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$\delta^{13}C$ and $\delta^{2}H$ isotope ratios in amphetamine synthesized from benzaldehyde and nitroethane†

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Previous work in these laboratories and by Butzenlechner *et al.* and Culp *et al.* has demonstrated that the $\delta^{2}H$ isotope value of industrial benzaldehyde produced by the catalytic oxidation of toluene is profoundly positive, usually in the range +300‰ to +500‰. Synthetic routes leading to amphetamine, methylamphetamine or their precursors and commencing with such benzaldehyde may be expected to exhibit unusually positive $\delta^{2}H$ values. Results are presented for $\delta^{13}C$ and $\delta^{2}H$ isotope values of 1-phenyl-2-nitropropene synthesized from an industrial source of benzaldehyde, having a positive $\delta^{2}H$ isotope value, by a Knoevenagel condensation with nitroethane. Results are also presented for $\delta^{13}C$ and $\delta^{2}H$ isotope values for amphetamine prepared from the resulting 1-phenyl-2-nitropropene. The values obtained were compared with $\delta^{13}C$ and $\delta^{2}H$ isotope values obtained for an amphetamine sample prepared using a synthetic route that did not involve benzaldehyde. Finally, results are presented for samples of benzaldehyde, 1-phenyl-2-nitropropene and amphetamine that had been seized at a clandestine amphetamine laboratory. Copyright © 2010 Commonwealth of Australia. Published by John Wiley & Sons, Ltd.

α-Phenethylamines are widely abused drugs in many countries including Australia.[1] Methylamphetamine is the most notable phenethylamine abused in Australia and its domestic clandestine production is dominated by synthetic routes employing ephedrine or pseudoephedrine as the immediate precursor with a much smaller amount being prepared from phenyl-2-propanone.[1,2] Amphetamine is a much less abused substance in Australia but is a major problem in Europe[3] and may be prepared by several synthetic routes including Leuckart synthesis, reductive amination of phenyl-2-propanone and the so-called 'nitrostyrene' route.[3,4] In Europe amphetamine is often produced by reactions employing phenyl-2-propanone as the immediate precursor and to a lesser extent benzaldehyde in the 'nitrostyrene' route.

One route to both amphetamine and methylamphetamine is the so-called 'nitrostyrene' route shown in Fig. 1.[4] The Knoevenagel condensation of benzaldehyde with nitroethane gives 1-phenyl-2-nitropropene which may be reduced directly to amphetamine or converted into phenyl-2-propanone to be used as a methylamphetamine precursor. Obvious manufacturing by-products which would be indicative of this particular route are the 1-phenyl-2-nitropropene intermediate, 1-phenyl-2-nitropropane, and the *cis* and *trans* oxime intermediates.[3,4] Conventional chemical profiling will detect these intermediates and by-products if they are present. Unfortunately, the 1-phenyl-2-nitropropene compound is often completely reduced to the desired amphetamine and the oxime intermediates have also been detected in samples of amphetamine known to have been synthesized by other synthetic routes. In addition, if the amphetamine has been highly purified prior to illicit sale there may not be any manufacturing by-products remaining on which to conduct conventional drug profiling.

While the purification processes employed in clandestine drug laboratories may remove all traces of route-specific marker compounds, the stable isotope ratios of carbon ($\delta^{13}C$), hydrogen ($\delta^{2}H$) and nitrogen ($\delta^{15}N$) that form the drug molecule can be measured. Although the purification processes such as recrystallization may affect the stable isotope ratios, a great deal of information may still be obtained from these measurements. Work conducted by the authors,[5] and by Kurashima *et al.*,[6] Butzenlechner *et al.*[7] and Culp and Noakes,[8,9] has shown that industrial benzaldehyde produced by the catalytic oxidation of toluene has $\delta^{2}H$ values that are positive, usually in the range +300 to +500‰ vs. VSMOW (Vienna Standard Mean Ocean Water). Previous work on the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ ratios in ephedrine and methylamphetamine performed by the authors[5] and by Makino *et al.*[10] demonstrated that ephedrine and pseudoephedrine that had been prepared by the semi-synthetic process that employs this type of industrial benzaldehyde as a precursor also had positive $\delta^{2}H$ values as did any methylamphetamine that was prepared from this source of ephedrine or pseudoephedrine.[5,6,10,11] The positive

