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Establishing the synthetic origin of amphetamines by ^{2}H NMR spectroscopy

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Nine samples of *N*-acetyl-3,4-methylenedioxyamphetamine (*N*-acetyl-MDA), prepared according to the most common synthetic procedures, are submitted to ^{2}H NMR spectroscopy. The relative deuterium content at the various sites of the molecule is shown to depend on its synthetic history. The technique provides a chemical fingerprint of *N*-acetyl-MDAs and it can be used to trace back the precursor materials and the synthetic pathways employed in the preparation of the samples.

Introduction

Each seizure of illicit drugs generally undergoes a thorough physical and chemical examination to obtain evidence of its probable origin for use in the criminal investigation. All the information is combined to identify the source of the product, in order to establish whether or not products from different seizures are from a common origin. Isotopic analysis has been recently introduced as an additional step in the systematic methodology of this origin assignment.

Stable isotope ratios, such as ^{2}H/^{1}H, ^{13}C/^{12}C, ^{18}O/^{16}O *etc*., are influenced by environmental factors and synthetic routes and they can be employed to trace back the origin of a certain compound. For example, isotopic methods have been widely exploited in the field of food analysis as tracers of the origin of food.[1,2] Two different techniques can be used to measure isotopic ratios: isotope ratio mass spectrometry (IRMS) and the SNIF-NMR® method (site-specific isotope natural fractionation measured by nuclear magnetic resonance). After several years of research and validation studies, a certain number of isotopic methods have been officially recognised by international institutions. In 1990, for example, the SNIF-NMR method applied to the detection of chaptalisation of wine with beet sugar was adopted by the EC as an official method of wine analysis (EC 2676/90).[3]

The simultaneous determination of the D/H ratios at the various sites of a molecule by SNIF-NMR allows multisite isotopic analysis to be performed, and greatly enhances the discriminating power of isotopic ratios. This approach has been useful, for example, in the determination of the origin (natural and synthetic) of amino acids,[4] of flavours, such as vanillin,[5] and in the recognition of geographical origin of natural drugs, such as heroin and cocain.[6]

3,4-Methylenedioxyamphetamine (MDA) and many *N*-substituted derivatives have become drugs of abuse, sold in tablets as "ecstasy".

δ^{13}C and δ^{15}N stable isotope analyses by GC-IRMS have been used in preliminary studies to link seizures made at different times or locations to a possible common clandestine origin.[7,8]

An examination of the δ^2H, δ^{13}C and δ^{15}N characteristics of fifty ecstasy tablets from police seizures in the Avon and Somerset area of the United Kingdom was published in 2002 in the *Analyst*.[9] The active ingredient (3,4-methylenedioxy-*N*-methyl-amphetamine—MDMA) extracted from each batch of ecstasy tablets was submitted to ^{2}H NMR analysis, to ascertain the position of deuterium substitution within the molecule. The signals corresponding to CH_2 and CH–N were integrated as a single entity, due to low resolution. The relative abundance of deuterium at CH_2 and CH sites was found to vary between 9–15%. This variation was tentatively attributed to the different synthetic origins of the ecstasy samples.[10]

We now report on a ^{2}H NMR study of nine samples of *N*-acetyl-3,4-methylenedioxyamphetamine (*N*-acetyl-MDA) prepared according to the main known synthetic routes, in order to show the possibility of establishing the synthetic history of a certain sample by the analysis of its deuterium pattern.

Experimental

We had in our hands nine samples of *N*-acetyl-MDA which had been prepared in the past in our laboratory according to the literature.[11] The origin of the chemicals was as follows: safrole **E** extractive, safrole **F** unknown, both available from the collection of chemicals of the department; heliotropin: commercial from Carlo Erba (**A**) and Fluka (**B**); 3,4-dihydroxybenzaldehyde, precursor of heliotropin **C**, from Fluka; diiodomethane from Carlo Erba; $LiAlH_4$ and $NaBH_4$ from Aldrich.

