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Electrochemical and spectroscopic characterisation of amphetamine-like drugs: Application to the screening of 3,4-methylenedioxymethamphetamine (MDMA) and its synthetic precursors

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Abstract

A complete physicochemical characterisation of MDMA and its synthetic precursors MDA, 3,4-methylenedioxybenzaldehyde (piperonal) and 3,4-methylenedioxy-β-methyl-β-nitrostyrene was carried out through voltammetric assays and Raman spectroscopy combined with theoretical (DFT) calculations. The former provided important analytical redox data, concluding that the oxidative mechanism of the *N*-demethylation of MDMA involves the removal of an electron from the amino-nitrogen atom, leading to the formation of a primary amine and an aldehyde. The vibrational spectroscopic experiments enable to afford a rapid and reliable detection of this type of compounds, since they yield characteristic spectral patterns that lead to an unequivocal identification.

Moreover, the rational synthesis of the drug of abuse 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy") from one of its most relevant precursors 3,4-methylene-dioxyamphetamine (MDA), is reported. In addition, several approaches for the *N*-methylation of MDA, a limiting synthetic step, were attempted and the overall yields compared.

Keywords: 3,4-Methylenedioxymethamphetamine; Synthesis; Raman spectroscopy; Voltammetry; Density functional theory calculations

1. Introduction

The amphetamine analogues 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") and 3,4-methylenedioxyamphetamine (MDA) are popular recreational drugs mainly due to their stimulant effects on the central nervous system and increased sense of well being [1–4]. These compounds are ring-substituted phenethylamines, structurally related to both psychomotor stimulant amphetamines and the hallucinogen mescaline. MDMA is nowadays a commonly misused drug, thus giving rise to a serious public health problem repeatedly associated with serotoninergic neurotoxicity [4–6].

All methylenedioxy-containing amphetamines became very popular after modification of the MDA structure in clandestine laboratories, by replacement of one or two hydrogens in the nitrogen atom by alkyl groups, yielding several distinct compounds with high potential for abuse. In fact, the simple addition of a *N*-methyl group is responsible for a decrease in the temporal course of action of the drug to less than half, as well as for a reduction (or even a disappearance) of the hallucinogenic effects associated to MDA [1].

MDA, besides other compounds, is frequently described as an impurity in the MDMA synthetic process. It is also produced

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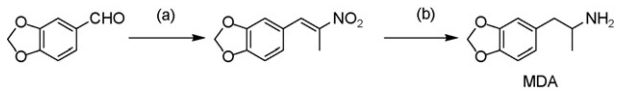

Fig. 1. General procedure for the synthesis of MDA. Chemical conditions: (a) $EtNO_2$, NH_4CH_3COO, reflux; (b) $LiAlH_4$, THF, reflux.

metabolically by *N*-demethylation of MDMA, hence becoming its major metabolite in a short time after ingestion. This biotransformation step is thought to proceed through a carbinolamine intermediate, formed by cytochrome P-450 mediated oxidation of the α-carbon to the *N*-methyl group, or through *N*-oxidation followed by rearrangement to a primary amine [7,8]. Accordingly, the knowledge of conceptual biotransformation, in which oxidation reactions play a major role [9], is absolutely critical to understand the toxicity of this kind of compounds.

The present work reports the total synthesis of MDA and MDMA. Firstly, the preparation of MDA was based on a well-known synthetic procedure, often described in the literature as one of the main routes for the manufacture of amphetamines [10–12], that relies upon the synthesis of the appropriate β-methyl-β-nitrostyrene precursor (Fig. 1). Secondly, several different pathways for *N*-methylation of MDA were investigated (Fig. 2), since this step is known to be limiting.

The oxidative electroactivity of MDMA and its synthetic precursors MDA, 3,4-methylenedioxybenzaldehyde (piperonal) and 3,4-methylenedioxy-β-methyl-β-nitrostyrene (Fig. 1) was studied using voltammetric techniques. Besides the putative analytical interest, the results thus gathered can hopefully help to clarify the hypothetical *in vivo N*-demethylation oxidation process. Actually, MDMA metabolism is known to comprise one or more oxidative pathways [13].

Furthermore, a conformational characterisation of MDMA and MDA was carried out through Raman spectroscopic experiments combined to quantum mechanical (DFT) calculations. Although there are some reported studies on the Raman analysis of amphetamine-like compounds [14–21], these experiments have only focused on particular regions of the vibrational spectra.

2. Experimental

2.1. Chemicals

Ethyl formate, lithium aluminium hydride (1 M solution in THF), pyridine, trifluoroacetic anhydride, formaldehyde (37% solution in water), sodium cyanoborohydride, sodium hydride (60% dispersion in mineral oil), methyl iodide, ethyl chloroformate, cesium carbonate and all the dry solvents were purchased from Sigma–Aldrich Química S.A. (Sintra, Portugal). All other reagents and solvents (*pro analysis* grade) were acquired from Merck (Lisbon, Portugal). All reagents were used without further purification. Deionised water (conductivity <0.1 μS cm^{-1}) was used in all experiments.

2.2. Synthesis

2.2.1. Structural elucidation

^1H and ^{13}C NMR data were acquired, at room temperature, on a Brüker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Dimethylsulfoxide-d_6 was used as a solvent; chemical shifts being expressed in δ (ppm) values relative to tetramethylsilane (TMS) (as internal reference); coupling constants (*J*) are in Hz. Spectral assignments were also made from DEPT (Distortionless Enhancement by Polarization Transfer) experiments (italicised values). Electron impact mass spectra (EI-MS) were obtained on a VG AutoSpec instrument; data are reported as *m/z* (% of relative intensity for the most important fragments). Melting points were measured on a Köfler microscope (Reichert Thermovar) and are uncorrected. All the intermediates of the following syntheses were identified by mass spectroscopy and were found to be the described compounds.

2.2.2. Chromatographic conditions

The analytical control was performed by thin-layer chromatography (TLC) on plates precoated with silica gel 60 F_{254} as stationary phase (0.2 mm as layer thickness) and the following mobile phases: petroleum ether/diethyl ether/formic

