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Emerging Use of Isotope Ratio Mass Spectrometry as a Tool for Discrimination of 3,4-Methylenedioxymethamphetamine by Synthetic Route

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Drug profiling, or the ability to link batches of illicit drugs to a common source or synthetic route, has long been a goal of law enforcement agencies. Research in the past decade has explored drug profiling with isotope ratio mass spectrometry (IRMS). This type of research can be limited by the use of substances seized by police, of which the provenance is unknown. Fortunately, however, some studies in recent years have been carried out on drugs synthesized in-house and therefore of known history. In this study, 18 MDMA samples were synthesized in-house from aliquots of the same precursor by three common reductive amination routes and analyzed for ^{13}C, ^{15}N, and ^{2}H isotope abundance using IRMS. For these three preparative methods, results indicate that ^{2}H isotope abundance data is necessary for discrimination by synthetic route. Furthermore, hierarchical cluster analysis using ^{2}H data on its own or combined with ^{13}C and/or ^{15}N provides a statistical means for accurate discrimination by synthetic route.

Drug profiling, or the ability to link batches of illicit drugs to a common source or synthetic route, has long been a goal of law enforcement agencies. Specifically, the use of synthetic drug profiles has been targeted as a law enforcement goal in the European Union Drugs Action Plan 2005−2008.[1] Analysis of drugs such as 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") is often performed by organic impurity profiling utilizing gas chromatography−mass spectrometry (GC−MS).[2−8] Problems with this strategy have been suggested. For instance, the clandestine laboratory may produce substances of high purity such that very few (if any) impurities remain.[9] Further, comparisons based on impurity profiling are often not sufficiently conclusive due to difficulty in achieving reproducible chromatograms as a result of low level impurities. It has also been suggested that chromatograms are often time and machine dependent, thereby confounding the comparison of results across laboratories,[9] although this type of problem may be encountered with other instrumental techniques.

Research in the past decade has explored isotope profiling with isotope ratio mass spectrometry (IRMS).[10−21] While the use of authentic street drugs has its advantages, due to the unknown

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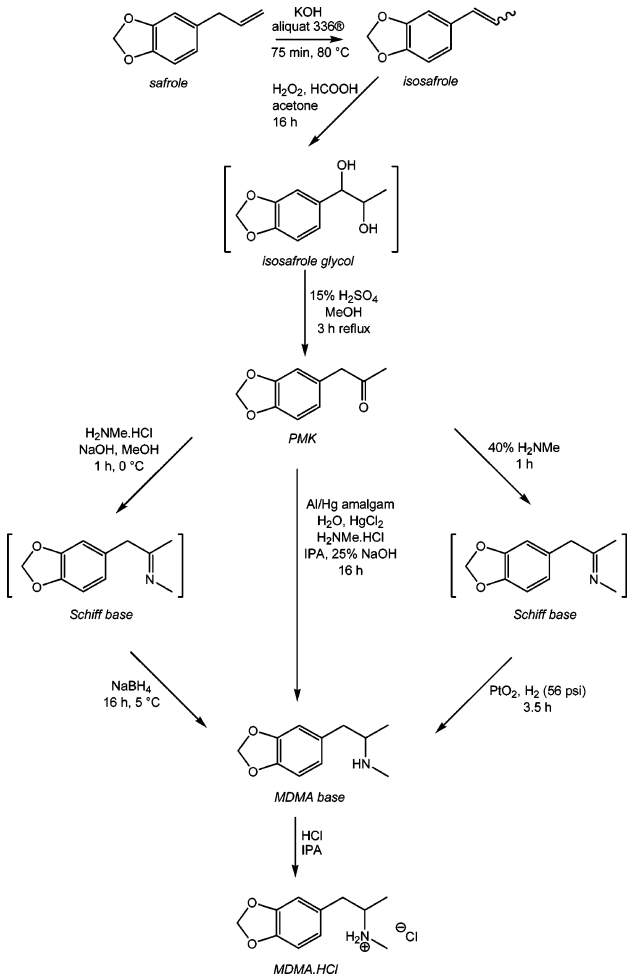

Figure 1. Synthetic pathway from safrole to PMK and the three reductive amination routes from PMK to MDMA·HCl.

provenance of the samples, this type of research can be limited. Thus, linkages and conclusions drawn from research on seized samples are tentative; although seized tablets can be "grouped" according to IRMS data, the verification of the groupings is not possible if the provenance of the samples is unknown. Fortunately, however, some studies in recent years have been carried out on drugs synthesized in-house and therefore of known history (methamphetamine,[12,14,16] cocaine,[18] heroin,[20] MDMA[10,17]). The work herein is the first study correlating ^{13}C, ^{15}N, and ^{2}H abundance of MDMA samples of known provenance.

The 18 MDMA samples in this study were synthesized via three commonly used reductive amination routes accessible to the clandestine chemist. The three reductive aminations of "PMK" (piperonyl methyl ketone) utilize different reducing agents: Al/Hg amalgam, $NaBH_4$, and Pt/H_2. Since this ketone has limited access without the appropriate licenses and thus cannot be easily diverted from trade,[22] clandestine synthesis of MDMA must start from pre-precursors such as safrole or isosafrole (see Figure 1). Often these pre-precursors are converted to PMK in Asia and then shipped to a separate location for MDMA synthesis.[23]

To mimic clandestine synthesis as closely as possible, a large batch (approximately 200 g) of PMK was synthesized from a single batch of safrole. The 18 batches of MDMA·HCl were subsequently synthesized from this single "pot" of PMK and then subjected to IRMS analysis for ^{13}C, ^{15}N, and ^{2}H isotope abundance.

