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ORIGINAL PAPER

Spectroscopic and electrochemical studies of cocaine–opioid interactions

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Abstract The drugs of abuse cocaine (C), heroin (H), and morphine (M) have been studied to enable understanding of the occurrence of cocaine–opioid interactions at a molecular level. Electrochemical, Raman, and NMR studies of the free drugs and their mixtures were used to study drug–drug interactions. The results were analyzed using data obtained from quantum-mechanical calculations. For the cocaine–morphine mixture (C–MH), formation of a binary complex was detected; this involved the 3-phenolic group and the heterocyclic oxygen of morphine and the carbonyl oxygen and the methyl protons of cocaine's methyl ester group. NMR studies conducted simultaneously also revealed C–MH binding geometry consistent with theoretical predictions and with electrochemical and vibrational spectroscopy results. These results provide evidence for the occurrence of a cocaine–morphine interaction, both in the solid state and in solution, particularly for the hydrochloride form. A slight interaction, in solution, was also detected by NMR for the cocaine–heroin mixture.

Keywords Cocaine · Opioids · Drug–drug interaction · Electrochemistry · Molecular spectroscopy

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Introduction

Drug abuse is a serious health problem in our society and is currently one of the greatest concerns of governments. Of particular interest is the increase in the number of drug addicts who report combined abuse of cocaine (C) and the opioid agonist heroin (H) ("speedball") [1, 2]. Apart from the harsher effects of cocaine [1, 3], this drug combination has been reported to cause a more pleasurable or rewarding experience than cocaine or heroin alone [3–6]. This enhanced effect may contribute to the reduced motivation of "speedball" users to stop their drug habit, and their greater probability of relapse compared with single drug users. Although the underlying biological basis for abuse of cocaine and opioid combinations is unclear, controlled clinical studies give insight into the desire for dual abuse of these substances.

Several drug-discrimination procedures have been used extensively to characterize the pharmacological mechanisms of action that mediate the abuse-related effects of

cocaine and mu opioids, either alone or in mixtures [1, 3, 7–17]. The objective of these studies was evaluation of the hypothesis that cocaine and mu agonists potentiate each other's discriminative stimulus (DS). The results of the studies have been contradictory, however, and in many instances results have varied for subjects within a study. Polettini et al. [18] have suggested the occurrence of a pharmacodynamic interaction between heroin and cocaine, but this has not been confirmed owing to the paucity of data and the many uncontrolled variables involved.

Human laboratory studies have enabled determination of the acute subjective and physiological effects of cocaine in combination with opioid mu agonists [2, 19, 20]. The subjective effects of different dose combinations of mu agonists and cocaine were found to be greater than those of either drug alone. The qualitative profile of such effects produced by the opioid–cocaine combination was found to be approximately equivalent to the sum of effects produced by the two drugs separately, rather than to novel and unique subjective effects [2, 3, 15, 16].

Although numerous biological studies of the subject have been reported, no conclusive data on drug–drug interactions is yet available in the literature [2, 14, 21–23]. Electrochemical investigation of the mechanisms of oxidation of cocaine, heroin, morphine, and several metabolites [24–26] led, however, to the observation of curious chemical behavior when cocaine and opioids (heroin and its metabolite morphine (M)) were combined in solution. These findings, and the lack of chemical data supporting the interpretation of this particular behavior (either in pure solution or in biological systems), prompted the authors to conduct a more thorough study. Consequently, detailed electrochemical and spectroscopic studies (both Raman and multidimensional NMR) were performed on cocaine (C), heroin (H), and morphine (M), and on 1:1 C–H and C–M mixtures both in the solid state and in solution (Fig. 1a). Complete conformational analysis (both geometry optimization and frequency calculations) was also conducted on cocaine and on the opioids heroin and morphine by quantum mechanical calculations (at the density functional theory (DFT) level) to enable a better understanding of their structural behavior at a molecular level, thus aiding interpretation of the electrochemical and spectroscopic experimental data.

