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A Detailed Mechanistic Fragmentation Analysis of Methamphetamine and Select Regioisomers by GC/MS

ABSTRACT: A novel ring-substituted methamphetamine regioisomer, *N*,α,4-trimethyl phenmethylamine, was synthesized in order to study the validity of proposed structures for various mass spectrometry (MS)-derived peaks in a methamphetamine fragmentation pattern. While other research efforts have studied aspects of methamphetamine in detail, a full fragmentation study has not been reported previously. In addition to showing molecular structures represented by fragment peaks, mechanisms for selected processes are detailed. An empirically derived procedure to easily determine by simple spectral peak pattern recognition the geometry of dimethyl- or ethyl-substituted immonium ions ($RRC = N^+RR$) where $m/z = 58$ is outlined. These results are platform independent for electron ionization (EI) instruments, but have also proven to be helpful in explaining spectral peaks observed in spectra from ion trap systems. The spectrum for the synthesized methamphetamine regioisomer was accurately predicted using this methodology. While this approach is useful in some casework, the converse may be more useful: when an unexpected or unusual peak pattern arises in a spectrum, being able to analyze it to determine the structure of the molecule. This paper gives an analyst the means to begin such retro-synthetic analyses.

KEYWORDS: forensic science, narcotics analysis, gas chromatography/mass spectrometry (GC/MS), methamphetamine isomers, fragmentation, mechanism, spectra interpretation

While numerous papers have been published involving the discrimination of various methamphetamine isomers by gas chromatography/mass spectrometry (GC/MS) (1–7), there are none that offer complete mechanistic explanations of the observed fragmentation patterns. For quantitative MS, deuterated analogs of internal standards are necessary, and as Ho et al. (8) states: "Before an isotopic analog is synthesized, the mass spectrometric fragmentation pattern of the analyte should be understood." In part to better address this issue, this paper provides detailed fragmentation pathways with mechanistic clarification for methamphetamine using scientifically accepted theories that have been in existence for decades (9–11). We assert that understanding the origins of more than just two or three peaks in the spectrum, based on reasonable fragmentation theory, is useful when generalized. Similar deconstruction studies have been reported for cannabinoid derivatives (12) but the same detailed mechanistic approach has not been performed for methamphetamine regioisomers. In fact, there are a few researchers who claim that MS alone is insufficient to distinguish between methamphetamine and phentermine (3,6). Thus, a structurally detailed map for methamphetamine fragmentation is presented and a GC/MS spectral analysis procedure is proposed that does in fact differentiate methamphetamine regioisomers. To illustrate the importance of this generalizable approach, spectral libraries (13–15) were searched for GC/MS-derived spectra of regioisomers of methamphetamine and six (Fig. 1, compounds **II–VII**) were found with spectra that can be explained using fragmentation theory. While other methamphetamine regioisomers exist, their spectra have not been reported. Upon studying the spectra in detail, it became apparent that the *m/z* region between 39 and 58 was crucial to differentiate methamphetamine regioisomers. Significantly, there was one interesting compound absent from the spectra available for isomeric methamphetamine, namely, *N*,α,4-trimethyl phenmethylamine (TMPMA; Fig. 1, compound **VIII**). This provided a real opportunity: predict the fragmentation that would be expected for **VIII** using the schema proposed, and then synthesize the compound and verify that the fragmentation theory is accurate. The results are shown below.

While this approach to spectral interpretation can be used to predict the spectrum of a new compound, perhaps more importantly to the forensic science community, the converse is also possible. That is, the analyst can predict the structure of an unfamiliar or unknown compound based on analyzing various peaks in an MS spectrum. For example, if an unusual spectrum is encountered and a spectral library suggestion is consequently not available, or the library suggestion is not correct, the informed analyst can nonetheless predict the structure of the unknown compound with the strategy presented here, or at the very least, dismiss the library spectrum as incorrect. Some may view this as an unnecessary approach for casework; however, a counterexample is the recent BALCO scandal where spectral interpretation of MS-derived data was pivotal in determining the parent structure of the synthetic steroid tetrahydrogestrinone (THG) (16–19). Another example of the importance of mass spectral analysis is a case in which ring-substituted amphetamine (para methyl—a regioisomer of compound **VIII** synthesized in this study) and *p*-methyl ring-substituted methamphetamine sold as amphetamine caused an adverse reaction in a seasoned amphetamine user (20). The approach of the laboratory to determine the culprit molecule was identification by GC/MS, followed by "analysis of authentic samples of *p*-methyl(meth)amphetamine prepared in the laboratory." Thus, it is not an unprecedented use of the tools of the forensic laboratory to use deconstructive fragmentation analysis of related compounds, followed by predictions of fragmentation and then synthesis of a compound to confirm the predictions. This said, the mechanistic detail shown below is not solely informative for

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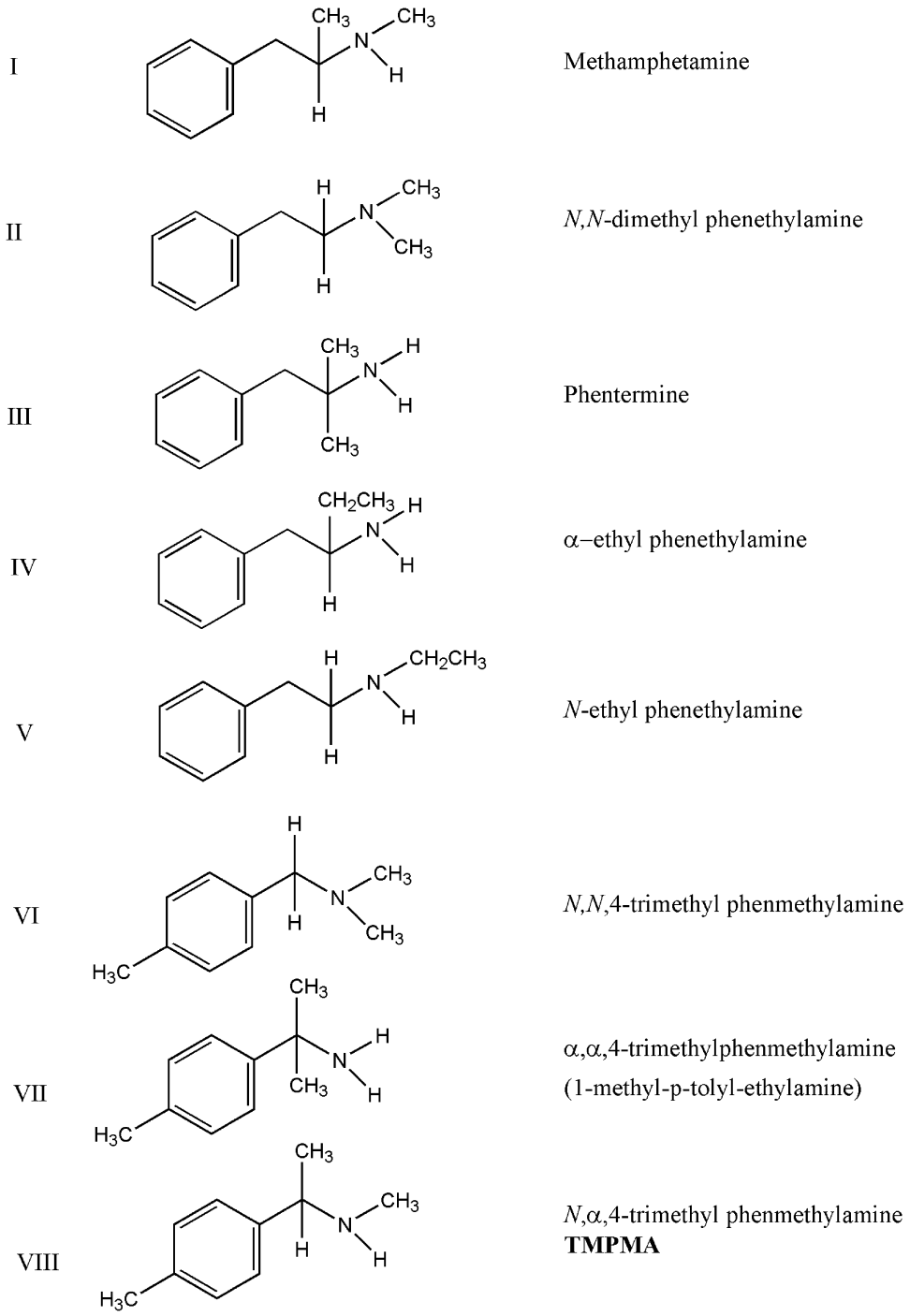


FIG. 1—*Methamphetamine and its isomers MW = 149 g/mol; the chemical names of the structures are indicated. Gas chromatography/mass spectrometry spectra available (13–15) for methamphetamine (***I***) and isomers* **II–VII**. *Isomer* **VIII** *was synthesized for this study.*

assigning peaks in a given spectrum, or for help with a retro-synthesis; it is also a useful approach to allow the analyst to dismiss spectral library "matches" that may be misleading or incorrect.