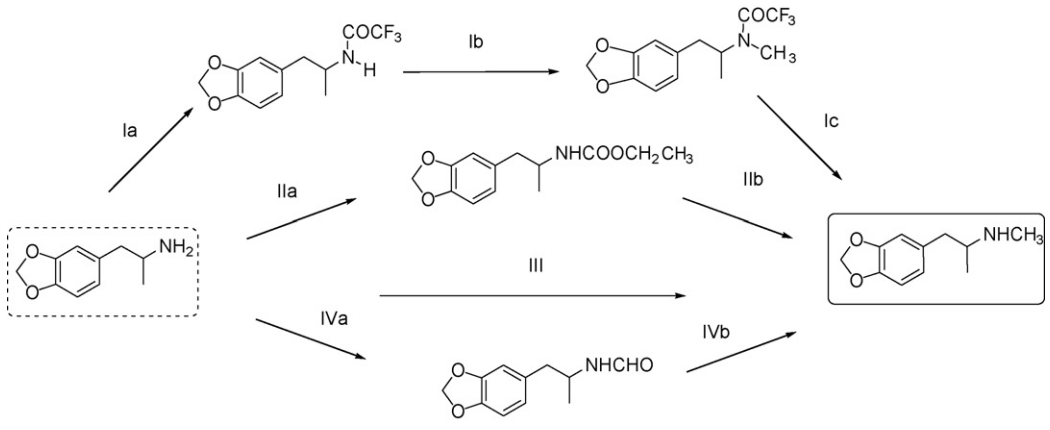

Fig. 2. Strategies used for the synthesis of MDMA using MDA as starting material. Method I—Ia: $O(COCF_3)_2$, Pyr, CH_2Cl_2; 0 °C, 2 h. Ib: NaH, CH_3I, DMF; 20 °C, 10 h. Ic: Cs_2CO_3, MeOH, H_2O; Δ, 2 h (overall yield: 41.6%). Method II—IIa: $ClCOOC_2H_5$, NaOH, H_2O; 0 °C, 1.5 h. IIb: $LiALH_4$, THF; Δ, 13 h (overall yield: 51.7%). Method III: HCHO, $NaBH_3CN$, MeOH, AcOH; rt, 3 h (overall yield: 33.3%). Method IV—IVa: $HCOOC_2H_5$; Δ, N_2, 6 h. IVb: $LiAlH_4$, THF; Δ, N_2, 4 h (overall yield: 37.1%).

acid (5:5:0.1); chloroform/acetone/formic acid (8:2:0.1); chloroform/methanol/formic acid (7:3:0.1). The spots were visualised under UV detection (254 and 366 nm) and iodine vapour.

The purity of the final products (>98% purity) was verified using a high-performance liquid chromatography (HPLC) system equipped with UV detector. HPLC-UV chromatograms were obtained in a Jasco instrument (pumps model 880-PU and solvent mixing model 880-30, Tokyo, Japan), equipped with a Nucleosil RP-18 column (250 mm × 4.6 mm, 5 μm, Macherey-Nagel, Düren, Germany), and UV detection at 280 nm (Jasco model 875-UV). pH 3 phosphate buffer/methanol (6:1) was used as the mobile phase, in isocratic flow rate of 1.2 mL min^{-1}. The chromatographic data was processed in a Compaq computer, fitted with CSW 1.7 software (DataApex, Czech Republic).

2.2.3. Synthesis of MDA (3,4-methylenedioxyamphetamine)

MDA was synthesised by an adaptation of a previous described method (Fig. 1) [11]. Briefly, a Henry reaction between nitroethane and piperonal (3,4-methylenedioxybenzaldehyde) was performed through basic catalysis of ammonium acetate followed by water elimination. The resulting β-methyl-β-nitrostyrene derivative was then reduced to the corresponding phenethylamine (MDA) by lithium aluminium hydride. The obtained free base oil was used straightforward into the next syntheses.

(*R,S*)-*3,4-Methylenedioxyamphetamine hydrochloride* (*MDA*).^{1}H NMR *δ*: 1.10 (3H, d, C*H*$_3$), 2.60 (1H, dd, *J* = 13.4; 8.5, C*H*$_2$), 2.89 (1H, dd, *J* = 13.4; 5.2, C*H*$_2$), 3.35 (1H, m, C*H*), 5.99 (2H, s, OC*H*$_2$O), 6.62 (1H, dd, *J* = 7.8; 1.2, H(6)), 6.74 (1H, d, *J* = 1.2, H(2)), 6.78 (1H, d, *J* = 7.9, H(5)), 8.08 (3H, bs, N*H*$_3^+$); ^{13}C NMR *δ*: *17.6* CH_3, *39.7* CH_2, *48.0* C*H*, *100.8* (OCH_2O), *108.3*, *109.5* C(2) and C(5), *122.4* C(6), 130.4 C(1), 146.0 C(3), 147.4 C(4); EI-MS *m/z* (%): 179 (*M*$^{\bullet+}$, 3), 162 (4), 136 (100), 105 (12), 82 (8), 77 (35), 65 (5), 58 (8); mp 186–188 °C.

2.2.4. Synthesis of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy")

2.2.4.1. Method I. (*Ia*) *N-Trifluoroacetyl-3,4-methylenedioxyamphetamine*: MDA as a free base (3.55 g, 19.8 mmol) was dissolved in dry dichloromethane (30 mL), under nitrogen, with the following addition of pyridine (2.3 mL, 28.5 mmol). The solution was ice-cooled and trifluoroacetic anhydride (3.8 mL, 27.3 mmol) was added dropwise to the reaction vessel. The mixture was allowed to reach room temperature and stirred for more 2 h. The solution was washed with water (30 mL) and the organic layer dried ($MgSO_4$) and evaporated under reduced pressure. The obtained residue (5.17 g, 18.8 mmol) was purified by column chromatography (silica gel; petroleum ether/diethyl ether (7:3)).

(*Ib*) *N-Trifluoroacetyl-3,4-methylenedioxymethamphetamine*: *N*-Trifluoroacetyl-3,4-methylenedioxyamphetamine (5.17 g, 18.8 mmol) was dissolved in DMF (25 mL) and a 60% dispersion in mineral oil of sodium hydride (0.80 g, 33.3 mmol) was added to the solution. The reaction was stirred during 2 h at room temperature. Then methyl iodide (1.3 mL, 20.9 mmol) was added and the mixture was stirred for further 8 h. After this time the reaction medium was transferred to a vessel containing a 0.1 M solution of acetic acid (15 mL). The product was extracted with dichloromethane (3× 50 mL) and the organic phase was dried ($MgSO_4$) and evaporated under reduced pressure. The product was subsequently purified by CC to remove the mineral oil (silica gel; dichloromethane/ethyl acetate in different proportions) (3.34 g, 11.5 mmol).

(*Ic*) *3,4-Methylenedioxymethamphetamine* (*MDMA*): To a solution of *N*-trifluoroacetyl-3,4-methylenedioxymethamphetamine (3.34 g, 11.5 mmol) in a mixture of methanol/water (1:1) (30 mL) cesium carbonate (8.48 g, 26.0 mmol) was added and the resulting mixture was refluxed for 1.5 h. After partial evaporation of the solvent and sequential addition of water (70 mL), the compound was extracted with ethyl acetate (2× 100 mL). The solvent was dried over magnesium sulphate and evaporated. The residue was recrystallised with methanol acidified with HCl and the MDMA hydrochloride was obtained as white solid product after addition of diethyl ether (1.89 g, overall yield: 41.6%).

