Profiling psychoactive tryptamine-drug synthesis by focusing on detection using mass spectrometry

Cláudia P.B. Martins, Sally Freeman, John F. Alder, Torsten Passie, Simon D. Brandt

The tryptamine nucleus is a building block for many biologically-active derivatives (e.g., neurotransmitter serotonin or antimigraine drugs of the triptan series). A variety of *N*,*N*-dialkylation of the nitrogen side chain can result in derivatives with psychoactive and hallucinogenic properties that are accessible by a large number of synthetic procedures.

The renewed interest in human clinical studies coincides with increased public interest and exchange of information on the Internet, including discussion in scientific, popular and clandestine literature. Over the past few years, an increasing number of case reports have attracted the attention of clinical, pharmaceutical, forensic and public-health communities, underlining the current lack of pharmaco-toxicological and analytical data.

This review assesses the current state of knowledge about the analytical profiling of drugs and by-products obtained from synthetic procedures discussed on Internet websites and scientific literature. Due to space considerations, we focus on detection using mass spectrometry (MS). We discuss commonalities and differences when considering fragmentation under a variety of ionization conditions and mass analysis using single-stage and multi-stage modes of MS.

Key features of mass-spectral fragmentation include formation of iminium-ion $C_nH_{2n+2}N^+$, normally assumed to be represented by appropriately substituted $CH_2{=}N^+(R^1R^2)$ species. Isomeric derivatives can often be differentiated by secondary and tertiary fragmentations that form $C_nH_{2n+2}N^+$ species after loss of neutrals. Soft-ionization techniques (e.g., electrospray) are often characterized by intense [3-vinylindole]$^+$-type species that reflect the extent of substitution on the indole ring. The fact that some tryptamines were found sensitive to halogenated solvents reminds the analyst to be aware of the potential for misinterpreting data when investigating the presence of route-specific impurities.

Keywords: Clinical; Drug synthesis; Forensic; Fragmentation; Hallucinogen; Mass analysis; Mass spectrometry; Pharmaceutical; Soft ionization; Tryptamine

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1. Introduction

The ability of humans to experience a wide range of altered states of consciousness has always been the subject of study throughout history. Alterations from what may be called a ''normal'' waking state may be induced by drugs and other non-drug-facilitated methods or may occur naturally [1]. As a consequence, the study of the human mind satisfies a range of diverse needs across the disciplines. One of the key molecules involved in regulation and modulation of fundamental processes within the central nervous system is neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) **1** (Fig. 1). This simple derivative is involved in a variety of functions (e.g., appetite, sex, sleep, cognition

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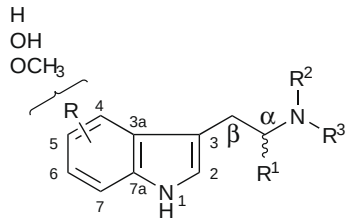

1 Serotonin: R = 5-OH; $R^1 = R^2 = R^3 = H$
2 DMT: R = R^1 = H; $R^2 = R^3 = CH_3$
3 Psilocybin: R = 4-OPO_3H_2; R^1 = H; $R^2 = R^3 = CH_3$
4 Psilocin: R = 4-OH; R^1 = H; $R^2 = R^3 = CH_3$
5 5-MeO-DMT: R = 5-OCH_3; R^1 = H; $R^2 = R^3 = CH_3$
6 5-OH-DMT: R = 5-OH; R^1 = H; $R^2 = R^3 = CH_3$
7 Sumatriptan: R = 5-$CH_2SO_2NHCH_3$; R^1 = H; $R^2 = R^3 = CH_3$
8 5,6-Br_2-DMT: R = 5,6-Br_2; R^1 = H; $R^2 = R^3 = CH_3$
9 5-Br-DMT: R = 5-Br; R^1 = H; $R^2 = R^3 = CH_3$
10 5-MeO-DIPT: R = 5-OCH_3; R^1 = H; $R^2 = R^3 = CH(CH_3)_2$

Figure 1. Generalized structure of a tryptamine derivative where the nature and the position of substituents determine the extent of psychoactivity. In addition, the α-carbon may be unsubstituted or carry a methyl or ethyl group, resulting in monoamine-oxidase-inhibiting properties. At the same time, the tryptamine nucleus serves as a template for many drugs used therapeutically.

and memory, sensory perception, mood, nociception, endocrine function, temperature regulation, motor activity and behavior) [2]. It is therefore not surprising that chemical modifications of the tryptamine nucleus result in the availability of a plethora of bioactive and neuroactive compounds ranging from highly toxic materials to medicinally important products.

N,*N*-dialkylation of the primary amine function of the tryptamine building block results in a large number of derivatives with psychoactive and/or hallucinogenic properties, and the impact on mood, cognition and perception appears to vary depending on the nature of substituents. Fig. 1 shows a generalized tryptamine structure, where psychoactivity is greatly affected by substitution on the 4 and 5 positions of the indole ring, the side-chain carbon and alkylation of the side-chain nitrogen [3]. Most of the psychoactive *N*,*N*-disubstituted derivatives known may show oral activity, but homologation of the *N*,*N*-dialkyl substituents appears to attenuate potency [4,5]. A number of naturally-occurring psychoactive tryptamines are *N*,*N*-dimethylated representatives: *N*,*N*-dimethyltryptamine (DMT) **2**, psilocybin **3**, psilocin **4** (4-OH-DMT), and 5-methoxy- and 5-hydroxy-DMT (bufotenin) (**5** and **6**, respectively). Psilocybin can be found in many mushroom species [6,7] whereas the remaining derivatives are found in many plants [8,9]. The pharmacology of these derivatives is complex but current knowledge points towards the involvement of 5-$HT_{1A\ \&\ 2A}$ receptor sub-types [10–12]. Recent findings also suggested that DMT **2** serves as an agonist at the sigma-1 receptor [13] and that a number of *N*,*N*-dialkylated tryptamines were found to be substrates at the plasma membrane serotonin transporter and the vesicle monoamine transporter [14].

