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Determination of the Synthetic Origin of Methamphetamine Samples by ^{2}H NMR Spectroscopy

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Samples of methamphetamine, prepared according to the most common synthetic pathways, were submitted to natural-abundance ^{2}H NMR spectroscopy. The deuterium content at the various sites of the molecule was found to depend on its synthetic history. The technique provides a chemical fingerprint of methamphetamine samples and can give hints to trace back the starting materials and the synthetic procedures.

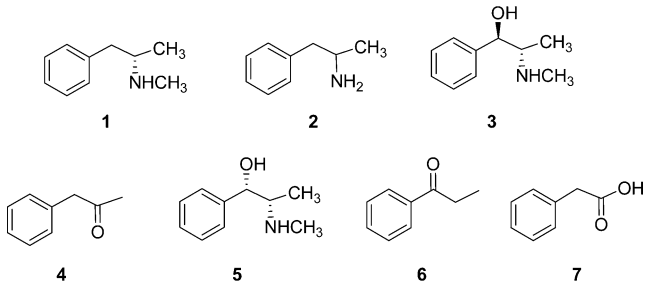
Methamphetamine (**1**) is a synthetic stimulant drug (Chart 1) that induces a strong feeling of euphoria and generates addiction. It was developed in the 1930s from its parent drug amphetamine (**2**) and was originally used in nasal decongestants, bronchial inhalers, and the treatment of narcolepsy and obesity. In the 1970s, it became a Schedule II drug—a drug with a high abuse risk, but also accepted medical uses in the United States. Nowadays, it is illegally produced in clandestine laboratories and sold in pill form, capsules, powder, and chunks.

Methamphetamine was first obtained in the work[1] of structural elucidation of ephedrine (**3**), the main active component isolated from *Ephedra vulgaris*. Structure **1** was tentatively assigned to the product obtained by reaction of ephedrine with red phosphorus and iodine. Compound **1** was synthesized by reduction of the condensation product of phenyl acetone (**4**) and methylamine, to verify the structural assignment. Methamphetamine is a chiral compound: (*S*)-**1** is a stimulant, while the (*R*)-enantiomer is a decongestant with no stimulant activity.

The synthetic methods, which are most widely employed in clandestine laboratories, are still making use of ephedrine or of phenyl acetone as starting materials. Also pseudoephedrine (**5**), which differs from ephedrine in the configuration of the benzylic stereocenter, can be used to prepare illegal stimulant methamphetamine.

Commercial ephedrine is produced in substantial amounts for medical purposes by extraction from *Ephedra* plants, by chemical synthesis starting from propiophenone (**6**) or by fermentation from benzaldehyde.

Chart 1



Phenyl acetone (**4**) is prepared by reaction of phenylacetic acid (**7**) with acetic anhydride. Pseudoephedrine is the active component of Sudafed, NyQuil, and several other over-the-counter decongestants. It is obtained together with ephedrine in the extraction from *Ephedra* or by treatment of ephedrine with boiling hydrochloric acid.

Ephedrine, pseudoephedrine, phenylacetic acid, acetic anhydride, and phenyl acetone are all substances included in the Red List,[2] i.e., the list of precursors and chemicals frequently used in the illicit manufacture of narcotic drugs and psychotropic substances under international control. This list has been prepared by the International Narcotics Control Board (INCB) as a tool to be used for the identification of substances scheduled in Tables 1 and 2 of the United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances (1988 Convention). The export−import of the substances belonging to the Red List is strictly controlled and is completely traceable (name and address of the exporter and importer and, when available, the consignee; quantity of the substance to be exported; expected point of entry and expected date of dispatch).

In the fight against mom-and-pop meth labs, it thus becomes extremely important to trace the synthetic path of seized methamphetamine samples, to establish whether products from different seizures are from a common origin, and to identify the reagents involved in the synthetic procedure. These are important clues for use in the criminal investigation.

The analysis of isotope ratios has been found to be a powerful tool to trace the origin of organic compounds. The distribution of the stable isotopes within the atomic species of an organic molecule is not random, but depends on the way the molecule was obtained.

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(2) http://www.incb.org/incb/red_list.html.

