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Role of Five Synthetic Reaction Conditions on the Stable Isotopic Composition of 3,4-Methylenedioxymethamphetamine

Hilary A. S. Buchanan,[†,§] Niamh Nic Daéid,[†,*] William J. Kerr,[†] James F. Carter,[‡] and Jenny C. Hill[‡]

Centre for Forensic Science, Department of Pure & Applied Chemistry, University of Strathclyde, 204 George Street, Glasgow G1 1WX, Mass Spec Analytical Ltd, Building 20F Golf Course Lane, P.O. Box 77, Filton, Bristol BS99 7AR, England, U.K.

The identification of links between seizures of illicit 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") has been a global target of law enforcement agencies in recent years. Previous work has shown that, when the reaction conditions are carefully repeated from batch to batch, stable isotope ratios allow the discrimination of MDMA·HCl batches according to synthetic route used for manufacture. In this study, the effects of altering five reaction conditions relating to the Pt/H_2 reductive amination synthesis were, for the first time, systematically investigated using a two level, five factor factorial design. Results indicate that the δ^2H values of MDMA·HCl are affected by the length of imine stir time, and the $\delta^{15}N$ values are affected by the degree of excess methylamine employed. Furthermore, the $\delta^{13}C$ and $\delta^{18}O$ values have been shown to be affected by the efficiency of the reaction, despite the similarity in carbon and oxygen composition of the starting material and product molecules. In addition to being of theoretical importance in this field of analytical science overall, this work is essential in order to more fully contextualize the interpretation of IRMS data which may be used as potential forensic evidence.

In recent years, drug profiling, or the ability to link batches of illicit drugs to a common source or synthetic route, has been a target for law enforcement agencies and researchers.[1] Relating to this, stable isotope ratio mass spectrometry (IRMS) has been investigated as an emerging technique of some value, which can provide information for the profiling of synthetic drugs, such as 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy").[2−8] Because much research using IRMS has been conducted on illicit MDMA of which the history is unknown, accurate evaluation of IRMS as an MDMA profiling technique cannot be fully undertaken.

By utilizing MDMA synthesized within our own laboratories, it has been shown[8] that IRMS analysis of MDMA·HCl batches can afford discrimination of the samples by the synthetic methods employed (i.e., reductive amination using Al/Hg, $NaBH_4$ or Pt/H_2). In particular, the δ^2H values were the most promising for such differentiation of the synthesized samples. Notably, in this previously published study, 18 batches of MDMA·HCl were synthesized using carefully controlled reaction conditions to ensure that any induced fractionation could be repeated from batch to batch. However, it is unlikely that clandestine chemists work in such a controlled manner and, even if they do, it is unlikely that two independent laboratories will employ identical reaction conditions. Consequently, if altering the reaction conditions has an effect on the δ values of MDMA·HCl, then discrimination by synthetic route may become more complicated.

Based on all of this and in order to assess the effect of altering specific synthetic reaction conditions on the stable isotope ratios of MDMA·HCl, the most commonly encountered[9,10] reductive amination with Pt/H_2 (Figure 1) was chosen for further investigation. In this preparative pathway, condensation of 3,4-methylenedioxyphenyl-2-propanone (PMK) and methylamine produce the corresponding imine, and reduction is achieved using PtO_2 catalyst under a hydrogen atmosphere at elevated pressure.

Five reaction conditions within the Pt/H_2 synthesis were systematically altered according to a full, two level (2^5) factorial design, resulting in the preparation of 32 MDMA·HCl batches. The five reaction conditions investigated in this study were quantity of methylamine, quantity of PMK, stir time for imine

* To whom correspondence should be addressed. Phone: +44-141-548-4700. Fax: +44-141-548-2532. E-mail: n.nicdaeid@strath.ac.uk.

† University of Strathclyde.

‡ Mass Spec Analytical Ltd.

§ Present address: Scottish Police Services Authority, Forensic Services (Edinburgh), 11 Howden Hall Road, Edinburgh EH16 6TL, Scotland, UK.