(*R,S*)-*3,4-Methylenedioxymethamphetamine hydrochloride* (*MDMA*). ^{1}H NMR *δ*: 1.08 (3H, d, C*H*$_3$), 2.54 (3H, s, NC*H*$_3$), 2.56 (1H, dd, *J* = 13.4; 8.7, C*H*$_2$), 3.04 (1H, dd, *J* = 13.4; 4.5, C*H*$_2$), 3.33 (1H, m, C*H*), 5.99 (2H, s, OC*H*$_2$O), 6.70 (1H, dd, *J* = 8.0; 1.6, H(6)), 6.86 (1H, d, *J* = 1.6, H(2)), 6.87 (1H, d, *J* = 7.9, H(5)), 8.78 (2H, bs, N*H*$_2^+$); ^{13}C NMR *δ*: *14.8* CH_3, *29.4* NCH_3, *37.7* CH_2, *55.1* C*H*, *100.7* OCH_2O, *108.1*, *109.3* C(2) and C(5), *122.2* C(6), 130.6 C(1), 146.3 C(3), 147.7 C(4); EI-MS *m/z* (%): 193 (*M*$^{\bullet+}$, 9), 178 (12), 163 (10), 135 (100), 105 (25), 89 (28), 83 (16), 77 (98); mp 148–150 °C.

2.2.4.2. Method II. (*IIa*) *N-Carbethoxy-3,4-methylenedioxyamphetamine*: MDA (0.81 g, 4.5 mmol) as a free base was dissolved in water (30 mL) at 0 °C. A solution of NaOH (0.3 g) in water (2.5 mL) was then added to the reaction with following addition of ethyl chloroformate (0.5 mL, 5.3 mmol). The suspension was stirred at 0 °C for 2 h and the reaction was then stopped by extraction with dichloromethane (3× 100 mL). The organic layer was dried (Na_2SO_4) and the solvent was evaporated to obtain the corresponding carbamate (0.95 g, 3.8 mmol).

(*IIb*) *3,4-Methylenedioxymethamphetamine* (*MDMA*): The carbamate (0.95 g, 3.8 mmol) was dissolved in THF (15 mL) and this solution was slowly added to lithium aluminium hydride (12 mmol) suspended in THF (25 mL), under N_2. The reaction was stirred, heated under reflux for 10 h and then allowed to cool to room temperature. The excess of $LiAlH_4$ was decomposed and the reaction quenched by smooth addition of cold water/ice with vigorous stirring. The inorganic residue was removed by vacuum filtration; the solvent was dried over anhydrous magnesium sulphate, evaporated, the residue diluted with diethyl ether and extracted with aqueous HCl (3× 100 mL of a 2 M solution). The acidic extract was alkalinised with a NaOH solution and extracted with diethyl ether (3× 100 mL). The organic layer was dried over anhydrous magnesium sulphate, filtered and concentrated to yield a light coloured oil turned into a white solid of MDMA hydrochloride by the addition of ethereal HCl (0.53 g, overall yield: 51.7%).

2.2.4.3. Method III. MDA (2.00 g, 11.7 mmol) as a free base was dissolved in methanol (50 mL) acidified with acetic acid until pH 5. Formaldehyde (1 mL of a 37% solution in water, 12.3 mmol) and sodium cyanoborohydride (0.98 g, 15.6 mmol) were then slowly added to the solution. The reaction was stirred at room temperature during 3 h. After that the solvent was evaporated and the resulting yellowish oil was dissolved in methanol, the solution was acidified with HCl and diethyl ether was added until insolubilisation. A white solid identified as MDMA hydrochloride was obtained (0.89 g, yield: 33.3%).

2.2.4.4. Method IV. (*IVa*) *N-Formyl-3,4-methylenedioxyamphetamine*: MDA (1.59 g, 8.9 mmol) as a free base was suspended in ethyl formate (50 mL) and the ensuing mixture was heated to reflux providing a homogeneous solution. After 6 h, the excess of ethyl formate was removed under reduced pressure, and the resulting yellow solid of the formamide (1.59 g, 7.7 mmol) was identified and used without further purification in the next reaction.

(*IVb*) *3,4-Methylenedioxymethamphetamine* (*MDMA*): A solution of *N*-formyl-3,4-methylenedioxyamphetamine (1.59 g, 7.7 mmol) in anhydrous tetrahydrofuran (25 mL) was added dropwise to a stirred solution of lithium aluminium hydride (15 mmol) in anhydrous tetrahydrofuran (35 mL), while cooling with ice-water in an inert environment (N_2). The mixture was allowed to warm up to room temperature and then refluxed for 4 h more. After cooling and decomposing the hydride excess by the cautious addition of water, the precipitated inorganic salts were eliminated by filtration. The combined organic solutions were dried over sodium sulphate and concentrated to yield a yellow oil that was dissolved in methanol and acidified with an ethereal solution of HCl to obtain a almost white solid identified as MDMA (0.76 g, overall yield: 37.1%).

2.3. Electrochemical measurements

For the electrochemical measurements, 10 mM stock solutions of MDMA, MDA, 3,4-methylenedioxy-β-methyl-β-nitrosyrene and piperonal were made by dissolving an appropriate amount of compound in water or ethanol, depending on their solubility. The voltammetric working solutions were prepared, in the electrochemical cell, by diluting 100 μL of the stock solution in 10 mL of supporting electrolyte, in order to get a final concentration of 0.1 mM.

The pH 7.3 supporting electrolyte used in the voltammetric determinations was prepared by diluting 6.2 mL of 0.2 M dipotassium hydrogen phosphate and 43.8 mL of 0.2 M potassium dihydrogen phosphate to 100 mL.

Voltammetric studies were performed using an Autolab PGSTAT 12 potentiostat/galvanostat (Eco-Chemie, Netherlands) and a one-compartment glass electrochemical cell. Voltammetric curves were recorded, at room temperature and without oxygen purging, using a three-electrode system. A glassy carbon working electrode (GCE) ($d = 2$ mm), a platinum wire counter electrode and an Ag/AgCl saturated KCl reference electrode were used. The working electrode was polished manually with aqueous slurry of alumina powder (BDH) on a microcloth pad and rinsed with water before use. A Crison pH-meter with glass electrode was used for the pH measurements (Crison, Spain).

2.4. Quantum mechanical calculations

The quantum mechanical calculations – geometry optimisation and calculation of the harmonic vibrational frequencies – were performed using the GAUSSIAN 98W program [22], within the Density Functional Theory (DFT) approach in order to properly represent the electron correlation effects. The B3LYP hybrid method, which includes a mixture of HF and DFT exchange terms and the gradient-corrected correlation functional of Lee et al. [23,24] as proposed and parameterised by Becke [25,26], was used, along with the double-zeta split valence basis set 6-31G* [27].

Molecular geometries were fully optimised by the Berny algorithm, using redundant internal coordinates [28]: the bond lengths to within *ca.* 0.1 pm and the bond angles to within *ca.* 0.1°. The final root-mean-square (rms) gradients were always less than 3×10^{-4} hartree bohr^{-1} or hartree radian^{-1}. No geometrical constraints were imposed on the molecules under study.

Frequency calculations were run, at the B3LYP/6-31G* level, for all the energy minima obtained for the molecules under study. In view of accounting for the anharmonicity, wavenumbers above 400 cm^{-1} were scaled using a factor of 0.9614 according to Scott and Radom [29], before comparing them with the experimental data.