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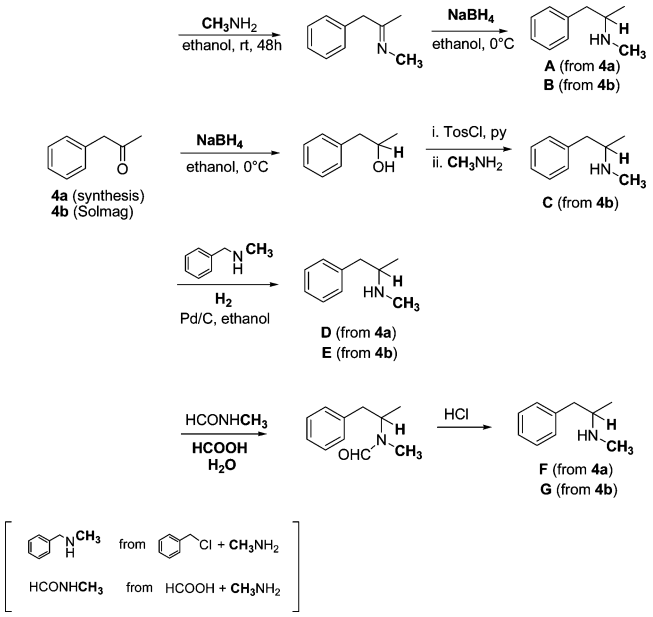
As for synthetic products, such as drugs, their isotopic composition is dependent on both the isotopic composition of the reactants and on the synthetic isotopic fractionation of the manufacturing process employed. A change in either of these variables produces a drug product having a different isotopic profile. Recent work has shown that when both the source of the starting materials and the manufacturing process are presumably held relatively constant during manufacture of the bulk drug substance, similar product isotope ratios are observed.[3] Two different techniques can be employed to determine isotope ratios: (i) isotope ratio mass spectrometry (IRMS) and (ii) Site-specific natural isotope fractionation measured by NMR spectroscopy (SNIF NMR). The latter is particularly advantageous because it allows the simultaneous determination of the deuterium content at each site of a molecule, expressed as D/H (ppm). This technique allows the D/H of each hydrogen atom to be directly correlated to the reagents employed to insert it. The information is very punctual: by combining the knowledge of the mechanisms of organic reactions with the determination of the deuterium enrichment or depletion of a certain position, it is possible to gain clues to trace the origin.

Isotope ratio analysis has been useful in the recognition of the geographical origin of natural extractive drugs such as heroin and cocain (SNIF NMR)[4] and in the linkage of different seizures of ecstasy tablets to a possible common origin (IRMS).[5−8] In the past,[9] we submitted nine samples of *N*-acetyl-3,4-methylenedioxyamphetamine, prepared according to the most common synthetic procedures, to ^{2}H NMR spectroscopy. We could show that the relative deuterium content at the various sites of the molecule was dependent on its synthetic history.

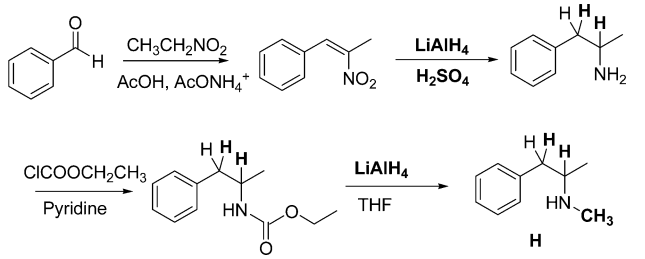
In a recent work published in this journal[10] carbon and nitrogen stable isotope analysis obtained by IRMS was used to determine the origin of ephedrine. The values of δ^{13}C and δ^{15}N were employed to discriminate natural, semisynthetic, or synthetic ephedrine samples.

We, now, report on the use of ^{2}H NMR spectroscopy to establish the synthetic origin of methamphetamine samples. Twelve samples of methamphetamine, prepared according to the most common routes, were submitted to ^{2}H NMR spectroscopy in order to establish whether the deuterium pattern of the molecule could retain a memory of the synthetic steps.