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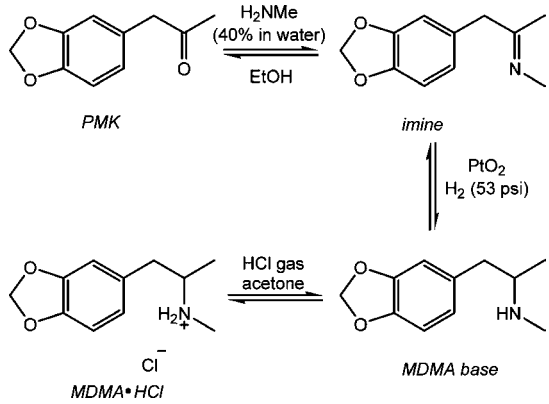

Figure 1. Illustration of the reductive amination (Pt/H_2) pathway to MDMA synthesis.

formation, quantity of PtO_2 catalyst, and length of time allowed for hydrogenation. These high and low levels were chosen such that all of the experiments could be comfortably undertaken using a single batch of homogenized PMK starting material, while still being able to obtain enough MDMA product from each run to accommodate the desired analyses. The resulting MDMA·HCl samples were then analyzed for $^2H/^1H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, and $^{18}O/^{16}O$ isotope ratios to assess what effect, if any, each preparative factor has on the isotopic composition of the MDMA·HCl product. The significance of each factor (and interactions between factors) was assessed using Design-Expert software (Version 6.0.10).[11]

The δ^2H values were previously shown to hold the best discriminating power of MDMA by synthetic route,[8] and the $\delta^{15}N$ values have been observed to vary widely within both in-house synthesized and illicit MDMA samples.[2,3,5,7,8] Because the two oxygen atoms and 10 of the 11 carbon atoms making up the MDMA molecule are contributed by the starting material (PMK),[8] the $\delta^{18}O$ and $\delta^{13}C$ values of MDMA·HCl are expected to be closely related to the batch of starting material used for synthesis.

EXPERIMENTAL SECTION

Reagents and solvents were sourced from chemical suppliers (such as Sigma Aldrich and Fisher Scientific, UK) and used without further purification. All MDMA·HCl batches in this study were synthesized from a single stock of homogenized PMK which had been prepared according to the procedure published previously.[8] Batches of MDMA base were prepared in-house according to the procedure published previously,[8] with the low and high levels detailed in Table 1a. The quantity of ethanol (2.5 mL) and the pressure in the hydrogenator (53 psi) were held constant. Furthermore, the workup procedure utilized for the isolation and purification of MDMA base had to be scaled to the level of PMK used in order to keep the method consistent enough to be considered a constant. For instance, the goal of the first step of the workup procedure is to acidify MDMA base so it moves into the aqueous layer. If the "usual" volume of acid is used for an experiment which required the high level of PMK, then the MDMA base may not be acidified completely and, in turn, would be discarded with the organic layer. Thus, the quantities of water, acid, and base used in the workup were scaled to the level of PMK employed as detailed in Table 1b.

Conversion of the MDMA base to the HCl salt was achieved as indicated.[12] To MDMA base (0.3 g, 1.5 mmol) was added acetone (1.9 mL). The solution was held at −10 to −15 °C. To a separate flask containing NaCl (5 g) was added 37% aqueous HCl (3 mL) with stirring. A volume (0.7−2.2 mL) of concd H_2SO_4 was added dropwise to the mixture. The HCl gas emitted from this mixture was allowed to pass via a cannula directly into the cooled MDMA base/acetone solution, with swirling. The reaction was stopped when most of the solution had turned to solid and/or the pH of the MDMA base/acetone solution was slightly acidic. (It was not possible to use a consistent amount of H_2SO_4 for each batch.) The solid was then washed with cooled acetone and dried under vacuum to reveal white or beige MDMA·HCl (0.33 g, 94% yield). IR, ^1H NMR, and ^{13}C NMR analysis of synthesized compounds was in agreement with published data reported previously;[8,13−16] see the Supporting Information (SI).

All permutations of the five factor levels were synthesized according to a random order generated by Design-Expert. Thirty-two MDMA·HCl batches resulted. Because only one replicate of each factor level permutation was carried out, an estimate of the variation of each observation (or the "pure error")[11,17] was not possible. To compensate for this, 11 replicate batches were synthesized using the baseline conditions: six were synthesized before the 32 experiment factorial design, and five were synthesized after. These replicate batches provided an estimate of the expected variation for each observation and, since their synthesis spans the factorial design study, incorporate variation over time. The variation in these 11 batches was automatically incorporated into the analysis of the 32 factorial design batches by Design-Expert software.