EXPERIMENTAL SECTION

Eighteen batches of MDMA·HCl were synthesized, six from each reductive amination route. Reagents and solvents were sourced from commercial suppliers (such as Sigma Aldrich and Fisher Scientific, U.K.) and used without further purification.

Precursor Synthesis. *Safrole to Isosafrole.*[2] To safrole (20 mL, 135 mmol) were added solid potassium hydroxide (KOH) pellets (9.0 g, 161 mmol) and Aliquat 336 (3.7 mL, 8.1 mmol, 6.0 mol %) with stirring for 20 min. The mixture was then heated to 80 °C for 75 min. After cooling to room temperature, the mixture was filtered through celite with dichloromethane (DCM) rinsing, and dried with magnesium sulfate ($MgSO_4$). Solvent was removed *in vacuo*. The mixture was filtered through a plug of silica with petrol. Removal of solvent gave isosafrole (19.2 g, 87% yield) as a colorless oil. On a larger scale, the silica purification step was not carried out, resulting in isosafrole as a yellow oil. Analysis was in agreement with that disclosed in the literature.[24−26] IR ν_{max} (CH_2Cl_2)/cm^{-1}: 1504, 1438, 1249 (C−O), 1193, 1041 (C−O), 965, 938. ^1H NMR (400 MHz, $CDCl_3$): δ 1.86 (dd, 3H, J 6.6 and 1.6 Hz, CH_3), 5.94 (s, 2H, OCH_2O), 6.07 (dq, 1H, J 15.6 and 6.6 Hz, CH), 6.32 (dq, 1H, J 15.6 and 1.6 Hz, CH), 6.73 − 6.75 (m, 2H, ArH), 6.89 ppm (s, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5, 101.1, 105.6, 108.4, 120.3, 124.2, 130.8, 132.8, 146.7, 148.2 ppm.

Isosafrole to PMK.[27] To 35% aqueous hydrogen peroxide (3.1 mL, 100 mmol) was added 88% formic acid (12.3 mL, 326 mmol) with stirring for 2 min. In a separate flask, isosafrole (2.9 mL, 20 mmol) was dissolved in acetone (12 mL) at room temperature, and the solution swirled. The isosafrole/acetone solution was then added dropwise to the hydrogen peroxide/formic acid solution with cooling (ice + NaCl bath) to keep the temperature from exceeding 40 °C. The mixture was allowed to warm to room temperature with overnight stirring. The solvent was removed *in vacuo* to reveal an orange oil. To the crude oil was added methanol (6.0 mL) and then 15% sulfuric acid (36 mL, 676 mmol). The mixture was heated to reflux for 3 h, producing a dark reddish-brown oil. After cooling, the mixture was extracted with ether (3 × 75 mL) and the organic layer washed with water until neutral pH.[28] The organic layer was then washed with 1M sodium hydroxide (50 mL) and water again until a neutral pH was realized, dried with $MgSO_4$, and the solvent removed *in vacuo* to reveal a brown oil. The crude oil was distilled under vacuum (3 mbar, 120−140 °C) to yield PMK as a yellow oil (1.6 g, 46% yield). Analysis was in agreement with published data.[29,30] IR ν_{max} (CH_2Cl_2)/cm^{-1}: 1710 (C═O), 1607, 1490, 1249 (C−O), 1040 (C−O). ^1H NMR (400 MHz, $CDCl_3$): δ 2.15 (s, 3H, CH_3), 3.61 (s, 2H, CH_2), 5.95

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(s, 2H, OCH_2O), 6.65 (d, 1H, *J* 7.9 Hz, ArH), 6.69 (s, 1H, ArH), 6.77 ppm (d, 1H, *J* 7.9 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.1, 50.6, 101.1, 108.5, 109.8, 122.5, 127.8, 146.7, 147.9, 206.5 ppm.

