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Isotopic characterisation of 3,4-methylenedioxyamphetamine and 3,4-methylenedioxymethylamphetamine (ecstasy)

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Combined δ^2H, δ^{13}C and δ^{15}N isotopic analysis of MDA and MDMA extracted from seized "ecstasy" tablets provides an isotopic "fingerprint" of the active ingredient allowing individual tablets to be linked to a common batch. Correlating these data with ^{2}H NMR analysis of the extracts has the potential to study both the natural precursor materials and synthetic pathways used in the preparation of MDA and *N*-substituted homologues.

Introduction

N-methyl 1-1-(3,4-methylenedioxyphenyl)-2-propanamine, also known as 3,4-methylenedioxy *N*-methylamphetamine (MDMA) or "ecstasy" was first synthesised by Merck Pharmaceuticals in 1912 and investigated as an appetite suppressant. This drug induces a state of tranquil euphoria and, since the late 1970s, has found wide recreational use. 3,4-methylenedioxyamphetamine (MDA) and many *N*-substituted derivatives have also become drugs of abuse, sold in tablet form as "ecstasy". At present, in the UK, any compound structurally derived from MDA is controlled under Schedule 1 of the Misuse of Drugs Regulations 1985; Misuse of Drugs Act 1971.

Techniques such as; IR spectroscopy,[1] surface Raman spectroscopy,[2] high performance liquid chromatography (HPLC),[3] gas chromatography-mass spectrometry (GC-MS),[4] HPLC-MS[5] and solid state NMR[6] have been used to provide evidence for the presence of MDMA (or related compounds) within a tablet formulation and as a means to characterise either bulk excipients or manufacturing impurities. Forensic science is frequently applied to infer a link between materials found at different times and locations. Whilst current techniques can provide some evidence to link batches of tablets, stable isotope analysis of the active ingredient has the potential to "fingerprint" the molecules of the active ingredient. In addition, it is often necessary to establish a connection between samples of seized drugs and trace residues. Trace materials are transferred to a substrate and retained by a variety of mechanisms[7] and due to preferential absorption and degradation the trace material is unlikely to be representative of the bulk material. Stable isotope ″fingerprinting″ of the active ingredient also has the potential to support this association.

Previous studies have applied δ^{13}C stable isotope analysis to establish geographical origins of natural and derivatised natural substances, *e.g.*, heroin,[8–12] cocaine[13] and caffeine.[14] Other studies have demonstrated that multielement isotope analysis (δ^2H, δ^{13}C and δ^{15}N) is needed to provide sufficient data for the discrimination of cocaine and heroin.[15] A limited study of both δ^{13}C and δ^{15}N of six ecstasy tablets[16] has already shown the potential of this technique to discriminate batches of tablets. In this study we present a comprehensive 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 UK.

Experimental

Five batches of seized ecstasy tablets, comprising ten visually similar tablets from each batch, were provided by Avon and Somerset Constabulary prior to official destruction. Each of the fifty tablets was extracted and analysed individually by GC-MS, gas chromatography-isotope ratio mass spectrometry (GC-irmMS) and elemental analysis-isotope ratio mass spectrometry (EA-irmMS) using a modification of the technique of Mas *et al.*[16] The active ingredient extracted from each batch of MDMA tablets was finally combined for ^{2}H NMR analysis to ascertain the position of deuterium substitution within the molecule.

All solvents used were supplied by Rathburn, Walkerburn, UK (HPLC grade), except $CDCl_3$ (Apollo Scientific Limited, Derbyshire, UK). Other reagents were supplied by Sigma-Aldrich, Poole, UK.

Individual tablets were crushed and dissolved in 2 mL of 2 M aqueous sodium hydroxide with vortex mixing. This amount of base was calculated to be sufficient to liberate the active ingredient present in a tablet as the free base (typically 50–150 mg of MDMA, present as the hydrochloride salt). The mixture was then centrifuged at 3000 rpm for 20 min and the supernatant transferred to a clean tube. This process was repeated twice and the supernatants combined. 2 mL of chloroform were added to the combined extract and following vortex mixing the organic phase was transferred to a clean tube. This process was also repeated twice. The combined extracts were evaporated to *ca.* 2 mL under a stream of dry nitrogen and the purity of the extract established by GC-MS and ^{1}H NMR.