All isotopic measurements were performed using a ThermoFinnigan Delta^{PLUS}XP isotope ratio mass spectrometer via a ConFlo III interface. Carbon and nitrogen isotopic measurements were performed using a ThermoElectron Flash 1112 elemental analyzer. Samples (approximately 0.2 mg) were crimped into tin capsules (Elemental Microanalysis, Okehampton, UK) and introduced to the instrument by an AS200 autosampler. The oxidation reactor, comprising chromic oxide and silver/cobaltous oxide, was maintained at a temperature of 950 °C, and the reduction reactor, comprising electrolytic copper, was maintained at 680 °C. Helium carrier gas was maintained at a flow of 150 mL min^{-1}. Water was removed from the evolved gases by anhydrous magnesium perchlorate, and the nitrogen and carbon dioxide formed in the reactor were separated using a gas chromatography column containing

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Table 1. High, Low, and Baseline Level Reaction Conditions Utilized for the Factorial Design Study[a]

label	factor	low level	high level	baseline level
(a)				
A	H_2NMe (mL)	0.5	2	1
B	PMK (mL)	0.6	2	1
C	stir time (h)	0.5	2	1
D	catalyst (mg)	15	38	25
E	hydrogen time (h)	1	4	2
(B)				
	PMK low level	PMK high level	PMK baseline	
water (mL)	50	165	83	
32% HCl (drops)	30	100	50	
25% NaOH (drops)	34	112	56	
25% NaOH (additional drops before second extraction)	3	10	5	
25% NaOH (additional drops before third extraction)	6	20	10	

[a] (a) The high, low, and baseline levels used for the five reaction conditions, and (b) the water, acid, and base quantities scaled to the level of PMK used in each synthesis.

PorapakQ maintained at a temperature of 70 °C. Isotopic compositions were referenced against gaseous carbon dioxide (Air Products 99.995%) and gaseous nitrogen (Air Products 99.9997%) which were, in turn, calibrated against IAEA-CH7 polyethylene, IAEA-C6 sucrose and IAEA N1 and USGS25 ammonium sulfates. Hydrogen and oxygen isotopic measurements were performed using a ThermoFinnigan TC/EA high temperature elemental analyzer. Samples (approximately 0.2 mg) were crimped into silver capsules and introduced to the instrument by an AS200 autosampler. The pyrolysis reactor, comprising an alumina tube lined with glassy carbon, was maintained at a temperature of 1450 °C with a helium carrier gas flow of 90 mL min^{-1}. Hydrogen and carbon monoxide pyrolytically formed in the reactor were separated using a gas chromatography column containing 5 Å molecular sieves maintained at a temperature of 90 °C. Isotopic compositions were referenced against gaseous hydrogen (Air Products 99.999%) and gaseous carbon monoxide (Air Products 99.97%) which were, in turn, calibrated against IAEA-CH7 polyethylene and NBS-19 calcium carbonate. Data were acquired and processed using ISODAT NT 2.0 software.

RESULTS AND DISCUSSION

The factor levels and isotope ratios for each batch of MDMA·HCl are available in SI Table S-1. The yield of MDMA base obtained for each reaction (based on the amount of PMK starting material employed) is also detailed in SI Table S-1.

Hydrogen Isotope Ratios. The half normal plot of the effects (SI Figure S-1) reveals that the stir time during imine formation (factor C) has the largest effect on the δ^2H values: as the stir time increases from low to high value, the δ^2H value decreases (SI Figure S-2). Analysis of Variance (ANOVA) performed with Design-Expert software confirms the significance of main effect C (C: $F(1, 41) = 42.52$, $p < 0.001$). With a reduced model including only the significant effect C, the lack of fit (LoF) of the model is not significant (LoF: $F(31, 10) = 0.77$, $p = 0.726$), indicating the reduced model adequately fits the data. Significant interactive effects are not present.