2.5. Raman spectroscopy

The Raman spectra were obtained at room temperature, on a triple monochromator Jobin-Yvon T64000 Raman system (0.640 m, *f*/7.5), with holographic gratings of 1800 grooves mm^{-1}. The detection system was a non-intensified CCD (Charge Coupled Device). The entrance slit was set to 200 μm and the slit between the premonochromator and the spectrograph was opened to 14.0 mm. The 514.5 nm line of an Ar^+ laser (Coherent, model Innova 300) was used as the excitation radiation, providing between 10 and 50 mW at the sample position. Samples were sealed in Kimax glass capillary tubes of 0.8 mm inner diameter. Under the above-mentioned conditions, the error in wavenumbers was estimated to be within 1 cm^{-1}.

3. Results and discussion

3.1. Chemistry

The synthesis of MDMA from MDA is outlined in Fig. 2 and the related structural elucidation data is presented in Section 2.

In general, amines can be *N*-alkylated through several pathways by the use of a key property of the nitrogen atom, its nucleophilicity. The alkylation methods are usually indirect, since the reaction of amines with haloalkanes is usually slow and not clean. Indeed, the *N*-methylation of a primary amine frequently results in a mixture of the secondary and the tertiary amine. Due to this difficulty four indirect alkylation processes

were designed, experimented and compared (methods I–IV), aiming at an improvement of the overall yield and of the purity of the final product.

Briefly, in the first procedure (method I), a simple modification of the direct method was chosen, in which a trifluoroacetyl group is used to generate an acetamide derivative (*N*-$COCF_3$, *N*-TFA). This group is readily attached to the nitrogen atom by reaction of the primary amine with trifluoroacetic anhydride ($(CF_3CO)_2O$) in the presence of a base (triethylamine or pyridine), at low temperature [30,31]. Then, after *N*-alkylation with methyl iodide, the resulting *N*-methyltrifluoroacetamide is easily cleaved (*N*-deacylation) in basic conditions [30].

On the other hand, method II is based on the synthesis of a carbamate intermediate (NCOOR), a classic example of protecting group for amines. The most common one is the ethylcarbamate ($NCOOCH_2CH_3$), a group obtained by reaction of the amine with ethyl chloroformate in the presence of a base (Method II). This group is usually reduced by most of hydrides, like $LiAlH_4$, giving the corresponding *N*-methylamines [32].

Method III seemed an attractive approach to MDMA synthesis from MDA since it corresponds to a one-pot reaction in which MDA is treated with an aldehyde (formaldehyde) in the presence of sodium cyanoborohydride as a catalyst. In these synthetic conditions the amine group undergoes a reductive methylation [31,33].

Method IV is based on a *N*-formylation of the primary amine (MDA) with ethyl formate (HCO_2Et) providing the corresponding formamide intermediate (IVa) which after reduction with lithium aluminium hydride gave the *N*-methyl compound (MDMA) [34,35] in relative good yields.

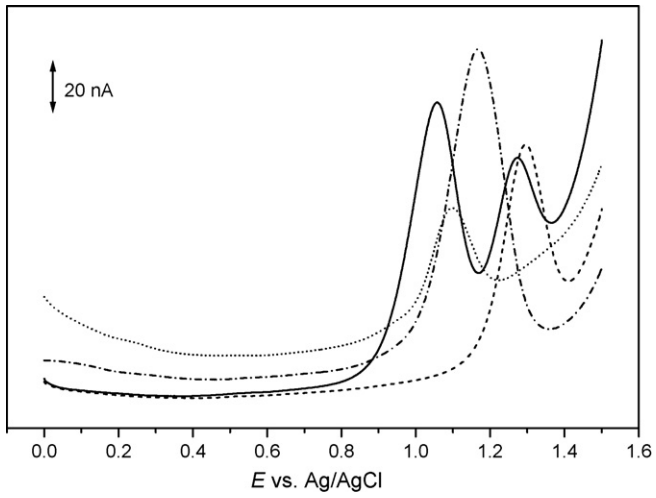

Fig. 3. Differential pulse voltammograms for 0.1 mM solutions of (—) MDMA, (···) MDA, (- - -) 3,4-methylenedioxybenzaldehyde and (- · - ·) 3,4-methylenedioxy-β-methyl-β-nitrostyrene, in physiological pH 7.3 supporting electrolyte. Scan rate: 5 mV s^{-1}.

It is worth mentioning that the synthesised amines were isolated and used in the analytical assays after obtention of their hydrochloride salts. However, prior to the *N*-methylation, the regeneration of the base from its salt form must be conducted after alkalinisation with ammonia. This step is important since

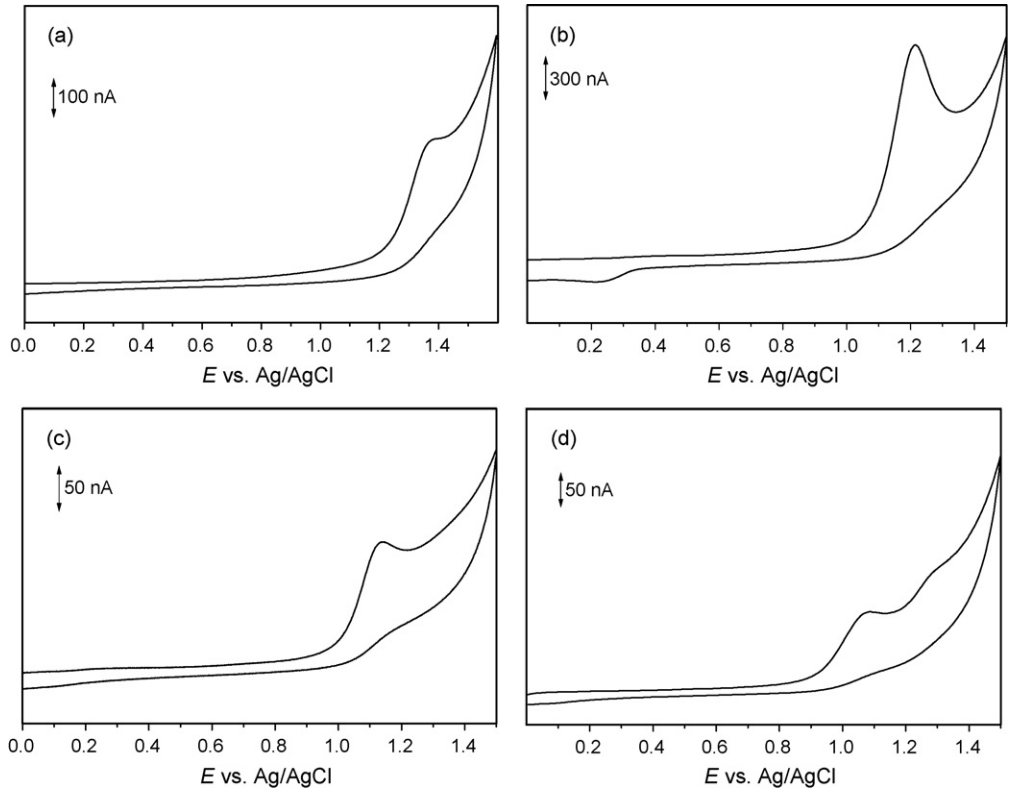

Fig. 4. Cyclic voltammograms for 0.1 mM solutions of (a) 3,4-methylenedioxybenzaldehyde, (b) 3,4-methylenedioxy-β-methyl-β-nitrostyrene, (c) MDA and (d) MDMA, in physiological pH 7.3 supporting electrolyte. Scan rate: 50 mV s^{-1}.

the lone pair of electrons on the nitrogen was unavailable to the usual reactions involving amines (alkylation and/or oxidation).