The ^{2}H NMR experiments were performed on a Bruker Avance 500 spectrometer equipped with a 10 mm probe head and a ^{19}F lock (C_6F_6) channel, under CPD (Waltz 16 sequence) proton decoupling conditions. The spectra were recorded at 298K. The spectra were recorded dissolving 0.4–1 g of material in 2.5–3.0 ml of solvent, adding 70 μl C_6F_6 for the lock. The solvents used were CH_2Cl_2 and acetone. At least four spectra were run for each sample collecting 7000–12000 scans to reach S/N > 100 (methyl signals) and using the following parameters: 5.9 s acquisition time, 1400 Hz spectral width and 16 K memory size. Each FID was Fourier transformed with a line broadening of 1.5–2 Hz, manually phased and integrated after an accurate correction of the spectrum base line. For partially overlapped signals the peak areas were determined through the deconvolution routine of the Bruker NMR software using a Lorentzian line shape.

Results and discussion

Two starting materials had been employed to obtain the *N*-acetyl-MDA samples in our hands: safrole and heliotropin. Two different commercial batches of heliotropin (heliotropin **A**, heliotropin **B**), and two synthetic batches, prepared from 3,4-dihydroxybenzaldehyde[12] (heliotropin **C**) and from safrole[12] (heliotropin **D**) according to Scheme 1, had been used. Two different commercial batches of safrole (safrole **E**, safrole **F**) had been employed.

The four samples of heliotropin had been converted into nitrostyrene derivatives **G** by condensation with nitroethane. Nitrostyrenes from heliotropin **A** and **C** had been submitted to

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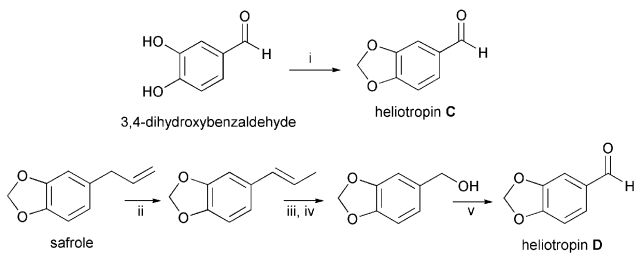
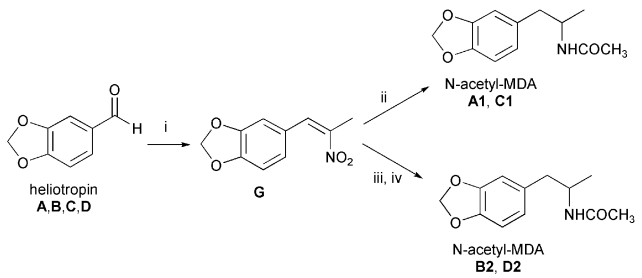
catalytic reduction with hydrogen, in the presence of Pd/C and acetic anhydride, to afford *N*-acetyl-MDAs (samples **A1** and **C1**, respectively). Nitrostyrenes from heliotropin **B** and **D** had been reduced with aluminium hydride and acetylated to give *N*-acetyl-MDAs (samples **B2** and **D2**, respectively) (Scheme 2).

The two samples of safrole had been treated with 3-chloroperbenzoic acid, and submitted to $LiAlH_4$ reduction. The alcoholic function of derivative **H** had been converted into the $NHCOCH_3$ moiety through a conventional route *via* azide intermediate. The *N*-acetyl-MDA samples **E3** and **F3** had been thus prepared (Scheme 3). Safrole **F** had also been submitted to oxymercuration and $NaBH_4$ reduction, to afford alcohol **H**. This sample of **H** had been converted into *N*-acetyl-MDA **F4**, *via* azide intermediate, and into *N*-acetyl-MDA **F5** *via* ketone **I**. Addition of HBr to safrole **F**, followed by NH_3 substitution, gave *N*-acetyl-MDA **F6**.

Fig. 1 shows the relative percent distribution of deuterium within the nine samples, evaluated on ^2H NMR integrals. The variations of the isotopic content at the various sites of the *N*-acetyl-MDA samples can be attributed to the employment of different precursors, and hence of different synthetic paths, and to kinetic isotopic effects involved in the corresponding synthetic steps.



Scheme 1 i. Diiodomethane, K_2CO_3; ii. NaOH; iii. O_3; CH_2Cl_2–MeOH; iv. $NaBH_4$; v. MnO_2.



Scheme 2 i. Nitroethane; ii. H_2; Pd/C, Ac_2O; iii. AlH_3, THF; iv Ac_2O, pyridine.