Materials and Methods

Chemicals

The two reactants for the synthesis of TMPMA, 4′-methylacetophenone (95% purity), and *N*-methylformamide (99% purity), were obtained from Aldrich (Milwaukee, WI) and used as received.

Nuclear magnetic resonance (NMR) spectroscopy was run in *d*-chloroform 99.9% D atom% with 0.03% (v/v). TMS were obtained from Aldrich and used as received.

Synthesis

A modified Leuckart synthesis (21) was used to make compound **VIII** in Fig. 1. 0.4 mol (23.3 mL) of *N*-methylformamide was added to a 250 mL round-bottom flask outfitted with a water-cooled reflux condenser, thermometer, and distillation head with a collector flask. 0.1 mol (13.0 mL) of 4-methylacetophenone was

added. The mixture was stirred in a sand bath at 190°C for a total of 24 h of reaction time—8 h at a time over 3 days. For safety reasons, we could not leave the reaction unattended. The cooled mixture was diluted with 3–4 vol. of water. Approximately 20 mL of concentrated HCl was added to the oil layer. This mixture was heated in the 190°C sand bath for a few hours over several days, and the reaction progress was monitored by GC/MS testing of small aliquots. Upon completion, water was added to the cooled mixture and the reaction was quenched by adding sodium hydroxide until basic. The product was extracted from an aqueous layer into ether using a separatory funnel. Upon addition of concentrated HCl, the product precipitated out and was collected and dried, yielding 0.39 g (~ 3%) of crude material. The low yield can be explained by the nonideal reaction conditions as a result of safety precautions. The product was recrystallized out of acetone; the melting point of the purified product was 159–160°C. Microcrystalline testing was pursued with a number of common reagents (22); only 5% (w/v) platinic bromide was found to form photodocumentable crystal forms (Fig. 2), which differ from methamphetamine. A number of other reagents were tested, with the result indicated square-parenthetically: 5% (w/v) platinic chloride [negative], 5% (w/v) gold chloride or hydrogen tetrachloroaurate [negative], 10% (v/v) hydrobromic acid [negative], 5% (w/v) mercuric chloride [oil], 5% (w/v) gold bromide or hydrogen tetrabromoaurate [oil], 5% (w/v) platinic iodide [oil], Wagner's (1.27 g iodine, 2.75 g KI in 100 mL water) [oil], saturated picric acid solution [oil], 5% (w/v) potassium iodide [negative].

Basified TMPMA product was allowed to dry to residue and diluted in CH_2Cl_2. GC/MS showed that no amine was present, although the wet product had contained the amine. This experimentation indicates that the base form of TMPMA is volatile; therefore, it should be stored as the salt form.

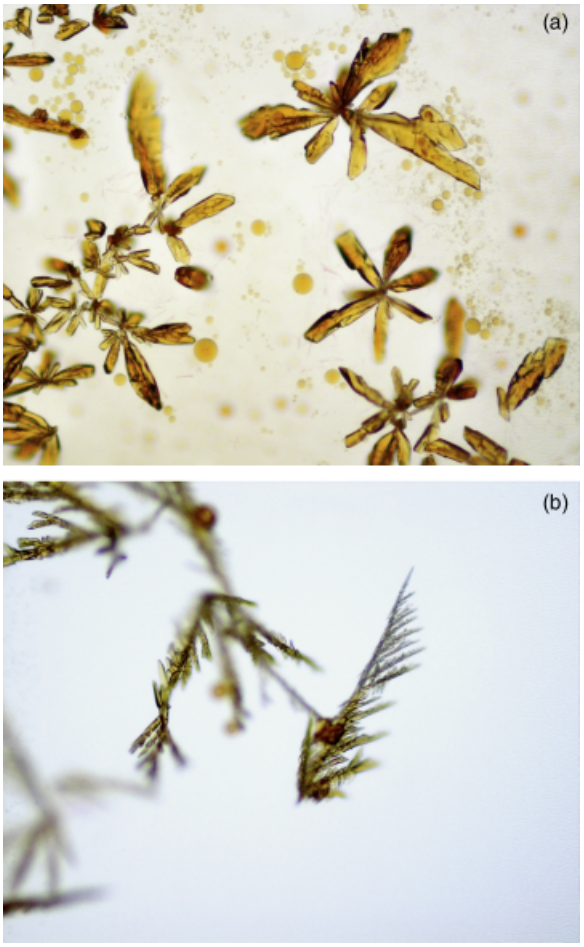


FIG. 2—*Microcrystalline forms of N,α,4-trimethyl phenmethylamine (***VIII***) with platinic bromide. (a) and (b) are representative forms of the resulting crystal structures. Magnification* c. *× 100 when photos are 2 × 2.5 in.*

GC/MS Instrument

Total ion chromatograms (TIC) were obtained using an HP 6890 Series GC connected to an HP 5972 Series Mass Selective Detector (MSD) with autosampling capability (Agilent 6890 Series injector [Santa Clara, CA]). Separation was achieved with an HP-5MS column (crosslinked 5% phenyl methyl siloxane 30 m × 0.25 mm × 0.25 µm film thickness) with helium carrier gas flowing at 0.9 mL/min (35 cm/s at 9 psi). The inlet was run in the split mode with a 25:1 ratio. The mass spectra were collected after a 2-min solvent delay over the mass range of 20–400 *m/z*. The injector was heated to 250°C and the MSD transfer line was maintained at 280°C. These instruments were interfaced with an HP Kayak Pentium 3 computer with enhanced ChemStation software G1701BA version B.01.00. The temperature program run on the GC column started at 100°C for 1.1 min, ramped at 40°C/min to 300°C, and held for 4.9 min. Chromatograms collected with this temperature program for regioisomers **I**, **III**, and **VIII** reported in this work contained peaks that were sufficiently resolved such that no problems were encountered with convolved spectra. Spectral library searches were conducted with the Agilent ChemStation software program (23), and the spectral library used was from the NIST Standard Reference Data Program (15). Background-corrected mass spectra were obtained by averaging data from the leading to trailing edges of the TIC peak of interest and subtracting averaged spectra from a flat region in the chromatogram (where no analyte was present, only eluent) over an approximately equal length of time. This approach minimizes the possible effects of spectral skewing (24,25) across the chromatographic peak; indeed, spectral variations from the leading edge, apex, and trailing edge do not affect the $m/z = 39$–58 region enough to invalidate the results in Table 1, although ratios in other regions may be slightly affected. While laboratories use different procedures to achieve an adequate lower limit of detection (for instance, some laboratories reference a presumed methamphetamine analyte against an amphetamine internal standard, while others may use deuterated methamphetamine as a reference), the protocol here was to concentrate on the spectral data itself and ensure that, at minimum,

TABLE 1—*Lookup chart of expected fragmentation patterns of dimethyl- and ethyl-substituted immonium ions with a parent peak of m/z = 58.*

	m/z →		
↓ Compound	41	42	56
α,*N*-dimethyl		≥ 39	≥ 39
α,α-dimethyl	≥ 39	≥ 39	
N,*N*-dimethyl		≥ 39	
α-ethyl	≥ 39		
N-ethyl		≤ 39	≤ 39

If the fragments present in the questioned spectrum match those in a row and have the indicated relative abundance with respect to the $m/z = 39$ peak, the arrangement of the dimethyl- or ethyl-substituted immonium structure is indicated in the far left column.

the spectrum showed either the molecular ion or M–H^+ (if there is an α hydrogen, otherwise the next largest α cleavage product) and that the peaks around $m/z = 51$ are discernible. Reference library spectra that are presented to illustrate various fragmentation patterns may not meet these minimum criteria as there was no way to control the conditions of those runs.