As far as human clinical studies are concerned, only DMT **2** and psilocybin **3** have been extensively studied in recent years (e.g., [15–20] and references therein). So far, only the *N*,*N*-dimethylated tryptamines are known to be present in nature and the term ''designer tryptamines'' is often used when referring to synthetically-accessible analogues, since many of the *N*,*N*-dialkylated tryptamines are prohibited by legislation. A corollary is the inability to exercise quality control over compounds prepared illegally, often leading to low-quality drugs with unpredictable biological activity and ill-defined impurity profiles.

From a molecular point of view, differentiation between legal and illegal compounds is not always straightforward if one considers, for example, that the so-called triptan-type antimigraine drugs (e.g., Sumatriptan **7**) are derivatives of DMT **2** substituted on the 5-position (Fig. 1). Interestingly, 5,6-dibromo-DMT **8** and 5-bromo-DMT **9** have been recently isolated from three marine sponges found in Florida, USA, where 5,6-dibromo-DMT **8** appeared to show antidepressant-like activities in rodents, whereas the 5-bromo-DMT **9** displayed potential sedative properties in the chick anxiety-depression model [21].

The renewed interest in exploring tryptamine-based hallucinogens within the clinical context and the availability of scientific and popular literature on Internet websites coincides with increased popularity within recreational communities. Most of the currently-known data on the psychoactive effects of higher *N*,*N*-dialkylated tryptamines are based on self experimentation and little is known about their pharmaco-toxicological properties, particularly with long-term use [4,5]. Dedicated websites such as *Erowid* (www.erowid.org) host a

wide range of useful information relating to every aspect of consumption of legal and illegal drugs. It provides a platform for existing users and those contemplating use. 5-Methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) **10** is one of the few tryptamine representatives that was subject to case reports and that has been implicated in toxic and fatal responses (e.g., [22–24]), so leading to increased public attention.

Compared with the vast amount of literature available on the analytical characterization and profiling of phenethylamine and amphetamine-type drugs, relatively little has been published in the area of psychoactive tryptamines. However, as mentioned above, the increasing interest in the so-called designer tryptamines has moved this area more into the spotlight of clinical, forensic and public-health-based investigations. In this review, we aim to provide an account of the key literature published on the characterization of synthetic routes obtained from Internet websites and research literature.

2. Mass-spectral features

Determination of psychoactive tryptamines relies heavily on implementation of separation technology coupled with mass spectrometry (MS), particularly when trace levels, biofluids and/or complex drug mixtures are involved. In the earlier days of mass-spectral characterization, only *N*,*N*-dimethylated DMT derivatives were primarily investigated and focus was placed on electron-ionization MS (EI-MS). More recent studies, involving the use of ion-trap (IT) mass analyzers, single-quadrupole, triple-quadrupole (QqQ) or time-of-flight (TOF) instruments, and a number of ionization techniques, extended the study to numerous less common *N*,*N*-dialkylated psychoactive tryptamine derivatives. Fig. 2 provides an overview of the fragmentation patterns derived from a generalized tryptamine **11**, resulting in two most commonly observed transitions.

Under EI conditions, base-peak formation is characterized by formation of the iminium ion **12** typically observed for aliphatic amines. Those $C_nH_{2n+2}N^+$ ions are therefore observed {e.g., at *m*/*z* 44, 58, 72, 86 and 100 [16+14n]}, which means, for example, that the base peak obtained after electron ionization of any *N*,*N*-dimethylated derivative of DMT **2** would be expected to appear at *m*/*z* 58, regardless of the substituent present on the indole ring. Iminium ions, also referred to as immonium ions, have also been found to play a key role when exposed to either chemical-ionization IT tandem MS (CI-IT-MS^2) and electrospray IT or QqQ tandem MS (ESI-IT-MS^2 or ESI-QqQ-MS^2), respectively [25–28]. Atmospheric pressure ionization (API) sources [e.g., ESI or atmospheric pressure chemical ionization (APCI)] afford formation of protonated molecule $[M + H]^+$, and, when subjected to collision-induced dissociation (CID) or in-source CID (increased capillary voltage), a [3-vinylindole]$^+$-type species **13** is commonly observed (Fig. 2) [29]. Tryptamines unsubstituted on both the indole ring and the α-carbon, and irrespective of their substitution

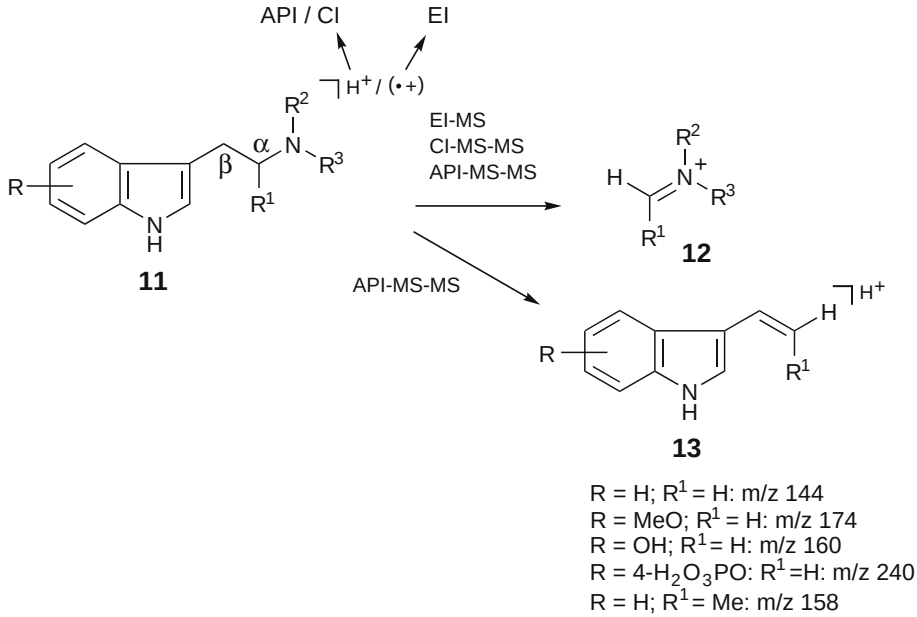

Figure 2. General mass-spectral fragmentation pattern of tryptamine derivatives **11**. Iminium ion **12** is normally observed to be dominant, independent of the ionization method used. Iminium ions are even-electron ions and can show secondary and tertiary fragmentations in alignment with the ion series characteristically found with aliphatic amines [16 + 14n], which can help to differentiate between isomeric derivatives (see also Fig. 3).

