GC–MS conditions. Synthesis and further characterisation of deuterated derivatives were included in order to gain mechanistic insights into the nature of the observed rearrangements. GC–MS analysis was carried out in electron (EI-MS) and chemical ionisation (tandem) MS mode (CI-MS–MS). NMR spectroscopy was also employed.

2. Experimental

2.1. Materials

Silica gel for flash chromatography (particle size 40–63 μm), silica gel aluminium TLC plates and reagents and solvents used for HPLC analysis were from VWR (UK). All other solvents and reagents were from Aldrich (UK) and were of analytical grade or equivalent if available.

2.2. Instrumentation

The investigation employed gas chromatography combined with electron- and chemical ionisation ion trap (single and double stage) mass spectrometry (GC-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 1 ml/min (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: 90 °C and held for 2 min, then heated at 20 °C/min to 260 °C and held at this temperature for 10.5 min; total run time was 21 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 and target TIC: 5000 counts. CI-MS–MS spectra were obtained by collision induced dissociation of the protonated molecule $[M+H]^+$ within the ion trap, using helium, by application of a waveform excitation amplitude in the non-resonant mode. Excitation storage level was set to 48.0 *m*/*z*. The excitation amplitude was set to 20 V. The number of ions in the trap was controlled by an automatic gain control function.

NMR spectra were recorded using a Bruker DPX 300 or Avance 300 at 300.1 MHz (^1H NMR) or 75.5 MHz (^{13}C NMR). The solvents used are indicated in the synthesis section. When d_6-DMSO was used, chemical shifts were determined relative to the residual solvent peak at $\delta = 2.51$ (^1H NMR) and $\delta = 39.6$ ppm (^{13}C NMR). In case of other solvents, chemical shifts are reported relative to TMS at $\delta = 0$ ppm. NMR spectra were obtained by ^1H, proton decoupled ^{13}C, DEPT-135 and DEPT-90, HSQC and HMBC experiments.

LC–MS analysis used a Waters Alliance 2695 HPLC separations module coupled to a Micromass LCT orthogonal acceleration time-of-flight (TOF) mass spectrometer (Waters, UK) equipped with an electrospray ionisation source in positive mode. Flow rate was set at 0.8 ml/min with a 10:1 post-column split. A flow of 80 μl/min was infused into the ESI source and the remaining flow was directed to a Waters 486 UV detector set at 280 nm. The column temperature was set by air conditioned surroundings at 21 °C. The aqueous mobile phase A consisted of 40 mM ammonium formate and 0.1% formic acid (pH 3.80). The organic component B was 0.1% formic acid in methanol. The mobile phase composition was set to 30% B and linearly increased to 90% B within 15 min, held for 5 min and returned to 30% B over 3 min. The column was left to equilibrate before the next injection for 12 min. Total run time was 35 min; total acquisition time was 20 min. The column used was a Phenomenex Synergi Max-RP (80 Å 250 mm × 4.6 mm, 4 μm). The sample was prepared at 1 mg/ml and 20 μl was injected onto column. Mass drift calibration and determination of exact masses were carried out with a sodium formate solution. Operation settings were: capillary voltage: 3000 V, sample cone voltage: 30 V, RF lens: 200 V, desolvation temperature: 150 °C, source temperature: 100 °C, acceleration: 200 V, cone gas flow: 22 l/h, desolvation gas flow: 602 l/h.

Microwave accelerated syntheses were carried out using a monomode CEM Explorer (UK) microwave system. Operation settings were: microwave power 200 W, temperature for synthesis of DMT derivatives 150 °C (140 °C for synthesis of tetrahydro-β-carbolines), maximum pressure 280 psi, ramp time 5 min, hold time 20 min. Reactions were performed in glass microwave tubes, closed with *Intellivent* caps (CEM) and contents of the vessel were continuously stirred by a Teflon-coated magnetic stirrer bar (10 mm × 3 mm). Temperature, pressure and power profiles were monitored using the ChemDriver software version 3.6.0.

*2.3. Microwave-accelerated synthesis of DMT derivatives (**1a** and **1b**)*

The identities of all synthesised compounds were confirmed by direct infusion ESI-TOF-MS exact mass measurements and NMR spectroscopy. Since they were needed only as reference standards no attempt was made to optimize the conditions during synthesis.

N,*N*-Dimethyltryptamine (DMT) **1a**: Tryptamines were synthesised by the reduction of indole-3-yl-*N*,*N*-dimethylglyoxalylamide with lithium aluminium hydride (LAH) [14] that was available from previous work [15]. To a microwave tube was added the stirrer bar and indole-3-yl-*N*,*N*-dimethylglyoxalylamide (216 mg, 1.0 mmol). Anhydrous THF (3 ml) was added under a stream of nitrogen and placed on ice. An ice-cold LAH solution (3 ml, 2 M in THF, 6 mmol) was added dropwise under nitrogen with vigorous stirring. The tube was capped after generation of hydrogen had ceased. The reaction mixture was subjected to the microwave system under the conditions described above. At the end of the reaction the mixture was transferred into a conical flask and cooled on ice. The tubes were then rinsed with 3 × 8 ml THF and the washings added to the flask. Excess hydride was destroyed by the dropwise addition of 5 ml water, followed by 4 ml 20% NaOH and 5 ml water. The volume of THF was increased by the addition of 20 ml. The precipitated inorganic salts were removed by filtration and washed with 30 ml THF. The filtrate was evaporated under reduced pressure and the resulting oily residue was dissolved in 60 ml chloroform, 1 ml 20% NaOH and 10 ml water and thoroughly shaken in a separating funnel. The organic layer was separated and two additional chloroform extractions (20 ml) from the remaining alkaline aqueous phases were carried out. The combined organic fractions were then pooled and washed two times with 40 ml distilled water and once with 40 ml saturated aqueous NaCl. The organic phase was evaporated under reduced pressure and the resulting product was purified by flash chromatography ($CHCl_3$/MeOH/NH_4OH: 8/2/0.1) to yield 154 mg (82%, 0.82 mmol) **1a** free base as a white solid. All analytical data were identical to those previously published [15].