NMR Instrument

Spectra were collected on a 90 MHz Anasazi instrument (Anasazi Instruments, Indianapolis, IN) (*c.* 2 T field strength) with a given compound diluted in *d*-chloroform with a TMS reference giving rise to a peak at 0 p.p.m. The concentration of the sample was not strictly controlled, rather it was dictated by how much sample could be spared for analysis. The sample was excited with a pulse width of 21.2 μs; 256 averages were taken. There were 8192 data points collected over the range 14.24 to −2.39 p.p.m. NMR spectra for methamphetamine and TMPMA are shown in Fig. 3.

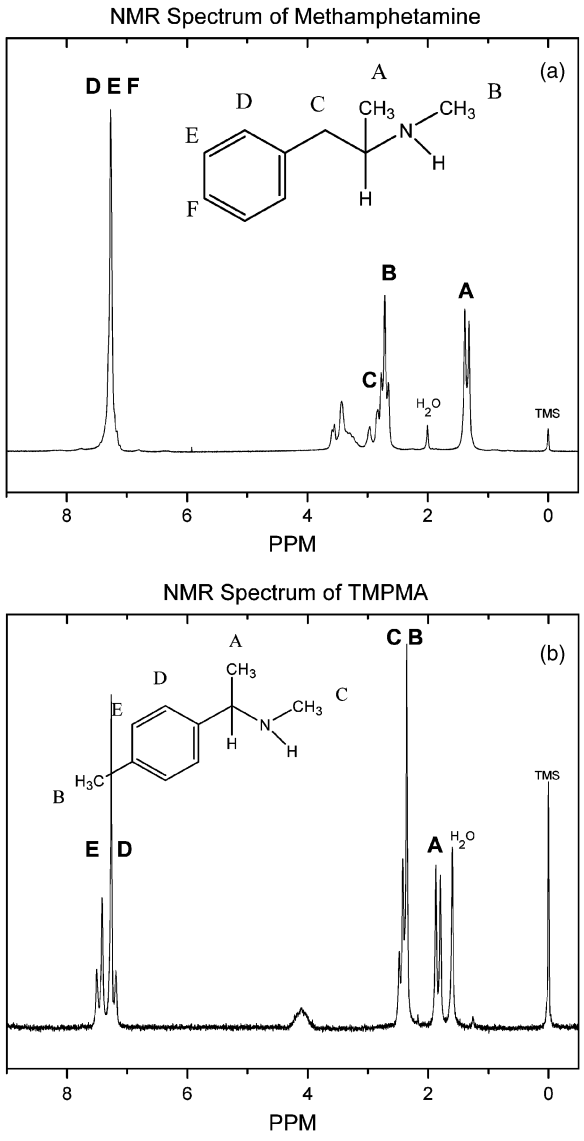


FIG. 3—*Nuclear magnetic resonance (NMR) spectrum of (a) methamphetamine and (b) N,α,4-trimethyl phenmethylamine (TMPMA) in $CDCl_3$. TMS reference with peak assignments shown. Field strength* c. *2 T resulting in 90 MHz proton resonance.*

Results

The series of regioisomers of methamphetamine considered in this study (**I–VII**) are presented in Fig. 1. One regioisomer of the series for which we could not find GC/MS data was TMPMA (**VIII**). This regioisomer was selected because it was targeted as a good synthetic candidate from easy-to-purchase starting materials and would offer spectral differences from methamphetamine. After synthesizing the compound, it was first characterized by microcrystalline tests shown in Fig. 2 and by NMR spectrometry shown in Fig. 3.

A synopsis of the GC/MS terminology used in the remainder of this article, and a review of some fundamental rules for fragmented ions will be established with the aid of Fig. 4. Only positively charged species can be seen by the electron ionization (EI) mass spectrometers used in most forensic settings. The rules stated here refer to EI spectra and will not apply to chemical ionization (CI) or electron capture (EC) techniques. A quadrupole MS system was used in this work, although ion trap data have been shown to follow the same detailed fragmentation patterns reported for methamphetamine in Fig. 5.

EI instruments cause a molecule to become a radical cation (odd electron ion [OE]) by removing an electron from somewhere in the molecule. Homolytic cleavage involves a radical site initiation of cleavage; upon fragmentation, the charge is retained on the original site or fragment (even electron ion [EE]) and a radical is ejected (Fig. 4*a*). Movement of single electrons characterizes the homolytic fragmentation mechanism. In this text, α cleavage is a very specific form of homolytic cleavage. Because the nonbonding electrons of nitrogen have fairly low ionization energies, the radical cation site is often assigned to nitrogen when it is present in a molecule. In the methamphetamine regioisomer series shown in Fig. 1, the carbon directly bonded to nitrogen is considered to be "α"; thus, an α cleavage will sever one of the three remaining bonds on the α carbon, thereby removing a radical and leaving behind a positive charge on the nitrogen. This process will be indicated with an α (three examples are shown in Fig. 5). The total abundance of the fragments formed from methamphetamine and its regioisomers is dominated by α cleavage processes, although this process only maximally represents three peaks in the spectrum.

Heterolytic cleavage involves movement of a pair of bonded electrons to the cationic site with a migration of charge to the atom attached to the former bond (Fig. 4*b*). This process is also known as induction, designated "i," or charge site initiation. One example of an energetically disallowed induction for methamphetamine (26) is given in the fragmentation scheme shown in Fig. 5. In fact, induction is a rare occurrence in the regioisomers of methamphetamine; indeed, in molecules containing an amine group, induction is far less favored than α cleavage. Induction does occur when more stable radicals such as *N*,*N*-dimethylamine and *N*-ethylamine are leaving groups; hence, it is appropriate to indicate the disallowed pathway for methamphetamine on the fragmentation map as Fig. 5 serves as a template for identifying fragmentation schemes for other molecules.

Rearrangement is defined as the movement of atoms that were not previously bonded in the radical cation to form a fragment with new bonds. This process ejects a neutral molecule; therefore, a radical cation is left behind (Fig. 4*c*). This type of fragmentation will be labeled "r" with three examples shown for methamphetamine in Fig. 5.