One possible explanation for this change in the δ^2H value as the stir time is varied may be suggested by considering the reaction mechanism that is believed to be followed during this stage in the preparative process. Figure 2 illustrates this (reversible) pathway toward formation of the imine. PMK and methylamine are reacting in ethanol to form the corresponding imine. The formation of the imine occurs efficiently, regardless of the length of the stir time (i.e., 0.5 or 2 h), as evidenced by the yields of MDMA base obtained. (The yields will be discussed in more detail in relation to the carbon results.)

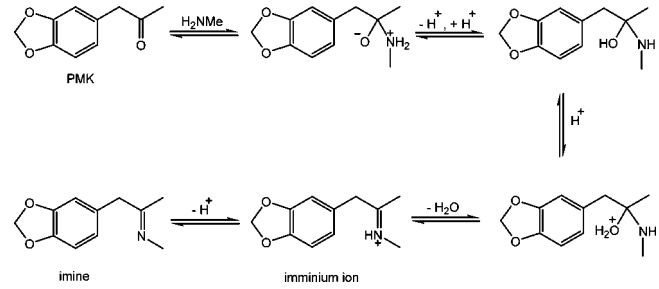

Figure 2. Mechanistic pathway for the formation of the corresponding imine from PMK.

It is clear that the intermediate imminium ion must be formed during this process. The carbon alpha to the imminium C=N bears relatively acidic protons, which may be replaced via a readily achieved deprotonation−reprotonation (imminium ion, enamine) process (Figure 3a). It is also possible that the corresponding protons on the imine product itself may be substituted by imine-enamine tautomerization (Figure 3b).

Because approximately 0.02% of hydrogen exists as the ^{2}H isotope, it is implicit that there are some ^{2}H atoms at the positions alpha to the imminium ion (or imine). Although isotopes of an element are chemically identical, they form bonds of differing strengths due to their differing masses; hence, a C—^{2}H bond is less reactive than a C—^{1}H bond. With increased stir time (from 0.5 to 2 h), the C—^{2}H bonds on the molecule have sufficient time to break (at the stages within the reversible mechanistic pathway illustrated above) and, once the bond is broken, a hydrogen atom in the vicinity will be incorporated when the bond is reformed. Since the vast majority of hydrogen atoms are the ^{1}H isotope, the reformed bond will almost certainly be C—^{1}H, thus resulting in depletion of ^{2}H in the product and more negative δ values as stir time increases.

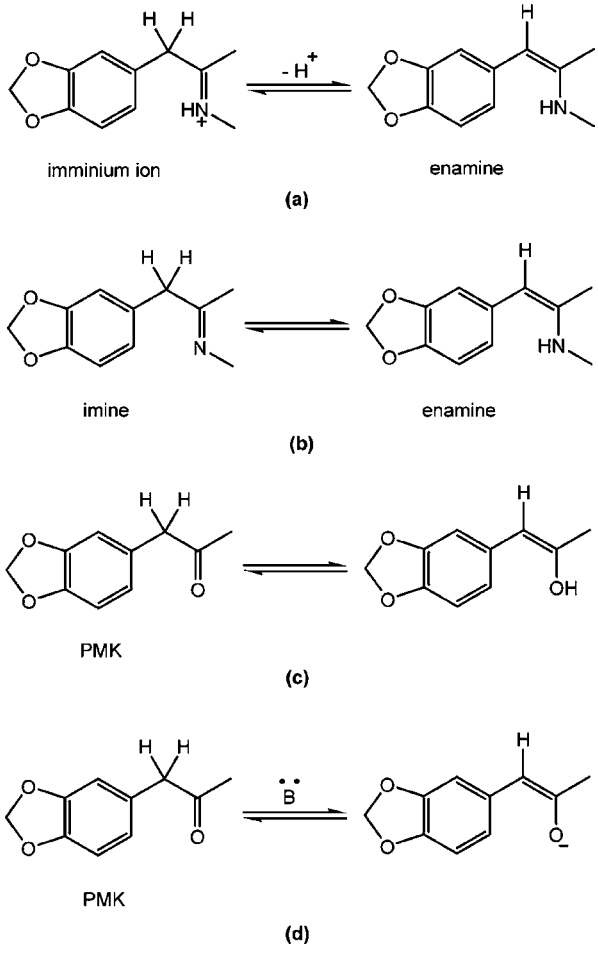

Figure 3. Illustration of the processes which are thought to contribute to the lowering of the δ^2H values as the stir time during imine formation increases: (a) the deprotonation-reprotonation process undergone by the imminium ion, (b) the imine-enamine tautomerization of the imine, (c) the keto−enol tautomerization of PMK, and (d) the enolization of PMK.