GC-MS analyses were performed using a FinniganMAT TSQ700 mass spectrometer coupled to a Varian 3400 gas chromatograph equipped with a septum-equipped temperature programmable injector (SPI). The gas chromatography column employed was a 30 m DB5-MS, 0.32 mm id 0.25 μm phase (J&W, supplied by Jones Chromatography, Hengoed, UK) with helium carrier gas at a constant head pressure of 10 psi. 1 μL aliquots of sample diluted in chloroform were injected in splitless mode using a CTC A200S autosampler. The injector was programmed from 35 °C–300 °C at a rate of 200 °C min^{-1} and maintained at this temperature for 25 min. The chromatographic column was programmed from 40 °C for 2 min to 300 °C at a rate of 10 °C min^{-1} and maintained at this temperature

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for 1 min. Spectra were recorded from *m*/*z* 50–500 at a rate of 1 scan s^{-1}.

^{1}H NMR spectra were obtained from one tablet extract randomly selected from each batch diluted in $CDCl_3$. Spectra were acquired using a JEOL GX/Delta 270 MHz instrument.

All isotopic analyses were performed using a ThermoFinnigan Delta^{Plus} XL stable isotope mass spectrometer with an ISODAT data system. δ^{13}C analyses were performed using an Agilent 6890A gas chromatograph coupled to the mass spectrometer *via* a ThermoFinnigan GCC III interface. The gas chromatography conditions were the same as used for GC-MS with the exception of a split/splitless injector, operated in splitless mode at a constant temperature of 300 °C and a constant carrier gas flow of 2 mL min^{-1}. The oxidation reactor comprised 25 cm lengths each of 0.1 mm copper wire, 0.15 mm nickel wire and 0.1 mm platinum wire operated at a temperature of 940 °C. A reduction reactor, comprising 3 × 0.1 mm copper wires, operated at a temperature of 600 °C was employed to ensure reduction of nitrogen oxides. Isotope ratios were referenced to gaseous carbon dioxide which was calibrated against ANU-sucrose (National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 8542).

δ^{15}N analyses were performed using a CarloErba NC2500 elemental analyser coupled to the mass spectrometer *via* a ThermoFinnigan ConFlo II interface. This technique was used in preference to GC-irmMS because of problems associated with δ^{15}N determination *via* a GC interface.[17] The tablet extracts were considered sufficiently pure that δ^{15}N contributions from other components would be minimal. The oxidation reactor comprised silver/silver–cobaltous oxide operated at 1150 °C. A subsequent copper reduction reactor was operated at 640 °C. Approximately 40 μL of tablet extract were placed in a tin capsule and allowed to dry at ambient temperature for approximately 5 min. Isotope ratios were referenced to gaseous nitrogen which had been calibrated against IAEA-N1-ammonium sulfate (NIST SRM 8547) and IAEA-N2-ammonium sulfate (NIST SRM 8548).

δ^2H analyses were performed using a ThermoFinnigan thermal conversion (GCTC) interface operated at a temperature of 1450 °C.[18] All other conditions were the same as for δ^{13}C analyses. Isotope ratios were referenced to gaseous hydrogen which had been calibrated against a suite of *n*-alkanes, independently calibrated against VSMOW-water (NIST SRM 8535).[19] The tablet extract already tested for purity by ^{1}H NMR was not analysed for δ^2H due to the probability of proton exchange between the solvent and the amine group.

Following mass spectral analyses, the ten extracts from each batch of tablets were combined in order to obtain sufficient material for analysis by ^{2}H NMR. The combined samples were evaporated to near dryness under a stream of dry nitrogen and dissolved in chloroform. 30,000 transients were acquired using a JEOL Alpha 500 MHz instrument at a frequency of 76.65 MHz.

Results and discussion

Morphology

Tablets from each of five batches supplied by Avon and Somerset Constabulary were described as 'visually similar'. Tablets are identified using the Avon and Somerset Constabulary reference numbers and description as detailed in Table 1.

GC-MS and ^{1}H NMR analysis

GC-MS and ^{1}H NMR analyses showed that the tablet extracts contained the free base active ingredient (MDA or MDMA) at >99% purity. GC-MS analyses identified a number of minor impurities (≪1%) in a number of the extracts, *e.g.* benzaldehyde, 3,4-methylenedioxyphenylacetone and caffeine.

GC-irmMS

Table 2 shows the upper and lower range for δ^{13}C, δ^{15}N and δ^2H for the five batches of tablets at 95% confidence. These values are calculated from duplicate analyses of the extracts of each of the tablets and, therefore, reflect the overall reproducibility of the technique. None of the individual tablets was isotopically distinct from other members of its batch in δ^{13}C, δ^{15}N and δ^2H.