A "-" is used to indicate secondary decomposition mechanisms, that is, rearrangement of atoms in a cation after a radical from the

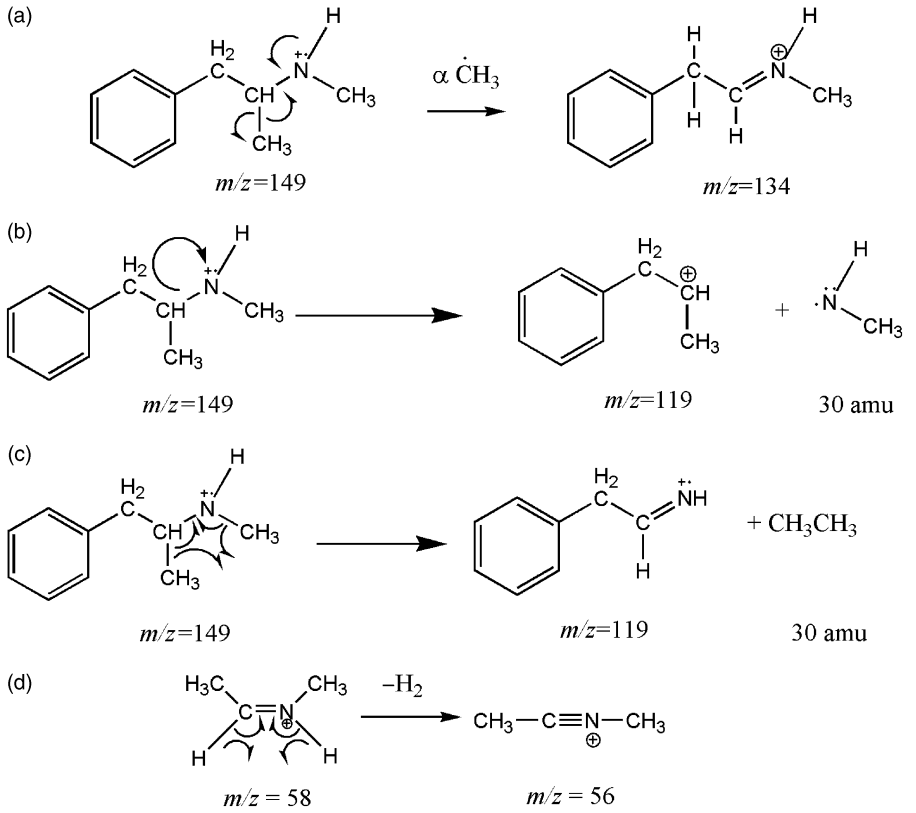


FIG. 4—*Example of mechanisms for (a) homolytic or radical site-initiated cleavage, (b) heterolytic or inductive cleavage, (c) rearrangement mechanism leaving a radical cation, and (d) secondary decomposition. Note that mechanism (b) does not occur in methamphetamine, rather mechanism (c) is correct (26).*

parent radical cation has been ejected (Fig. 4*d*). Movements of single or pair of electrons are acceptable. This movement of electrons may cause charge migration, but it is not by definition induction. Additionally, these losses are also neutral and may involve rearrangement of bonding, but they do not come from a radical cation; therefore, this type of fragmentation is not considered a rearrangement as defined above. There are many examples of this type of loss for methamphetamine shown in Fig. 5. While the number of secondary decomposition peaks is large and the losses describing these peaks are typically the key to understanding a spectrum, the total abundance represented by them is often a small fraction of the total abundance of other fragments. These mechanisms are most difficult to analyze and often require an isotopic study for verification (25).

As alluded to above, a map proposing the structures of the main GC/MS-derived fragments of methamphetamine is given in Fig. 5. The fragments are assigned to the methamphetamine GC/MS spectrum in Fig. 6. Notable entries are peak F for $m/z = 115$ where we propose an indene cation (26) and peaks J ($m/z = 65$) and N ($m/z = 51$) whose structures are detailed in Fig. 7 below. A schema for making these peak assignments and for predicting fragmentation of unknown compounds will be proposed and reviewed in "Discussion."

After culling the literature for GC/MS-derived spectra of methamphetamine regioisomers and running standards of available compounds in our laboratory, a clear, consistent pattern of fragments began to emerge. The traditional patterns of phenyl and benzyl secondary decompositions were observed (Fig. 7*b* and *c*). One notable variation proposed here is for the phenyl-derived $m/z = 51$ and benzyl-derived $m/z = 65$ and $m/z = 63$ fragments. As shown in Figs. 5 and 7, it is suggested that the structures of each of these fragments contain a straight-chain component. One resonance form of the phenyl-derived $m/z = 51$ fragment has a conjugated straight-chain structure: the benzyl-derived $m/z = 65$ and $m/z = 63$ ethylene- and acetylene-terminated cyclopropenyl-cationic configurations, respectively; some texts (10) and indeed "conventional wisdom" have designated cyclobutadiene and cyclopentadiene structures. These are contraindicated by analyzing the populated π-orbitals in the structures, that is, cyclobutadiene and cyclopentadiene each contain a diradical, or two unpaired electrons. While diradicals are not necessarily inherently unstable, a given structure gains stability in cyclizing only if it achieves aromaticity. By the $4n+2$ Hückel rule, cyclobutadiene and cyclopentadiene are not aromatic (each has four π electrons); in fact, they are antiaromatic (antiaromaticity is defined by four conditions: (1) $4n$ π electrons are present, (2) each atom in the cyclized structure has a *p* orbital perpendicular to the ring, (3) the cyclic structure is planar, and (4) the structure is less stable [higher energy] than the open chain). Conversely, each of our proposed structures has stability: the straight chain $m/z = 51$ possesses resonance stabilization and aromaticity in its structures, while the proposed $m/z = 63$ and 65 fragments contain cyclopropenyl ion functional groups and are aromatic by the $4n+2$ Hückel rule, where $n = 0$.

One topic that should be covered is the concept of ionization energy. This leads directly to an understanding of mechanisms and how most of the proposed fragments follow from a nitrogen radical cation. The lower the ionization energy of a given substituent, the greater the likelihood an electron is removed from its structure versus another site with a higher ionization energy. In general, the series of ionization potentials progresses as follows: nonbonding electrons < π electrons $\ll$ σ. Specific to groups relevant to