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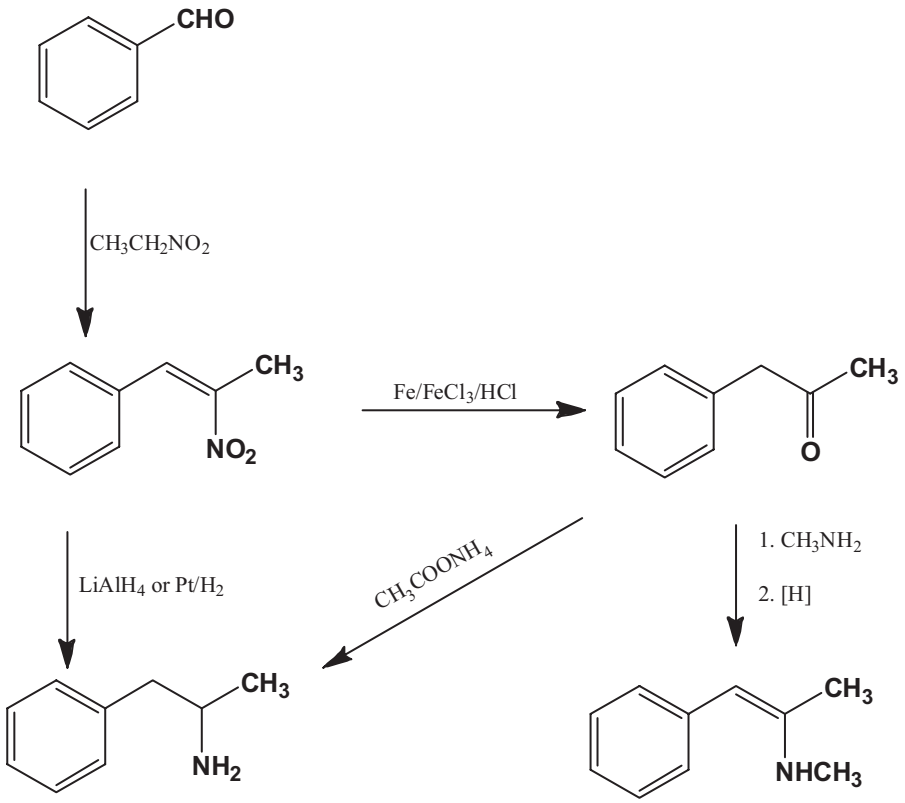

Figure 1. Synthetic scheme for the production of amphetamine and methylamphetamine commencing with benzaldehyde.

δ^2H values obtained for industrial benzaldehyde were most unusual and allowed for discrimination between methylamphetamine samples prepared from semi-synthetic ephedrine/pseudoephedrine and methylamphetamine produced from any other source of ephedrine/pseudoephedrine.

It therefore seemed likely that 1-phenyl-2-nitropropene synthesized from such a deuterium-enriched source of benzaldehyde should also have a positive δ^2H value and these positive values may be reflected in any amphetamine or phenyl-2-propanone prepared from the 1-phenyl-2-nitropropene. This paper describes the synthesis of 1-phenyl-2-nitropropene from benzaldehyde, produced via the catalytic oxidation of toluene, and nitroethane, the subsequent syntheses of amphetamine and the determination of δ^{13}C and δ^2H ratios for each product. The paper also examines the δ^{13}C and δ^2H ratios obtained from samples seized at a clandestine facility known to have been manufacturing amphetamine from benzaldehyde and nitroethane.

EXPERIMENTAL

Standards and reagents

Certified reference standards of 1-phenyl-2-nitropropene, phenyl-2-propanone, and amphetamine were obtained from the National Measurement Institute Australia's Chemical Reference Materials Laboratory (Pymble, NSW, Australia). Acetanilide, hexatriacontane, eicosanoic acid methyl ester and phenanthrene standards for elemental analyzer/thermal conversion-isotope ratio mass spectrometry (EA/TC-IRMS) calibration were purchased from Arndt Schimmelmann at the Department of Geological Sciences, Indiana University (Bloomington, IN, USA). A high-purity (94.9% as HCl form) methylamphetamine quality control sample obtained from the Australian Federal Police (Weston, ACT, Australia) was used to verify EA/TC-IRMS performance.