N,*N*-Dimethyl-[α,α,β,β-d_4]-tryptamine (d_4-DMT) **1b**: The synthetic procedure was essentially carried out as described above except that a slurry of 6 mmol lithium aluminium deuteride (252 mg in 3 ml anhydrous THF) was added instead of the LAH solution. A pale yellow solid of free base **1b** was obtained in 76% yield (146 mg, 0.76 mmol). ^{1}H NMR ($CDCl_3$): 8.45 (1H, brs, NH), 7.60 (1H, d, H-4, *J* 7.2 Hz), 7.31 (1H, d, H-7, *J* 7.5 Hz), 7.17 (1H, td, H-6, *J* 7.7, 1.0 Hz), 7.10 (1H, td, H-5, *J* 7.5, 1.2 Hz), 6.96 (1H, d, H-2, *J* 2.3 Hz), 2.35 (6H, s, CH_3). ^{13}C NMR: 136.4 (C-7a), 127.5 (C-3a), 121.8 (C-6), 121.6 (C-2), 119.1 (C-5), 119.7 (C-4), 113.9 (C-3), 111.2 (C-7), 45.3 (CH_3). HRESIMS-theory: 193.1643; observed: 193.1632.

*2.4. General procedure for the synthesis of N-halogenated-alkyl DMT chloride/bromide derivatives (**2a–2g**)*

DMT or d_4-DMT (1.1 mmol each) was dissolved in the corresponding halogenated solvent, i.e. dichloromethane (DCM or d_2-DCM), dibromomethane (DBM) or 1,2-dichloroethane (DCE) to give a concentration of 10 mg/ml. The solution was left sealed and stored at ambient temperatures for 0.5–4 weeks until precipitation of the product, either as an oil or solid, was complete. The crystalline white needles were filtered, washed with the solvent and dried under vacuum over P_2O_5. Melting points could not be determined since attempts to recrystallise the solids were not successful. Oils were isolated by decanting, rinsed with solvent and dried under vacuum over P_2O_5.

N-Chloromethyl-DMT chloride **2a** has been reported previously [12,13].

N-Chloro-deuteromethyl-DMT chloride **2b** (166 mg, 0.60 mmol, 55%), d_2-DCM was used as solvent, and **2b** was isolated as pale yellow needles: ^{1}H NMR (d_4-MeOD): 7.62 (1H, d, H-4, *J* 7.5 Hz), 7.40 (1H, d, H-7, *J* 7.8 Hz), 7.23 (1H, s, H-2), 7.14 (1H, td, H-6, *J* 7.5, 1.4 Hz), 7.07 (1H, td, H-5, *J* 7.3, 1.2 Hz), 3.70–3.64 (2H, m, CH_2-α), 3.27 (6H, s, CH_3), 3.27–3.20 (2H, m, CH_2-β). ^{13}C NMR: 138.1 (C-7a), 128.0 (C-3a), 124.7 (C-2), 123.0 (C-6), 120.3 (C-5), 119.0 (C-4), 112.7 (C-7), 108.8 (C-3), 64.3 (CH_2-α), 50.2 (CH_3), 19.9 (CH_2-β). HRESIMS-theory for ^{35}Cl isotope cation: 239.1284; observed: 239.1278.

N-Chloroethyl-DMT chloride **2c** (87 mg, 0.35 mmol, 32%), DCE was used as solvent, and **2c** was isolated as a pale yellow thick oil which solidified under vacuum: ^{1}H NMR (d_4-MeOD): 7.62 (1H, d, H-4, *J* 7.3 Hz), 7.39 (1H, d, H-7, *J* 8.1 Hz), 7.24 (1H, s, H-2), 7.14 (1H, td, H-6, *J* 7.7, 1.2 Hz), 7.07 (1H, td, H-5, *J* 7.4, 1.1 Hz), 4.01 (2H, t, CH_2Cl, *J* 6.6 Hz), 3.82 (2H, t, NCH_2, *J* 6.6 Hz), 3.67–3.59 (2H, m, CH_2-α), 3.28–3.21 (8H, m, overlapping CH_2-β and CH_3). ^{13}C NMR: 138.1 (C-7a), 128.1 (C-3a), 124.6 (C-2), 122.9 (C-6), 120.3 (C-5), 119.1 (C-4), 112.7 (C-7), 109.2 (C-3), 66.5 (CH_2-α), 65.4 (NCH_2), 51.8 (CH_3), 36.7 (CH_2Cl), 20.0 (CH_2-β). HRESIMS-theory for ^{35}Cl isotope cation: 251.1315; observed: 251.1321.

N-Bromomethyl-DMT bromide **2d** (169 mg, 0.53 mmol, 48%), DBM was used as solvent, and **2d** was isolated as a brown thick oil which solidified under vacuum: ^{1}H NMR (d_4-MeOD): 7.64 (1H, d, H-4, *J* 8.0 Hz), 7.38 (1H, d, H-7, *J* 8.0 Hz), 7.23 (1H, s, H-2), 7.14 (1H, td, H-6, *J* 7.6, 1.1 Hz), 7.07 (1H, td, H-5, *J* 6.8, 1.1 Hz), 5.38 (2H, s, CH_2-Br), 3.75–3.68 (2H, m, CH_2-α), 3.33 (6H, s, CH_3), 3.28–3.21 (2H, m, CH_2-β). ^{13}C NMR: 138.1 (C-7a), 128.0 (C-3a), 124.7 (C-2), 123.0 (C-6), 120.3 (C-5), 119.1 (C-4), 112.7 (C-7), 108.8 (C-3), 65.3 (CH_2-α), 58.1 (CH_2-Br), 51.3 (CH_3), 20.1 (CH_2-β). HRESIMS-theory for ^{79}Br isotope cation: 281.0653; observed: 281.0626.

N-Chloromethyl-D_4-DMT chloride **2e** (133 mg, 0.48 mmol, 44%), DCM was used as solvent, and **2e** was isolated as yellow needles: ^{1}H NMR (d_4-MeOD): 7.62 (1H, d, H-4, *J* 7.2 Hz), 7.39 (1H, d, H-7, *J* 7.8 Hz), 7.23 (1H, s, H-2), 7.14 (1H, td, H-6, *J* 7.5, 1.4 Hz), 7.07 (1H, td, H-5, *J* 7.3, 1.2 Hz), 5.37 (2H, s, CH_2-Cl), 3.29 (6H, s, CH_3). ^{13}C NMR: 138.2 (C-7a), 128.0 (C-3a), 124.6 (C-2), 122.9 (C-6), 120.3 (C-5), 119.0 (C-4), 112.7 (C-7), 108.7 (C-3), 69.6 (CH_2Cl), 50.2 (CH_3). HRESIMS-theory for ^{35}Cl isotope cation: 241.1410; observed: 241.1400.