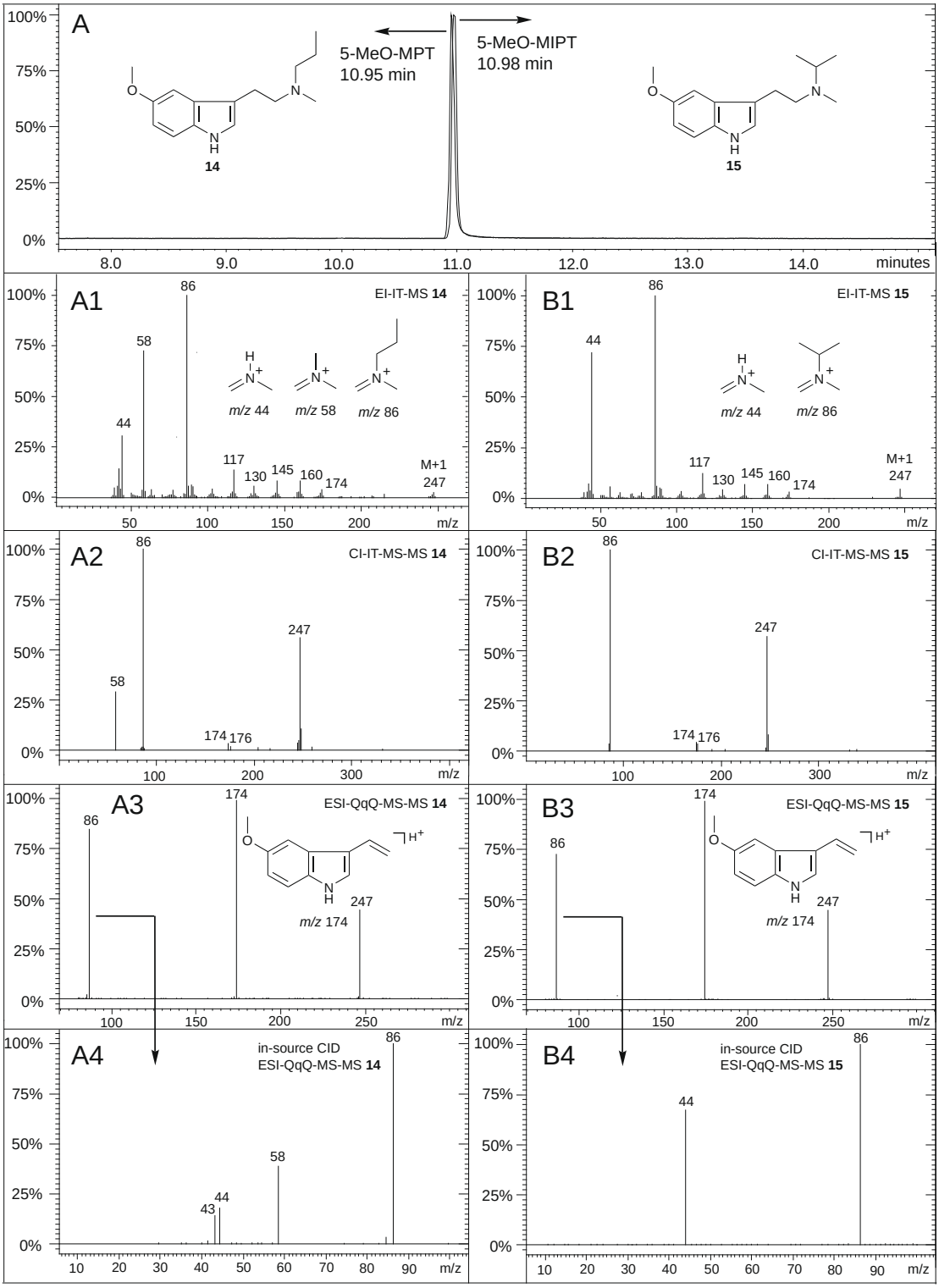

Figure 3. (A) Representative example where separation of two isomeric tryptamines **14** and **15** was impossible under a variety of GC conditions but differential fragmentations facilitated unambiguous identification due to secondary fragmentation of the iminium-ion species at *m/z* 86 (see also Fig. 2). (A1) and (B1): ion trap electron ionization mass spectra. (A2) and (B2): ion trap chemical ionization tandem mass spectra. (A3) and (B3): electrospray triple quadrupole tandem mass spectra obtained from direct infusion. (A4) and (B4): application of in-source CID using increased capillary voltage. This was subsequently subjected to MS^2 analysis of the *m/z* 86 base peak, hence leading to a *quasi* MS^3 spectrum that facilitated differentiation.

pattern at the ethylamine side chain, would be observed to form the [3-vinylindole]$^+$-type species **13** at *m/z* 144.

Isomeric tryptamine drugs can often be chromatographically separated and therefore differentiated, provided that reference standards are available. However, differentiation of isomers by mass spectral methods may be possible, depending on the ionization method involved. This is of particular importance in cases where insufficient chromatographic resolution is encountered (e.g., Fig. 3 shows two asymmetrically substituted isomers that were found to co-elute under GC-IT-MS conditions in the authors' laboratory).

Both 5-methoxy-*N*-methyl-*N*-propyltryptamine **14** (5-MeO-MPT) and 5-methoxy-*N*-methyl-*N*-isopropyltryptamine **15** (5-MeO-MIPT) could not be separated successfully (Fig. 3A), but inspection of Fig. 3 reveals that mass spectral differentiation was possible under certain conditions. The intensive nature of EI-induced fragmentation can often facilitate sufficient differentiation due to secondary and tertiary fragmentation of the iminium base peak. A comparison of both EI-IT-mass spectra (Fig. 3A1 and B1) shows that the fragmentation of the $CH_2{=}N^+(CH_3)C_3H_7$ iminium base peak ion (*m/z* 86, derived from 5-MeO-MPT **14**) is characterized by the loss of propene and ethene. By contrast, the iminium ion that corresponds to 5-MeO-MIPT **15** only eliminates propene, so no fragment at *m/z* 58 is observed. A third isomeric candidate was 5-MeO-*N*,*N*-diethyltryptamine (5-MeO-DET), which also displayed the *m/z* 86 base peak represented by corresponding iminium species $CH_2{=}N^+(C_2H_5)_2$. However, the loss of ethene from $CH_2{=}N^+(C_2H_5)_2$ only produced a secondary iminium ion at *m/z* 58, $CH_2{=}N^+H(C_2H_5)$, in moderate intensity and was therefore distinguishable from both 5-MeO-MPT **14** and 5-MeO-MIPT **15**, since it lacked the $C_2H_6N^+$ fragment at *m/z* 44 [25].