Reductive Amination of PMK. *Al/Hg Amalgam (Route 1)*.[27] To aluminum foil (4.5 g) cut into 2 cm squares was added water (159 mL) containing mercuric chloride (0.11 g, 0.41 mmol, 1.2 mol %). The amalgamation was allowed to proceed with swirling for 3 min, and fine bubbles and a light gray precipitate were observed. The water was decanted and the foil washed with fresh water (317 mL). The water from the last wash was removed from the foil as thoroughly as possible by shaking. Added to the remaining foil in succession were methylamine hydrochloride (6.8 g, 100 mmol, 3 equiv) dissolved in water (6.8 mL), isopropyl alcohol (20 mL), 25% aqueous NaOH (16 mL, 588 mmol), PMK (6 g, 33 mmol), and isopropyl alcohol (39 mL). The mixture was immersed in an ice bath as necessary to keep the temperature below 50 °C. Once thermally stable, the mixture was left stirring overnight. The resultant mixture was then filtered through celite, rinsing with methanol, and the volatiles removed *in vacuo*. The residue was suspended in water (272 mL), and 32% aqueous hydrochloric acid added to make the phase acidic (pH 1). Excess PMK was extracted with DCM (3 × 75 mL). The remaining aqueous layer was made basic (pH 9) with 25% aqueous NaOH and extracted with DCM (3 × 75 mL). A further 10 drops of 25% aqueous NaOH were added before the second and third DCM extractions, respectively. The combined organic layers were dried over magnesium sulfate and the solvent removed *in vacuo* to reveal MDMA base as an amber oil (4.2 g, 65% yield). Analysis was in agreement with published data.[31,32] IR ν_{max} (CH_2Cl_2)/cm^{-1}: 1489, 1444, 1249 (C−O), 1041 (C−O), 938. ^1H NMR (400 MHz, $CDCl_3$): δ 1.05 (d, 3H, *J* 6.2 Hz, CH_3), 2.40 (s, 3H, NCH_3), 2.52 − 2.65 (m, 2H, CH_2), 2.69 − 2.77 (m, 1H, CH), 5.93 (s, 2H, OCH_2O), 6.63 (dd, 1H, *J* 7.8 and 1.4 Hz, ArH), 6.68 (d, 1H *J* 1.5 Hz, ArH), 6.73 ppm (d, 1H, *J* 7.8 Hz, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.9, 34.2, 43.4, 56.7, 101.0, 108.4, 109.7, 122.4, 133.5, 146.1, 147.9 ppm.

Conversion of MDMA base to the HCl salt was achieved by dissolving the base (1.5 g, 7.7 mmol) in isopropyl alcohol (2.5 mL) and acidifying with 37% aqueous HCl (30 drops). The solvent was removed *in vacuo* with heat to reveal a precipitate (ranging in color from white to pink to brown) which was homogenized and washed with a 2:1 isopropyl alcohol:ether solution before a final wash with ether. The resulting white MDMA hydrochloride was dried under high vacuum (1.7 g, 95% yield). Analysis was in agreement with published data.[31,33,34] IR ν_{max} (CH_2Cl_2)/cm^{-1}: 1491, 1447, 1246 (C−O), 1040 (C−O), 932. ^1H NMR (400 MHz, D_2O): δ 1.32 (d, 3H, *J* 6.6 Hz, CH_3), 2.74 (s, 3H, NCH_3), 2.85−2.91 (m, 1H, CH_2) 3.00−3.05 (m, 1H, CH_2), 3.53 (m, 1H, CH), 6.01 (s, 2H, OCH_2O), 6.83 (dd, 1H, *J* 7.9 and 1.4 Hz, ArH), 6.89 (d, 1H, *J* 1.4 Hz, ArH), 6.93 ppm (d, 1H, *J* 8.0 Hz, ArH). ^{13}C NMR (100 MHz, D_2O): δ 15.4, 30.5, 39.0, 57.1, 101.7, 109.3, 110.3, 123.4, 130.3, 147.0, 148.4 ppm.

$NaBH_4$ (Route 2).[2] To a well stirred solution of methylamine hydrochloride (3 g, 44 mmol, 1.7 eq) in methanol (25 mL) at −20 °C was added a solution of solid sodium hydroxide (1.8 g, 45 mmol, 1.7 equiv) dissolved in methanol (35 mL). Addition was portionwise so as to keep the temperature of the solution at −20 °C in an acetone bath containing chips of dry ice. PMK (4.6 g, 25 mmol) was then added and the mixture allowed to warm to 0 °C. Stirring was continued for 1 h. To the solution was added sodium borohydride (0.46 g, 12 mmol), portionwise, so as to keep the temperature at 0 °C. The mixture was stirred overnight at 5 °C in a cryogenic bath. Methanol was removed *in vacuo* and the residue dissolved in water (330 mL). The mixture was acidified with 32% aqueous HCl (pH 1) and extracted with DCM (3 × 75 mL) to remove excess PMK. The aqueous phase was basified with 25% aqueous NaOH (pH 11) and the organics extracted with DCM (3 × 75 mL). A further 20 drops of 25% aqueous NaOH were added before the second and third DCM extractions, respectively. The combined organic layers were dried over $MgSO_4$ and concentrated, resulting in clean MDMA (3.4 g, 68% yield). The MDMA base was converted to the HCl salt according to the procedure detailed above. Analyses were as described previously.

Pt/H_2 (Route 3).[35] To ethanol (5 mL) was added a solution of 40% methylamine in water (2 mL, 58 mmol, 4.4 equiv). PMK (2 mL, 13 mmol) was slowly added to the mixture and allowed to stir at room temperature for 1 h. To the mixture was added PtO_2 (0.05 g, 0.2 mmol, 1.7 mol %) under a stream of nitrogen. The resultant mixture was placed under a hydrogen atmosphere at 56 psi for 3.5 h. The pressure in the device was returned to 56 psi at the 1 and 2 h marks. After filtration through celite with ethanol to remove PtO_2, the volatiles were removed *in vacuo*. The residue was dissolved in water (165 mL) and acidified with 32% aqueous HCl (pH 1) and extracted with DCM (3 × 75 mL). The aqueous phase was basified with 25% aqueous NaOH (pH 10) and the organics extracted with DCM (3 × 75 mL). A further 10 and 20 drops of 25% aqueous NaOH were added before the second and third DCM extractions, respectively. The combined organic layers were dried over $MgSO_4$ and concentrated to give clean MDMA as an amber oil (2.0 g, 78% yield). The MDMA base was converted into its HCl salt according to the procedure above. Analyses were as described previously.