N-Chlorodeuteromethyl-D_4-DMT chloride **2f** (101 mg, 0.36 mmol, 33%), d_2-DCM was used as solvent, and **2f** was isolated as a white solid: ^{1}H NMR (d_4-MeOD): 7.61 (1H, d, H-4, *J* 7.2 Hz), 7.37 (1H, d, H-7, *J* 7.9 Hz), 7.23 (1H, s, H-2), 7.14 (1H, td, H-6, *J* 7.2, 1.2 Hz), 7.07 (1H, td, H-5, *J* 7.5, 1.2 Hz), 3.33 (6H, s, CH_3). ^{13}C NMR: 138.2 (C-7a), 128.0 (C-3a), 124.5 (C-2), 122.9 (C-6), 120.3 (C-5), 118.9 (C-4), 112.7 (C-7), 108.7 (C-3), 50.1 (CH_3). HRESIMS-theory for ^{35}Cl isotope cation: 243.1535; observed: 243.1534.

N-Chloroethyl-D_4-DMT chloride **2g** (75 mg, 0.26 mmol, 24%), DCE was used as solvent, and **2g** was isolated as a pale yellow thick oil which solidified under vacuum: ^{1}H NMR (d_4-MeOD): 7.61 (1H, d, H-4, *J* 7.9 Hz), 7.37 (1H, d, H-7, *J* 7.5 Hz), 7.23 (1H, s, H-2), 7.13 (1H, td, H-6, *J* 7.5, 1,4 Hz), 7.06 (1H, td, H-5, *J* 7.6, 1.2 Hz), 4.02 (2H, t, CH_2Cl, *J* 6.6 Hz), 3.86 (2H, t, NCH_2, J 6.7 Hz), 3.27 (6H, s, CH_3). ^{13}C NMR: 138.2 (C-7a), 128.0 (C-3a), 124.5 (C-2), 122.9 (C-6), 120.2 (C-5), 119.0 (C-4), 112.6 (C-7), 109.2 (C-3), 65.4 (NCH_2), 51.7 (CH_3), 36.6 (CH_2Cl). HRESIMS-theory for ^{35}Cl isotope cation: 255.1566; observed: 255.1576.

*2.5. Synthesis of 3-(2-chloroethyl)-indole (**3**)*

Adapted from ref. [16]: A solution of thionyl chloride (1 ml) and pyridine (5 ml) in benzene (15 ml) was added dropwise to an ice-cold solution of 3-(2-hydroxyethyl)indole (500 mg, 3.1 mmol) in anhydrous pyridine (5 ml). The mixture was stirred at room temperature until the disappearance of starting material was confirmed by TLC ($CHCl_3$/MeOH/NH_4OH: 9.5/0.5/0.1). The reaction was placed on ice and water (30 ml) was added. After two washes of the organic layer with saturated $NaHCO_3$ (40 ml) and evaporation under reduced pressure, the crude residue was subjected to flash chromatography (same solvent system as TLC). After evaporation of solvent and storage over P_2O_5, a dark-yellow oil was obtained (367 mg, 2.0 mmol, 65%). ^{1}H NMR ($CDCl_3$): 7.91 (1H, brs, NH), 7.59 (1H, d, H-4, *J* 8.5 Hz), 7.35 (1H, d, H-7, *J* 7.3 Hz), 7.20 (1H, td, H-6, *J* 7.6, 1.1 Hz), 7.13 (1H, td, H-5, *J* 7.3, 1.1 Hz), 7.02 (1H, d, H-2, *J* 1.9 Hz), 3.76 (2H, t, CH_2-α, *J* 7.5 Hz), 3.22 (2H, t, CH_2-β, *J* 7.3 Hz). ^{13}C NMR: 136.2 (C-7a), 127.1 (C-3a), 122.4 (C-2), 122.2 (C-6), 119.6 (C-5), 118.5 (C-4), 112.6 (C-3), 111.3 (C-7), 44.6 (CH_2-α), 29.1 (CH_2-β). Compound **3** did not ionise under the HR-ESI-MS conditions used. EI/CI-MS data were consistent with **3** as discussed in the text.

*2.6. Synthesis of tetrahydro-β-carbolines (**4–6**)*

2-Methyltetrahydro-β-carboline (2-Me-THBC) (**4**) (adapted from ref. [17]): Sodium cyanoborohydride (278 mg, 4.4 mmol) and tetrahydro-β-carboline (500 mg, 2.9 mmol) were cooled to 0 °C on ice in a mixture of methanol

(40 ml) and glacial acetic acid (525 mg, 0.5 ml, 8.7 mmol). A 37% (w/v) aqueous solution of formaldehyde (7.3 mmol, 0.59 ml) in 10 ml methanol was added dropwise to the solution over 10 min. The reaction was allowed to return to room temperature and monitored by TLC ($CHCl_3$/MeOH/NH_4OH: 8/2/0.1) until starting material disappeared (~2 h). Upon completion, the solution evaporated under reduced pressure and work-up was carried out similarly to the procedure described above for **1a** and **1b**. Recrystallisation from MeOH yielded 335 mg of white crystals (1.8 mmol, 62%). ^{1}H NMR (d_6-DMSO/d_6-acetone): 10.68 (1H, br s, N-9H), 7.35 (1H, d, H-5, *J* 7.7 Hz), 7.28 (1H, d, H-8, *J* 7.3 Hz), 7.01 (1H, td, H-7, *J* 7.5, 1.2 Hz), 6.94 (1H, td, H-6, *J* 7.5, 1.2 Hz), 3.54 (2H, s, CH_2-1), 2.70 (4H, s, CH_2-3, CH_2-4), 2.41 (3H, s, N-2 CH_3). ^{13}C NMR: 135.9 (C-8a), 132.7 (C-9a), 126.6 (C-4b), 120.1 (C-7), 118.1 (C-6), 117.1 (C-5), 110.7 (C-8), 106.0 (C-4a), 52.5 (CH_2-3), 51.9 (CH_2-1), 45.2 (CH_3), 21.1 (CH_2-4). HRESIMS-theory: 187.1235; observed: 187.1219.