Isopropanol, absolute ethanol and diethyl ether were obtained from Merck (Kilsyth, VIC, Australia). Analytical grade methanol, toluene and dichloromethane (DCM) were obtained from Mallinckrodt Chemicals (Philipsburg, NJ, USA). Sodium hydroxide (NaOH) pellets, potassium carbonate, glacial acetic acid, sulfuric acid (98%), sodium sulfate anhydrous granular and hydrochloric acid (HCl, 36%) were obtained from UNIVAR Ajax Finechem (Seven Hills, NSW, Australia). Phenyl-2-propanone (98%) was obtained from Fluka (Steinheim, Germany). Benzaldehyde (purified by re-distillation ($\geq$99.5%)), n-butylamine, nitroethane, platinum(IV) oxide, tetrahydrofuran (THF), lithium aluminium hydride and sodium borohydride (98%) were obtained from Sigma-Aldrich (Castle Hill, NSW, Australia). Iron powder was obtained from BDH Chemicals (Poole, UK) and hydrogen peroxide (35%) was obtained from Riedel-deHaen (Seelze, Germany). All reagents were used without further purification.

Synthetic chemistry

1-Phenyl-2-nitropropene from benzaldehyde[12]

To a flask were added sequentially absolute ethanol (67 mL), n-butylamine (3.4 mL), benzaldehyde (68 mL) and nitroethane (48 mL). The mixture was placed under a nitrogen atmosphere with a condenser and drying tube attached, and then refluxed on a steam bath for 8 h. The solution was transferred to a conical flask and allowed to

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cool. Crystal formation was encouraged by scratching the cooled solution with a glass rod. The crystals were filtered, washed with water and cold methanol and dried, yielding a yellow crystalline solid (65.6 g). The solid was identified by gas chromatography/mass spectrometry (GC/MS), against a certified reference standard, as 1-phenyl-2-nitropropene.[13]

Catalytic reduction of 1-phenyl-2-nitropropene to amphetamine with hydrogen gas[14]

1-Phenyl-2-nitropropene (2 g), ethanol (70 mL), glacial acetic acid (200 μL) and platinum oxide (1.0 g) were placed in a hydrogenation flask which was attached to a Parr hydrogenator. The flask was evacuated and flushed with hydrogen three times, charged to a pressure of 45 psi with hydrogen and mechanically shaken for 3 h until uptake of hydrogen had ceased. The reaction mixture was filtered to remove catalyst and the residue was washed with ethanol (100 mL). Solvent was removed by rotary evaporation leaving a dark-yellow oil. To the oil was added water (50 mL) which was then acidified to pH 3 with concentrated HCl (10 M). The resulting mixture was washed with DCM (3 × 100 mL) and the washings discarded. The aqueous phase was basified to pH 12 with dilute sodium hydroxide solution (1 M) and extracted with DCM (3 × 150 mL). The extracts were combined and the DCM was removed by rotary evaporation leaving a clear colourless oil (0.8 g). The oil was dissolved in cold ethanol (10 mL) and acidified with concentrated sulfuric acid (18 M) to pH 3, resulting in a white precipitate. The precipitate was filtered and washed with a 50:50 mixture of cold diethyl ether and ethanol leaving white crystals (0.65 g) which were identified by GC/MS, against a certified reference standard, as amphetamine.

Reduction of 1-phenyl-2-nitropropene to amphetamine with lithium aluminium hydride[15]