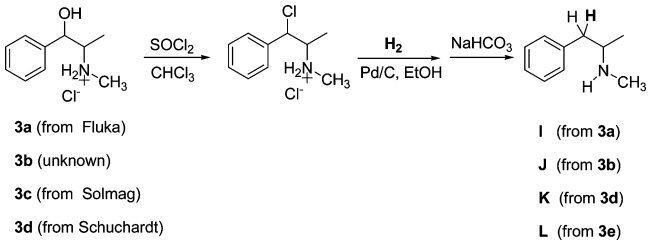
Scheme 1. Methamphetamine Samples from Phenyl Acetone



Scheme 2. Methamphetamine from Benzaldehyde



Scheme 3. Methamphetamine Samples from Ephedrine



EXPERIMENTAL SECTION

Materials and Methods. Twelve samples of methamphetamine were prepared in our laboratory according to the most common synthetic procedures, as shown in Schemes 1−3. The origins of the chemicals were as follows: phenyl acetone commercial from Solmag (**4b**), phenyl acetone prepared by synthesis from phenylacetic acid and acetic anhydride (**4a**); ephedrine unknown (**3b**) available from the collection of chemicals of the department; ephedrine commercial from Fluka (**3a**), Schuchardt (**3c**), and Solmag (**3d**); benzaldehyde commercial from Fluka; $LiAlH_4$ and $NaBH_4$ from Aldrich.

The ^{2}H experiments were performed on a Bruker Avance 500 spectrometer equipped with a 10-mm probe head and a ^{19}F lock channel (C_6F_6), under CPD (Waltz 16 sequence) proton decoupling conditions at the temperature of 300 K. The spectra were

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recorded dissolving 0.7−0.8 g of material in ~3.0 mL of CH_2Cl_2 adding ~50 mL of C_6F_6 for the lock and 130−150 mg of hexamethyldisiloxane as internal standard. Hexamethyldisiloxane was chosen because the signal of the six equivalent methyl groups occurs in a well-isolated region of the spectrum allowing precise peak integration. It shows a D/H ratio of 130.7 ppm determined by calibration against the official standard TMU (Community Bureau of References, BCR) with a certified D/H ratio. Hexamethyldisiloxane is rather volatile and thus extreme care had to be taken for precise weighing. The solutions were prepared introducing and weighing directly in the NMR tube the methamphetamine followed by the standard and the solvent, maintaining the tube carefully closed. The signal assignment of the spectrum of methamphetamine, obtained from the analysis of the proton spectrum taken in the same conditions of the deuterium spectrum, is the following (δ, ppm): 7.34−7.25 (5H, m, Ph), 2.81 (1H, m, J = 6.5 Hz, CH), 2.75 (1H, dd, J = 6.5 and 13.2 Hz, Ha), 2.62 (1H, dd, J = 6.4 and 13.2 Hz, Hb), 2.40 (3H, s, NCH_3), 1.06 (3H, d, J = 6.2 Hz. CH_3). Four spectra were run for each sample, collecting 3000−4000 scans using the following parameters: 5.9-s acquisition time, 1380-Hz spectral width, 16 K time domain, and 2−3-s delay. In these conditions, the signal/noise ratio calculated for the smallest peaks using a FID exponential multiplication of 0.5 Hz is always superior to 15. An estimation of the relaxation times (T_1) of the deuterium nuclei showed that the longest T_1 is that of the reference material (~1.0 s). Thus, the total time of 7−8 s between successive pulses is certainly sufficient to ensure a complete nuclear relaxation. Each FID was Fourier transformed with a line broadening of 0.5−1.0 Hz, manually phased, and integrated after an accurate correction of the spectral baseline. For partially overlapped signals, the peak areas were determined through the deconvolution routine of the Bruker TopSpin NMR software using a Lorentzian line shape.

The absolute values of the site-specific D/H ratios were calculated according to the formula

$$(D/H)_i = n_{WS} g_{WS} (MW)_L S_i (D/H)_{WS} / (n_i g_L (MW_{WS}) S_{WS})$$

where WS stands for the working standard with a known isotope ratio $(D/H)_{WS}$ and L for the product under examination; n_{WS} and n_i are the number of equivalent deuterium atoms of the standard and of the ith peak; g_{WS} and g_L are the weights of the standard and the sample; MW_L and MW_{WS} are the corresponding molecular weights; S_i and S_{WS} are the areas of the ith peak and of the standard, respectively.