The deuterium content of the CH positions clearly allows the discrimination of *N*-acetyl-MDAs prepared from heliotropin *via* nitrostyrene from those prepared from safrole. This hydrogen is inserted by reduction of the double bond in samples **A1**, **C1**, **B2** and **D2**, while it belongs to the parent safrole skeleton in samples **E3**, **F3**, **F4** and **F6**. The relative abundance of deuterium at CH position is 2–3% in *N*-acetyl-MDAs from heliotropin, while it reaches 7–8% in those obtained from safrole. The difference between these two groups is found to be highly significant by two tails t test (p = 1.05 10^{-5}).

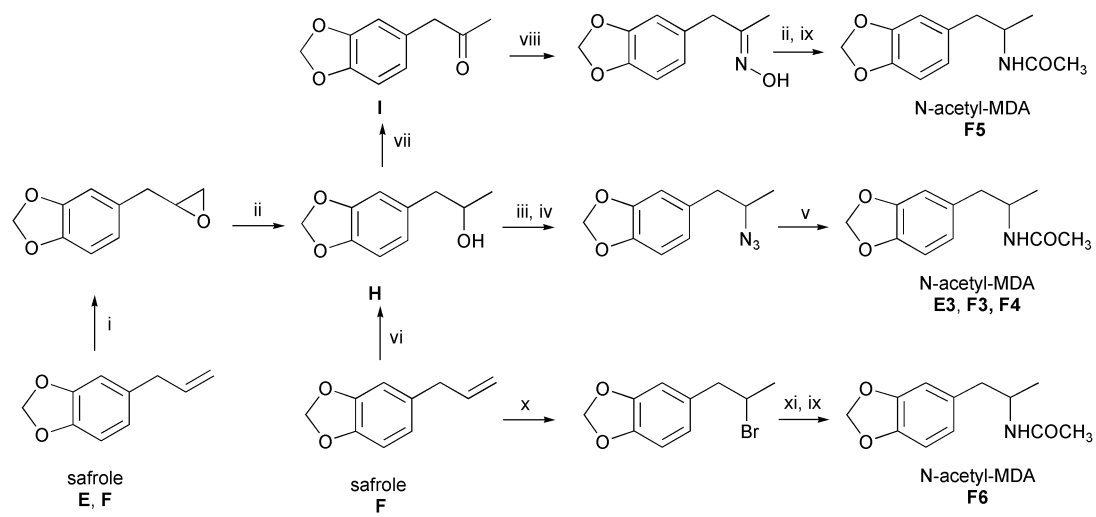
An exception is sample **F5** prepared from safrole *via* ketone **I**. No deuterium is detected at the CH position: this hydrogen comes from $LiAlH_4$ reduction of the oxime intermediate.

Within the samples obtained from nitrostyrene, discrimination of the double bond reduction methodology is possible. As a matter of fact, through a mathematical deconvolution of the spectra of *N*-acetyl-MDAs the peak areas of the two hydrogen atoms of the methylenic group could be determined separately. Fig. 2 shows the relative deuterium content of these two diastereotopic hydrogens. The catalytic reduction of intermediates **G** with H_2 gives rise to a lower deuterium content for the low-field resonating hydrogen in samples **A1** and **C1**. The opposite situation is observed in *N*-acetyl-MDAs **B2** and **D2**, and in most of the samples prepared from safrole. Addition of H_2 to double bonds is stereospecific, and it occurs with a *syn* mechanism: the stereochemical control on the hydrogen insertion at the benzylic position can influence the isotopic content of that specific hydrogen atom. The same situation is found in sample **F5**: the change of the deuterium distribution of the diastereotopic hydrogens with respect to the other samples prepared from safrole can be tentatively attributed to the keto-enolic tautomerisation of ketone **I**.

The deuterium fraction of CH_3 can be rationalised at the light of the employed synthetic procedures. The deuterium content at the CH_3 position is similar in the four samples prepared from heliotropin, being the methyl group, the one belonging to nitroethane, while it bears information on the synthetic pathway for those prepared from safrole.