1-Phenyl-2-nitropropene (2 g) in anhydrous THF (15 mL) was slowly added to a stirred mixture of lithium aluminium hydride (2.8 g) in anhydrous THF (35 mL) under nitrogen at 0 to 5°C. The mixture was gently refluxed for 7 h, allowed to cool and isopropyl alcohol (3 mL), dilute NaOH (1 M, 3 mL) and water (6 mL) were added. The reaction mixture was filtered to remove particulate matter and the residue washed with THF (100 mL). The solvent was removed by rotary evaporation leaving a yellow oil. To the oil was added water (50 mL) and it was then acidified to pH 3 with concentrated HCl (10 M). The mixture was washed with DCM (3 × 150 mL) and the washings were discarded. The aqueous phase was basified to pH 12 with dilute NaOH solution (1 M) and extracted with DCM (3 × 150 mL). DCM was removed from the combined extracts by rotary evaporation leaving a clear colourless oil (1.81 g). The oil was dissolved in cold ethanol (10 mL) and acidified with concentrated sulfuric acid (10 M) to pH 3, resulting in a white precipitate. The precipitate was filtered and washed with a 50:50 mixture of cold diethyl ether and ethanol leaving white crystals (1.6 g) which were identified by GC/MS, against a certified reference standard, as amphetamine.

Amphetamine synthesis by reductive amination of phenyl-2-propanone with $NaBH_3CN$[16]

To a solution of ammonium acetate (11.42 g) and methanol (15.5 mL) was added phenyl-2-propanone (2 g) and then sodium cyanoborohydride ($NaBH_3CN$) (1.8 g). The mixture was stirred for 36 h at room temperature after which water (1000 mL) containing concentrated HCl (5 mL) was added. The unreacted phenyl-2-propanone was extracted with DCM (3 × 100 mL). The aqueous layer was basified to pH 12 with dilute NaOH solution (1 M) and extracted with DCM (3 × 150 mL). The DCM was removed using a rotary evaporator leaving a yellow oil (~1.6 g). The oil was dissolved in cooled isopropanol (10 mL) and acidified with concentrated hydrochloric acid (18 M) to pH 3. Diethyl ether (10 mL) was added resulting in precipitation of a crystalline material. The crystals were filtered, washed with a 50:50 mixture of isopropanol and diethyl ether and dried yielding white crystals (1.4 g) which were identified by GC/MS, against a certified reference standard, as amphetamine.

Stable isotope ratio mass spectrometry

Sample preparation

Approximately 0.5 mg of drug material was weighed into tin foil capsules (3.3 mm × 5 mm, IVA Analysentechnik, Meerbusch Germany) for $\delta^{13}C$ analysis and 0.2 mg into silver foil capsules for $\delta^{2}H$ analysis. Capsules were crimped and placed with forceps into 96-well plates with the position recorded against the sample identification number.

Isotopic calibration and quality control of EA/TC-IRMS measurements

A Flash elemental analyzer (EA) 1112, with dual combustion and thermal conversion (TC) capabilities (Thermo, Delft, The Netherlands), connected to a ConFlo IV interface and Delta V Plus Mass Spectrometer (Thermo, Bremen, Germany), was used to determine $\delta^{13}C$ and $\delta^{2}H$ values by continuous flow for samples synthesized in this laboratory. Data was acquired using ISODAT 2.5 software (Thermo, Bremen, Germany). Prior to sequence acquisitions, zero enrichment was performed using each of the two reference gases. The standard deviation of nine 20-s gas pulses was determined to be less than 0.1‰ for CO_2 and 0.5‰ for H_2.

Sample sequences for $\delta^{13}C$ analysis were bracketed by triplicates of a certified reference standard of acetanilide ($\delta^{13}C = -29.5 \pm 0.1$‰). EA-IRMS performance was verified by analysis of the high-purity methylamphetamine HCl quality control ($\delta^{13}C = -27.9 \pm 0.4$‰) every five samples. The $\delta^{13}C$ values, reported as per mille (‰) differences from the Vienna Pee Dee Belemnite (VPDB) international standard, were measured relative to high purity (>99.5%) CO_2 gas (BOC gases, Sydney, NSW, Australia; $\delta^{13}C = -6.8 \pm 0.4$‰) that had been calibrated against NBS 19 (Environmental Isotopes Pty. Ltd., Sydney, NSW, Australia).