RESULTS AND DISCUSSION

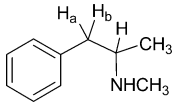
We have examined samples of methamphetamine prepared from phenyl acetone (Scheme 1), benzaldehyde (Scheme 2), and ephedrine (Scheme 3). Two different batches of phenyl acetone had been used, a commercial one (**4b**) received from Solmag (Italy) and one prepared in our laboratory (**4a**) from phenylacetic acid according to the same industrial synthesis. The conversion of phenyl acetone into methamphetamine requires the transformation of the carbonyl group into the $CHNCH_3$ moiety. This can be achieved according to the four different paths reported in Scheme 1: the hydrogen atom at C(2) in final compound **1** comes from the reducing agent ($NaBH_4$, H_2, HCOOH) and the methyl group is essentially that of methylamine.

The reaction starting from benzaldehyde needs the addition of two carbon atoms, and this can be obtained by condensation with nitroethane (Scheme 2). The two hydrogen atoms at C(1) and C(2) of **1** are inserted by reduction with $LiAlH_4-H_2SO_4$ and the methyl group is generated by $LiAlH_4$ reduction of the urethane intermediate.

In the transformation of ephedrine into methamphetamine (Scheme 3), the only modification in the pattern of the hydrogen atoms is the insertion of a hydrogen atom in the benzylic position by means of molecular hydrogen.

The values of D/H (ppm) for the different positions of the 12 samples of methamphetamines are reported in Table 1. The most

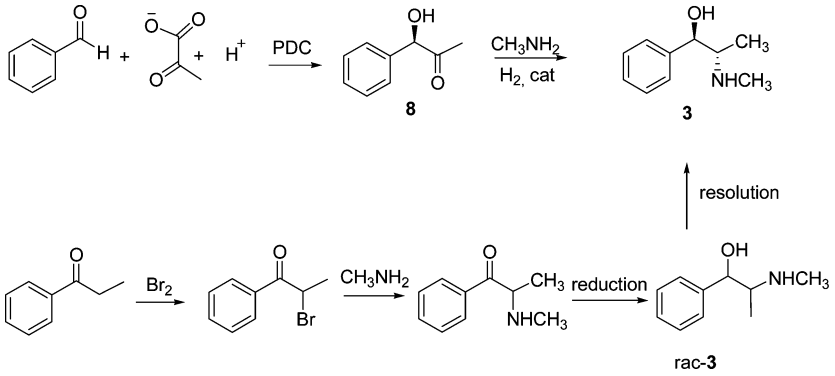
Table 1. $(D/H)_i$ Isotope Ratios (ppm)[a]



origin	samples	D/H(CH_a)	D/H(CH_b)	D/H(CH)	D/H(CH_3)	D/H(NCH_3)	D/H(Ph)
from phenyl acetone (see Scheme 1)	**A**	138.7 (3.1)	139.4 (2.1)	111.0 (3.2)	115.9 (2.8)	129.4 (1.5)	152.8 (1.5)
	B	140.7 (3.2)	137.0 (2.9)	107.7 (3.8)	116.7 (0.8)	130.4 (0.8)	151.0 (1.5)
	C	168.4 (3.7)	158.6 (3.0)	150.4 (3.4)	137.7 (1.3)	130.6 (0.7)	152.9 (2.2)
	D	134.8 (3.2)	143.8 (3.2)	**36.5** (4.2)	125.6 (3.2)	135.6 (0.6)	153.1 (1.6)
	E	128.4 (3.5)	136.7 (3.5)	**26.3** (2.9)	117.2 (1.9)	131.6 (1.2)	152.3 (1.0)
	F	137.1 (2.9)	139.4 (3.5)	135.6 (5.7)	121.1 (2.3)	134.8 (1.4)	150.9 (1.9)
	G	141.9 (3.2)	140.8 (3.7)	114.6 (3.4)	122.9 (2.1)	137.8 (1.7)	151.6 (2.5)
from benzaldehyde (see Scheme 2)	**H**	102.0 (3.1)	102.4 (1.2)	**38.2** (3.4)	139.9 (1.4)	**44.6** (0.6)	153.1 (2.0)
from ephedrine (see Scheme 3)	**I**	59.3 (3.5)	88.3 (2.4)	**35.3** (3.8)	102.1 (2.9)	130.5 (1.2)	145.1 (1.8)
	J	61.9 (1.9)	83.2 (1.8)	**28.9** (1.0)	101.9 (1.1)	127.5 (1.8)	147.9 (1.5)
	K	161.9 (4.1)	131.1 (2.4)	**62.3** (5.0)	125.3 (1.4)	102.1 (1.7)	129.6 (1.6)
	L	160.6 (3.1)	158.3 (3.6)	**27.9** (3.1)	98.8 (1.6)	118.9 (1.6)	154.9 (2.1)