Stable Isotope Analysis by Isotope Ratio Mass Spectrometry. *^{13}C and ^{15}N Isotope Analysis by EA-IRMS:* Carbon and nitrogen isotope abundance analyses were carried out using an automated nitrogen−carbon analyzer (ANCA) coupled to an automated breath carbon analyzer (ABCA) isotope ratio mass spectrometer (SerCon Ltd, Crewe, U.K.). Typically 0.4 mg of sample material was weighed into tin capsules (Elemental Microanalysis, Devon, U.K.) and introduced via a solid Costech Zero-Blank autosampler (Pelican Scientific Ltd, Alford, U.K.). The Elemental Analyzer (EA) reactor tubes were comprised of two quartz glass tubes filled with chromium(III) oxide / copper oxide and reduced copper, held at 1020 °C and 620 °C for combustion and reduction, respectively. A water trap filled with magnesium perchlorate was used to remove water from combustion gases thus generated, and a postreactor GC column was kept at 65 °C

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for separation of evolved N_2 and CO_2. Data were processed using proprietary software (SerCon Ltd, Crewe, U.K.). Measured isotope ratios are expressed in the δ notation [‰] (eq 1) relative to the appropriate international isotope standard material anchoring the isotope scale (e.g., VPDB for ^{13}C or VSMOW for ^{2}H).

$$\delta\ [‰] = \left[\frac{RSample - RStrd}{RStrd}\right] \times 1000 \qquad (1)$$

Isotopic Calibration and Quality Control of EA-IRMS Measurements: Each batch of samples was bracketed by two blanks (empty tin capsules) and two sets of laboratory certified standards of known isotopic composition (Iso-Analytical, Crewe, U.K.). This standard was leucine ($\delta^{13}C_{VPDB} = -30.52‰$, $\delta^{15}N_{AIR} = +10.77‰$). Quality of isotope abundance measurement was monitored by participation in annual inter-laboratory exercises organized by the Forensic Isotope Ratio Mass Spectrometry Network (FIRMS).

^{2}H Isotope Analysis by TC/EA-IRMS. A Delta^{Plus}-XP isotope ratio mass spectrometer (IRMS) coupled to a high-temperature conversion / elemental analyzer (TC/EA; both Thermo-Fisher Corporation, Bremen, Germany) was used for $^{2}H/^{1}H$ isotope ratio measurement of synthesized MDMA samples. Typically, 0.2 mg of solid sample was weighed into a silver capsule and placed in a desiccator for one week before the samples were introduced into the TC/EA by means of a solid Costech Zero-Blank solid autosampler (Pelican Scientific Ltd, Alford, U.K.). The reactor tube was self-packed and comprised of an Alsint ceramic tube containing a glassy carbon tube filled with glassy carbon granulate, silver and quartz wool (SerCon, Crewe, Cheshire). The reactor temperature was set to 1425 °C while the postreactor GC column was maintained at 85 °C. Helium (99.99% purity, Air Products plc, Crewe, Cheshire) pressure was set to 1.45 bar. Data were processed using proprietary Isodat NT software, version 2.0 (Thermo-Fisher Corporation, Bremen, Germany). The run time per analysis was 350 s. Measured $^{2}H/^{1}H$ isotope ratios are expressed as δ values in ‰ relative to VSMOW as per eq 1.

Isotopic Calibration and Quality Control of TC/EA-IRMS Measurements: The working reference gas, H_2 (BOC, Guilford, Surrey, U.K.), was calibrated against VSMOW ($\delta^{2}H = 0.0‰$) and checked against the international reference material (IRM), IAEA-CH-7 polyethylene ($\delta^{2}H_{VSMOW} = -100.3‰$; IAEA, Vienna, Austria). Cross-checking the $\delta^{2}H_{VSMOW}$-value obtained for the working reference gas H_2 against a further international reference material (IRM) for H^2 isotope analysis, namely GISP, yielded a measured $\delta^{2}H_{VSMOW}$-value for GISP of −194.6‰ (accepted $\delta^{2}H_{VSMOW} = -189.73‰$; IAEA, Vienna, Austria). The H^{3+} factor was determined on reference H_2 gas pulses of different signal size and was found to be 4.43‰/nA. A typical batch analysis comprised 10 samples run in triplicate, preceded and followed by a set of standards as reported previously.[36] This consisted of in-house standards (coumarin, $\delta^{2}H_{VSMOW} = +62.56‰$) and one IRM (IAEA-CH-7) as calibration controls at the beginning and end of the set. Each batch was preceded and followed by a blank silver capsule. Precision of ^{2}H isotope analysis as monitored by the IRMs and lab standards was ±1.15‰ or better. Measured $\delta^{2}H$-values were normalized according to the method described by Sharp et al.[37] with stretch factors typically being of the order of 1.027 to 1.053.