Low values of deuterium relative percentage (less than 20%) are found when the methyl group is created by $LiAlH_4$ opening of the epoxide ring (**E3**, **F3**) or by addition of HBr to the double bond of safrole (**F6**). Higher values (nearly 25%) are observed in both *N*-acetyl-MDAs prepared *via* oxymercuration followed by *in situ* $NaBH_4$ reduction.

No relevant variations can be detected in the deuterium content of the aromatic hydrogens, and of the methylenedioxy moiety.



Scheme 3 i. 3-Chloroperbenzoic acid; ii. $LiAlH_4$; iii. *p*-toluenesulphonyl chloride, pyridine; iv. NaN_3; v. H_2, Pd/C, Ac_2O; vi. $Hg(OCOCH_3)_2$, $NaBH_4$; vii. CrO_3; viii. NH_2OH; ix. Ac_2O; x. HBr, AcOH; xi. NH_3.

Indeed, most of commercial heliotropin is prepared from safrole *via* isomerisation to isosafrole in alkaline medium, followed by ozonolysis.

Figs. 3 and 4 show at a glance the difference between *N*-acetyl-MDAs synthesised from safrole (**E3**) and from heliotropin (**C1**). The ^{2}H NMR spectrum of **E3** is characterised by an intense peak corresponding to the CH position at δ = 4.19 ppm and by the fact that the two methylenic hydrogens *a* and *b* show the same intensity. In the ^{2}H NMR spectrum of **C1** the signal of the methyne group is scarcely visible at 4.19 ppm, and the different intensity of protons *a* and *b* can be clearly appreciated.

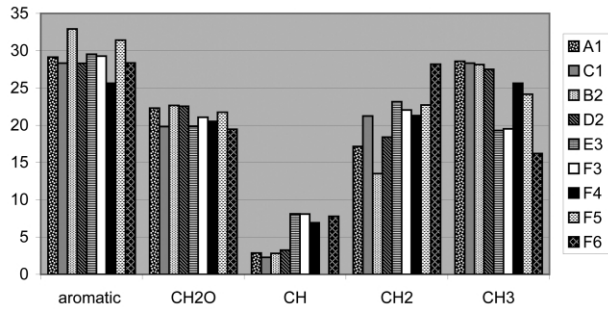


Fig. 1 Relative distribution of deuterium by SNIF-NMR.

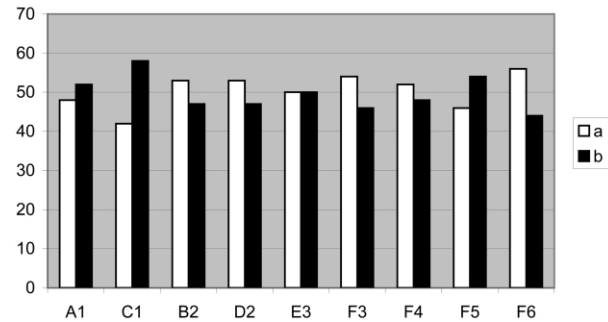


Fig. 2 Relative deuterium content of the diastereotopic hydrogens *a* and *b* of CH_2.

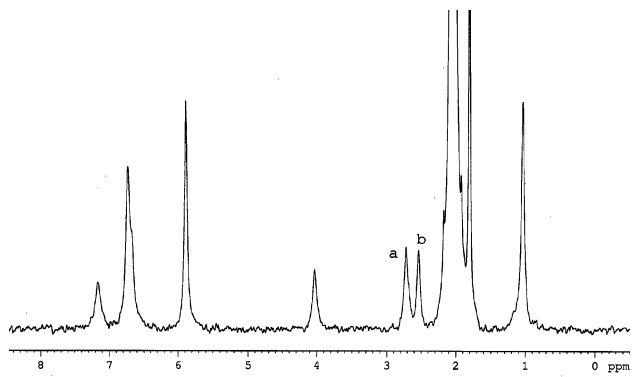


Fig. 3 Natural abundance deuterium NMR spectrum in acetone solvent of sample **E3** prepared from safrole according to Scheme 3.