Experimental

Apparatus

Electrochemical studies

Electrochemical studies were performed using an Autolab PGSTAT 12 potentiostat/galvanostat (EcoChemie, Netherlands) and a one-compartment glass electrochemical cell. Voltammetric curves were recorded at room temperature 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. A Crison (Spain) pH-meter with glass electrode was used for pH measurement.

DFT calculations

Quantum mechanical calculations—full geometry optimization and calculation of the harmonic vibrational frequencies—were performed using the Gaussian 98W program [27], within the density functional theory (DFT) approach, to account properly for electron correlation effects. The widely employed hybrid method denoted by B3LYP [28, 29], which includes a mixture of HF and DFT exchange terms and the gradient-corrected correlation functional of Lee, Yang, and Parr [30, 31], as proposed and parameterized by Becke [32, 33], was used, with the double-zeta split valence basis sets 6-31G* [34] and 6-31G** [35]. Molecular geometries were fully optimized (bond lengths to within ca 0.1 pm and bond angles to within ca 0.1°) by use of the Berny algorithm, using redundant internal coordinates [36]. The final root-

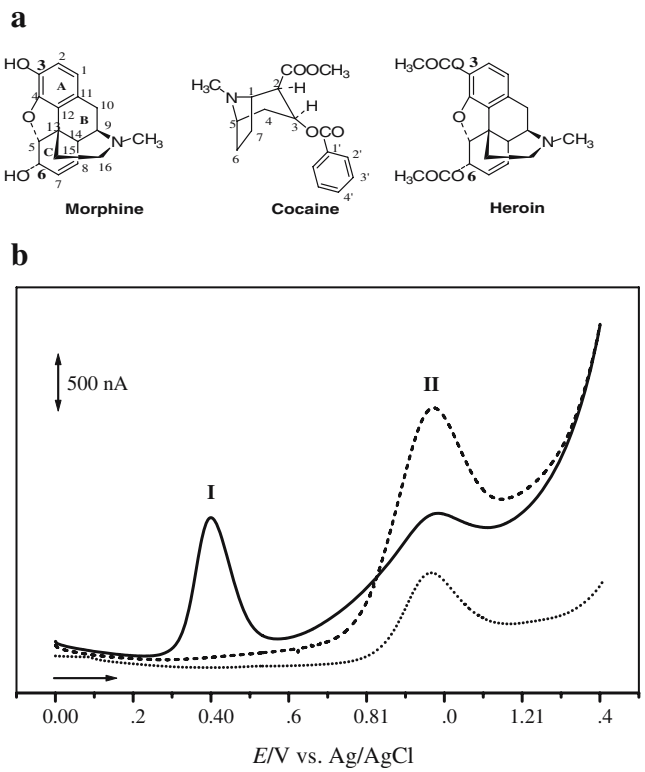


Fig. 1 (**a**) Schematic representation of morphine, cocaine and heroin and (**b**) differential pulse voltammograms obtained from 100 μmol L^{-1} solutions of morphine (*unbroken line*), cocaine (*dashed line*), and heroin (*dotted line*). (pH 7, 0.2 mol L^{-1} phosphate buffer, scan rate 5 mV s^{-1}, pulse amplitude 50 mV, pulse width 20 ms)

mean-square (rms) gradients were always less than 3×10^{-4} hartree bohr^{-1} or hartree radian^{-1}. No geometric constraints were imposed on the molecules under study. All frequency calculations were run at the B3LYP/6-31G* level and wavenumbers above 400 cm^{-1} were scaled [37] before comparing them with the experimental data.

Raman spectroscopy

The Raman spectra of the solid samples, at 20 °C, were recorded on a triple-monochromator Jobin–Yvon T64000 Raman system (0.640 m, *f*/7.5) with holographic gratings of 1,800 grooves mm^{-1}. The premonochromator stage was used in the subtractive mode. The detection system was a non-intensified CCD (charge-coupled device). The 514.5 nm line of an argon laser (Coherent, model Innova 300) was used as excitation radiation, providing ca 80 mW at the sample position, and a 90° geometry was used. The entrance slit was set to 200 μm and the slit between the premonochromator and the spectrograph was opened to 13.2 mm. An integration time of 5 s and 20 to 30 scans were used in all the experiments. Samples were sealed in Kimax glass capillary tubes with an inner diameter of 0.8 mm. Under these conditions the error in wavenumbers was estimated to be within 1 cm^{-1}.