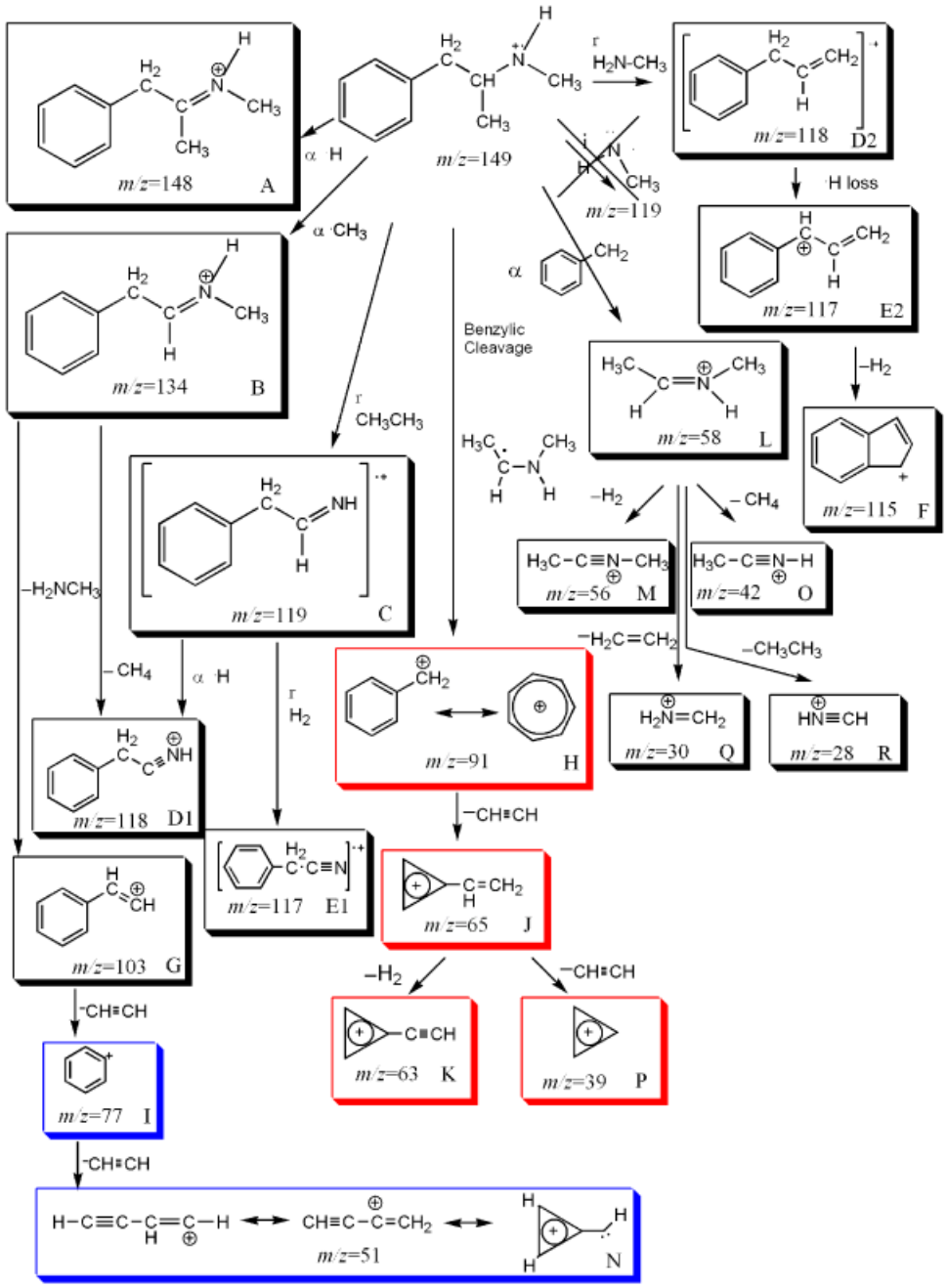


FIG. 5—*Structure of methamphetamine fragments observed by mass spectrometry (MS). Letters are used to refer to fragments present in MS spectrum of methamphetamine in Fig. 6. Benzyl fragments are H, J, K, and P; phenyl fragments are I and N.*

methamphetamine regioisomers is as follows (9): R_2N–H < RHN–H ≈ benzyl π electrons < benzene π electrons ≪ $R(H_2C–CH_2)R$. It is not unlikely to remove a benzene π electron, simply less likely than removing a nonbonding electron from the nitrogen in the amine; thus, the different pathways represent competing forms of the generated radical cation. Therefore, as detailed in the mechanism shown in Fig. 7*c*, benzylic cleavage occurs after ionizing the benzene ring. It should be noted that σ-bond cleavage occurs rarely for compounds with other, more ionizable, functional groups so that it is not a reasonable mechanism for fragmentation of the compounds studied here.

Yet, the most exciting offshoot of Fig. 7*a* has to do with the secondary decomposition mechanisms of the $m/z = 58$ immonium ions. The mechanisms are proposed to explain the GC/MS-derived peaks observed for isomers involving dimethyl- and ethyl-substituted amines. Table 1 summarizes the empirical observations that arose from analyzing hundreds of spectra and now allows an analyst to use it as a lookup chart to determine the configuration of the two methyl or single ethyl groups on an immonium ion fragment, and therefore the original amine configuration. It treats the $m/z = 39$ peak as a reference; thus, it is primarily useful for compounds that have both an amine and benzene ring in the parent molecule. Because it uses a peak generated at the time of the data collection as a pseudo-internal standard, this procedure has proven to be platform independent. In addition to the presented data that were collected under the conditions reported above, published data (1,2,4–7,13,15,27,28) demonstrate that spectra collected on different instruments under different conditions all support the

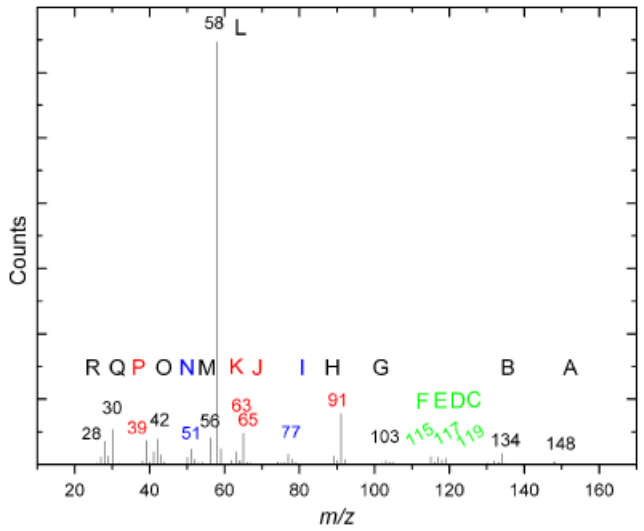


FIG. 6—*Mass spectrometry spectrum for methamphetamine. Masses of important m/z fragments are indicated. Letters refer to the fragment structures shown in Fig. 5.*

results of this lookup table (refer to Fig. 8). Table 1 allows an analyst to determine the substitution pattern on the immonium ion fragment if the peaks and intensities observed are among those listed by finding all of the $m/z = 41$, 42, and 56 peaks and comparing the relative abundance of each with the $m/z = 39$ peak. The isomeric dimethyl- or ethyl-substituted methylamine structures can be recognized by looking in the $m/z = 39$–58 range.

After inspecting the mechanisms in Fig. 7*a* for the fragmentation of $m/z = 58$ immonium ions, the reason why the α,*N*-dimethyl $m/z = 56$ and 42 fragments have a higher relative abundance than the *N*-ethyl $m/z = 56$ and 42 peaks reported in Table 1 becomes clear: the extra rearrangement step that the *N*-ethyl fragment must take lessens the amount of the $m/z = 58$ dimethyl arrangement available for further fragmentation. It is important to note that the magnitude of the α,*N*-dimethyl-derived $m/z = 56$ and 42 populations exceeds the relative abundance of $m/z = 39$ reference peak while the $m/z = 56$ and 42 peaks are lower than the $m/z = 39$ peak for *N*-ethyl-derived compounds. This crossover with respect to the internal standard of $m/z = 39$ is a fortuitous empiricism that is exploited in the differentiation analysis shown in Table 1.

While the structural configuration of the ubiquitous amine functional group of methamphetamine isomers is easily determined from the lookup chart in Table 1, the structure of the remainder of the molecule must be ascertained from other spectral decomposition analyses. Figure 8 shows GC/MS-derived spectra for somewhat common illicit compounds having amine termini with substitution patterns in Table 1. Each example illustrates empirically that the data in Table 1 hold true; as some of the spectra reported in Fig. 8 were from other laboratories, it shows that this procedure is not just for special regioisomeric methamphetamine standards on a specific instrument under certain conditions in our laboratory, but that it is transportable across platforms and is indeed program independent. As an additional test of platform independence, mass spectral data obtained from an ion trap instrument for methamphetamine and phentermine also adhere to the fragmentation patterns reported in Table 1.

The GC/MS-derived spectra of compounds **VI** and **VII** (Fig. 9) are useful guides for predicting what peaks the spectrum for TMPMA (**VIII**) will contain. One observation is that the relative abundance of the peak at $m/z = 105$ for **VI** is much higher than for **VII** (refer to Fig. 6). The reason for this is twofold: the inductive cleavage leaving the fragment $m/z = 105$ [H_3C–(C_6H_5)–CH_2^+ or tolyl cation] is benzylic for compound **VI**, and thus fairly facile; secondly, the leaving radical [:N.(CH_3)$_2$] is stabilized by the presence of two alkyl groups (a secondary amine). This is contrasted with disallowance of the inductive pathway for methamphetamine (see Fig. 5, fragment E2): as it is not a benzylic cleavage and it leaves behind a relatively unstable primary amine radical, it does not occur via the induction route (26). The formation of the $m/z = 105$ fragment for structure **VI** is expected to be abundant due to the tolyl leaving cation as well as the stabile amine radical; furthermore, as the α carbon position in compounds **VI–VIII** becomes more substituted, it is expected and observed that α-cleavage is preferential, resulting in the change of base peak of $m/z = 58$ for **VI** to $m/z = 134$ for **VII**. Additionally, the stability of the $m/z = 134$ fragment is enhanced due to the delocalization of charge through the resulting conjugated network.

The columns of Table 2 show the observed mass spectral peaks of **VI** and **VII** and predicted mass spectral peaks of TMPMA (**VIII**). The last three rows of Table 2 are derived from the lookup chart in Table 1: in the case of **VI** and **VII**, these values were verified and for TMPMA (**VIII**) they were predicted.