A second, similar process may contribute to the observed stir time effect on the δ^2H values. Because the formation of the imine is a reversible process, it is likely that a small amount of PMK is always present in the solution. The relatively acidic protons alpha to the carbonyl are also likely to exchange, through keto−enol tautomerization (or enolization; see Figure 3c and d), with the more abundant ^{1}H atoms. As with exchange at the imminium/imine stage, this is increasingly likely as the stir time of the reaction increases.

Because the yields of MDMA base do not appear to be affected by the stir time (0.5 or 2 h), the formation of the imine must be efficient at both levels. As such, it is more likely that the exchange of protons responsible for the more negative δ^2H values occurs on the imminium ion (or the imine itself), rather than on the PMK molecule, although the latter process could make a small contribution.

Nitrogen Isotope Ratios. The half normal plot of the effects (SI Figure S-3) reveals that the quantity of methylamine (factor A) and the quantity of PMK (factor B) have the largest effects on the δ^{15}N values: as the quantity of methylamine increases, the δ^{15}N value increases, and as the quantity of PMK increases, the δ^{15}N value decreases (SI Figure S-4a and S-4b). ANOVA confirms the significance of factors A and B (A: $F(1, 40) = 119.48$, $p < 0.001$; B: $F(1, 40) = 49.50$, $p < 0.001$). The lack of fit is not significant (LoF: $F(30, 10) = 1.15$, $p = 0.429$), indicating that the reduced model fits the data adequately. Significant interactive effects are not observed.

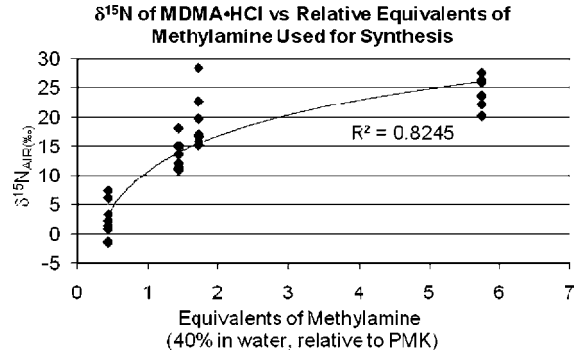

Figure 4. δ^{15}N values of MDMA·HCl as related to the relative equivalents of methylamine used for each synthesis. A trend is observed: as the degree of excess methylamine increases, the δ^{15}N values increase.

As the quantity of methylamine increases, the ^{15}N/^{14}N ratio of the product increases. A possible explanation for this observation involves the step in the synthesis in which the methylamine adds to the carbonyl group (Figure 2). When the methylamine molecule contains the ^{15}N isotope, a C—^{15}N bond is formed. This bond is stronger and, in turn, less reactive than the corresponding C—^{14}N bond. Consequently, if ^{15}N is present in the intermediate molecule, the likelihood of this step reversing is lowered. Crucially, when a greater quantity of methylamine is in use, more ^{15}N is available; as such and especially due to the reversibility of this key first step in the reaction mechanism, a greater opportunity exists for ^{15}N to be present within the combined substrate molecules, thus increasing the overall δ^{15}N value in the final MDMA product.

These effects can be better understood by examining the δ^{15}N results in relation to the equivalents of methylamine *relative* to those of PMK for each factor level combination, which were as follows: 0.43 equiv (methylamine low, PMK high), 1.4 equiv (methylamine low, PMK low), 1.7 (methylamine high, PMK high), 5.7 equiv (methylamine high and PMK low).

If the δ^{15}N results are plotted versus relative equivalents of methylamine (Figure 4), a trend is clearly observed: as the equivalents of methylamine increase, the δ^{15}N value increases.