3.2. Electrochemical oxidation

To increase the understanding of the redox profile of the drugs, important for the advance of the knowledge of molecular toxicological mechanisms and the development of new analytical assays, the electrochemical behaviour of MDMA, MDA and their synthetic precursors 3,4-methylenedioxybenzaldehyde (piperonal) and 3,4-methylenedioxy-β-methyl-β-nitrostyrene was studied at physiological pH, at a glassy carbon working electrode (GCE) using differential pulse and cyclic voltammetry.

For MDMA, two well-defined anodic peaks can be observed at physiological pH using differential pulse voltammetry (Fig. 3). The first oxidation peak, $E_p = +1.05$ V, is probably due to the removal of one electron from the aromatic nucleus. The second wave, $E_p = +1.26$ V, corresponds to the oxidation of the secondary amine present in the MDMA molecule. Cyclic voltammograms were also recorded at different sweep rates. Two well-defined anodic peaks were observed for MDMA at physiological pH (Fig. 4). The fact that no peaks were observed in the reversed scan suggests that the oxidation processes are irreversible. These peaks correspond to the above-described waves observed by differential pulse voltammetry.

The electrochemical study of MDMA synthetic impurities, MDA, 3,4-methylenedioxybenzaldehyde (piperonal) and 3,4-methylenedioxy-β-methyl-β-nitrostyrene enabled to get insight the established mechanistic hypothesis that the groups involved in the oxidation of MDMA are the aromatic electrophore and the secondary amine. For these compounds, only a single and well-defined anodic oxidation wave is noticed at physiological pH that can be attributed to the removal of one electron from the aromatic nucleus. Cyclic voltammetric measurements performed showed the irreversible nature of the oxidation waves for these compounds (Fig. 4). The differences observed for these compounds in terms of peak potential and peak current may be due to the nature and electrophilic properties of the substituent in the aromatic ring.

The only structural difference between MDMA and MDA lies in the amine group, MDMA has a secondary and MDA a primary amine group. Thus, the second wave observed for MDMA could only be related with the oxidation of the secondary amine group. The appearance of a peak at this E_p value resulting from the oxidation of aliphatic secondary amines has also been described in the literature for other aliphatic amines [36,37]. Previous published works using compounds structurally related with MDMA also showed the occurrence of an anodic wave that has been undoubtedly attributed to the oxidation of the secondary amine [37–39].

The first peak observed for MDMA, $E_p = +1.05$ V, ascribed to the oxidation of the aromatic electrophore also occurs for MDA, $E_p = +1.06$ V. The inexistence of other groups in the molecule capable of being oxidised could itself justify this attribution. In addition, it could be found in literature some studies involving compounds possessing similar functionalities that present a wave at similar potentials to that established for MDMA and MDA and that were also directly related to the oxidation of the aromatic ring [40,41].

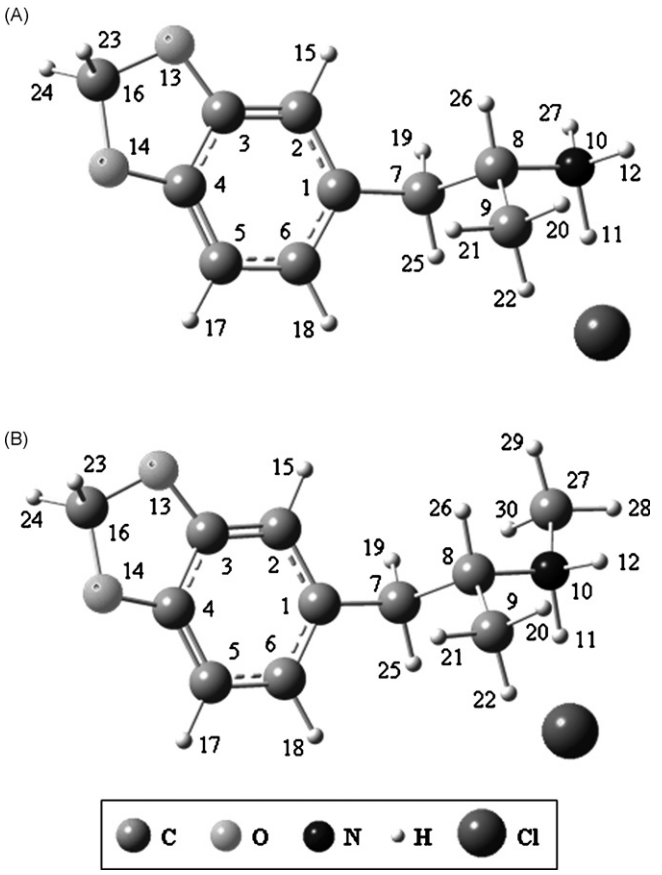

Fig. 5. Schematic representation of the most stable calculated (B3LYP/6-31G*) conformers for the hydrochloride salts of 3,4-methylenedioxyamphetamine (MDA) (A) and 3,4-methylenedioxymethamphetamine (MDMA) (B). (The atom numbering is included.)

Besides the importance of redox data for toxicological studies, in which metabolism is an important pathway, its importance is noteworthy for analytical forensic assays in which an electrochemical detector is often used due to its sensitivity and selectivity (*e.g.* HPLC).

3.3. Conformational analysis

The geometry optimisation carried out for both MDA and MDMA ("ecstasy") hydrochlorides allowed to obtain their low energy conformers represented in Fig. 5. The corresponding theoretical vibrational spectra were yielded by the harmonic frequency calculations (which also confirmed these geometries as real minima in the potential energy surface). In MDA and MDMA the unsaturated aliphatic ($C_7C_8N_{10}$) side chain was found to be almost perpendicular relative to the coplanar aromatic and methylenedioxy rings – ($C_2C_1C_7C_8$) equal to 73.9° for "ecstasy" and 75.4° for MDA (Table 1) – thus minimising steric repulsions between the C_8-methyl and aromatic hydrogens (H_{15} or H_{18}).