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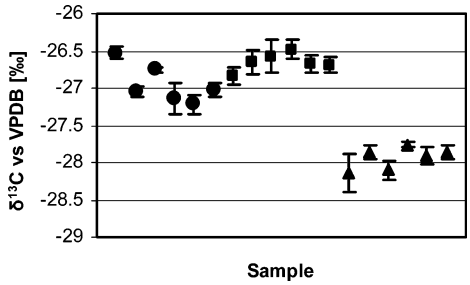

Figure 2. $\delta^{13}C$ results for the 18 batches: (●) Al/Hg amalgam; (■) $NaBH_4$; (▲) Pt/H_2. Visual discrimination of the Pt/H_2 set is possible. Error bars indicate one standard deviation around the mean for each individual sample ($n = 3$).

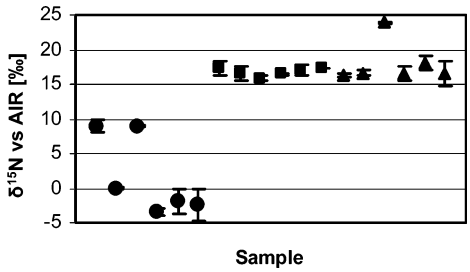

Figure 3. $\delta^{15}N$ results for the 18 batches: (●) Al/Hg amalgam; (■) $NaBH_4$; (▲) Pt/H_2. Visual discrimination of the Al/Hg amalgam set is possible, although the variation within that route is large. Error bars indicate one standard deviation around the mean for each individual sample ($n = 3$).

Sample Preparation: Upon receipt by the Stable Isotope Forensics Facility at Queen's University, sample ID and weight of synthesized MDMA samples were entered into a chain of custody sheet as well as into the lab's drug book. Aliquots sufficient for stable isotope analysis were weighed out and dried in a desiccator to remove any traces of moisture (*in vacuo* over P_4O_{10}). Weights of the remaining drug samples were recorded in the lab's drug book and the samples locked into the drug safe.

To prepare samples for isotope analysis, drug aliquots were removed from the desiccator and approximately 0.2 and 0.4 mg samples were weighed out in triplicate into silver and tin capsules (SerCon Ltd, Crewe, U.K.) for analysis by TC/EA-IRMS and EA-IRMS, respectively. Capsules were subsequently crimped and placed into 96 well-plates already prepared with blanks and appropriate reference materials. Batch run ready well-plates were placed into another desiccator, where they were kept *in vacuo* over P_4O_{10} until analysis.

RESULTS AND DISCUSSION

A large stock of PMK was first synthesized from one bottle of safrole, according to the scheme in Figure 1. Once a sufficient stock of PMK had been attained, MDMA·HCl was synthesized according to the three reaction pathways in Figure 1. The $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ data for the 18 MDMA samples are graphically represented in Figures 2−4. Each data point represents the mean of triplicate analyses, and the error bars indicate one standard deviation around the mean for each individual sample. The $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ data of the 18 batches are given in Table 1. These results are in line with published IRMS values of MDMA.[10,13,15−17,21]

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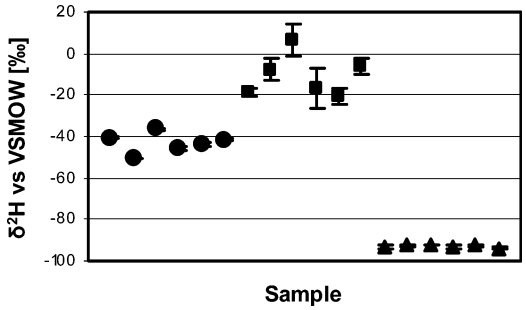

Figure 4. δ^2H results for the 18 batches: (●) Al/Hg amalgam; (■) $NaBH_4$; (▲) Pt/H_2. Visual discrimination of all routes is possible, but the separation between the Al/Hg amalgam and $NaBH_4$ sets is small. Error bars indicate one standard deviation around the mean for each individual sample ($n = 3$).

Table 1. δ^{13}C, δ^{15}N, and δ^2H Values for the MDMA·HCl Samples Synthesized from the Same Precursor (PMK) Using Three Reductive Amination Routes[a]

route	δ^{13}C [‰]	δ^{15}N [‰]	δ^2H [‰]
Al/Hg amalgam	−26.5	8.9	−40.7
Al/Hg amalgam	−27.1	−0.1	−50.5
Al/Hg amalgam	−26.7	8.9	−36.5
Al/Hg amalgam	−27.1	−3.6	−45.6
Al/Hg amalgam	−27.2	−1.9	−43.8
Al/Hg amalgam	−27.0	−2.3	−41.6
$NaBH_4$	−26.8	17.4	−18.8
$NaBH_4$	−26.7	16.5	−7.6
$NaBH_4$	−26.6	15.9	6.3
$NaBH_4$	−26.5	16.4	−16.7
$NaBH_4$	−26.7	17.0	−20.6
$NaBH_4$	−26.7	17.3	−5.8
Pt/H_2	−28.1	16.3	−93.4
Pt/H_2	−27.9	16.6	−92.7
Pt/H_2	−28.1	23.8	−92.5
Pt/H_2	−27.8	16.6	−93.5
Pt/H_2	−27.9	18.1	−92.6
Pt/H_2	−27.9	16.5	−93.9

[a] Each value is the mean of triplicate analyses; standard deviation around the mean ranged from 0.10 to 0.25‰, from 0.15 to 0.95 ‰, and from 0.5 to 10.0 ‰ for δ^{13}C, δ^{15}N, and δ^2H values, respectively. Precision for measured δ^{13}C, δ^{15}N, and δ^2H values of the reference materials was ± 0.15‰, ± 0.20‰, and ± 1.0‰ or better.