MDMA may be synthesised from a number of naturally occurring materials, *e.g.* safrole, *iso*-safrole or piperonal,[20] ultimately *via* reductive amination of 3,4-methylenedioxyphenylacetone with methylamine.[21] Potential synthetic routes are summarised schematically in Fig.1. Since the δ^{13}C content of natural products is determined, primarily, by the carbon fixation mechanism of the parent organism[22] little variation has been observed in compounds with identical biosynthetic pathways. Isotopic variation between MDMA samples is, therefore, expected to reflect the synthetic reactions undergone

Table 1 Reference number and description of tablets

RN/1491/00	10 tablets taken from a seizure of 25 tablets bearing a 'Mitsubishi' logo, off-white in colour	Known to contain MDA
RN/6108/00	10 tablets taken from a seizure of 33 tablets bearing a tulip logo, white in colour	Smaller than other tablets, known to contain MDMA hydrochloride
RN/2932/99	10 tablets taken from a seizure of 248 tablets bearing a 'Mitsubishi' logo, beige in colour	Known to contain MDMA
RN/883/00	10 tablets taken from a seizure of 29 tablets bearing a 'Mitsubishi' logo, white in colour	Known to contain MDMA
RN/1061/01	10 tablets bearing a 'TT' logo, beige in colour	Positive response to Marquis reagent confirmed by GC-MS

Table 2 95% confidence limits for δ^{13}C, δ^{15}N and δ^2H based on duplicate analyses of the extract of each tablet

	δ^{13}C (‰) *vs.* VPDB[a] (*n* = 10)	δ^{15}N (‰) *vs.* air (*n* = 10)	δ^2H (‰) *vs.* VSMOW[b] (*n* = 9)
RN/1491/00	−24.04 to −20.98	−4.02 to −2.20	−73.00 to −63.00
RN/6108/00	−28.59 to −26.64	1.80 to 3.50	−117.00 to −106.00
RN/2932/00	−28.90 to −27.43	9.11 to 11.17	−68.00 to −65.00
RN/883/00	−30.02 to −29.78	18.31 to 20.85	−78.00 to −61.00
RN/1061/01	−30.65 to −29.96	0.24 to 1.27	−84.00 to −73.00

[a] VPDB, Vienna PeeDee Belemnite. [b] VSMOW, Vienna Standard Mean Ocean Water.

by the precursor materials. Isotopic differences may, therefore, result either from variations in the isotopic composition of the synthetic reagents employed or from kinetic isotope effects (KIEs) during synthesis.[23]

Batches of tablets form three isotopically distinct $\delta^{13}C$ groupings at 95% confidence. Two groupings (RN/6108/00–RN/2932/00 and RN/883/00–RN/1061/01) differ by $<1‰$ and require analyses with high precision to identify differences. In contrast, tablets from batch RN/1491/11 were isotopically heavier in $\delta^{13}C$ than all other tablets by $>2.5‰$. Significantly, GC/MS analysis showed this batch of tablets to contain MDA rather than MDMA and it is, therefore, possible that the variation in $\delta^{13}C$ results from the contribution of the *N*-methyl group to the overall isotopic value. For the single carbon atom of the *N*-methyl group to cause this effect would require it to have an isotopic value of *ca*. 80‰. Further studies are in progress to elucidate this anomaly.

$\delta^{15}N$ analysis revealed a wide overall range of isotopic values, with each batch of tablets forming a distinct group, at 95% confidence. This variability can be attributed to the reductive amination process.

$\delta^{2}H$ analysis revealed four of the five tablet batches to be isotopically indistinct at 95% confidence. Batch RN/6108/00 was, however, approximately 22‰ lighter in ^{2}H. $\delta^{2}H$ is known to reflect moisture availability during biosynthesis and exhibits greater variations than other stable isotopes in natural products.[24] This batch of tablets was not distinct from other batches in $\delta^{13}C$, nor particularly distinct in $\delta^{15}N$, so it appears unlikely that this difference results from reductive amination. $\delta^{2}H$ data may, therefore imply that batch RN/6108/00 was synthesised from a different starting material from that used in the manufacture of the other batches of tablets.

Fig. 2 shows plots of $\delta^{13}C/\delta^{15}N$, $\delta^{13}C/\delta^{2}H$ and $\delta^{15}N/\delta^{2}H$. The significant variations observed in $\delta^{15}N$ of MDMA and MDA provides a means of linking ecstasy tablets to a common batch, and discriminating between batches of tablets. Combining these data with the smaller variations in $\delta^{2}H$ or $\delta^{13}C$ provides a further means to characterise and discriminate batches. Individual isotopic groupings are observed for a batch of ecstasy tablets, at 95% confidence. Combining all these data in a triangular plot (Fig. 3) reveals distinct isotopic groupings for each batch of tablets providing an isopotic "fingerprint" of the active ingredient. The isotopic signature of MDMA will be affected by: the natural starting material, the reagents used for synthesis and KIEs during synthesis. The combined technique could be applied to samples as small as 200 ng and, therefore, has the potential to link trace residues to whole tablets.