1,2-Dimethyltetrahydro-β-carboline (**5**) (adapted from ref. [17]): This synthesis was carried out as described above for compound **4** starting with 300 mg (1.6 mmol) **5a** (see synthesis procedure below) and identical equivalents of MeOH, $NaCNBH_3$, AcOH and CH_2O solution. Purification was carried out by flash chromatography ($CHCl_3$/MeOH/NH_4OH: 8/2/0.1) to yield a light brown oil that solidified after storage over P_2O_5 (145 mg, 0.7 mmol, 45%). ^{1}H NMR (d_6-DMSO): 10.70 (1H, br s, N-9H), 7.39 (1H, d, H-5, *J* 7.7 Hz), 7.31 (1H, d, H-8, *J* 7.7 Hz), 7.05 (1H, td, H-7, *J* 7.5, 1.0 Hz), 6.97 (1H, td, H-6, *J* 7.3, 1.0 Hz), 3.50 (1H, q, CH, *J* 6.5 Hz), 3.08–3.01 (1H, m, CH_2-3), 2.71–2.67 (2H, m, CH_2-4), 2.64–2.57 (1H, m, CH_2-3), 2.44 (3H, s, N-CH_3), 1.42 (3H, d, CH_3, *J* 6.6 Hz). ^{13}C NMR: 137.0 (C-8a), 136.0 (C-9a), 126.6 (C-4b), 120.3 (C-7), 118.2 (C-6), 117.4 (C-5), 110.8 (C-8), 106.0 (C-4a), 55.4 (CH), 51.0 (CH_2-3), 42.2 (N-CH_3), 20.5 (CH_2-4), 18.0 (CH_3). HRESIMS-theory: 201.1392; observed: 201.1383.

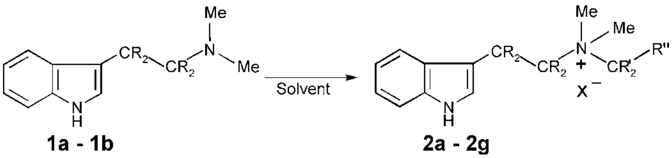
1-Methyltetrahydro-β-carboline (**5a**): To a microwave tube was added the stirrer bar and 600 mg (3.7 mmol) tryptamine; toluene (3 ml), trifluoroacetic acid (0.4 ml, 614 mg, 5.4 mmol) and acetaldehyde (1 ml, 785 mg, 17.8 mmol) were subsequently added. Microwave irradiation was applied for 20 min at 140 °C. A dark-brown oil was obtained at the bottom of the tube which was separated by decantation of the supernatant liquor. The residue was dissolved in a minimum amount of dichloromethane and subjected to flash chromatography ($CHCl_3$/MeOH/NH_4OH: 7/3/0.1). Evaporation of the collected fractions yielded a dark-brown oil that solidified after storage over P_2O_5 (335 mg, 1.8 mmol, 49%). ^{1}H NMR (d_6-DMSO): 11.14 (1H, br s, N-9H), 7.44 (1H, d, H-5, *J* 7.0 Hz), 7.35 (1H, d, H-8, *J* 8.1 Hz), 7.11 (1H, td, H-7, *J* 7.5, 1.0 Hz), 7.01 (1H, td, H-6, *J* 7.3, 0.6 Hz), 4.58 (1H, q, CH, *J* 6.8 Hz), 3.54–3.47 (1H, m, CH_2-3), 3.28–3.19 (1H, m, CH_2-3), 2.91–2.85 (2H, m, CH_2-4), 1.57 (3H, d, CH_3, *J* 6.8 Hz). ^{13}C NMR: 136.0 (C-8a), 132.4 (C-9a), 126.0 (C-4b), 121.4 (C-7), 118.8 (C-6), 117.9 (C-5), 111.2 (C-8), 105.4 (C-4a), 48.2 (CH), 40.7 (CH_2-3), 18.9 (CH_2-4), 17.7 (CH_3). HRESIMS-theory: 187.1235; observed: 187.1232.

3. Results and discussion

3.1. Impact of halogenated solvents on DMT free base *1a* and *1b*

Typically, sample preparation and work-up procedures employ organic solvents, for example during acid–base extractions where the compound partitions between aqueous and organic layers. Halogenated solvents are frequently used for this purpose and are generally considered to be inert. The fact that DMT **1a** was found to give rise to by-product formation when exposed to DCM raised questions regarding the possibility that nucleophilic substitution may occur with other halogenated solvents. It was therefore decided to dissolve DMT **1a** and its tetradeutered analogue **1b** in DCE and DBM in order to investigate this further (Table 1).

Table 1
Exposure of *N*,*N*-dimethyltryptamine (DMT) to a variety of halogenated solvents led to the precipitation of quaternary ammonium salt derivatives **2a–2g**



DMT **1**	R	Solvent	Product **2**	*R′*	*R″*
1a	H	DCM[a]	**2a**[b]	H	Cl
1a	H	D_2-DCM[c]	**2b**	D	Cl
1a	H	DCE[d]	**2c**	H	CH_2Cl
1a	H	DBM[e]	**2d**	H	Br
1b	D	DCM	**2e**	H	Cl
1b	D	D_2-DCM	**2f**	D	Cl
1b	D	DCE	**2g**	H	CH_2Cl

X = Cl or Br.

[a] Dichloromethane.

[b] Characterisation of this compound has been discussed in Ref [13].

[c] Dideutero-DCM.

[d] 1,2-Dichloroethane.

[e] Dibromomethane.

3.2. Characterisation of N-halogenated-alkyl-DMT derivatives (*2a–2g*)

3.2.1. NMR spectroscopy

All ^{1}H and ^{13}C NMR spectra of the precipitated quaternary ammonium salt derivatives (**2a–2g**) were consistent with their structures and confirmed that chloro/bromo-alkylation occurred at the side chain. Substitution at the indole nitrogen could be excluded by analysis of the NMR spectrum: in d_6-DMSO, the typical broad NH singlet was still visible, which would not have been the case had the proton been replaced by any alkyl substituent. Aromatic proton resonances did not change. In the case of the chloromethylated derivatives an additional methylene singlet appeared in the ^{1}H NMR spectrum at $\delta = 5.39$ ppm (in d_4-methanol) integrating for 2 protons. Interestingly, when spectra of the *N*-chloromethyl (**2a**) and *N*-bromomethyl (**2d**) derivatives were compared, it was found that the ^{1}H NMR spectra were virtually identical, with no significant shift changes observed. One difference was observed in the ^{13}C NMR spectrum, where the *N*-CH_2Br methylene group resonated at 58.1 ppm, whereas inspection of the HSQC spectrum revealed an upfield shift for *N*-CH_2Cl of **2a** at 69.7 ppm. Correspondingly, the DEPT-135 spectrum for **2a** displayed a negatively phased peak at ca. 69.7 ppm. As expected, both ^{1}H NMR and DEPT spectra of deuterated derivatives were characterised by the disappearance of the corresponding resonances. For example, *N*-CD_2Cl-DMT **2b** did not show the methylene singlet in the ^{1}H NMR spectrum. The negatively phased peak in the DEPT-135 spectrum was also absent when compared with **2a**.