NMR spectroscopy

^{1}H-and ^{13}C NMR (^{1}H decoupled) spectra were acquired, at room temperature, on a Bruker Avance 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Chemical shifts are expressed as δ (ppm) values relative to tetramethylsilane (TMS), used as internal reference, and coupling constants (*J*) are given in Hertz (Hz). CD_3OD was used as the sample solvent. Unequivocal ^{1}H assignments were made by using 2D *g*COSY and NOESY (800-ms mixing time) experiments, and ^{13}C assignments were made with the aid of ^{13}C NMR DEPT and 2D *g*HSQC and *g*HMBC experiments (delays for one bond and long-range *J* C/H couplings having been optimized for 145 and 7 Hz, respectively).

Reagents and solutions

Morphine free base (M) and morphine hydrochloride (MH) were obtained from Uquipa (Lisbon, Portugal) and were used without further purification. Cocaine hydrochloride was kindly supplied by Policia Judiciária (Lisbon, Portugal). Heroin hydrochloride was synthesized as described elsewhere [25]. All other chemicals and solvents were reagent grade and were used as received. Deionized water (conductivity less than 0.1 μS cm^{-1}) was used throughout.

The pH 7 phosphate buffer used for voltammetric determinations was prepared by mixing 40.5 mL 0.2 mol L^{-1} dipotassium hydrogen phosphate and 9.5 mL 0.2 mol L^{-1} potassium dihydrogen phosphate and diluting to 100 mL.

NMR studies were performed on solutions containing equal concentrations (0.1 mol L^{-1}) of either single drugs or mixtures.

Results and discussion

Electrochemical studies

The electrochemical oxidation behavior of cocaine and the two opioids heroin and its main metabolite morphine