In general terms, EI-induced secondary and tertiary fragmentations of the iminium-base-peak $C_nH_{2n+2}N^+$ species result in potentially distinguishable fragmentation pathways resulting in $C_nH_{2n+2}N^+$ species of lower masses, induced by the loss of an appropriate neutral species [30]. Under CI-IT-MS^2 conditions, both **14** (Fig. 3A2) and **15** (Fig. 3B2) appeared to be differentiated in a similar manner. However, as may be expected, in comparison to the EI mass spectra, less fragmentation was observed. The use of soft API procedures allows for coupling with separation devices (e.g., capillary electrophoresis or liquid chromatography). As a consequence, the mass-spectral information content based on CID procedures, either via MS^2 or in-source CID of the protonated molecule, is normally limited to the two dissociations mentioned above. For example, 5-MeO-MPT **14** and 5-MeO-MIPT **15** could not be differentiated under ESI-TQ-MS^2 conditions, since both showed the $[M + H]^+ > [5\text{-MeO-3-vinylindole}]^+$ (*m/z* 174) and $[M + H]^+ > C_5H_{12}N^+$ (*m/z* 86) transitions (Fig. 3A3 and B3). However, isomeric differentiation may be achieved by implementing an ESI-IT-MS^n approach, where the iminium-ion species can be fragmented further at the MS^3 stage, as Rodriguez-Cruz demonstrated for a number of derivatives [28]. Under QqQ conditions, limitations incurred by the MS^2 stage can sometimes be overcome, as shown in Fig. 3A4 and B4. Differentiation between 5-MeO-MPT **14** and 5-MeO-MIPT **15** was possible when subjecting $[M + H]^+$ (*m/z* 247) to increased capillary voltage (in-source CID) resulting in dissociation into the two $[5\text{-MeO-3-vinylindole}]^+$ and $C_5H_{12}N^+$ ions before reaching the first quadrupole. Under these conditions, MS^2 was then applied to the *m/z* 86 fragment to afford the differentiating dissociations.

3. Fingerprint analysis of synthetic routes

Tryptamine derivatives are synthetically accessible by a countless number of synthetic routes and the ubiquitous occurrence of tryptamine and indole species in nature also leaves great scope for preparing and concentrating the key precursors en route to these psychoactive compounds. The main synthetic routes may be classified into methods that:

- create the indole nucleus by cyclization;
- start with indole and substituted indoles; and,
- modify a commonly available molecule, which contains the tryptamine moiety [31–34].

Choices of synthetic routes selected by clandestine chemists are often determined by precursor availability through unwatched or unwatchable channels but also information available on the Internet and other public or scientific sources of literature [35–37].

Many of the commonly used synthetic routes to the psychoactive tryptamines are based on relatively old literature, reflecting the maturity of many synthetic methods. It also reflects the conservatism of clandestine synthetic chemists and their dependence on certain precursor chemicals. However, systematic analytical characterization of these synthetic approaches is still largely unexplored. Many indole-containing derivatives show biological activity, which means that the presence of starting materials, intermediates and by-products within a poorly purified product needs to be considered, because the potential clinical implications of these derivatives are unknown.

One of the most commonly used preparative methods for psychoactive tryptamines is based on the procedure of Speeter and Anthony [38]. Fig. 4A shows a representative reaction sequence for the synthesis of DMT **2**. The indole starting material **16** reacts with oxalyl chloride to give the indole-3-yl-glyoxalylchloride **17**. Exposure to *N*,*N*-dimethylamine, dissolved in a number of solvents or in gaseous form, yields the indole-3-yl-glyoxalylamide