The mass spectrum acquired for TMPMA is shown in Fig. 10. Each of the peaks indicated is explained with predictions by analogy to **VI** and **VII** (see Table 2), the α,*N*-dimethyl-substituted immonium fragments from the lookup chart in Table 1, or by benzyl and phenyl fragments (Fig. 7*b* and *c*). As can be seen, all of the significant fragment contributors to the mass spectrum were identified *a priori* without much effort.

Discussion

The determination of the structure implied by important peaks in a GC/MS-derived spectrum is what we propose in the following schema. The schema is useful for any compound, not just methamphetamine or TMPMA (**VIII**). For a known compound, the first step is to identify large functionalities present. If benzene rings are present, peaks at $m/z = 77$ and 51 should be expected (Fig. 7*b*). If there is a CH_2 group adjacent to a benzene (benzylic configuration), peaks at $m/z = 91$, 65, 63, and 39 are probable (Fig. 7*c*). For an alkyl-substituted benzene ring with an adjacent CH_2 group connected to the rest of the molecule R–C_6H_4–CH_2–R', peaks at $m/z = 91 + R - 1$, and possibly $65 + R - 1$ and $39 + R - 1$ are likely (see as an example Fig. 9*a*; $m/z = 105$, 79, and 53 are observed although the R group leaves on a substituted acetylene causing a lower population of $m/z = 79$ and 53, thus complicating the spectrum slightly). For an amine, it can be expected that each of the three C–R bonds adjacent to the nitrogen may undergo α cleavage. The next step is to address "logical losses" such as H_2 ($m/z = 2$), CH_4 ($m/z = 16$), CH_3CH_3 ($m/z = 30$), $H_2C = CH_2$ ($m/z = 28$), and HC ≡ CH ($m/z = 26$), which can arise from combining groups from two adjacent atoms that easily form double or triple bonds such as C–C and C–N without sterically hindering groups (Fig. 5, L → M, O, Q, R, G → I). The last, and perhaps the most difficult part, is to propose multistep rearrangements that result in chemically stable products (refer to Fig. 5, E2 → F, and even H → J → K, P, or I → N as examples).

The scheme for "retro-synthesis," that is, figuring out the structure of the analyte based on the spectrum, is undoubtedly more complicated as the answer is often unknown. Furthermore, it is difficult to propose a generalized schema for the vast number of possible compounds that could arise in casework. However, the notion of describing "logical losses" from the spectrum has merit.

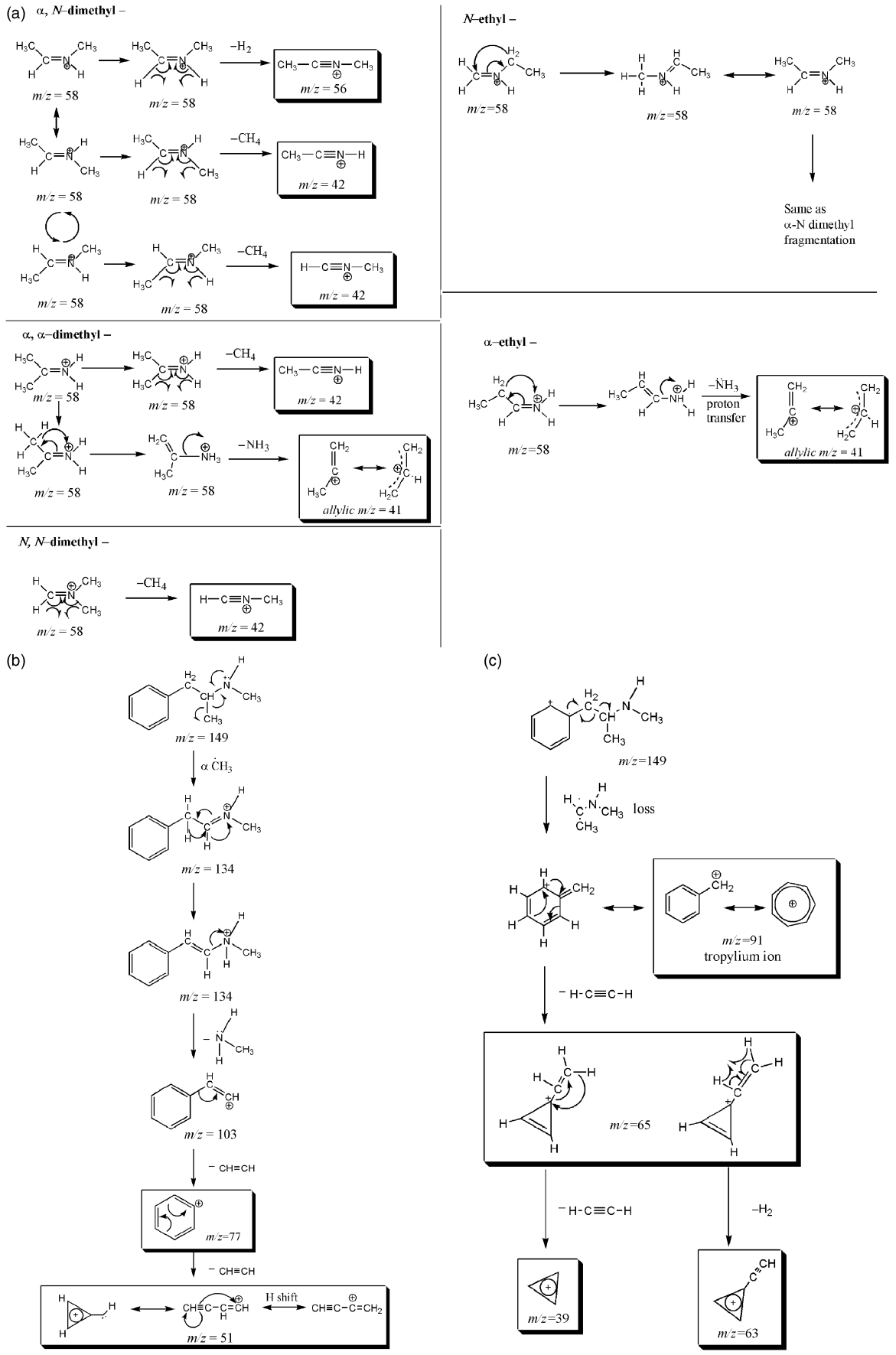


FIG. 7—*Secondary decomposition mechanisms of (a) immonium ions where m/z = 58, (b) phenyl products m/z = 77 and 51, and (c) benzyl products m/z = 91, 65, 63, and 39.*