N-Methylation of MDA, yielding its *N*-methyl analogue "ecstasy" (MDMA), occurs in a *trans* position relative to the

Table 1
Relevant calculated (B3LYP/6-31G*) geometric parameters for the most stable conformers of MDA and MDMA

	MDA	MDMA
μ (D)[a]	(8.4)	(8.8)
Bond lengths (pm)		
C_1–C_2[b]	141.4	141.4
C_3–C_4	139.4	139.4
C_5–C_6	140.8	140.8
C_1–C_7	151.4	151.5
C_7–C_8	154.4	154.4
C_8–C_9	152.7	152.9
C_3–O_{13}	137.4	137.4
C_4–O_{14}	137.4	137.5
C_{16}–O_{13}	143.3	143.3
C_8–N_{10}	150.3	150.9
C_{27}–N_{10}	–	148.2
C_2–H_{15}	108.6	108.6
C_{16}–H_{23}	109.4	109.4
C_7–H_{19}	110.0	109.7
C_8–H_{26}	109.8	109.9
C_9–H_{20}	109.7	109.7
C_9–H_{21}	109.2	109.2
N_{10}–H_{11}	122.0	116.3
N_{10}–H_{12}	102.2	102.2
C_{27}–H_{28}	–	109.2
C_{27}–H_{29}	–	109.4
Bond angles (°)		
C_6–C_1–C_2	119.7	119.7
C_6–C_1–C_7	120.5	120.6
C_1–C_7–C_8	113.2	112.8
C_7–C_8–C_9	113.9	113.2
O_{13}–C_{16}–O_{14}	108.1	108.0
C_4–C_3–O_{13}	109.7	109.7
C_3–O_{13}–C_{16}	105.7	105.6
C_7–C_8–N_{10}	107.6	109.7
C_8–N_{10}–C_{27}	–	117.3
C_1–C_7–H_{19}	109.4	108.9
C_7–C_8–H_{26}	109.3	109.5
C_8–C_9–H_{20}	111.1	111.2
O_{13}–C_{16}–H_{23}	109.4	109.4
N_{10}–C_{27}–H_{28}	–	108.3
N_{10}–C_{27}–H_{29}	–	110.8
H_{20}–C_9–H_{21}	107.9	107.9
H_{23}–C_{16}–H_{24}	110.9	111.0
Dihedral angles (°)		
C_1–C_2–C_3–C_4	0.0	−0.1
C_3–C_2–C_1–C_7	−179.4	−179.8
C_2–C_1–C_7–C_8	75.4	73.9
C_1–C_7–C_8–C_9	64.6	62.6
C_1–C_7–C_8–N_{10}	−175.6	−177.6
C_7–C_8–N_{10}–C_{27}	–	65.2
C_7–C_8–N_{10}–H_{11}	−61.2	−57.0
C_7–C_8–N_{10}–H_{12}	179.5	−170.7
C_2–C_3–O_{13}–C_{16}	174.2	173.6
C_3–O_{13}–C_{16}–O_{14}	10.6	11.6
C_3–O_{13}–C_{16}–H_{23}	129.6	130.7
C_6–C_1–C_2–H_{15}	−179.1	−179.1
C_6–C_1–C_7–H_{19}	134.9	132.3
C_1–C_7–C_8–H_{26}	−58.7	−60.5
C_7–C_8–C_9–H_{20}	−177.9	−177.3
C_8–N_{10}–C_{27}–H_{28}	–	171.3

[a] $D = 1/3 \times 10^{-2}$ C m.
[b] Atoms are numbered according to Fig. 5.

methyl group in C_8 (($C_9C_8N_{10}C_{27}$) = −171.5°, Fig. 5), in order to avoid repulsion effects between the two adjacent CH_3 groups. Moreover, inclusion of the methyl group in the terminal amino moiety of the molecule is associated to a slight decrease of the ($C_1C_7C_8C_9$) dihedral angle – from 64.6° in MDA to 62.6° in "ecstasy" – coupled to an increase for ($C_1C_7C_8N_{10}$) – from −175.6° in MDA to −177.6° in "ecstasy" (Table 1). *N*-Methylation also leads to a slight variation of the relative orientation of the C_8–C_9 bond relative to the plane of the rings – ($C_9C_8C_1C_6$) = −26.4° in MDA to −29.9° in MDMA – as well as to a reorientation of the amino group – ($C_7C_8N_{10}H_{11}$) and ($C_7C_8N_{10}H_{12}$) equal to −61.2° and 179.5° in MDA *versus* −57.0° and −170.7° in "ecstasy" (Table 1).

3.4. Raman spectroscopy

The Raman spectra of MDA and MDMA are represented in Fig. 6 (from 100 to 1750 cm^{-1}), along with the spectra of their synthetic precursors 3,4-methylene-dioxybenzaldehyde (piperonal) and 3,4-methylenedioxy-β-methyl-β-nitrostyrene. The corresponding vibrational wavenumbers were assigned in the light of the theoretical results presently carried out (Table 2) and the spectroscopic data previously reported for β-methyl-β-nitrostyrene derivatives [42–45] and similar compounds [46–52].

As previously verified [42], Raman spectroscopy proved to be quite adequate for a rapid and reliable characterisation of this type of system. In fact, the spectral patterns obtained in this work allow an unequivocal identification of the amphetamine MDA and the corresponding *N*-methyl derivative MDMA ("ecstasy") (Figs. 6 and 7). The former displays typical Raman bands at 836/861 cm^{-1} (CH_2 in-plane deformation, $\delta(CH_2)$/CH_2 wagging, $\omega(CH_2)$), 985–965 cm^{-1} (NH_3 rocking, $r(NH_3)$), 1024 cm^{-1} (OCO ring deformation, δ(OCO)), 1310/1319 cm^{-1} (NH_3 in-plane deformation, $\delta(NH_3)$), and 1355 cm^{-1} ($r(NH_3)$). MDMA, in turn, presents characteristic signals at 381–479 cm^{-1} (skeletal CCC, COC and CCN out-of-plane deformations, Γ(CCC/COC,CCN)), 855 cm^{-1} (NH_2 rocking, $r(NH_2)$), 888/1040/1081 cm^{-1} (rocking of the *N*-methyl group, $r(CH_3)$), 1199 cm^{-1} (NH_2 twisting, $t(NH_2)$), and 1443 cm^{-1} (NH_2 scissoring, $sciss(NH_2)$) (Table 2).

The NH_3 stretching (for MDA) is calculated at 3405 cm^{-1}, while $\nu(NH_2)$ (for "ecstasy") is calculated at 3339 cm^{-1}. Since no significative intramolecular hydrogen-type interactions may occur in these molecules, the NH_2/NH_3 stretching vibrations can only be affected by interaction with the chloride counterion, which may explain why they are not detected in the experimental Raman spectra (added to the typical low intensity of these modes in Raman). Apart from the amino group stretching modes, a quite good agreement was found between the experimental and calculated frequencies for both MDA and MDMA (Fig. 8).