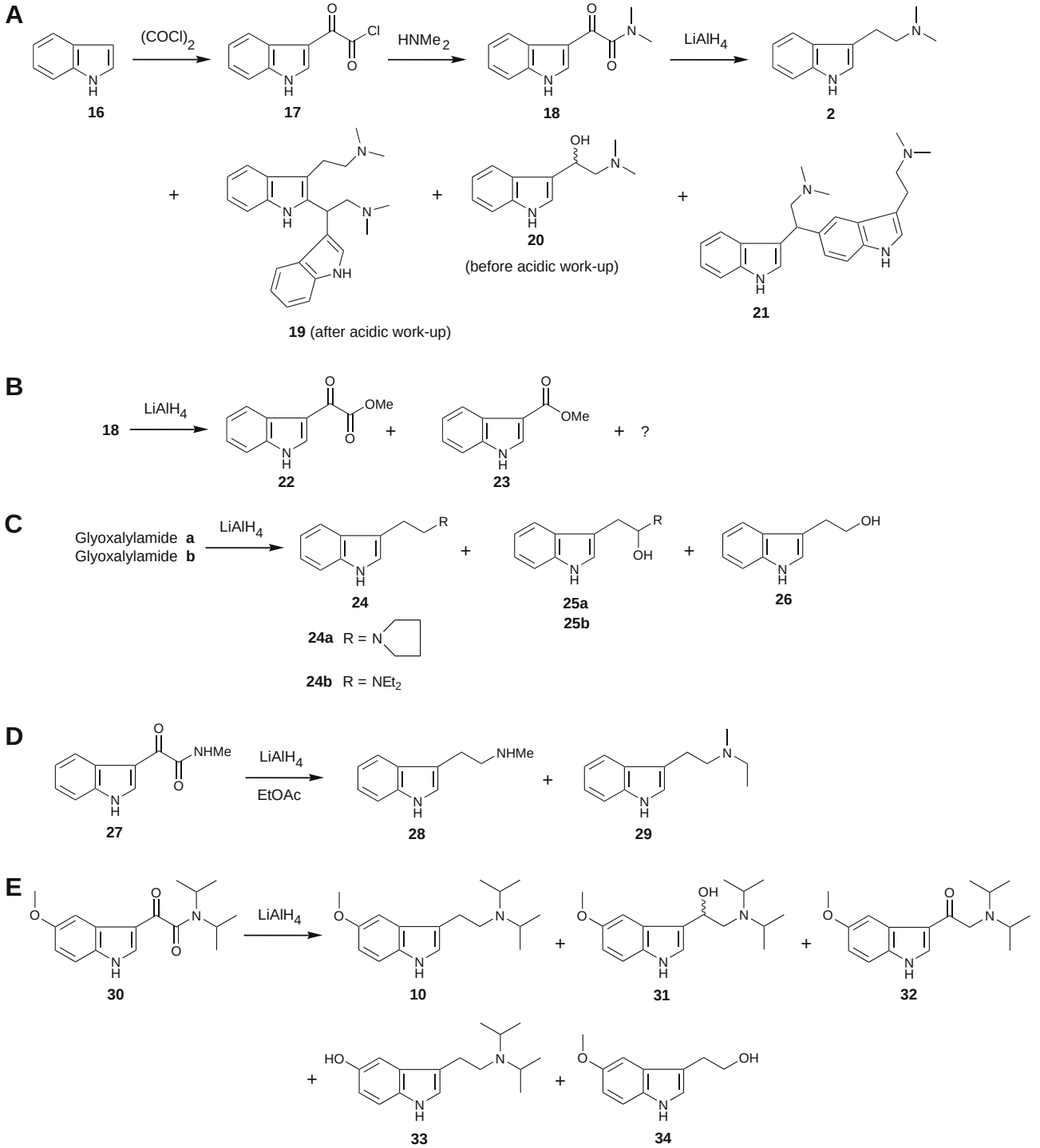

Figure 4. The Speeter and Anthony route [38] and the reported formation of side products. (A) Synthesis of DMT **2** led to the identification of dimeric side products (**19** + **21**) during acidic workup [39]. (B) Two by-products, observed during the first step of DMT, have been characterised as (**22** + **23**) [41]. (C) Synthesis of **24a** and **24b**. Incompletely reduced side products (**25a** + **25b**) and tryptophol **26** have been identified [42]. (D) The reduction of indole-3-yl-*N*-methylglyoxalylamide **27** to *N*-methyltryptamine (NMT) **28**. Quenching of $LiAlH_4$ with ethyl acetate was found to result in *N*-ethylation which led to the detection of *N*-methyl-*N*-ethyltryptamine **29** [44]. (E) $LiAlH_4$ reduction of **30** formed 5-MeO-DIPT **10** and several by-products (**31–34**) have also been characterised [45].

18. Subsequent reduction with lithium-aluminum hydride ($LiAlH_4$) produces the desired DMT **2**. This simple, versatile procedure can be employed for the synthesis of a large number of psychoactive derivatives [4], provided the appropriate (substituted) indole precursor and primary or secondary amine are available.

$LiAlH_4$ usually allows for easy reduction of these amides, but a fingerprint analysis and/or profiling for incompletely reduced tryptamine intermediates has rarely been reported in the literature. One of the few examples included the report by Crookes and co-workers, who could isolate a crystalline by-product during DMT **2**