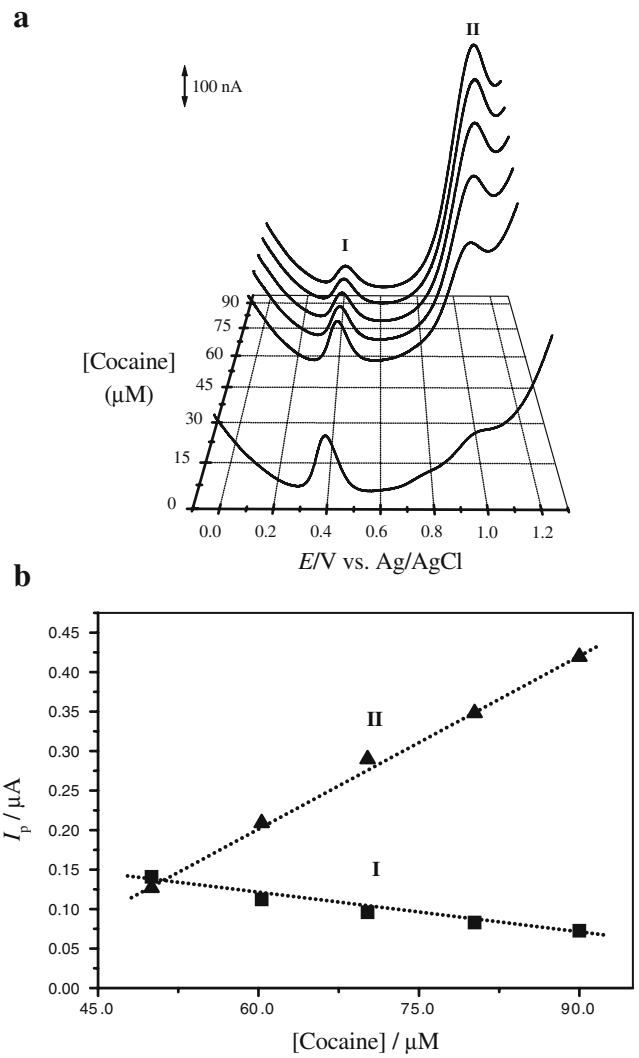
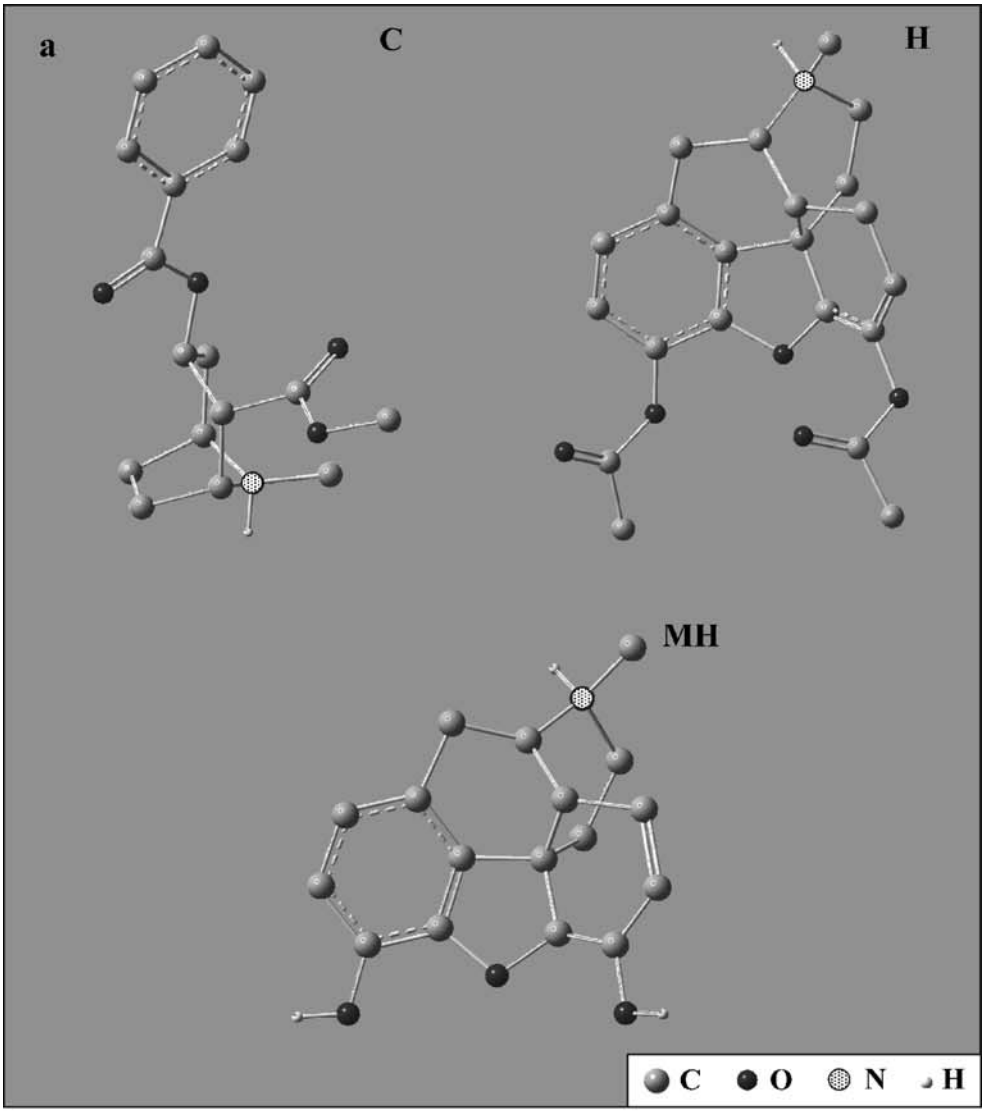


Fig. 2 (**a**) 3D plot and (**b**) plot of I_p against concentration obtained from successive differential pulse voltammograms (*I*, phenolic anodic oxidation peak; *II*, tertiary amine anodic oxidation peak), for cocaine at concentrations of 0.0, 50.0, 60.3, 70.2, 80.2, and 90.0 μmol L^{-1} and morphine at a concentration of 60 μmol L^{-1} (pH 7, 0.2 mol L^{-1} phosphate buffer, scan rate 5 mV s^{-1}, pulse amplitude 50 mV, pulse width 20 ms)