Raman spectroscopy was described to be a valuable technique for a complete characterisation of drugs of abuse, either in seizure or biological samples. In that way it was applied to easily identify MDA and MDMA, as well as to differentiate them from their synthetic precursors piperonal and 3,4-

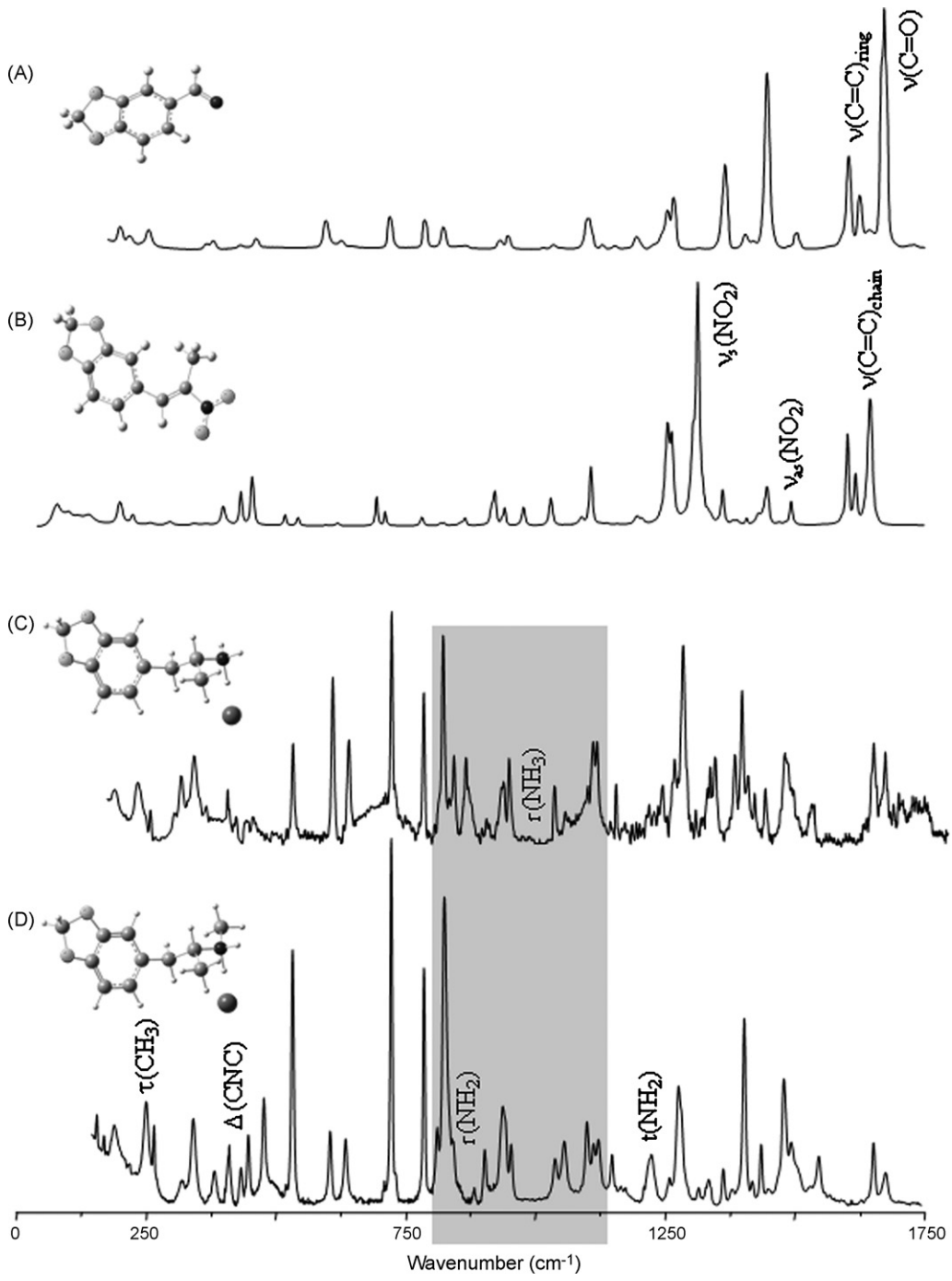

Fig. 6. Experimental Raman spectra (100–1750 cm^{-1}, solid state, at 25 °C) for piperonal (A), 3,4-methylenedioxy-β-methyl-β-nitrostyrene (B), 3,4-methylenedioxyamphetamine hydrochloride (MDA) (C), 3,4-methylenedioxymethamphetamine hydrochloride (MDMA) (D). (δ: in-plane deformation; Δ: in-plane deformation of skeleton atoms; ν: stretching; ν_s: symmetric stretching; ν_{as}: antisymmetric stretching; r: rocking; t: twisting; τ: torsion. Atoms are represented as in Fig. 5. The shaded area is expanded in Fig. 7.)

methylenedioxy-β-methyl-β-nitrostyrene, through the signals at 1445 cm^{-1} (aldehyde deformation, δ(CH)) and 1647 cm^{-1} (chain C=C stretching, ν(C=C)) – typical of piperonal – and at 1316 cm^{-1} (symmetric stretching of the nitro group, $\nu_s(NO_2)$) and 1672 cm^{-1} (carbonyl stretching, ν(C=O)) – characteristic of 3,4-methylenedioxy-β-methyl-β-nitrostyrene (Fig. 6).

This study is, to the authors' knowledge, the first attempt to perform a complete Raman spectral assignment of these amphetamine-like systems, based on a structural analysis carried out by theoretical methods. It follows a similar conformational analysis performed for β-methyl-β-nitrostyrenes [42], which are synthetic precursors of several amphetamines. These compounds can be detected as impurities or side-products in illicitly produced MDMA [21,53], which are likely to lead to severe adverse effects when contaminated with such products.

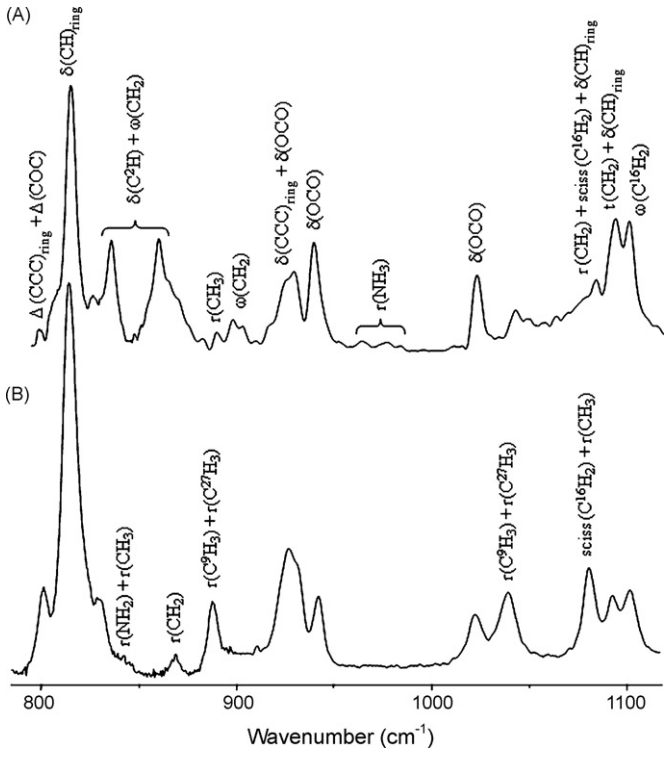

Fig. 7. Experimental Raman spectra (780–1120 cm^{-1}, solid state, at 25 °C) for MDA (A) and "ecstasy" (B) hydrochlorides. (Atoms are numbered according to Fig. 5. δ: in-plane deformation; γ: out-of-plane deformation; r: rocking; ω: wagging; sciss: scissoring; t: twisting.)