The $\delta^{2}H$ values, reported as per mille (‰) differences from the VSMOW international standard, were measured relative to ultra-high-purity (>99.99%) H_2 gas (BOC gases; $\delta^{2}H = -340 \pm 4$‰) calibrated in-house and normalized to certified reference materials: hexatriacontane, ($\delta^{2}H = -247 \pm 1$‰); phenanthrene, ($\delta^{2}H = -84 \pm 1$‰); and

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eicosanoic acid methyl ester ($\delta^2H = +76 \pm 1‰$).[17] The TC-IRMS performance was verified by analysis of the high-purity methylamphetamine HCl quality control sample ($\delta^2H = -195 \pm 4‰$) every five samples. The H_3^+ factor (3.39 ppm/nA) was determined by ISODAT 2.5 software using regression analysis performed on the *m/z* 2 and *m/z* 3 ion voltages for reference H_2 gas pulses with signal size linearly incremented.

$\delta^{13}C$ analysis by EA-IRMS

A folded tin foil capsule containing no sample material was the first and final analysis performed in a sequence to demonstrate that the system was void of contamination. A typical sequence comprised of ten samples run in triplicate, preceded and followed by a triplicate analysis of acetanilide standard. Crimped tin capsules containing sample material were introduced into a Thermo Scientific *No Blank* chamber and pressurized with high-purity oxygen gas (BOC gases) to exclude the contribution of nitrogen from ambient air. The sample entered the combustion furnace operated at 980°C where it was burnt exothermically in a stream (250 mL/min, 3 s) of high-purity (>99.5%) oxygen. Water was removed from the resultant gas stream using a trap filled with magnesium perchlorate. A post-reactor GC column, operated at 65°C, separated evolved N_2 and CO_2. The pressure of the ultra-high-purity (>99.99%) helium (BOC Gases) was set to 100 kPa to enable a run time of 650 s per sample.

δ^2H analysis by TC-IRMS

Silver foil capsules containing sample material were introduced into the TC reactor consisting of an alumina ceramic outer containing a glassy carbon tube with glassy carbon granulate and silver wool packing. The thermal conversion furnace was operated at 1450°C and the post-reactor GC column at 85°C. The helium pressure was set to 330 kPa to enable a run time of 300 s. A typical sequence comprised of ten samples run in triplicate, preceded and followed by a triplicate analysis of hexatriacontane and eicosanoic acid methyl ester standards. To minimize the influence of memory effects on δ^2H values, samples recording positive δ^2H values were each re-analyzed with the methylamphetamine HCl quality control ($\delta^2H = -195 \pm 4‰$) run between the samples.

Measurement uncertainty for $\delta^{13}C$ and δ^2H measurements

An estimation of measurement uncertainty was performed by combining the contributions of bias and precision using the square root of the sum of squares method.[18] The bias for $\delta^{13}C$ (0.1‰) and δ^2H (1‰) measurements was determined from the mean difference between observed and reference values for certified hexatriacontane (n = 9). The precision for the $\delta^{13}C$ (0.2‰) and δ^2H (2‰) measurements was determined from the pooled standard deviation of the high-purity methylamphetamine HCl control sample. The combined standard uncertainty (u) for the $\delta^{13}C$ and δ^2H measurements was calculated to be ±0.2‰ and ±2‰, respectively. To provide a 95% confidence interval (k = 2) an expanded uncertainty (U) for the $\delta^{13}C$ and δ^2H measurements was estimated to be ±0.4‰, and ±4‰, respectively. These uncertainty estimates were considered to be fit-for-purpose based on the range of values recorded for the quality control sample. The estimate of measurement uncertainty (±4‰) provided for δ^2H measurements is valid for the range from −247‰ to +76‰. An expanded uncertainty of ± >4‰ is estimated for the δ^2H values recorded outside this range.

RESULTS AND DISCUSSION

The major aim of conventional chemical profiling of illicit drugs has been the determination of the geographical origin of cultivated and semi-cultivated drugs such as cocaine and heroin, and the synthetic pathway and precursor chemicals employed in clandestine drug manufacture of fully synthetic drugs such as amphetamine, methylamphetamine and 3,4-methylenedioxymethylamphetamine (MDMA or 'Ecstasy'). Chemical profiling of drug seizures is also used to link separate seizures to a common source. Identification of precursors, intermediates, manufacturing by-products, solvents and catalysts by chromatographic and spectroscopic techniques has been used successfully to this end.