It can be assumed that when methylamine is not in excess (0.43 equiv), *all* of the available methylamine reacts with PMK; thus, the ^{15}N/^{14}N ratio of the nitrogen contributing reagent is carried through to the product without appreciable effects during the addition to the carbonyl group stage of the mechanistic process. Figure 4 reveals that as the equivalents of methylamine increase, the δ^{15}N values also increase logarithmically. This demonstrates that when the amount of *excess* methylamine increases, more ^{15}N is observed in the product, thus lending support to the earlier suggestion that the PMK has a greater chance of incorporating ^{15}N-containing methylamine when there is more methylamine available.

While both the factorial design analysis and the plot of δ^{15}N versus equivalents of methylamine indicate the same conclusions, it is perhaps more appropriate, chemically, to view the

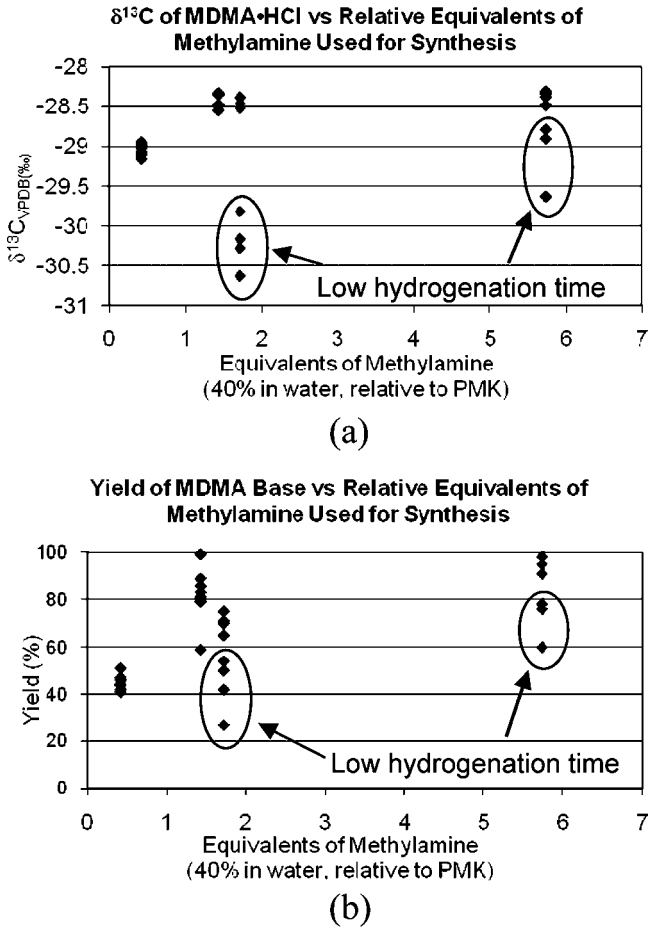

Figure 5. The $\delta^{13}C$ of MDMA·HCl and yield of MDMA base as related to the relative equivalents of methylamine used for each synthesis. (a) $\delta^{13}C$ values plotted versus the relative equivalents of methylamine used. The (circled) more negative $\delta^{13}C$ values at 1.7 and 5.7 equivalents correspond to the use of the short hydrogenation time. (b) The yield of MDMA base plotted against the relative equivalents of methylamine used. The (circled) low yielding batches at 1.7 and 5.7 equivalents correspond to the circled batches in Figure 5a utilizing the low hydrogenation time.

levels of methylamine and PMK in terms of relative equivalents rather than absolute volume.

Carbon Isotope Ratios. The half normal plot of the effects (SI Figure S-5) reveals that three interactive effects are present involving the quantity of methylamine (factor A), the quantity of PMK (factor B), and the hydrogenation time (factor E); the interactive effects are AE, BE, and ABE. While ANOVA confirms that factors A, B, E, and interactions AE, BE, and ABE are all significant (A: $F(1, 35) = 14.65$, $p < 0.001$; B: $F(1, 35) = 83.40$, $p < 0.001$; E: $F(1, 35) = 56.42$, $p < 0.001$; AE: $F(1, 35) = 54.32$, $p < 0.001$; BE: $F(1, 35) = 14.12$, $p < 0.001$; ABE: $F(1, 35) = 14.12$, $p < 0.001$), the lack of fit statistic is also significant (LoF: $F(25, 10) = 11.86$, $p < 0.001$), thus indicating that the reduced model does not adequately fit the data. As a result, the model was abandoned.