When examining first the δ^{13}C data, the least variation is shown by this element (1.6‰); see Figure 2. The small variation in δ^{13}C values of the samples in this study is unsurprising given that 10 of the 11 carbon atoms on the final MDMA molecule are contributed by the safrole starting material, and all of these samples were synthesized using the same bottle of safrole. The *N*-methyl carbon on the molecule is contributed by methylamine hydrochloride (Al/Hg amalgam and $NaBH_4$) or aqueous methylamine (Pt/H_2); see Figure 5. In general, the δ^{13}C data points *within* a synthetic route cluster together visually, indicating the fractionation induced within the synthetic process is reproducible by the same chemist using the same method and materials. By δ^{13}C data, the Pt/H_2 route can be discriminated from the other two, but the δ^{13}C values of the Al/Hg amalgam and $NaBH_4$ routes overlap.

Examination of the δ^{15}N data points reveals variation over 27‰, and the Al/Hg amalgam batches exhibit the widest variation within any synthetic route with two of the batches showing considerable ^{15}N enrichment relative to the remaining four (Figure 3). By δ^{15}N data, the Al/Hg amalgam route can be discriminated from the

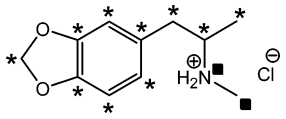

Figure 5. Origin of the marked carbon and nitrogen atoms on the MDMA·HCl molecule synthesized by three reductive amination routes are as follows: (∗) safrole; (■) methylamine hydrochloride or aqueous methylamine.

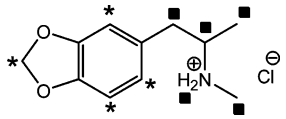

Figure 6. Origin of the marked hydrogen atoms on the MDMA·HCl molecule synthesized by three reductive amination routes are as follows: (∗) safrole; (■) a proton source throughout the synthetic process such as safrole, KOH, H_2O_2, HCOOH, MeOH, H_2SO_4, H_2O, CH_3NH_2, HCl, H_2, etc.

other two sets, although the variation within that set is large. Notably, MDMA synthesis with the Al/Hg amalgam was the hardest to replicate as the temperature of the exothermic reaction is difficult to control exactly, and a series of reagents must be added by hand in quick succession; thus, temperature reached and rate of addition of reagents are likely to vary slightly from batch to batch. From this study, the δ^{15}N data appears to be the most sensitive to these inadvertent differences in preparative method, confirming the observations by Carter et al.[16] during the synthesis of methamphetamine. It should be noted, however, that results were published by Billault et al.[10] in which a different Al/Hg amalgam method produced MDMA with consistent δ^{15}N values.

It is also interesting to examine the δ^{15}N data in relation to the nitrogen contributing precursor used. The nitrogen atom on the MDMA molecule is contributed, along with the *N*-methyl carbon, by methylamine hydrochloride (Al/Hg amalgam and $NaBH_4$) or aqueous methylamine (Pt/H_2). If the δ^{15}N of the nitrogen contributing reagent was solely responsible for the δ^{15}N of the MDMA·HCl, then the δ^{15}N values would be expected to fall into two groups: Al/Hg amalgam and $NaBH_4$ data points in one group and Pt/H_2 data points in the other. Instead (with the exception of one Pt/H_2 batch which is enriched in ^{15}N relative to the rest of the set) the $NaBH_4$ and Pt/H_2 data points fall within the same range, thus indicating the synthetic process itself is responsible for fractionation of the nitrogen isotopes. This observation confirms previous studies suggesting that δ^{15}N values are largely the result of fractionation due to the synthetic process rather than the precursor.[10,16]

The δ^2H data points show the most variation (100‰); see Figure 4. This is perhaps unsurprising due to the number of potential proton contributors. Furthermore, hydrogen atoms at select positions under certain conditions may be prone to exchange. For the synthetic routes in this study, a few of the possible hydrogen atom contributors are safrole, KOH, H_2O_2, HCOOH, MeOH, H_2SO_4, H_2O, CH_3NH_2, HCl, H_2, etc; see Figure 6. The observed variation of the δ^2H values is therefore expected due to the large number of possible hydrogen contributors, but fractionation due to synthetic process is also likely to contribute. Interestingly, the δ^2H data points corresponding to the Pt/H_2 set cluster together remarkably well relative to those of the other two sets. Practically, the Pt/H_2 synthesis was the easiest to

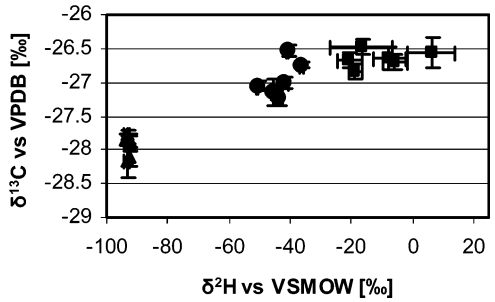

Figure 7. Two variable plot of $\delta^{13}C$ and $\delta^{2}H$ data. Here, the Pt/H_2 route can be clearly separated from the other two sets, but the Al/Hg amalgam and $NaBH_4$ sets cannot be accurately discriminated. Error bars indicate one standard deviation around the mean for each individual sample ($n = 3$).