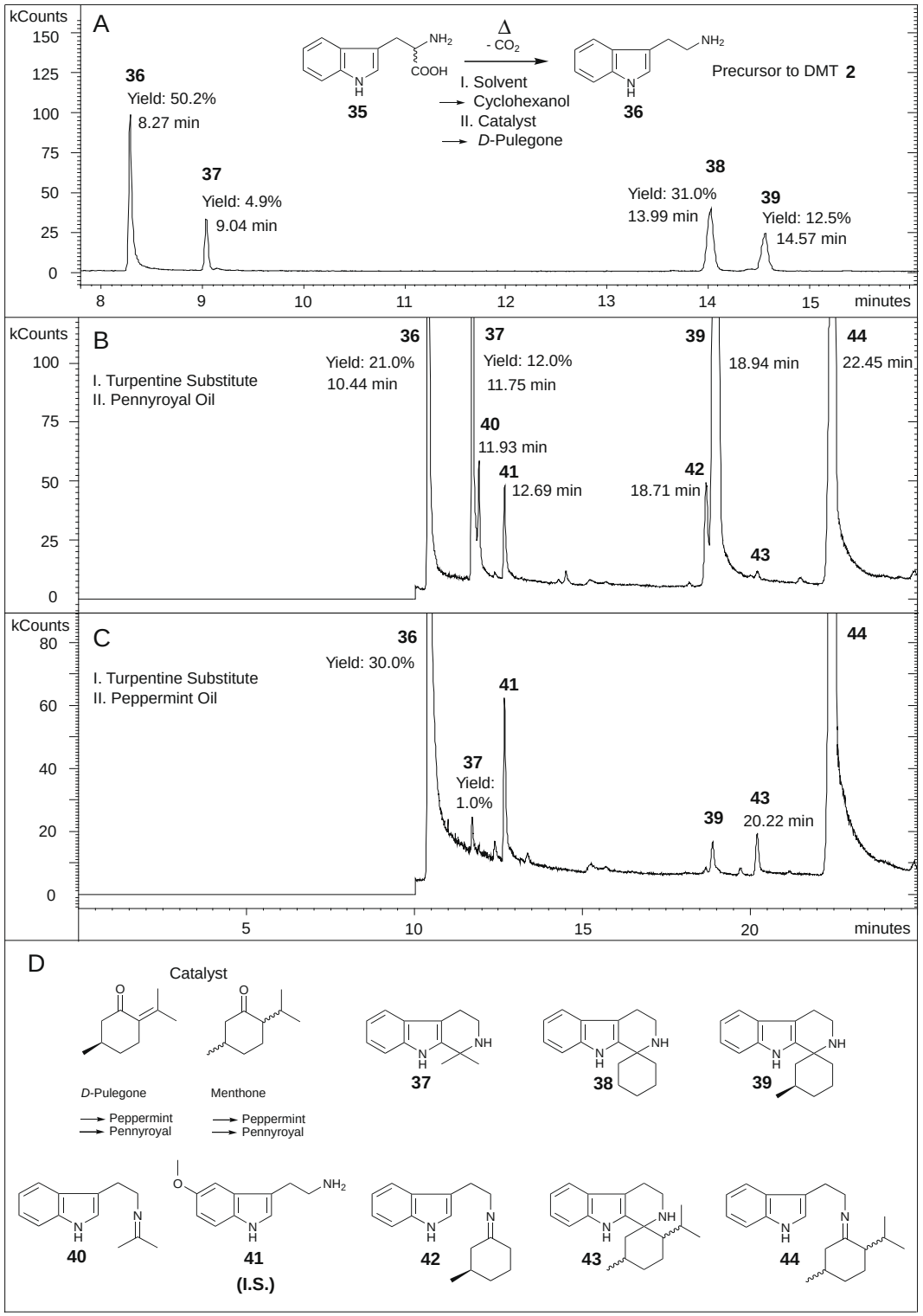

Figure 5. Representative GC-EI/CI-IT-MS^2 traces obtained after thermal decarboxylation of *D,L*-tryptophan **35** to tryptamine **36** using high-boiling point solvents and ketone catalysts [46,47]. Structures of THBC and imine derivatives detected allowed determination of a ''fingerprint'' of the catalyst employed. Both *D*-pulegone and menthone are also major constituents of peppermint and pennyroyal oils.

synthesis after acidic work-up when using the Speeter and Anthony procedure [39]. This dimeric product was characterized as **19** (Fig. 4A) based on elemental analysis, UV, ^{1}H and ^{13}C nuclear magnetic resonance (NMR) and EI-MS. Analysis of the crude DMT product before acidic work-up did not reveal the presence of dimer **19** but instead showed significant amounts (8–10%) of the incompletely reduced β-hydroxy-DMT **20**. It was suggested that exposure of β-hydroxy derivative **20** to acid would lead to water cleavage and formation of a reactive indolium species that effects electrophilic substitution at carbon-2 of another DMT molecule, resulting in dimer **19** [39]. The identity of the incompletely reduced **20** was confirmed by synthesis and characterization by thin layer chromatography (TLC), ^{1}H NMR, elemental analysis and EI-MS. The identity of dimer **19** was confirmed by synthesis when reacting β-hydroxylated species **20** with nine-fold molar excess of DMT **2** and 3 M aqueous HCl in methanol. Analysis revealed the presence of 76% of dimer **19** and 18% of a 3:1 mixture (HPLC) of two other isomeric dimers. The major isomer was suggested to be represented by dimer **21** [39]. A structurally-related, incompletely reduced β-hydroxy derivative was isolated by Troxler and colleagues when working on preparation of psilocybin derivatives where $LiAlH_4$ reduction of 4-benzyloxyindole-3-yl-*N*,*N*-dimethylglyoxalylamide was carried out in THF [40].

Gielsdorf and co-workers employed GC-MS for the analysis of DMT **2** and precursors **17** and **18** obtained *via* the Speeter and Anthony procedure [41]. Only few experimental and analytical details were given, but two side-products were characterized by GC-MS during the first synthetic step. These were identified as indole-3-glyoxylic acid methyl ester **22** and indole-3-carboxylic acid methyl ester **23** (Fig. 4B) based on EI-MS. During the next step, a third compound was detected ($M^{\cdot+}$ at *m*/*z* 216) but not identified [41].

Cowie and colleagues characterized the synthesis of *N*,*N*-tetramethylene tryptamine **24a** and *N*,*N*-diethyltryptamine (DET) **24b** using the same route. Analytical techniques included the use of TLC, ^{1}H NMR, IR and GC-EI/CI-MS [42]. Both syntheses yielded the corresponding hydroxylated derivatives **25a** and **25b**, but the position of the hydroxy-group was assigned to the α-carbon (Fig. 4C) based on mass spectral fragmentation [42], although this assignment has been questioned by Soine when reviewing the work [43]. Cowie and colleagues also presented an EI-MS of an unknown compound (base peak at *m*/*z* 143) and attributed this to the presence of tryptophol **26** [42]. However, it is worth noting that we characterized tryptophol detection by the presence of a base peak at *m*/*z* 130 under EI-MS conditions instead of *m*/*z* 143, pointing towards erroneous identification.

Traditionally, $LiAlH_4$ reductions are carried out in excess, which means that a careful quenching procedure is required before work-up. This is often done by adding water/organic-solvent mixtures that are considered to be inert. It was once reported that the use of ethyl acetate during the quenching procedure resulted in side-product formation. That is, after the reduction of amide **27** the non-psychoactive *N*-methyltryptamine **28** was prepared as planned, but the presence of the psychoactive, dialkylated *N*-methyl-*N*-ethyltryptamine (MET) **29** has been isolated as the oxalate salt (Fig. 4D). Detailed analytical data were not given, apart from elemental analysis and a comment on NMR data that were, however, not included [44].

One recent example of the detection of by-products occurring during application of the Speeter and Anthony procedure was reported for the reduction of 5-methoxyindole-3-yl-*N*,*N*-diisopropylglyoxalylamide **30**. 5-Methoxy-*N*,*N*-diisopropyltryptamine (5-MeO-DIPT) **10** was obtained, as expected, and several key impurities were identified [45]. These included two incompletely-reduced derivatives 5-MeO-β-OH-DIPT **31** and 5-MeO-β-keto-DIPT **32**, together with compounds 5-OH-DIPT **33** and 5-MeO-tryptophol **34**, respectively (Fig. 4E). Identification was carried out by ^{1}H and ^{13}C NMR and 2D-NMR experiments, orthogonal acceleration ESI-TOF (*oa*-ESI-TOF) and ESI-QqQ-MS^2 studies, and further confirmation was obtained by organic synthesis [45].