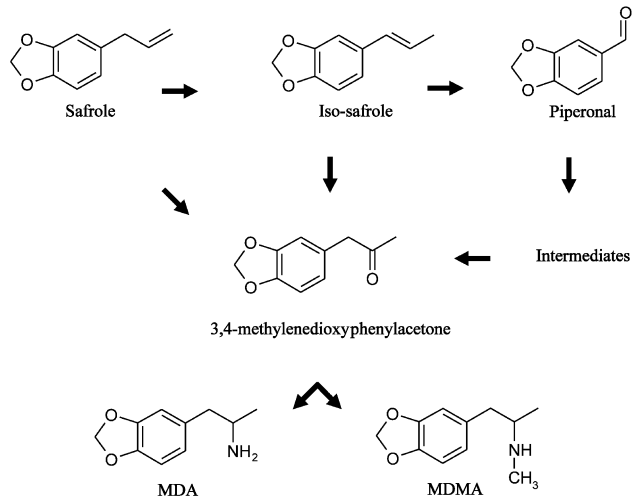

Fig. 1 Potential synthetic pathways of MDA and MDMA.

SNIF-NMR

Site-specific natural isotopic fractionation NMR (SNIF-NMR)[25] has been applied to study the origins of simple natural

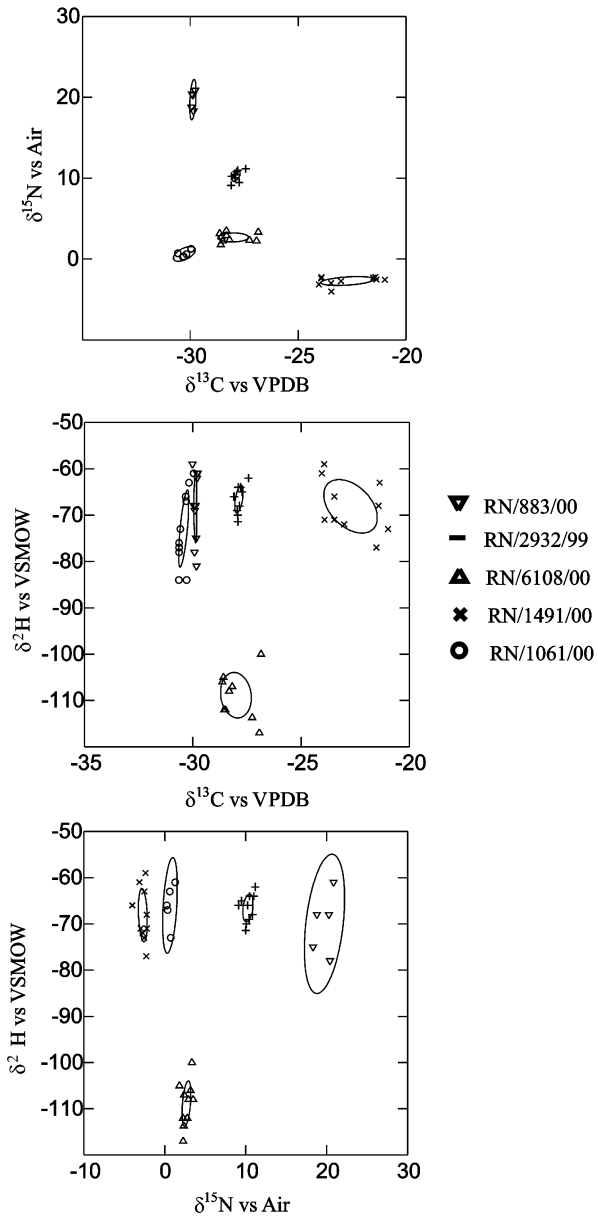

Fig. 2 Combined isotopic data for five tablet batches with 95% confidence ellipses.