The identification of manufacturing by-products that are only formed in a drug prepared by a specific route has been the mainstay in the identification of synthetic routes for amphetamine-type stimulants. Examples of this approach include the pyrimidine compounds formed during the Leuckart synthesis of amphetamine,[19] the naphthalene by-products typically found in methylamphetamine produced by the hydriodic acid reduction of ephedrine or pseudoephedrine,[20,21] the cyclohexadienyl products formed during the Birch reduction of pseudoephedrine to methylamphetamine,[21] chloroephedrine formed during the 'Emde' synthesis of methylamphetamine,[22] and dibenzylketone and its reduction product that may be seen in methylamphetamine prepared from phenyl-2-propanone which in turn had been prepared from phenylacetic acid.[3,4]

The GC/MS detection of trace amounts of 1-phenyl-2-nitropropene or 1-phenyl-2-nitropropane in a sample of illicit amphetamine is routine and indicates that the synthetic route shown in Fig. 1 has been employed. Unfortunately, traces of these compounds may not always be observed after the reduction step and salting out to produce amphetamine sulfate for the illicit market. The reduction of the nitropropene intermediate is an efficient reaction usually going to completion and even when trace amounts remain they may be lost during any subsequent recrystallization step. However, law enforcement agencies and other national and international agencies rely on forensic investigation to determine when this synthetic approach is being used. Benzaldehyde and nitroethane are common legitimate industrial chemicals produced in large quantities and it is desirable to disrupt drug trafficking operations engaged in the diversion of these chemicals for illegitimate use.

Stable isotope ratio measurements are being increasingly used in forensic science including the field of illicit drug profiling. This technique has been shown to be very useful in helping to geo-locate drugs such as cocaine[23,24] and heroin[24–28] and has been used to help link seizures of fully synthetic drugs such as methylamphetamine and MDMA. Stable isotope ratio analysis of benzaldehyde has shown that it is possible to discriminate between industrial sources of benzaldehyde and

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those that are botanically derived.[7–9] Catalytic oxidation of toluene would be the most likely source of the benzaldehyde precursor for the so-called 'nitrostyrene' route as this is the major industrial manufacturing method for benzaldehyde. Other processes such as the benzal chloride route are not as predominant. Industrial benzaldehyde has been reported to be heavily deuterium-enriched, with $\delta^{2}H$ values as high as +720‰.[7–9] Therefore, a synthetic scheme commencing with an industrial source of benzaldehyde ($\delta^{2}H = +552$) and comprising only two steps, with few exchangeable hydrogen atoms, could be expected to result in a product with a positive $\delta^{2}H$ value. Amphetamine was prepared from such a benzaldehyde precursor using the nitrostyrene method shown in Fig. 1. The $\delta^{13}C$ and $\delta^{2}H$ values for the benzaldehyde precursor, 1-phenyl-2-nitropropene intermediate and amphetamine produced from the 1-phenyl-2-nitropropene intermediate by two different reduction procedures are given in Table 1. The results demonstrate deuterium depletion during the course of the Knoevenagel condensation to 1-phenyl-2-nitropropene and its subsequent reduction to amphetamine. This is to be expected as the benzaldehyde moiety becomes part of a larger molecule with additional hydrogen atoms contributed by nitroethane and lithium aluminum hydride or hydrogen gas. However, as expected, the $\delta^{2}H$ values were still profoundly positive in the amphetamine product. This would not be the case if a natural source of benzaldehyde had been used. Results for $\delta^{13}C$ and $\delta^{2}H$ values for amphetamine produced from a natural benzaldehyde ($\delta^{2}H = -125$‰) are presented in Table 2. The $\delta^{2}H$ value of the amphetamine product is depleted relative to the starting material. While it is conceivable that clandestine manufacturers might employ natural sources of benzaldehyde (i.e. extracts from almonds, cherries, etc.) it is more likely that fully synthetic benzaldehyde would be used as it is abundantly produced. Table 3 presents the $\delta^{13}C$ and $\delta^{2}H$ data for amphetamine synthesized by the reductive amination of phenyl-2-propanone, a common method for the production of amphetamine. This particular phenyl-2-propanone had not been prepared from benzaldehyde but rather from phenylacetic acid. The carbon isotope ratio is, within measurement uncertainty, unchanged. Enrichment in the hydrogen isotope value has occurred in this instance, with the value increasing from −90‰ to −55‰ but still remaining negative.