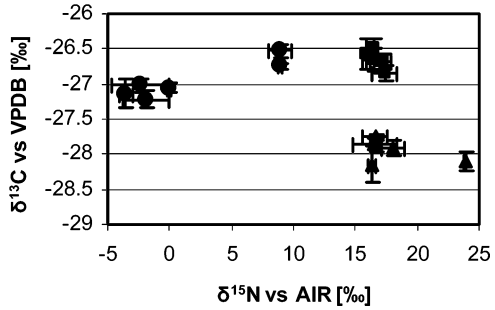

Figure 8. Two variable plot of $\delta^{13}C$ and $\delta^{15}N$ data. The samples fall into five groups because two Al/Hg amalgam samples and one Pt/H_2 sample are separated from their respective sets. Error bars indicate one standard deviation around the mean for each individual sample ($n = 3$).

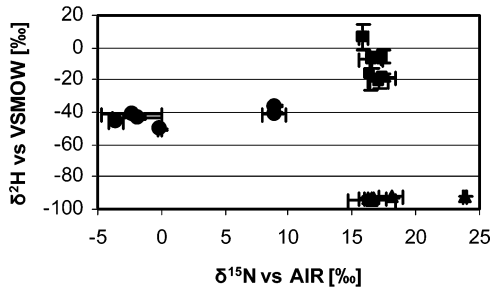

Figure 9. Two variable plot of $\delta^{2}H$ and $\delta^{15}N$ data. The samples fall into five groups because two Al/Hg amalgam samples and one Pt/H_2 sample are separated from their respective sets. Error bars indicate one standard deviation around the mean for each individual sample ($n = 3$).

replicate since once the imine is formed, the reduction occurs in a hydrogenation device under controlled pressure. By $\delta^{2}H$ data, it is tentatively possible to discriminate between the three synthetic routes, but the separation between the Al/Hg amalgam and $NaBH_4$ routes is small given the spread of data within each group.

Two variable plots were then assessed to determine if two elements might afford better visual discrimination than single variable plots. The resulting groups, however, prove somewhat misleading in terms of discrimination according to synthetic route. It is clear from visual interpretation of Figures 7−9 that the 18 samples do not cluster into three groups according to synthetic route, and therefore the single variable plot using $\delta^{2}H$ data provides the best visual discrimination of these MDMA·HCl samples according to synthetic route.

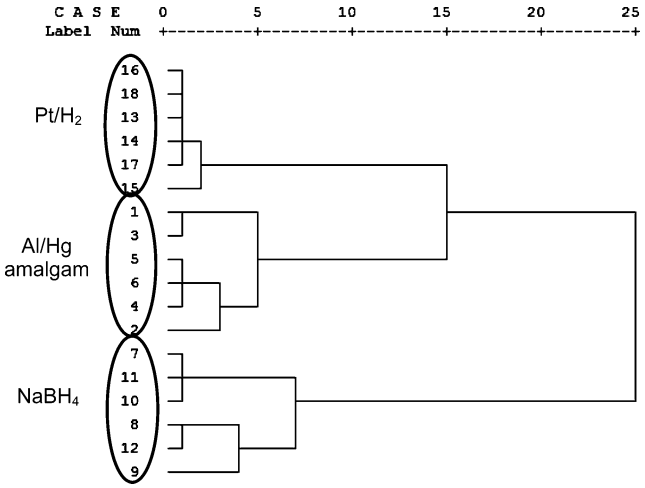

Figure 10. HCA using $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ data of 18 synthesized MDMA batches. Accurate clustering by synthetic route is evident using SPSS 14.0 (furthest neighbor and Euclidean distance).

Chemometric Analysis. Because visual discrimination may lend itself to subjectivity and has not allowed irrefutable discrimination of the sets (note the marginal separation of the $NaBH_4$ and Al/Hg batches in Figures 4 and 7), cluster analysis was employed to find an algorithm that could accurately link the data according to synthetic route. Hierarchical cluster analysis (HCA) has been used in similar published works, but in this study, the validity of the resulting clusters can be assessed because the samples are of known provenance. Remarkably, HCA analysis of the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ data allows the samples to be grouped correctly according to synthetic route (Figure 10). HCA analysis was performed using SPSS 14.0, furthest neighbor clustering method, and Euclidean distance measure. In the resulting dendogram, samples that are found to be most similar are linked by a bracket near the 0 end of the scale. HCA generates three clusters in the data, each one corresponding to a synthetic route. By this algorithm, samples from the Pt/H_2 route are more similar to the Al/Hg amalgam set than the $NaBH_4$ set.

To ascertain the discriminating power of each of the elements, HCA was performed on all combinations of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ taken one or two at a time. Accurate discrimination of the synthetic routes was only possible when $\delta^{2}H$ data was included, that is when $\delta^{2}H$ data was subjected to HCA on its own or in conjunction with $\delta^{13}C$ and/or $\delta^{15}N$ (Figures 11 and 12). Clearly, this work indicates that $\delta^{2}H$ analysis of MDMA is sufficient and necessary for the discrimination of samples by synthetic route, as $\delta^{13}C$ and $\delta^{15}N$ data cannot discriminate all three routes accurately when examined visually or with HCA.

