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

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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 CnH2n+2N+, normally assumed to be represented by appropriately substituted CH2—N(R1R2) species. Isomeric derivatives can often be differentiated by secondary and tertiary fragmentations that form CnH2n+2N+ 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.

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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...
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-HT1A & 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 and 5-bromo-DMT 9 have been shown to display 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) \( \text{10} \) 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 \( \text{11} \), resulting in two most commonly observed transitions.

Under EI conditions, base-peak formation is characterized by formation of the iminium ion \( \text{12} \) typically observed for aliphatic amines. Those \( \text{C}_n\text{H}_{2n+2}N^+ \) 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 \( \text{2} \) 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 \([3\text{-vinylindole}]^+ \) type species \( \text{13} \) is commonly observed (Fig. 2) [29]. Tryptamines unsubstituted on both the indole ring and the \( \alpha \)-carbon, and irrespective of their substitution

![Figure 2](http://example.com/figure2.png)

**Figure 2.** General mass-spectral fragmentation pattern of tryptamine derivatives \( \text{11} \). Iminium ion \( \text{12} \) 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 MS² analysis of the m/z 86 base peak, hence leading to a quasi MS³ 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 CH2=N(CH3)C4H9 iminium base peak ion (m/z 86, derived from 5-MeO-MPT) is characterized by the loss of propene and ethene. By contrast, the iminium ion that corresponds to 5-MeO-MIPT 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 CH2=N(C2H5)2. However, the loss of ethene from CH2=N(C2H5)2 only produced a secondary iminium ion at m/z 58, CH3=NH(C2H5), in moderate intensity and was therefore distinguishable from both 5-MeO-MPT 14 and 5-MeO-MIPT 15, since it lacked the C5H12N+ fragment at m/z 44 [25].

In general terms, EI-induced secondary and tertiary fragmentations of the iminium base peak CnH2n+2N+ species result in potentially distinguishable fragmentation pathways resulting in CnH2n+2N+ species of lower masses, induced by the loss of an appropriate neutral fragment at 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 CH2=N(C2H5)2. However, the loss of ethene from CH2=N(C2H5)2 only produced a secondary iminium ion at m/z 58, CH3=NH(C2H5), in moderate intensity and was therefore distinguishable from both 5-MeO-MPT 14 and 5-MeO-MIPT 15, since it lacked the C5H12N+ fragment at m/z 44 [25].

In general terms, EI-induced secondary and tertiary fragmentations of the iminium-base-peak CnH2n+2N+ species result in potentially distinguishable fragmentation pathways resulting in CnH2n+2N+ species of lower masses, induced by the loss of an appropriate neutral species [30]. Under CI-IT-MS2 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 MS2 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-MS2 conditions, since both showed the [M + H]+ > [5-MeO-3-vinylindole]^+ (m/z 174) and [M + H]+ > C4H12N+ (m/z 86) transitions (Fig. 3A3 and B3).

However, isomeric differentiation may be achieved by implementing an ESI-IT-MSn approach, where the iminium-ion species can be fragmented further at the MS3 stage, as Rodriguez-Cruz demonstrated for a number of derivatives [28]. Under QqQ conditions, limitations incurred by the MS2 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-MeO-3-vinylindole]^+ and C4H12N+ ions before reaching the first quadrupole. Under these conditions, MS2 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
Subsequent reduction with lithium-aluminum hydride (LiAlH₄) produces the desired DMT₂. 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.

Figure 4. The Speeter and Anthony route [38] and the reported formation of side products. (A) Synthesis of DMT₂ 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 LiAlH₄ with ethyl acetate was found to result in N-ethylation which led to the detection of N-methyl-N-ethyltryptamine 29 [44]. (E) LiAlH₄ reduction of 30 formed 5-MeO-DIPT 10 and several by-products (31–34) have also been characterised [45].
LiAlH₄ 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-MS² 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-benzoxylindole-3-yl-N,N-dimethylglyoxylalamide 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$^+$ at m/z 216) but not identified [41].

Cowie and colleagues characterized the synthesis of N,N-tetramethylethylene 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-methoxy-indole-3-yl-N,N-diisopropylglyoxalylalamide 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,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

![Diagram of chemical reactions](image)

**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-MS² 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-MS² (GC-IT-MS²) 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-MS² 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-HT₁A receptor (1.9 nM) over the 5-HT₂A receptor (1696 nM). The binding affinity for the 5-HT₂C receptor was determined at 612 nM, and characterization of this product was carried out by UV, HPLC, ¹H and ¹³C 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-MS², 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-MS² 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 exist 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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References


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