[a] Values in parentheses are $(D/H)_i$ standard deviations.

Scheme 4. Commercial Syntheses of Ephedrine



interesting isotope ratio values are those of the CH, CH_2 (CH_a and CH_b),[1] and NCH_3 positions, because these hydrogen atoms were manipulated during the synthetic procedure.

On the basis of the values of $(D/H)_{CH}$, the samples can be divided into two groups: one (**A**, **B**, **C**, **F, G**) with $(D/H)_{CH}$ > 100 ppm (from 107.7 to 150.4 ppm, underscored values in Table 1) and the other (**D**, **E**, **H**−**L**) with $(D/H)_{CH}$ < 100 ppm (from 26.3 to 62.3 ppm, highlighted values in Table 1). The analysis of the $(D/H)_{NCH_3}$ shows that all the samples have comparable values except sample **H** (D/H = 44.6 ppm). The values of $(D/H)_{CHa}$ and of $(D/H)_{CHb}$ appear to be randomly dispersed.

Isotope Ratios $(D/H)_{CH}$. Table 1 shows that samples **A**, **B**, **C**, **F**, and **G**, with $(D/H)_{CH}$ > 100 ppm can be distinguished from samples **D, E**, **H**, **I**, **J**, **K**, and **L,** characterized by $(D/H)_{CH}$ < 100 ppm. Samples **A**−**G** are prepared starting from phenylacetone, sample **H** is prepared from benzaldehyde, samples **I**−**L** are obtained from ephedrine.

For the samples obtained from phenyl acetone, the deuterium content of the hydrogen atom of this position was found to depend on the reduction reagent. The use of molecular hydrogen produced a deuterium depletion (samples **D** and **E**), compared to the reduction performed with sodium boron hydride and formic acid (samples **A**−**C**, **F**, **G**). The evidence in our hands[9] is as follows: (i) the reduction of C═N by H_2 or $LiAlH_4$, and the reduction of double bonds by H_2 or $LiAlH_4$−H_2SO_4 (addition to sp^2 carbon atoms) leads to the introduction of deuterium-depleted hydrogen atoms; (ii) the use of $NaBH_4$ in carbonyl reductions gives the insertion of hydrogen atoms with high deuterium content. In the meth series, these observations are also confirmed by the low D/H of the CH position of **H**. This latter was obtained by $LiAlH_4$−H_2SO_4 reduction of the condensation product between benzaldehyde and nitroethane.

For the samples prepared starting from ephedrine, the $(D/H)_{CH}$ values were found to be rather low. This hydrogen belongs to the starting ephedrine molecule; thus, the synthetic methods employed to prepare ephedrine should be considered. The samples of methamphetamine examined had been prepared from four different batches of ephedrine, but we have no idea of their synthetic history.

The largest manufacturer of (1*R*,2*S*)-**3** is Knoll Pharmaceuticals, a division of BASF. The key intermediate is (*R*)-phenylacetylcarbinol (**8**), which is prepared by biotransformation of benzaldehyde by pyruvate decarboxylase in whole cells of fermenting baker's yeast (Scheme 4). Compound **8** is then submitted to stereoselective reductive amination by reaction with methylamine and molecular hydrogen.