3.2.2. Gas chromatography–mass spectrometry

Identification of **3** (Fig. 2(A1 and A2)) and **4** (Fig. 2(B1 and B2)) was based on the interpretation of mass spectral data in EI-

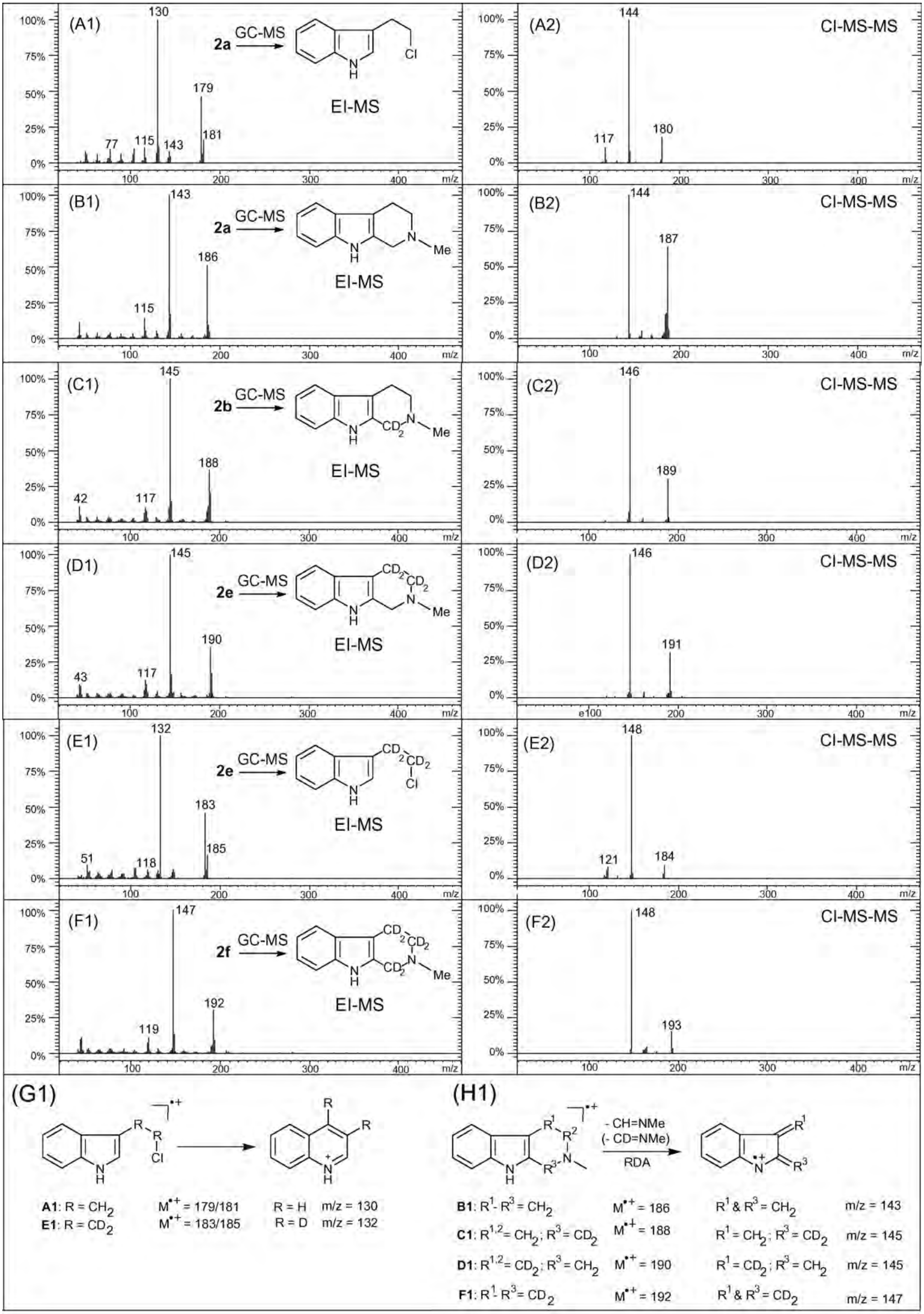


Fig. 2. Single stage electron ionisation mass spectra (EI-MS) **A1**–**F1** and chemical ionisation tandem mass spectra (CI-MS–MS) **A2**–**F2** of 3-(2-chloroethyl)indole and 2-Me-THBC derivatives which have been artificially formed during GC–MS analysis of *N*-chloromethylated derivatives **2a**, **2b**, **2e** and **2f**, respectively (Fig. 2(A)). The use of deuterated analogues revealed 2-Me-THBC formation depending on the *N*-chloromethylene substituent to facilitate cyclisation (C(1) and C(2)). Formation of the corresponding 3-(2-chloroethyl)indoles remained unaffected by this substituent.

MS and CI-MS–MS mode as previously discussed in detail [13] and verification was obtained by synthesis which is reported here. The key EI-MS fragmentation steps for 3-(2-chloro/bromoethyl)indoles and tetrahydro-β-carbolines (THBCs) are summarised in Fig. 2. In the former case, quinolinium base peak formation was the dominating principle (*m/z* 130) whereas in the case of THBC fragmentation, an odd-electron *retro*-Diels-Alder fragmentation (RDA) product was observed (Fig. 2(H1)).