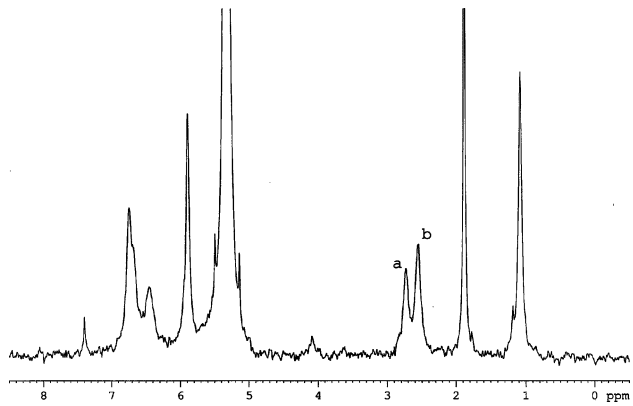


Fig. 4 Natural abundance deuterium NMR spectrum in CH_2Cl_2 solvent of sample **C1** prepared from heliotropin according to Scheme 2.

Our results, if compared with those obtained by Carter *et al.* for five batches of seized ecstasy tablets, could help to recognize the synthetic route of production of MDMA in clandestine laboratories. We have found that some of our deuterium fractions were similar to those found by Carter (we have not considered the deuterium content of N–CH_3, being almost constant for all the seized samples). In particular, the samples prepared from safrole seem to be more similar than those from heliotropin. For the comparison, we have considered principally the relative difference between the four groups ("aromatic", "OCH_2", "CH–CH_2", "CH_3") rather than the absolute value of deuterium fraction in each one. Fig. 5 shows the analogies we have checked.

The correspondence of the deuterium fractions between RN/2902/00 and **F4** is very sharp and intriguing as the corresponding columns of the histogram are superimposable.

The analogies between our analysed samples and those seized infer us to believe that the preferable raw material to prepare ecstasy in clandestine laboratories is safrole. This statement is also supported by the fact that safrole is a starting material that requires neither a large number of steps to produce ecstasy nor any special equipment.

Conclusions

The SNIF-NMR method is a powerful tool for the investigation of the synthetic history of a certain compound. The distribution of deuterium in a molecule can give valuable information on the chemical reactions employed to build up its structure. This work shows that the deuterium content at the various sites of *N*-acetyl-MDA bears memory of the corresponding synthetic procedure. The deuterium fractions at CH, CH_2 and CH_3 sites can provide a chemical fingerprint of the amphetamines, and help in tracing back the synthetic path. A first distinction between *N*-acetyl-MDAs prepared from heliotropin and from safrole can be obtained by the analysis of the deuterium content at the CH position. A further discrimination between catalytic and chemical reduction of nitrostyrene intermediates is possible by determining the relative deuterium content of the diastereotopic hydrogens of CH_2 moiety. The conversion of safrole into alcohol **H** *via* epoxydation or oxymercuration is written in the deuterium fraction of CH_3. The use of ketone **I** as an intermediate is revealed by the total lack of deuterium at the CH position.

The comparison of Figs. 3 and 4 highlights one of the advantages of the method: a simple visual inspection of the ^{2}H NMR spectrum can give suggestions on the origin of the sample before any calculation of relative deuterium content is done.

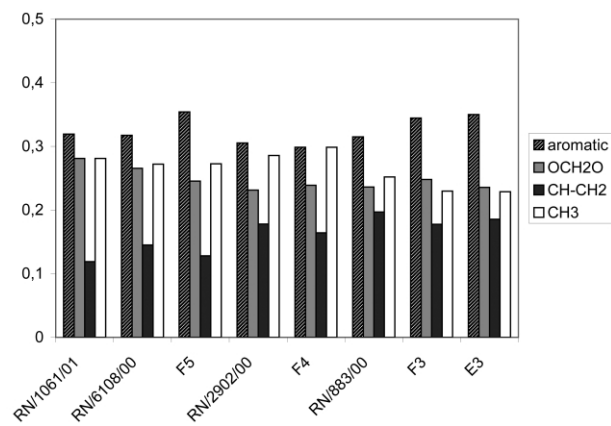


Fig. 5 Comparison between our data and those of Carter *et al.*(ref. 9).

The analysis of the deuterium pattern obtained by Carter *et al*. on seized ecstasy tablets allows to draw preliminary considerations on the most usual procedures employed by clandestine laboratories. Correlation of seized ecstasy tablets to a common origin becomes thus possible.

Acknowledgement

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