Optically active ephedrine can be obtained by resolution of the racemate prepared by bromination of propiophenone (**6**), reaction with methylamine, and reduction of the carbonyl group. Natural ephedrine is obtained by extraction from *Ephedra* plants. According to the synthetic procedure, the hydrogen atom at C(2) of the final ephedrine is that of the starting propiophenone or is generated by reductive amination. The low deuterium content at the CH position of samples **I**−**L** could be tentatively attributed to the use of H_2 as a reducing agent.

Isotope Ratios $(D/H)_{NCH_3}$. The D/H values for the methyl group linked to the nitrogen atom are very similar. The only exception is sample **H**: the D/H is 44.6 ppm. This low value is in accord with the fact that the methyl group is generated by $LiAlH_4$ reduction of the urethane intermediate. This low value is diagnostic to distinguish this method of insertion of the methyl group at the nitrogen atom from those involving methylamine or compounds prepared thereof ($PhCH_2NHCH_3$ from $PhCH_2Cl$ and CH_3NH_2 $HCONHCH_3$ from HCOOH and CH_3NH_2 in Scheme 1).

Isotope Ratios $(D/H)_{CHa}$ and D/H_{CHb}. Figure 1A shows the D/H values of samples **A**−**C**, **F**, and **G**, with $(D/H)_{CH}$ > 100 ppm. The six samples are almost superimposed: no relevant differences are noted between samples **A** and **F**, obtained from phenyl acetone **4a**, and samples **B**, **C**, and **G**, obtained from phenyl acetone **4b**. In all these samples, the benzylic CH_2 is that of the parent phenyl acetone and the corresponding values of $(D/H)_{CHa}$ and $(D/H)_{CHB}$ are rather similar. Sample **C**, which is the one prepared from phenylacetone by reduction followed by amination, shows the highest D/H values for H_a and H_b. This might be tentatively attributed to a scrambling of the hydrogen atoms in the benzylic position due to a keto−enolic equilibrium promoted by the basic conditions of $NaBH_4$ carbonyl reduction.

Figure 1B shows the D/H values of samples **D**, **E**, and **I**−**L**, with $(D/H)_{CH}$ < 100 ppm (sample **H** excluded). Samples **I** and **J** are almost identical: they were obtained from two different batches of ephedrine. Samples **D**, **E**, **K**, and **L** appear to be similar: **D** and **E** were prepared from phenyl acetone by reaction with benzyl methylamine and molecular hydrogen; **K** and **L** were obtained from two different ephedrines.

The experimental results obtained for samples **I**−**L** are rather intriguing. All the samples were prepared by converting ephedrine into the chloro intermediate, which was then submitted to H_2 reduction: hydride substitution at a sp^3 carbon atom was involved

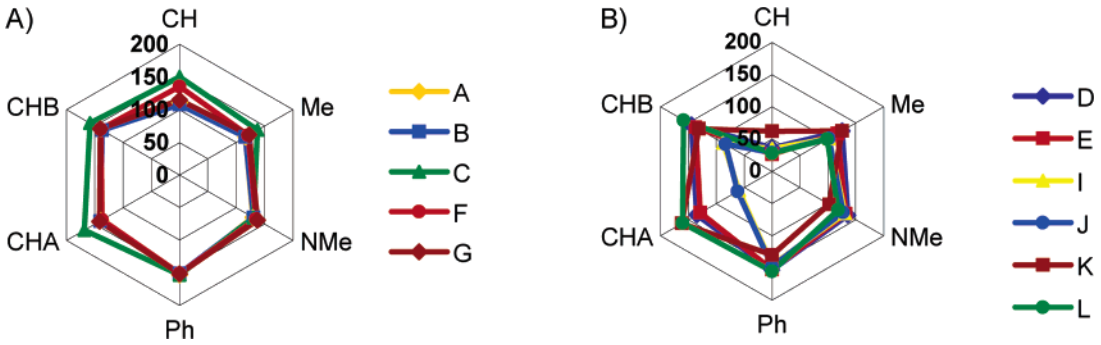


Figure 1. (A) Graphical representation of the D/H values of samples **A**−**C**, **F**, and **G**, with $(D/H)_{CH}$ > 100 ppm; (B) Graphical representation of the D/H values of samples (**D**, **E**, **I**−**L**, except **H**) with $(D/H)_{CH}$ < 100 ppm.