The conversion of **2a** into compounds **3** and **4** appeared to be thermally induced by contact with the GC injection port. Additional indication for the involvement of heat derived from the exposure of the quaternary salts to microwave irradiation at 150 °C for 5 min when added to the appropriate solvent. Direct infusion LC–MS showed the disappearance of the salts and formation of the rearrangement products (data not shown). This raised some questions about the mechanism for their formation. For this purpose, DMT free base (**1a**) was dissolved in deuterated DCM (d_2-DCM) and a pale yellow crystalline precipitate was obtained and subsequently characterised as the d_2-derivative of **2a**, namely *N*-chloro-dideuteromethyl-DMT **2b**. Table 1 shows that a variety of deuterated *N*-chloro-methyl DMT salts have been prepared (**2b**, **2e** and **2f**). In all cases, the same number of degradation products has been detected in the total ion chromatograms and were represented as two peaks at identical retention times (10.68 min for **3** and 11.66 min for **4**, respectively). This was expected because the corresponding decomposition products only differed by the presence of deuteriums. EI and CI-MS–MS spectra of the resulting rearrangement products are summarised in Fig. 2(C1–F2) in order to illustrate the mass shifts according to the position of deuteriums.

When **2b** was exposed to GC–MS conditions, both EI and CI spectra showed the presence of both rearrangement products. Inspection of their mass spectra revealed that formation of 3-(2-chloroethyl)indole **3** did not involve the deuterated methylene group that derived from the *N*-chloromethyl substituent since the spectrum remained unchanged (not shown). In contrast, 2-Me-THBC was observed to be shifted by two mass units which pointed towards incorporation of both deuteriums. Furthermore, inspection of the corresponding mass spectra (Fig. 2(C1 and C2)) indicated that both deuteriums may have been found at position C-1 which meant that the *N*-chloromethylene substituent served as the carbon source for cyclisation in order to form the 1,1-dideutero derivative. Fig. 2(C1) shows that, under EI conditions, both the molecular ion (d_2-$M^{\bullet+}$ *m/z* 188) and the RDA fragment (*m/z* 145) incorporated both deuteriums (see also Fig. 2(H1)). This particular mass shift was also present under CI-MS–MS conditions (protonated RDA fragment at *m/z* 146 and $[d_2\text{-}M+H]^+$ at *m/z* 189, Fig. 2(C2)). In order to consider or rule out any possible involvement of the *N*,*N*-dimethyl groups in this cyclisation reaction however, the hexadeuteromethylated version of **2a** (*N*-chloromethyl-d_6-DMT) may have to be prepared and subjected to GC–MS analysis. Participation of any of the deuterated methyl groups would correspondingly lead to the detection of the same mass-shifted RDA fragment which has been found in **2b**. It follows that the occurrence of the usual RDA fragment at *m/z* 143 (EI-MS) and 144 (CI-MS–MS) would then exclude this participation.

A further attempt was made to probe some steric requirements for a possible nucleophilic substitution where DMT **1a** and **1b** were dissolved in 1,2-dichloroethane (DCE). Interestingly, the quaternary *N*-chloroethyl-DMT **2c** and **2g** were formed in a similar manner. However, under GC–MS conditions, some differential mass spectral features were observed when compared with the decomposition of the *N*-chloromethyl derivative **2a/2b**. Fig. 3(A) displays the resulting TIC after submission of **2c** to GC–MS. In addition to the occurrence of both **3** and **4**, four additional degradation products were detected. One co-eluted with the THBC derivative **4** which was subsequently identified as 1,2-dimethyl-THBC **5**. This was based on the presence of a $[M+H]^+$ at *m/z* 201 which indicated a molecular weight of 200 Da. The CI-MS–MS spectrum displayed a major fragment at *m/z* 158 which suggested the protonated RDA fragment to carry an additional CH_3 group. Correspondingly, EI-MS did not yield a *m/z* 143 base peak but instead *m/z* 185 which pointed towards mono-methyl substitution at position C-1 (see discussion above). The remaining methyl group was therefore thought to be located at N-2 which was subsequently verified by synthesis of **5** and chromatographic and mass spectral comparison. The EI and CI mass spectra of the fourth decomposition product was consistent with tertiary *N*-chloroethyl-*N*-methyltryptamine **7** at 13.11 min, which was considered to be formed by dequaternisation *via* methyl loss from the quaternary *N*-chloroethyl-DMT **2c**. Support for this tentative assignment came from the single stage CI-MS $[M+H]^+$ at *m/z* 237 with corresponding ^{37}Cl contributions at *m/z* 239. Further dissociation in tandem mode led to base peak formation (*m/z* 106 and *m/z* 108) which pointed towards the existence of the chloroalkylated iminium ion ($CH_2 = N^+(Me)C_2H_4Cl$). Iminium ion formation is one of the most characteristic features in EI-MS and CI-MS–MS spectra of dialkylated tryptamine derivatives and it provides information on the nature of the side-chain substituents [15,18]. An ion with high abundance was also detected at *m/z* 201 which may have been formed after loss of HCl. Another relatively intense species was detected at *m/z* 144 under CI-MS–MS conditions. The EI-MS of the dialkylated tryptamine species **7** did not yield highly intense fragment ions apart from the aforementioned *m/z* 106 and 108 iminium ion base peak (not shown). Another dequaternisation was observed with the detection of DMT **1a** at 10.80 min by loss of the chloroethyl substituent. Fig. 3(A) also shows a remaining decomposition product **6** at 10.42 min but its identity is currently unknown. The CI-MS indicated a potential $[M+H]^+$ at *m/z* 201 and its tandem mass spectrum with an excitation amplitude of 30 V was as follows: *m/z* 201 (44%), 170 (30%), 158 (100%), 144 (20%) and 58 (19%). The EI spectrum showed a potential molecular ion at *m/z* 200 with a 38% relative abundance. Key fragment ions included *m/z* 199 (17%), 156 (16%), 129 (11%), 128 (10%), 115 (14%), 58 (100%) and 42 (74%).

A possible mechanism for the ethylchloride quaternary ammonium salt **2c** rearrangement is shown in Fig. 4(A). After *N*-demethylation and formation of the tertiary amine **7**, an aziridine (**i**) can be generated which is a potent electrophile.