If the $\delta^{13}C$ values are plotted against the equivalents of methylamine relative to PMK (Figure 5a), an important aspect of the data is revealed. Most of the $\delta^{13}C$ values at each equivalent level show little variation; however, some of the points at 1.7 and 5.4 equivalents are more negative than the others.

Considering first the eight $\delta^{13}C$ values at 1.7 equivalents of methylamine, the four circled δ values all have the low hydrogenation time (factor E), whereas the other four points at 1.7 equivalents have a high hydrogenation time (Figure 5a). At 5.7 equivalents, the same pattern is observed except that only three of the four low hydrogenation time points are distinct.

This effect can be explained by looking at the yields of MDMA base obtained for each synthetic run. As illustrated in Figure 5b, when the yield of MDMA base achieved for each batch is plotted against relative equivalents of methylamine, it is observed that the batches which had the anomalous, more negative $\delta^{13}C$ values in Figure 5a also have lower yields in Figure 5b. Based on the yields, it is evident that the low hydrogenation time is too short to allow reduction of all of the imine to the product amine. While in the hydrogenation device, the imine is reduced under a high pressure hydrogen atmosphere to MDMA base (the second transformation shown in Figure 1); thus, a C—N π-bond is broken. It is anticipated that imines comprised of $^{12}C{=}N$ will be reduced in preference to those comprised of the stronger (and less reactive) $^{13}C{=}N$ unit, resulting in the more negative $\delta^{13}C$ values observed at the low hydrogenation time.

Further examination of Figure 5b reveals that all eight data points at 0.43 relative equivalents of methylamine are lower yielding than the majority of the points at 1.4, 1.7, and 5.7 equivalents. This is easily explained by the simple mass balance that one molecule of PMK requires one molecule of methylamine to form one molecule of the imine. Since only 0.43 equivalents of methylamine are available, a maximum of 43% of the PMK can be brought through the synthesis to form the product amine. The yields of these 0.43 equivalents (methylamine low, PMK high) batches range from 41 to 51% (SI Table S-1) and are the result of using PMK oil containing some impurities as the starting material. Thus, the quoted amount of PMK used for these reactions was, in reality, marginally less than anticipated, with the relative quantity of methylamine being slightly greater than the quoted 0.43 relative equivalents.

It is interesting to note from Figure 5b that the highest yielding batches of MDMA are those which had either 1.4 or 5.7 relative equivalents of methylamine. While the reasons for the low yields when employing only 0.43 equivalents of methylamine have been discussed already, it is worthy of note that the reaction runs with 1.7 equivalents of methylamine also have generally lower yields. In relation to this it is important to note that the 1.4 and 5.7 equivalents batches are those in which the lower level of PMK was used; thus, these syntheses had to convert only 4.0 mmol of PMK to the final product. The 0.43 and 1.7 equivalents batches, on the other hand, correspond to the high level of PMK in which 13.4 mmol had to be converted to the final product. It appears, therefore, that the high levels of the other factors were not sufficiently elevated to bring all of this material through the synthesis.

While the interpretation of the $\delta^{13}C$ values is more complicated than that for the $\delta^{2}H$ and $\delta^{15}N$ values, some revealing patterns in the data have been identified which have implications for MDMA profiling. The $^{13}C/^{12}C$ ratio of MDMA was expected to be similar to the $^{13}C/^{12}C$ ratio of the PMK, since this starting material contributes 10 of the 11 carbon atoms in the product. For this reason, it has been suspected that the $\delta^{13}C$ values of MDMA might reveal the geographic location of the origin of the plant-derived safrole starting material. This

concept has been exploited successfully for heroin and cocaine samples.[18−21]

This study, however, has shown that fractionation of the carbon isotopes can occur during the synthesis due to the efficiency of the reaction. With substoichiometric amounts of methylamine, the product is depleted in the heavy isotope. With hydrogenation times which cut short the reduction of the imine, the product, again, has a reduction in the heavy isotope. Having stated this, the latter effect was less consistent and reproducible. Nonetheless, using the batches at 1.7 equivalents as an example, a clandestine chemist who does not carefully control the hydrogenation time could conceivably produce batches of MDMA with $\delta^{13}C$ values differing by more than 2.25‰, even though the same batch of starting material and reagents were used. This has significant implications for the interpretation of $\delta^{13}C$ values for sample to sample linkages.