A number of synthetic routes are described on Internet websites and are often based on published literature. One procedure commonly discussed on Internet websites involved a two-step synthesis to DMT **2** that became known as *The Breath of Hope Synthesis*. It suggested

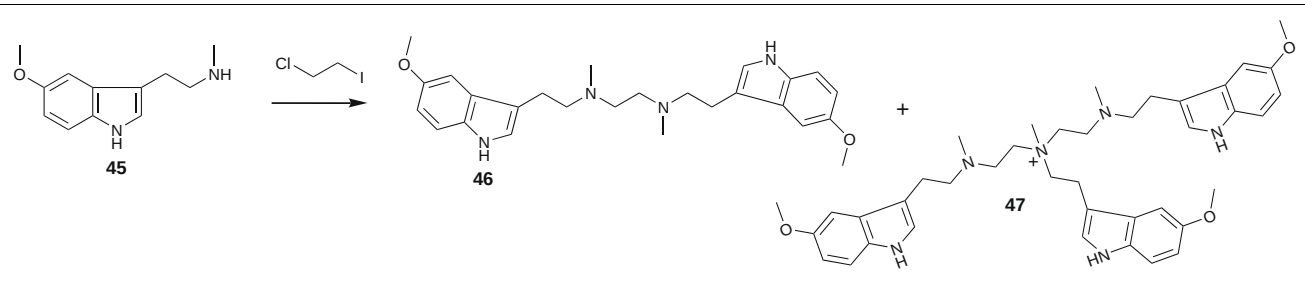

Figure 6. The reaction of 5-MeO-*N*-methyltryptamine (5-MeO-NMT) **45** with 1-chloro-2-iodoethane has been reported to yield pharmacologically-active side products **46** and **47**, as judged by receptor-binding studies [53].

employing the widely-available amino acid *D,L*-tryptophan **35** (Trp) as the starting material. In the presence of high-boiling solvents and a number of ketone catalysts, heating at reflux was proposed to result in decarboxylation and formation of tryptamine **36** (Fig. 5A). The second stage was based on the synthesis of DMT **2** using

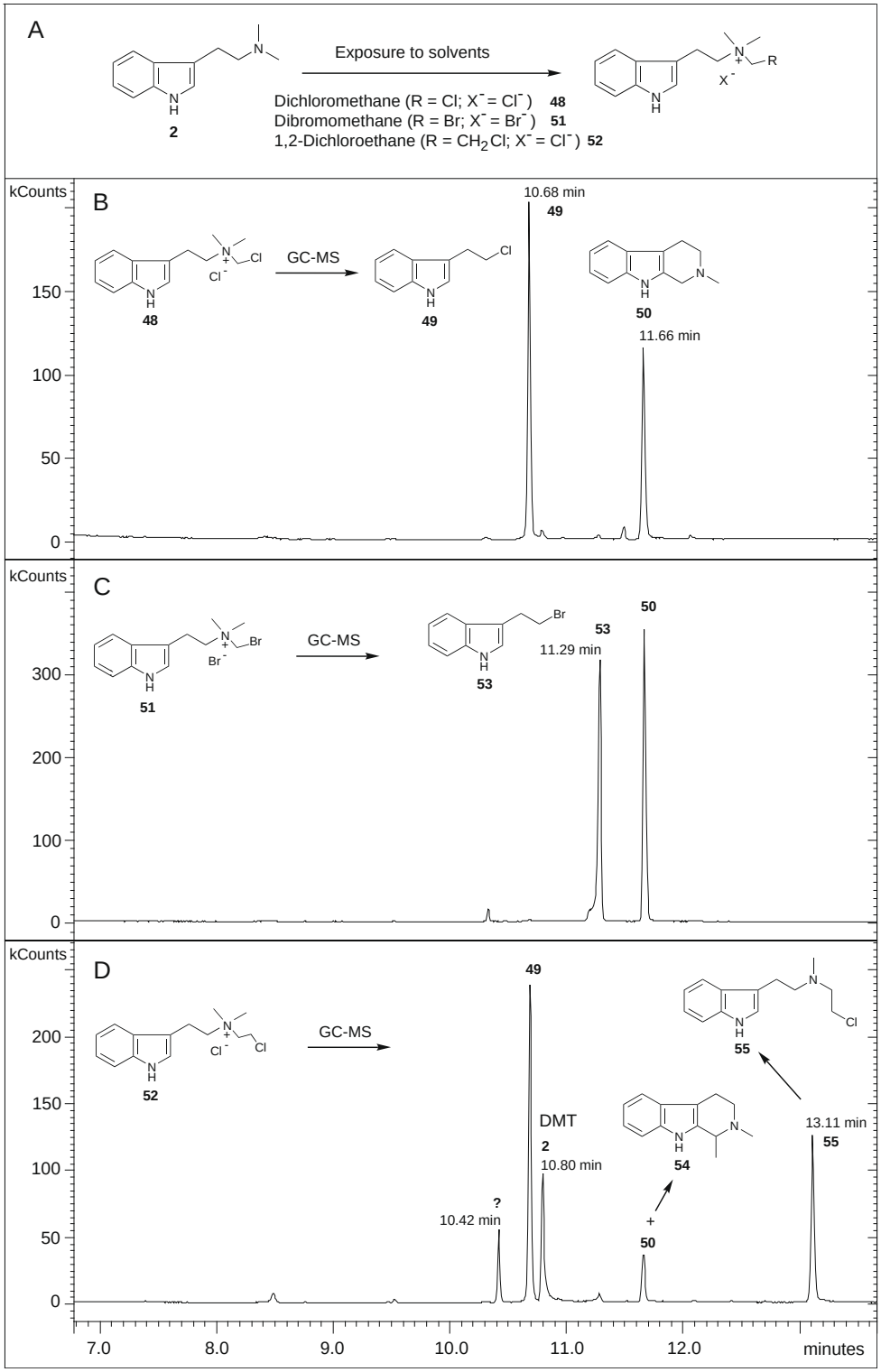

Figure 7. (A) Short-term and long-term exposure of DMT free base **2** to a number of halogenated solvents led to formation of quaternary ammonium salts. Heat-induced rearrangements led to detection of several artificially-formed by-products when subjected to GC-EI/CI-IT-MS^2 analysis [55,56].

methyl iodide, benzyltriethylammonium chloride/NaOH phase-transfer catalyst and dichloromethane (DCM) as the solvent [46,47]. Analytical characterization of the decarboxylation step involved the application of GC-EI/CI-IT-MS^2 (GC-IT-MS^2) and NMR. This led to isolation and identification of 1,1-disubstituted-tetrahydro-β-carbolines (THBCs) **37**–**39** formed as major impurities in the tryptamine **36** product (Fig. 5A and D). Formation of THBC derivatives originated from the reaction with both the solvent (e.g., cyclohexanol) and the ketone catalysts (aliphatic or cyclic), often at significant levels. Confirmation was obtained by organic synthesis of the THBC derivatives from tryptamine using the Pictet-Spengler cyclization [46].