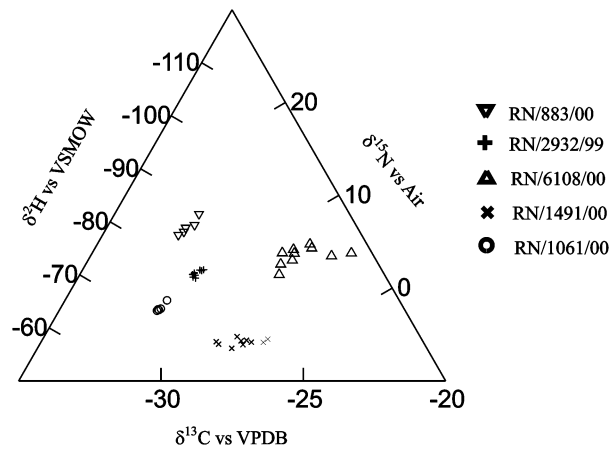

Fig. 3 Combined isotopic data for five tablet batches.

products such as ethanol[26] and glucose.[27] Fig. 4 shows a typical ^2H NMR spectrum of MDMA isolated from a batch of tablets. Despite band broadening, caused by the quadrupole of the ^2H nucleus, the signals due to the main proton environments are clearly distinguished. Due to insufficient resolution the signals corresponding to positions 4 and 5 were integrated as a single entity. Data from tablets RN/1491/11 are not included as this batch contained MDA rather than MDMA. Fig. 5 shows the relative distribution of ^2H within the four batches of MDMA tablets. Date from the N–D signal are not included since these deuterons are in chemical exchange with traces of H_2O and HOD in the sample.

^2H substitution in the majority of unique environments varies by 1–2% reflecting the experimental precision of this technique.[28] In contrast, the relative abundance of ^2H at positions 4 and 5 is significantly different for all batches and varies between 9 and 15%. These differences in ^2H distribution are clearly not reflected in the overall δ^2H value determined by irmMS.There are many possible explanations for the observed variation. ^2H substitution at position 5 will reflect hydrogen addition during reductive amination. ^2H substitution at positions 4 and 5 may also reflect exchange during keto–enol tautomorism. This variation may also reflect the use of different starting materials employed in the synthesis of MDMA. 3,4-Methylenedioxyphenylacetone may be synthesised directly from safrole, *iso*-safrole or piperonal. Safrole may be isomerised to *iso*-safrole which may in turn be converted to piperonal prior to formation of 3,4-methylenedioxyphenylacetone *via* a number of synthetic intermediates. These reactions may involve additions of hydrogen at positions 4, 5 and 8 and would be expected to have a significant effect on the ^2H distribution. The limited variation in δ^2H observed by irmMS may simply reflect an overall averaging of natural and synthetic isotope effects.

Study of ^2H substitution within MDMA molecules may, therefore, provide a means to characterise the early synthetic origins. Further studies are also in progress to examine this possibility.

In contrast to the limited variation in δ^2H determined by irmMS, SNIF-NMR reveals significant relative differences in ^2H substitution at certain positions involved in the synthetic route. These variations are likely to reveal more information about the synthetic preparation of MDMA than δ^2H analysis of the whole molecule. Whilst applicable to batches of tablets, this technique requires relatively large amounts of material (> 500 mg) and will not be applicable to trace analysis.

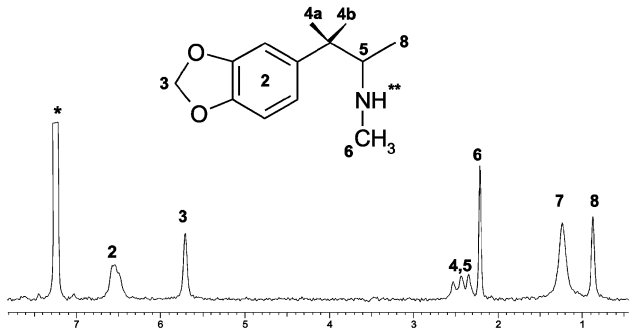

Fig. 4 Typical ^2H NMR spectrum of MDMA from combined tablet extracts indicating the position of ^2H environment (* indicates solvent/reference shift $CDCL_3$; **ND signal not seen due to exchange with HOD; 7 HOD).

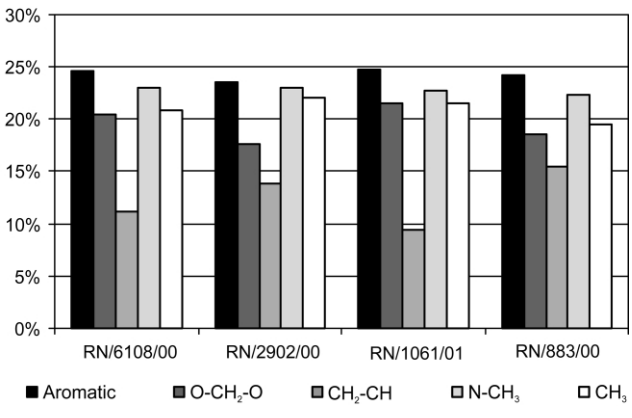

Fig. 5 Relative distribution of ^2H substitution by SNIF-NMR.

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