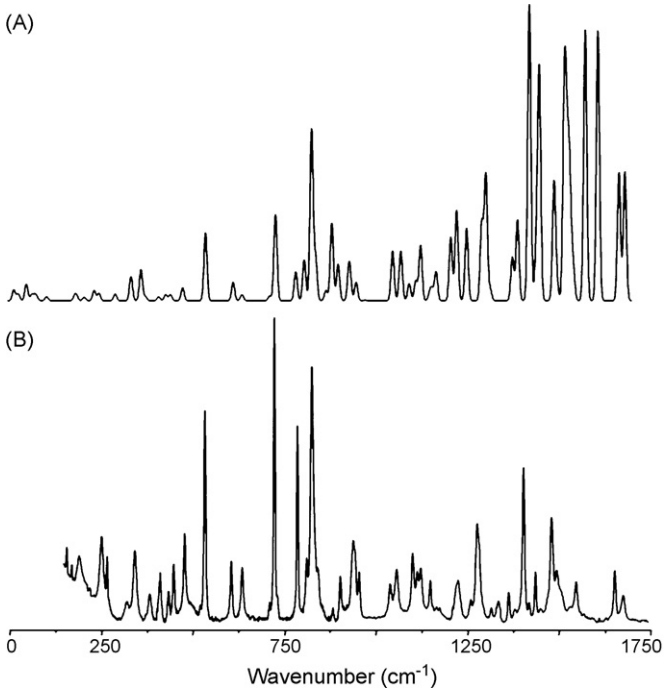

Fig. 8. Calculated (A) *vs.* experimental (B) Raman spectra (0–1750 cm^{-1}) for 3,4-methylenedioxymethamphetamine hydrochloride.

Table 2
Selected experimental (solid state) and calculated (B3LYP/6-31G*) Raman wavenumbers (cm^{-1}) for the most stable conformer of "ecstasy" (MDMA)

Experimental	Calculated[a]	Approximate description[b]
	3339	$\nu(NH_2)$
	3105	$\nu(C^5H)$
3083	3082	$\nu(C^2H)$
	3079	$\nu(C^5H) + \nu(C^6H)$
3060	3072	$\nu(C^{27}H_3)$
3054	3044	$\nu(C^{27}H_3)$
3023	3038	$\nu(C^9H_3)$
2994	2997	$\nu(C^{16}H_2)$
2981	2992	$\nu(C^7H_2)$
1634		$\nu(CC)_{ring}$
1611	1617	$\nu(CC)_{ring}$
	1546	$\omega(NH_2)$
1516	1514	$t(C^{16}H_2)$
	1512	$r(NH_2)$
1459	1458	$r(C^{27}H_3) + r(C^9H_3) + t(C^7H_2)$
1443	1440	$r(C^{27}H_3) + sciss(NH_2)$
1403	1395	$\gamma(C^{27}H_3) + \gamma(C^9H_3) + sciss(C^{16}H_2) + r(NH_2)$
1371	1367	$t(C^7H_2)$
1249	1243	$\omega(CH_2) + \delta(C^8H) + t(NH_2)$
1199	1204	$t(CH_2) + r(NH_2)$
1196	1178	$r(C^{16}H_2)$
1102	1108	$\omega(C^{16}H_2)$
1094	1095	$t(CH_2) + \delta(CH)_{ring}$
1081	1084	$sciss(C^{16}H_2) + r(C^{27}H_3)$
1040	1054	$r(C^9H_3) + r(C^{27}H_3)$
1023	1030	$\delta(OCO)$
931	939	$\delta(OCO)$
927	916	$\delta(OCO)$
888	896	$r(CH_3) + r(CH_2) + r(NH_2) + \delta(C^5H) + \delta(C^6H)$
869	868	$r(NH_2)$
855	852	$r(CH_3) + r(NH_2)$
829	837	$\delta(C^2H)$
814	810	$\delta(C^8H)$
801	780	$\delta(CH)_{ring}$
777	758	$\nu(C{=}C) + \nu(CO)$
725	706	$\nu(CO)_{ring}$
717	690	$\nu(C{=}C)$
633	617	$\Delta(CCC)_{ring}$
606	594	$\Delta(CCC)_{ring}$
535	522	$\Delta(CCC)_{ring}$
479	481	$\Gamma(CCC) + \Gamma(CCN)$
449	448	$\Gamma(COC) + \Gamma(CCC) + \Gamma(CCN)$
411	416	$\Gamma(COC) + \Gamma(CCC) + \Gamma(CCN)$
381	380	$\Gamma(COC) + \Gamma(CCC)$
341	368	$\Gamma(COC) + \Gamma(C^8NC^{27})$
267	254	$\tau(C^9H_3)$
252	241	$\tau(C^9H_3) + \Gamma(COC)$
	214	$\tau(C^9H_3)$
193	193	$\tau(C^{27}H_3)$

[a] Wavenumbers above 400 cm^{-1} are scaled by a factor of 0.9614^{P6}.

[b] Atoms are labeled according to Fig. 5; δ: in-plane deformation; γ: out-of-plane deformation; Δ: in-plane deformation of skeleton atoms; Γ: out-of-plane deformation of skeleton atoms; ν: stretching; ν_s: symmetric stretching; ν_{as}: anti-symmetric stretching; r: rocking; sciss: scissoring; t: twisting; τ: torsion; ω: wagging.

4. Conclusions

The results of this work evidence a reliable interplay between different areas of research (synthesis, electrochemistry and spectroscopy, coupled with DFT calculations) which all together

allow an insight into the physicochemical properties of a drug of abuse (MDMA) and its synthetic impurities, obtaining valuable information for forensic or toxicological studies.

The development of new synthetic strategies for *N*-methylation is of the utmost importance, both chemically and toxicologically. In fact, the pharmacological and toxicological profile of a drug is often a consequence of its metabolism, being *N*-methylation one of the most important pathways. For instance, *N*-methylated metabolites of amphetamine-like drugs of abuse (*e.g.* 3,4-dihydroxymethamphetamine) were found to be important to understand the toxicological profile of "ecstasy", at a molecular level [54,55].

In the present work, along with the development of synthetic strategies of *N*-methylation the characterisation of the compounds was performed by electrochemical assays, as well as by Raman spectroscopy experiments and quantum mechanical (DFT) calculations. From the voltammetric results for MDMA, it was verified that the main anodic waves of the oxidation profile are associated with the aromatic moiety and the secondary amine group. A similar oxidative pattern was found for "ecstasy" synthetic impurities.

The vibrational (Raman) spectra, interpreted in the light of DFT calculations, allowed a rapid and unequivocal identification of the compounds investigated, which is of the utmost relevance for clinical, forensic and toxicological purposes. Raman spectroscopy has lately been shown to be a valuable tool for an unambiguous detection of amphetamine-like products [14,17]. Actually, this technique provides a spectral fingerprint of the compounds tested and can thus be used to identify even chemically similar intermediates, as well as to trace back the precursor materials and the synthetic pathways employed in the preparation of the samples. In a near future, one will hopefully be able to rely on a Raman database for both forensic and toxicological studies.

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