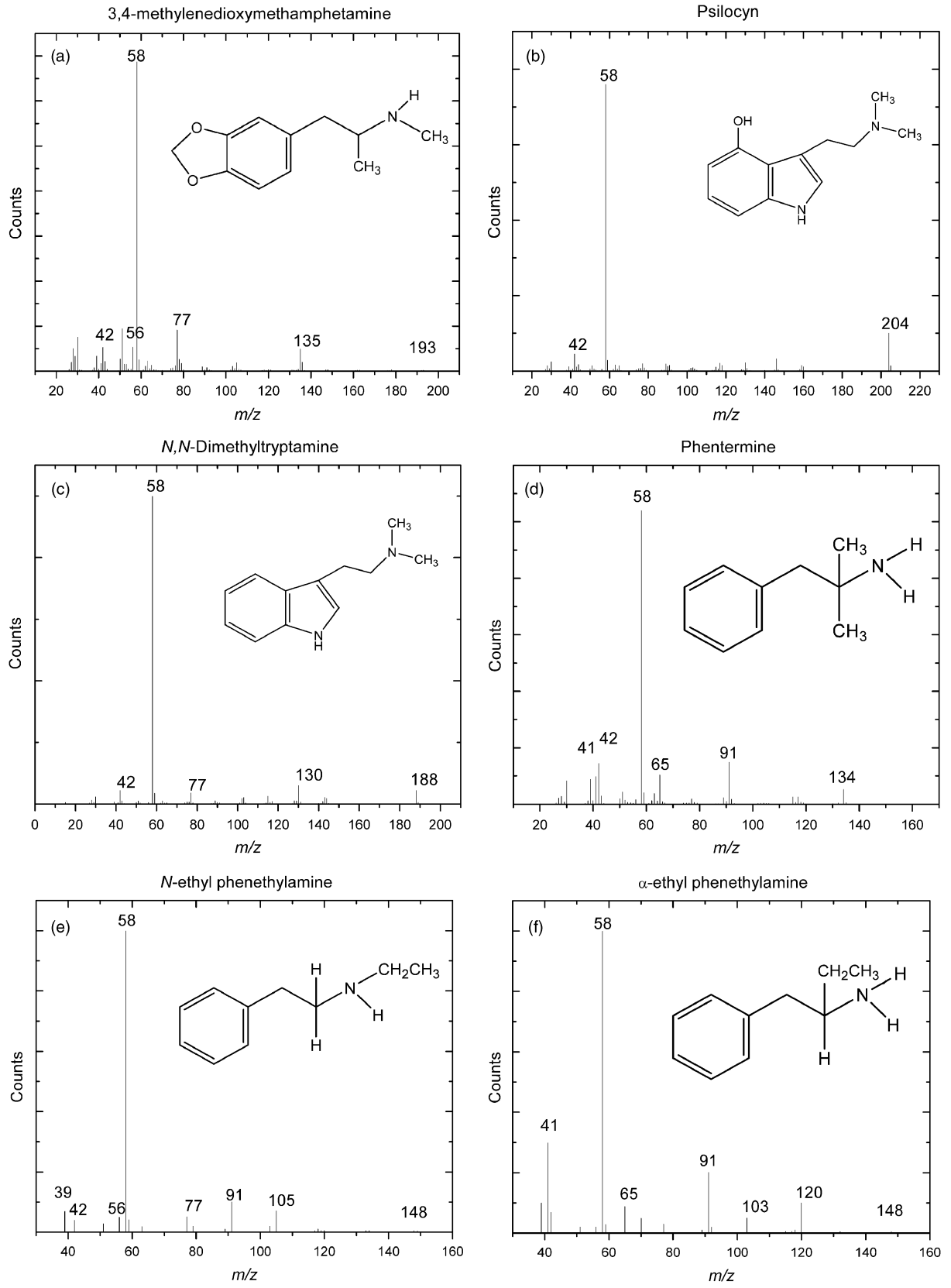


FIG. 8—*Examples of illicit substances generating dimethyl- or ethyl-substituted immonium ions upon fragmentation. (a) 3,4-methylenedioxymethamphetamine, (b) psilocyn, (c)* N,N*-dimethyltryptamine (15), (d) phentermine, (e)* N*-ethyl phenethylamine (15), and (f)* α*-ethyl phenethylamine (15). Each exhibits fragmentation following the criteria in Table 1.*

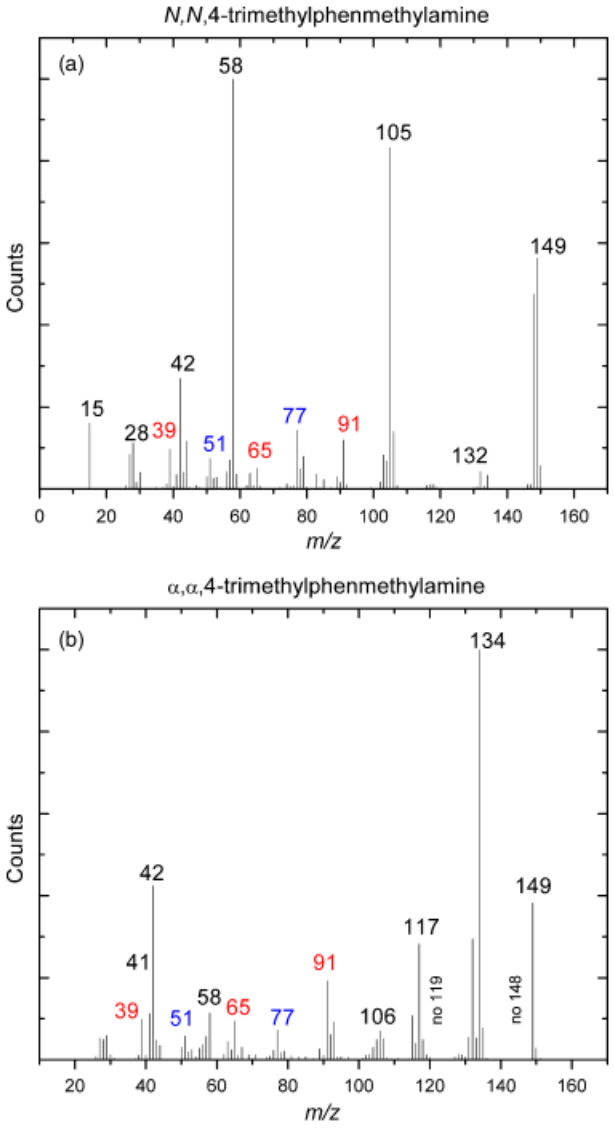


FIG. 9—*Mass spectra of known ring-substituted phenmethylamines* **VI** *(a) and* **VII** *(b) (15). Important mass fragments are indicated; notable absences are labeled. m/z values for the peaks of the tropylium ion and its fragments are m/z = 91, 65, and 39 (H, J, P in Fig. 5); phenyl cation and its decomposition product are m/z = 77 and 51 (I, N in Fig. 5).*

Some of those detailed in the previous paragraph are typical for controlled substances.

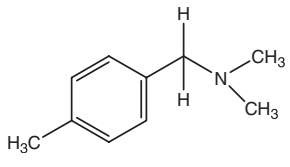
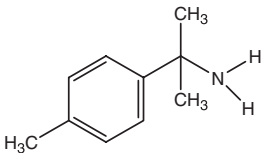
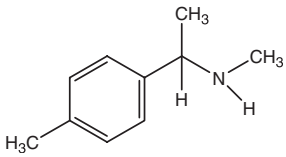
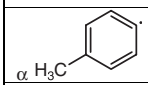
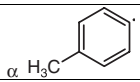
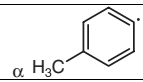
The nitrogen rule can be useful in such retro-synthetic analyses and is summarized as follows: compounds containing an odd number of nitrogen will have an odd nominal mass (this includes all radical cations before fragmentation). Upon fragmentation, if the positively charged fragment retains the nitrogen, it will have an even nominal mass. If, however, the radical lost contains the nitrogen, the positively charged ion without the nitrogen will have an odd nominal mass. This rule does not apply to deuterated compounds. Reliance on the nitrogen rule is important to show whether or not the proposed suggestion is physically possible. For instance, structure D2 of Fig. 5 has an even mass, but no nitrogen. It is therefore necessary to realize that it must still be a radical cation. Only when it loses a hydrogen radical, $^{\cdot}H$, with an odd mass to make the mass of the cation fragment odd, can the nitrogenless structure be shown as a cation.