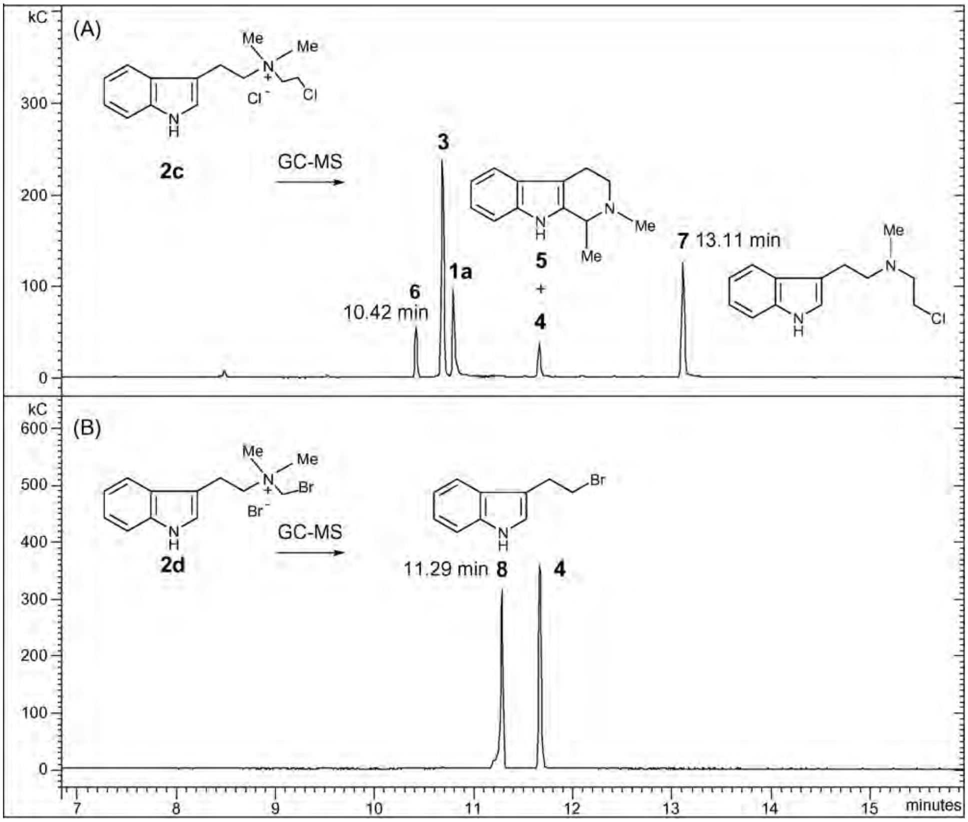


Fig. 3. (A) The chloroethyl derivative **2c** was observed to show more complex decompositions: In addition to **3** and **4**, the demethylated **7**, DMT **1a**, 1,2-dimethyl-THBC **5**, which co-eluted with **4**, and a currently unknown **6** at 10.42 min were detected. (B) *N*-Bromomethyl-DMT **2d** decomposed in analogy to **2a** and resulted in the detection of 3-(2-bromoethyl)-indole **8** and THBC **4**. The deuterated analogues, refer to Table 1, exhibited a similar decomposition behaviour under the conditions used and exhibited the same number of peaks with identical retention times (not shown). The corresponding mass shifts were also observed.

The observed product was 1,2-dimethyl-THBC **5**, although it is also possible that compounds (**ii**) and (**iii**) may form from nucleophilic attack on the aziridine. As indicated above in Fig. 3(A), a currently unknown compound **6** with a $[M+H]^+$ at *m*/*z* 201 has been detected after rearrangement of **2c**. The question arises whether either the spiro piperidine derivative (**iii**) or the azepinoindole (**ii**) could represent this unknown candidate, provided the suggested pathways are correct. At present however, this must remain speculative until these derivatives are available as standards.

The use of DBM was based on the question whether formation of a corresponding quaternary salt was possible since bromide would be considered to be a better leaving group than chloride. Interestingly, *N*-bromomethyl-DMT bromide **2d** precipitated as a dark-brown oil, which solidified after storage over P_2O_5. Precipitation however, was observed to take around 3 weeks which may be accounted for by increased solubility of the bromide salt in DBM due its increased lipophilic character when compared with the chloride salt **2a**.

When **2d** was subjected to GC–MS, similar rearrangements were observed, resulting in the formation of two peaks in the TIC. Fig. 3(B) shows that both peaks were identified as 3-(2-bromoethyl)indole **8** at 11.29 min and 2-Me-THBC **4**. As expected, the EI-MS of **8** displayed the quinolinium base peak at *m*/*z* 130 and a molecular ion at *m*/*z* 223 and 225 of equal intensity which reflected the presence of both bromine isotopes (not shown). The CI-MS–MS spectrum was also comparable with its chlorine counterpart as far as the presence of the *m*/*z* 144 base peak and *m*/*z* 117 species were concerned. The $[M+H]^+$ of **8** was observed at *m*/*z* 224 and 226 (not shown).

A mechanism for the formation of rearrangement products under GC–MS conditions is proposed in Fig. 4(B). 3-(2-Bromoethyl)indole **8** is formed by pathway (**b**) where the bromide anion displaces the quaternary ammonium salt. In pathway (**a**), a methyl group is first displaced by the bromide anion to give the tertiary amine (**iv**), which can give the iminium salt (**vi**). The iminium salt is a good electrophile and can react with the adjacent indole ring to give the 2-Me-THBC **4** as verified by synthesis (see discussion above). The spiro-pyrrolidine derivative (**vi**) is theoretically possible [19], although aromaticity would be lost.

3.3. Summary

In summary, it was found that DMT **1a**, and its deuterated analogue **1b**, formed quaternary ammonium salt by-products when dissolved in three different halogenated solvents, although different timescales were observed. When DCM was used, precipitation of crystals appeared to occur within several days which was comparatively rapid in comparison with DBM where the oily precipitate appeared after 2–4 weeks at ambient conditions. As mentioned before, this may have