Unsurprisingly, carbon isotope data was shown to be relatively poor for discrimination among the batches as the dendogram produced from HCA of $\delta^{13}C$ and $\delta^{2}H$ data was found to be exactly identical to that produced from HCA of $\delta^{2}H$ data alone (Figure 11). Furthermore, the dendogram produced from HCA of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ data (Figure 10) is nearly identical to that produced from HCA analysis of $\delta^{15}N$ and $\delta^{2}H$ (Figure 12). The slight difference in these two dendograms is evidenced by comparison of the order of the first three Pt/H_2 batches along the vertical column, i.e., 16, 18, 13 vs 13, 16, 18. This "difference" is of course meaningless as the level of similarity and, thus, grouping are the

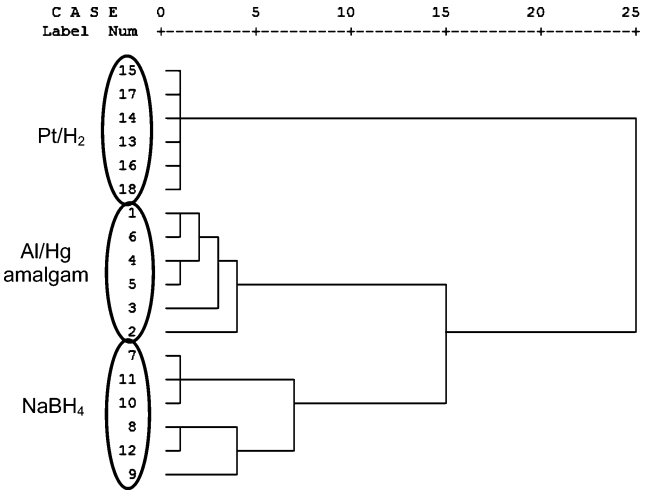

Figure 11. HCA of δ^2H data of 18 synthesized MDMA batches. Accurate clustering by synthetic route is evident using SPSS 14.0 (furthest neighbor and Euclidean distance). HCA analysis of δ^2H and δ^{13}C data results in an identical dendogram.

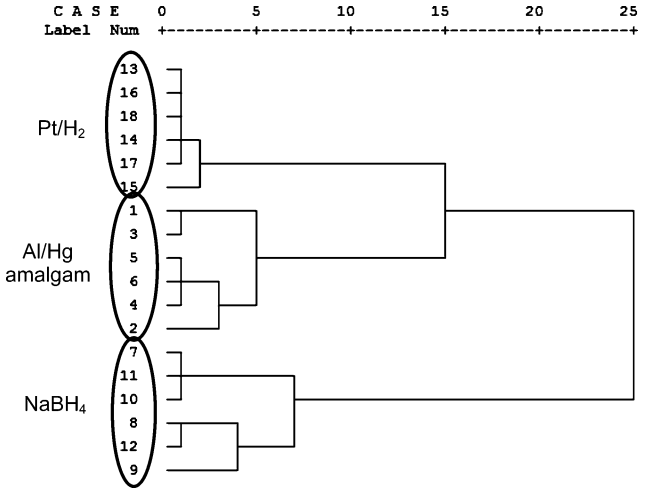

Figure 12. HCA of δ^{15}N and δ^2H data of 18 synthesized MDMA batches. Accurate clustering by synthetic route is evident using SPSS 14.0 (furthest neighbor and Euclidean distance).

same, again illustrating that the carbon isotope data do not add discriminating power on the basis of the synthetic route.

CONCLUSIONS

Despite the careful control exercised during each synthesis, the δ^{15}N data varied between batches, even *within* a synthetic route. For this reason, δ^{15}N data may be of little use in grouping batches synthesized by the same preparative route or even laboratory; instead, δ^{15}N data may be so affected by reaction conditions that it is useful only for discriminating samples according to the production batch.

If these MDMA·HCl samples had been seized on the street and subjected to IRMS analysis, δ^2H data would have allowed tentative visual discrimination into three groups corresponding to the synthetic route used for manufacture, although the separation between the $NaBH_4$ and Al/Hg amalgam sets is narrow and therefore disputable. It should be noted that each synthetic route in this study was as carefully controlled as possible so that it could be "exactly" repeated. In reality, however, clandestine chemists are likely to work in a less controlled fashion during the manufacture of MDMA·HCl, although one might argue that repetitive large scale syntheses over long periods of time would develop a degree of "control-by-routine" on the part of the clandestine chemist. It is not yet known if slight alterations to the conditions of a synthetic route (such as temperature, stirring time, pressure achieved in the hydrogenator, *etc.*) would change the magnitude of fractionation to such a degree that data points lie on a continuum rather than in discrete groups. This is the subject of ongoing further studies within our laboratories.

Regardless, the present study shows that hierarchical cluster analysis of δ^2H data of MDMA·HCl is powerful enough to accurately discriminate between three common synthetic routes, and the addition of carbon and/or nitrogen data does not diminish or enhance this. Because visual discrimination may be subjective, HCA has been shown as an effective method for accurately discriminating among synthetic routes when δ^2H data is considered on its own or in conjunction with δ^{13}C and/or δ^{15}N data.

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