It is also important to reiterate that α cleavage is by far the largest contributor to abundant peaks for compounds containing amines. Proposed fragmentation patterns that rely heavily on induction reactions probably are not reasonable. Secondary decomposition routines are also key to describing peaks; however, chemically reasonable structures must be posited. Offering predictions for other methamphetamine regioisomers that have as yet unreported mass spectra (ββ-dimethyl-phenethylamine, β-methylamphetamine, and *N*-ethyl-4-methyl-phenmethylamine) might yield interesting directions for future research. For instance, ββ-dimethyl-phenethylamine would most likely exhibit α cleavage with loss of α $^{\cdot}H$ ($m/z = 148$), α dimethylbenzyl radical ($m/z = 30$), benzene radical cation-initiated losses of $^{\cdot}CH_3$ ($m/z = 134$) and $^{\cdot}CH_2NH_2$ ($m/z = 119$), no $m/z = 58$, phenylic components of $m/z = 77$ and 51, and the base peak would most likely be $m/z = 30$. β-methylamphetamine: α $^{\cdot}H$ ($m/z = 148$), α $^{\cdot}CH_3$ ($m/z = 134$), the ring excitation losses of $^{\cdot}H$, $^{\cdot}CH_3$, and methylamine radical leaving the $m/z = 148$, 134, and 105 methylbenzyl cation fragments and the base peak would probably be $m/z = 44$ from an α methylbenzyl radical loss exhibiting $m/z = 42$ and 28 satellites. *N*-ethyl-4-methylphenmethylamine would probably show a peak at $m/z = 148$ from $\alpha \cdot H$ loss that is larger than that in methamphetamine due to the extended conjugation of the remaining cation (similar to TMPMA), $m/z = 134$ from α $^{\cdot}CH_3$, an $m/z = 58$ peak from α methylbenzyl radical with satellites at $m/z = 42$ and 56 that are less intense than the $m/z = 39$ peak (see Table 1), and peaks at $m/z = 148$, $m/z = 134$, and $m/z = 105$ from $^{\cdot}H$, $^{\cdot}CH_3$, and $^{\cdot}NHCH_2CH_3$ radical losses from the benzene ring-initiated fragmentation. For each of these compounds, the resulting spectrum would be distinguishable from methamphetamine by the presence of additional peaks not apparent in methamphetamine ($m/z = 105$ for β-methylamphetamine and *N*-ethyl-4-methyl-phenmethylamine) or by obvious absences of major peaks (no $m/z = 58$ for ββ-dimethyl-phenethylamine). Similar predictive exercises for other compounds are possible using the fundamentals outlined in this paper.

Additionally, alternative approaches to traditional spectral library search routines may be warranted. Tackett (29), Steeves et al. (30), and Stromberg and Wistedt (6) each recommend methods that suppress the base peak and perform searches on the remaining, normalized, fragmentation pattern. Another fruitful approach is the proposal by Gan et al. (31) to perform searches based on peak positions rather than peak intensity to pull out related compounds when the spectrum of a questioned compound has a low match value from the spectral library search. Notably, every peak present in the methamphetamine spectrum (Fig. 6) is present in the regioisomeric TMPMA spectrum (Fig. 9), the relative abundance of each being the only variable. This observation lends credence to the latter type of modified spectral library search routine to give structurally related compounds. Interestingly, the spectral library search (15,23) of the TMPMA spectrum gave structural suggestions based on the intense $m/z = 134$ peak and did not suggest methamphetamine as a possible match.

Reiterating the point about multiplatform applicability of immonium fragment rearrangements: spectra collected from our quadrupole EI system under differing conditions, reference library spectra, and data from literature and spectra collected on an ion trap mass spectrometer, all show the fragmentation pattern proposed in Table 1. Occasionally, a library spectrum does not report m/z values < 40 (13), which makes application of this method more challenging by removing the pseudo-internal standard, although it can still be used to differentiate α,α-dimethyl, *N*,*N*-dimethyl, and α-ethyl but not between α,*N*-dimethyl

TABLE 2—A priori *predictions of MS fragmentation for new synthetic product, TMPMA (***VIII***), versus ring-substituted* **VI** *and* **VII***; each parent has a molecular weight of 149 g/mol.*

Observed MS Peaks for  **VI**		Observed MS Peaks for  **VII**		Predicted MS Peaks for  **VIII**	
α H	148	no α H	no 148	α H	148
no α CH_3	no 134	α CH_3	134	α CH_3	134
r HCH_3	small 133	r HCH_3	small 133	r HCH_3	small 133
no r CH_3CH_3	no 119	no r CH_3CH_3	no 119	r CH_3CH_3	119
i $^{\cdot}N(CH_3)_2$ somewhat stable, leaves benzylic = large driving force	105	i $^{\cdot}N(H)_2$ not very stable	v. small to no 133	i $^{\cdot}NH(CH_3)$ intermediate stability	v. small 119
	58		58		**58**
$-CH_4$	42	$-CH_4$	42	$-$ **H_2**	**56**
		$-NH_3$	41	$-$ **CH_4**	**42**

α, r, and i—used to indicate fragment losses as in text. Bold entries for TMPMA predictions come from the lookup chart in Table 1.

and *N*-ethyl. Short of running the standard to obtain a spectrum with a broader *m/z* range, the lookup chart of Table 1 can aid analysis.

It should be mentioned that another technique that has been used to get around "regioisomer indistinguishibility" by GC/MS is GC/IR/MS. These clever experiments (32,33) combine a spectroscopic technique with the GC/MS method detailed here. Additional data allow for indisputable differentiation of regioisomers. Unfortunately, for most laboratories, this expensive instrumentation is unavailable. In this case, being able to extract the most out of GC/MS-derived data to which most forensic laboratories already have access is critical and additionally emphasizes the importance of this work, although by no means does it suggest that GC/IR/MS is invalid.

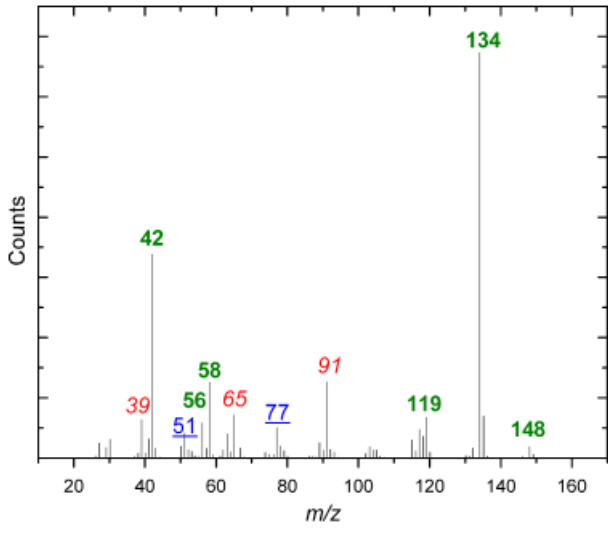


FIG. 10—*Mass spectrometry spectrum for N,α,4-trimethyl phenmethylamine (***VIII***). m/z values of important fragments shown with tropylium ion fragments are italicized, phenyl cation fragments are underlined, and fragments predicted in Table 2 are indicated in bold.*

Soine et al. (34) state that some side chain position isomers of amphetamine are difficult to distinguish by GC/MS. Abercrombie (28) claims MS indistinguishability between dimethylamphetamine and mephentermine. These claims, along with the present study, provide a segue to future study of variously substituted amines including methyl (amphetamine-like), trimethyl (mephentermine-like), or methyl/ethyl-substituted phenethylamines.

Conclusions

Methamphetamine regioisomers can be differentiated with the use of GC/MS. This paper adds confidence to allow the analyst to make these identifications by MS instead of by derivitization techniques, GC–IR, or chromatographic separations. We have outlined a total fragmentation analysis of methamphetamine based on fundamental principles of cleavage, rearrangement, and secondary decomposition and supported by the available experimental data. The present analysis may be used as a template for determining the molecular structures of other compounds. We also showed that spectrum prediction capability exists when compounds similar to that of the new chemical variant are analyzed. These principles may assist in the situations where an unfamiliar, unknown, or low spectral library match to a spectrum exists. This paper helps the analyst approach the next step of the problem, which may be to perform a peak-position search for related structures, spectral decomposition analysis, or synthesis or purchase of the deduced molecular candidate.

It is essential to state that by writing this paper, we do not discredit the use of spectral libraries as tools for analysts of controlled substances; rather, we suggest that augmenting match routines with a knowledge of fragmentation mechanisms is of prime importance to be able to: (1) understand why a given spectrum has a low(er) match value; (2) identify or discount a possible library error; (3) predict what fragments to expect from a new

compound; or (4) correctly interpret chemical variants analyzed in the laboratory.

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