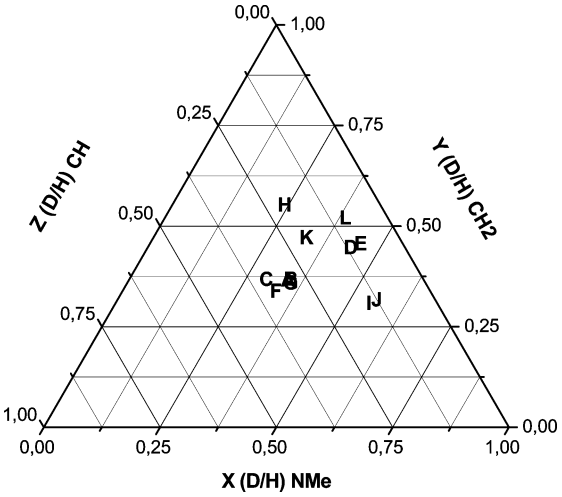


Figure 2. Ternary plot $(D/H)_{CH}$ vs $(D/H)_{CH_2}$ vs $(D/H)_{NCH_3}$.

(Scheme 3). Low D/H were determined for samples **I** and **J**: $(D/H)_{CHA}$ = 59.3−61.9 ppm and $(D/H)_{CHB}$ = 88.3. −83.2 ppm, respectively. Samples **K** and **L** showed, on the contrary, a relevant deuterium enrichment at the CH_2 position, $(D/H)_{CHA}$ = 161.9−160.6 ppm and $(D/H)_{CHB}$ = 131.1−158.3 ppm. These values are comparable to those of the samples obtained from phenyl acetone.

No conclusion can be drawn from these data. First, we do not know the synthetic origin and the deuterium pattern of the starting ephedrines. Second we expect that, the mechanism of the substitution reaction of an hydride at a sp^3 carbon atom being different from that of the addition to a sp^2 carbon atom, a different kinetic isotope fractionation could occur during this reaction step.

A comprehensive pictorial view of the diagnostic D/H values is given Figure 2, which was obtained by plotting $(D/H)_{CH}$ versus $(D/H)_{NCH_3}$ versus $(D/H)_{CH_2}$ (these latter data were the mean values of $(D/H)_{CHa}$ and $(D/H)_{CHb}$). The plot highlights the similarity of samples **A**−**C** and **F**−**G** and of samples **I**−**J** and **D**−**E**. It shows also the clear different origin of the ephedrine batches employed as starting materials for samples **I**−**L**. Sample **H** has its own synthetic history from benzaldehyde.

CONCLUSIONS

Interesting information can be obtained from the ^2H NMR spectra of methamphetamine samples. A high value of $(D/H)_{CH}$ (>100 ppm) allows the identification of samples prepared from phenylacetone using $NaBH_4$ or $HCOOH-HCONHCH_3$. When low values of $(D/H)_{CH}$ are found (<100 ppm), the deuterium content of the benzylic position becomes discriminant and should be considered. Deuterium depletion of H_a and H_b is correlated to the use of ephedrine as a starting material. If high values of $(D/H)_{CHa}$ and $(D/H)_{CHb}$ are found, the choice of the synthetic procedure is between phenyl acetone/H_2 reduction or ephedrine dehalogenation. The use of benzaldehyde and nitroethane gives a very typical and easily recognizable deuterium pattern. The $(D/H)_{NMe}$ is diagnostic to discriminate the use of methylamine as a source of the NCH_3 moiety.

Such knowledge of the reagents and starting materials and the definition of the synthetic procedure are valuable tools in the fight against synthetic illicit drugs. All the reagents are traceable, and the probable suppliers of specific compounds can be detected. The deuterium pattern of the methamphetamine molecule can evidently provide a useful "fingerprint" to be considered in criminal investigations.

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