Discussions on websites suggested the use of several natural oils in order to accelerate formation of the desired tryptamine product **36**. This proposal was based on the occurrence of ketone constituents in these natural products, and aliphatic ketones were known catalysts for this decarboxylation. Fingerprint analysis of the thermolytic decarboxylation was also extended to use of household solvents (e.g., turpentine substitute and white spirit). The use of essential oils as a source of naturally-occurring catalysts led to the detection of a variety of additional THBC and imine derivatives **40**–**44** (Fig. 5B–D) [47]. Some THBC derivatives can show a variety of biological activities, including the inhibition of monoamine oxidase [48,49] or interaction with imidazoline-binding sites [50], but detailed investigations about these particular 1,1-disubstituted derivatives and their potential interactions with the tryptamines have yet to be carried out.

The second step of *The Breath of Hope* procedure involved the methylation of tryptamine **36** to give the desired DMT **2** product and discussion on the Internet and, separately, work in the author's laboratory repeating the proposed method by Drone #342, indicated that it did not work well [51]. Considering the fact that the thermolytic decarboxylation step was found to yield a range of THBC by-products, it was decided to reproduce this route in the laboratory first using pure tryptamine. The reaction product was characterized by LC-ESI-QqQ-MS^2 and *oa*-ESI-TOF. Quantitative determinations were carried out in positive multiple reaction monitoring (MRM) mode, which included synthesis of the identified reaction products. MRM screening of the products did not lead to the detection of DMT **2**. When pure tryptamine **36** was used as the starting material, 21.0% *N*,*N*,*N*-trimethyltryptammonium iodide (TMT) and 47.4% 1-*N*-methyl-TMT (1-Me-TMT) were detected. Also, 11.1% tryptamine starting material and 0.5% trace of the monomethylated *N*-methyltryptamine (NMT) were found to be present, indicating that the reaction did not go to completion [52].

The identification of impurities, especially within the context of illegal hallucinogenic drug synthesis, plays a key role in clinical and forensic investigations. However, pharmaco-toxicological screenings of newly-discovered side-products are normally not carried out, although one might expect to discover potentially new chemical entities of pharmacological interest that might otherwise be missed during drug design. One of the very few examples was reported during the evaluation of a number of *N*,*N*-dialkylated tryptamines targeting several 5-HT receptor subtypes [53]. From the reaction between 5-methoxy-*N*-methyltryptamine **45** and 1-chloro-2-iodoethane, the dimeric *N*-bridged ethylene-bis-tryptamine **46**, contaminated with a quaternary by-product **47**, was isolated as an impurity (Fig. 6). For example, when subjected to receptor-binding assays, the unpurified compound was found to display an 893-fold selectivity for the 5-HT_{1A} receptor (1.9 nM) over the 5-HT_{2A} receptor (1696 nM). The binding affinity for the 5-HT_{2C} receptor was determined at 612 nM, and characterization of this product was carried out by UV, HPLC, ^{1}H and ^{13}C NMR and ESI-MS [53].

4. Interactions with solvents and artifact formation

The use of organic solvents is often required during the isolation of synthetic or natural products. Halogenated solvents (e.g., DCM) are also frequently employed for extraction and purification, which require these solvents to be inert. Interestingly, DMT **2** was found to be reactive towards DCM, during work up or long-term storage, which led to the unexpected formation of quaternary ammonium salt *N*-chloromethyl-DMT chloride **48** as a by-product (Fig. 7A) [54,55]. Furthermore, when **48** was subjected to analysis by GC-EI/CI-IT-MS^2, two rearrangement products were detected instead and characterized as 3-(2-chloroethyl)indole **49** and 2-methyltetrahydro-β-carboline **50** (Fig. 7B) [55].

The interesting point to note here was that both rearrangement products were artificially formed during analysis that involved heat. The extent of *N*-chloromethyl-DMT chloride **48** formation appeared to depend on exposure time of DMT **2** to the halogenated solvent. However, the occurrence of thermally-induced degradation of impurities and the potential toxicological properties of inhaled **49** and **50** remain to be investigated.

A subsequent study revealed that DMT free base **2** appeared to form the corresponding quaternary salts **51** and **52** after long-term exposure to other halogenated solvents [e.g., dibromomethane (DBM) and 1,2-dichloroethane (DCE) (Fig. 7A)] [56]. Both *N*-bromomethyl- and *N*-chloroethyl quaternary ammonium derivatives **51** and **52** showed artificially-induced rearrangement reactions under GC-EI/CI-IT-MS^2 conditions that resulted in the identification of **2**, **49**, **50**, **53**–**55** and one unidentified product, respectively (Fig. 7C and D). Organic synthesis and further characterization of

deuterated derivatives were also included in this study in order to gain some insights into the nature of rearrangements. Organic solvents are normally considered inert, and the observation that these solvent-DMT interactions existed reminds the analyst to be aware of potentially misleading interpretation of data.

5. Conclusion

The complex nature of psychoactive tryptamine chemistry provides great scope for exciting, challenging and interdisciplinary research opportunities. The implementation of traditional separation techniques may soon expand to two-dimensional chromatography, ion mobility and microfluidic technology in order to facilitate rapid analysis. The characterization of so-called ''research chemicals'' and other products obtained from Internet websites places high demand on accurate identification of novel derivatives to help to inform health-care providers, forensic scientists and clinicians, who deal with frontline exposure to products that are often unknown. The detection of route-specific impurities should also be of interest within the pharmaceutical context where a number of tryptamines might be prepared for human clinical studies.

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