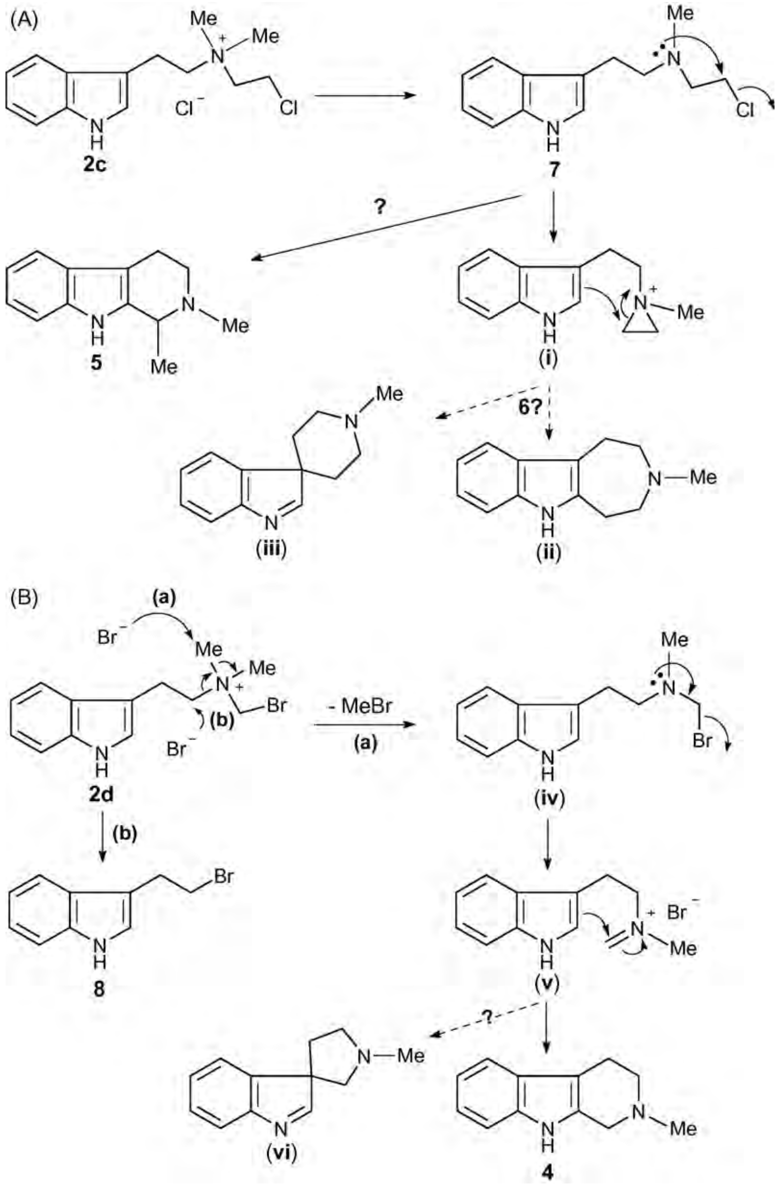


Fig. 4. Proposed mechanism for the rearrangements of *N*-chloro/bromo-alkylated quaternary ammonium salts of DMT **2a**-**2d** under GC–MS conditions. Quaternary ammonium salt **2c** may become demethylated giving the tertiary amine **7**. This provides the precursor to the generation of **5**. The aziridine (**i**) serves as a potent electrophile and may provide the entry to (**ii**) and (**iii**) as possible candidates for the detected unknown **6** (Fig. 3(A)). A simple dealkylation could account for the presence of DMT **1a**.

indicated higher solubility in the solvent. The reason for long-term exposure was also based on the desire to isolate and characterise these by-products by NMR and track their rearrangement behaviour under GC–MS conditions. Formation of quaternary ammonium salts was also observed before precipitation occurred and their analyses were accessible *via* HPLC–UV/MS where rearrangement did not occur. Precipitation seemed to be concentration-dependent and concentrations of ~10 mg/ml were found beneficial for this purpose. At significantly higher concentrations precipitation was not always observed. As described previously, even a short-term contact of DMT with DCM during work-up, yielded detectable amounts of **2a** [13]. Contact of DMT with DCE over a period of 2–3 weeks facilitated precipitation of *N*-chloroethyl DMT chloride derivatives **2c** and **2f** which showed extensive dealkylation and rearrangements, Fig. 3(A). Dequaternisations however, were not observed after submission of **2a** to GC–MS. The fact that no third peak was observed in the TIC was unexpected [13]. For example, demethylation was expected to occur to some degree which would have resulted in the detection of the *N*-chloromethyl derivative of **7** that was formed *via* injection of **2c** but this was not the case. For example, it has previously been observed by the authors that *N*,*N*,*N*-trimethylammonium salts of DMT were formed synthetically after overalkylation during the reaction of tryptamine with methyl iodide. However, when these derivatives were subjected to GC–MS analysis, only DMT could be detected due to demethylation (unpublished results).

The instance that a drug product reacts with an inert solvent, either during a synthetic procedure or work-up adds an additional complexity to profiling or fingerprinting analyses of illegally manufactured compounds. It can also provide the analyst with some further insights into the nature of a performed synthesis, which includes the use of a specific solvent, particularly when the presence of a variety of detected compounds does not agree with a previously characterised profile. The knowledge of these solvent-specific interactions is therefore of interest. An additional reason derives from the fact that a variety of common solvents are not easily obtainable anymore from manufacturers which may force a clandestine chemist to obtain less usual alternatives which again may introduce a characteristic trace. For example, it has recently been reported that solvent and catalyst-specific side products were detected in significant amounts where an internet-based procedure was fingerprinted for the analysis of a tryptamine synthesis *via* tryptophan decarboxylation [11]. A number of other examples are known where artefact formation was observed when amphetamine or phenethylamine derivatives were exposed to GC–MS conditions, either with or without contact to methanolic solutions used for the preparation of liquid sample injection [20–23].

Preliminary investigations have indicated that a variety of mono and dialkylated tryptamine derivatives, other than dimethyl, may not display this behaviour to such a comparable extent unless exposure is extended over a period of several months. One reason for the particular sensitivity of DMT towards DCM may derive from both inductive and steric effects where the dimethyl pattern increases electron density towards the nitrogen by (+)-inductive effects which may render this more reactive towards nucleophilic substitution. Elongation of the alkyl chain length attached to the nitrogen in other tertiary amines, on the other hand, may prevent nucleophilic attack on the delta positive carbon of DCM. The extent of this, however, is currently under investigation.

4. Conclusion

DMT has been found to form previously unreported quaternary ammonium salt halides when dissolved in a selection of halogenated solvents. Although these were detectable by HPLC–UV/MS analysis, rearrangements did occur under GC–MS conditions which resulted in the formation of a variety of artificially generated by-products. Detection of these solvent-drug interactions makes the analyst aware of a potentially misleading interpretation of data, particularly when forensic sample materials are involved. The presence of potentially characteristic marker molecules, however, also enables one to consider the question whether specific solvents have been used during manufacturing of controlled substances which is often neglected since these are considered inert.

Acknowledgements

The School of Pharmacy and Chemistry (LJMU) is gratefully acknowledged for financial contributions to the project. The synthetic work was carried out under a Home Office licence.

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