Oxygen Isotope Ratios. The half normal plot of the effects (SI Figure S-6) reveals that the quantity of PMK (factor B) and an interactive effect between the imine stir time and the hydrogenation time (factors C and E, respectively) have the largest effects on the $\delta^{18}O$ values (SI Figure S-7a and S-7b). ANOVA reveals that factor B and an interaction between C and E are significant effects (B: $F(1, 38) = 7.06$, $p = 0.011$; CE: $F(1, 38) = 7.16$, $p = 0.011$). The lack of fit of this model is not significant (LoF: $F(28, 10) = 1.36$, $p = 0.316$).

Because the two oxygen atoms on the MDMA molecule are in situ at the outset of the synthesis, and bonds to these two atoms are not broken or formed during the synthesis, it is difficult to explain why the $^{18}O/^{17}O$ ratios of MDMA·HCl may be affected by the reaction conditions.

The $\delta^{18}O$ results generally fall within 8−12‰, although three data points have $\delta^{18}O$ values of 6‰ or less. The three lowest $\delta^{18}O$ values correspond to batches in which the yield of MDMA base was only 41−42%, and these three batches utilized the high level of PMK (factor B), high level of stir time for imine formation (factor C), and high level of hydrogenation time (factor E). Theoretically and for example, when only a 42% yield is achieved only 42% of the original oxygen atoms make it through to the product. Since the composition of the product molecules appears to be dictated by the mass discriminating effects involving the isotopes at the C—N bond, as discussed previously, the specific oxygen isotopes within these molecules are arbitrary. Consequently, this might explain why a pattern cannot be detected in the $\delta^{18}O$ results.

CONCLUSIONS

This is the first study which has systematically altered the reaction conditions within the synthesis of MDMA by the commonly employed Pt/H_2 reductive amination route. Rationalizations for the observed shifts in δ values have been proposed. Based on all of this, it can be seen that drug profiling of MDMA in relation to specific synthetic route is complicated when utilizing the δ values of MDMA·HCl. Even the isotope ratios which should be very similar to those of the starting material, namely the $^{13}C/^{12}C$ and $^{18}O/^{17}O$, have been shown to vary on the basis of the reaction conditions and the associated reaction efficiency. The δ^2H values, which were previously identified as holding the most power for discriminating MDMA by the synthetic route used for manufacture,[8] have importantly been shown to be affected by the length of the stir time allowed for imine formation. Furthermore, the nitrogen isotope ratios are affected by the degree of excess methylamine (the nitrogen-contributing reagent) utilized within the synthetic protocol.

It should be pointed out that the MDMA syntheses in this study were undertaken on a small scale relative to those encountered in large clandestine MDMA laboratories; as such, it is possible that the kinetic isotope effects vary depending on the scale in use. However, it has been shown that the scale up (from milligram to one gram quantity) of the acetylation of morphine base to heroin base, as reported by Casale et al[22] and Besacier et al,[21] produced the same fractionation of the carbon isotopes.

The work detailed in this paper is a crucial first step toward uncovering the reasons behind isotope fractionation during MDMA synthesis and, importantly, highlighting its complicating effect on MDMA profiling using isotope ratios. It should be stated that, in a previous study,[22] Rayleigh fractionation has been shown to occur during the conversion of heroin and cocaine base to their respective HCl salts; if this fractionation also occurs during the conversion of MDMA base to HCl salt, and it is not reproducible and/or depends on reaction conditions, then MDMA profiling using isotope ratios becomes even more convoluted.

Overall, we believe that this study makes a distinct and valuable contribution to the growing body of research relating to the application of IRMS methods within drug profiling endeavors. Based on the results described here, the emerging situation in this area appears to be complex. Having stated this, it may be possible to employ sophisticated data analysis techniques in order to elicit accurate synthetic route information from the δ values of MDMA·HCl, and this is the subject of ongoing studies within our laboratories.

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SUPPORTING INFORMATION AVAILABLE

Spectral data, factor levels, reaction yields, IRMS δ values, half normal plots of the effects for the δ values, plots of the significant effects and/or interactions for the δ values, and a brief explanation of factorial design. This material is available free of charge via the Internet at http://pubs.acs.org.

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