Table 1. $\delta^{13}C$ and $\delta^{2}H$ values for industrial source of benzaldehyde and 1-phenyl-2-nitropropene, amphetamine, nitroethane and phenyl-2-propanone produced from it

Precursor	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)
Benzaldehyde; purified by re-distillation (≥99.5%) (industrial source)	−26.5	+552
Nitroethane	−24.5	−34
1-Phenyl-2-nitropropene intermediate	−26.1	+279
Amphetamine (Pt_2O/H_2 gas)	−26.7	+90
Amphetamine ($LiAlH_4$)	−26.0	+93

Table 2. $\delta^{13}C$ and $\delta^{2}H$ values for a natural source of benzaldehyde and the 1-phenyl-2-nitropropene and amphetamine produced from it

Precursor	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)
Benzaldehyde; purified by re-distillation (≥98%) (Sigma 'Kosher')	−28.3	-125
Nitroethane	−24.5	-34
1-Phenyl-2-nitropropene intermediate	−28.9	-111
Amphetamine (Pt_2O/H_2 gas)	−28.5	-157
Amphetamine ($LiAlH_4$)	−28.2	-157

Table 3. $\delta^{13}C$ and $\delta^{2}H$ values for phenyl-2-propanone and amphetamine: a 'non-benzaldehyde'-derived amphetamine source

Precursor	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)
Phenyl-2-propanone	−27.9	−90
Amphetamine	−28.4	−55

Table 4. $\delta^{13}C$ and $\delta^{2}H$ values for samples seized at a clandestine laboratory

Precursor	$\delta^{13}C_{VPDB}$ (‰)	$\delta^{2}H_{VSMOW}$ (‰)
Benzaldehyde precursor sample 1	−23.9	+437
Benzaldehyde precursor sample 2	−24.6	+489
Nitroethane	−18.8	+25
1-Phenyl-2-nitropropene intermediate sample 1	−25.3	+265
1-Phenyl-2-nitropropene intermediate sample 2	−25.3	+257
1-Phenyl-2-nitropropene intermediate sample 3	−25.2	+267
Amphetamine product	−24.3	+133

Samples of amphetamine sulfate, 1-phenyl-2-nitropropene and benzaldehyde seized at a clandestine laboratory in northern New South Wales, Australia, in 2006, were also subjected to stable isotope ratio analysis and the $\delta^{13}C$ and $\delta^{2}H$ values are presented in Table 4. The results are very similar to those values presented in Table 1 and consistent with a heavily deuterium-enriched industrial source of benzaldehyde as the precursor. Obviously, from the materials found at the clandestine laboratory, the 'nitrostyrene' route had been employed and on this occasion conventional chemical profiling of the amphetamine did reveal low levels of 1-phenyl-2-nitropropene and palladium catalyst. However, had chemical profiling failed to provide sufficient information to determine the synthetic route the stable isotope ratio analysis would have strongly suggested the 'nitrostyrene' route.

CONCLUSIONS

The careful measurement of hydrogen stable isotope ratios has been demonstrated to be useful in discriminating between amphetamine produced from industrial benzaldehyde and nitroethane on one hand and amphetamine manufactured using synthetic routes that do not employ benzaldehyde. Very few common industrial compounds other than benzaldehyde have such large positive $\delta^{2}H$ values. It is likely then that an amphetamine sample possessing a large positive $\delta^{2}H$ value

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would have been produced by the condensation of industrial benzaldehyde, produced from the catalytic oxidation of toluene, and nitroethane to give the 1-phenyl-2-nitropropene intermediate, and the subsequent reduction of this intermediate. However, an amphetamine sample with a negative δ^2H value might also have been produced in the same way if a natural source of benzaldehyde, such as almond oil, had been used or if benzaldehyde produced via the benzal chloride process was used. It is more likely, however, that the clandestine production of amphetamine using the so-called 'nitrostyrene' route will result in a product that has a positive δ^2H value as the majority of industrial benzaldehyde produced is manufactured via the catalytic oxidation of toluene and is heavily deuterium-enriched. In the absence of 'route-specific' molecules in the final product, a positive δ^2H value would be consistent with the 'nitrostyrene' route to amphetamine.

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