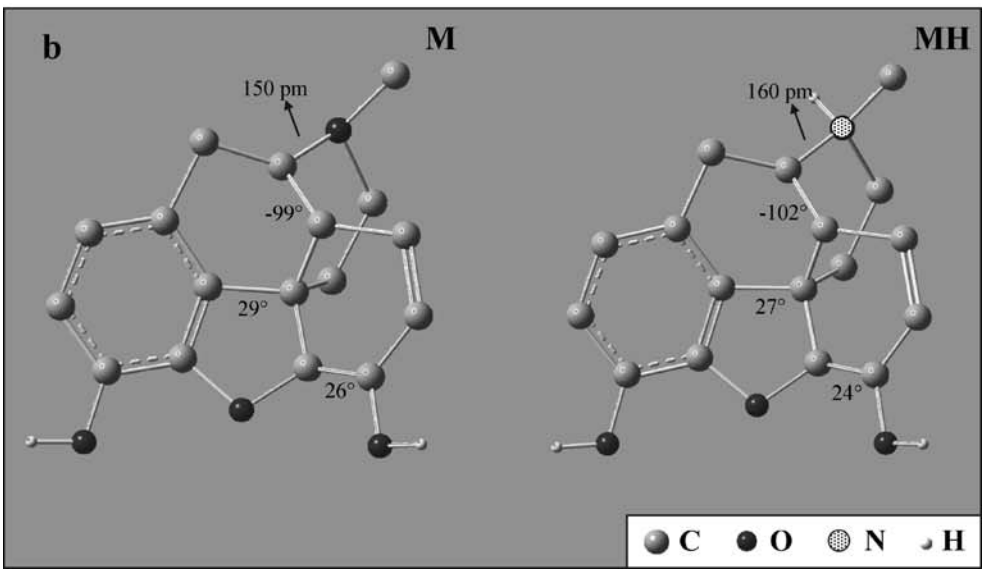


Fig. 3 (**a**) Schematic representation of the calculated (B3LYP/6-31G**) lowest-energy conformations for cocaine (C), heroin (H), and morphine (MH) salts, and (**b**) main conformational differences between morphine base (M) and *N*-protonated (MH) morphine

(Fig. 1b) was studied at physiological pH at a glassy carbon electrode using differential pulse voltammetry. The study showed that anodic oxidation of both the phenolic (E_p= +0.42 V) and tertiary amine (E_p=+1.0 V) groups was possible for morphine whereas oxidation of the tertiary amine group, only, is possible for cocaine (E_p=+1.0 V) and heroin (E_p=+0.9 V) (Fig. 1b) [25, 26].

To gain insight into the molecular oxidative mechanisms of the drugs under study the electrochemical behavior of binary mixtures was also investigated.

Cocaine and heroin each have only one oxidation peak, corresponding to oxidation of the tertiary amine group present in both molecules; this occurs at very similar potentials, meaning that electrochemical study of the cocaine–heroin interaction is not possible because of convolution of the peaks. Because study of the cocaine–opioid interaction by use of voltammetric methods depends on the ability to measure the oxidation potentials of each compound separately only the cocaine–morphine mixture can be investigated. Considering the described pharmacological effects, this mixture was also studied.

To study the cocaine–morphine interaction the following experimental procedure was used. First, standard calibration plots were obtained for each drug separately (no significant adsorption processes were observed). Second, using the standard addition method the cocaine–morphine mixture was studied by fixing the concentration of morphine and adding cocaine (Fig. 2a). The results obtained were surprising because they showed a marked interaction between the two drugs.

When the concentration of morphine was fixed, the peak for the oxidation of the phenolic group of morphine (E_p=+0.42 V) unexpectedly decreased as the concentration of cocaine was increased (Fig. 2b). Considering the dilution effect as negligible, this peak current should have remained constant.

From study of the cocaine–morphine mixture (hydrochloride salts; C–MH) important conclusions can be drawn. The results obtained clearly suggest that a strong interaction

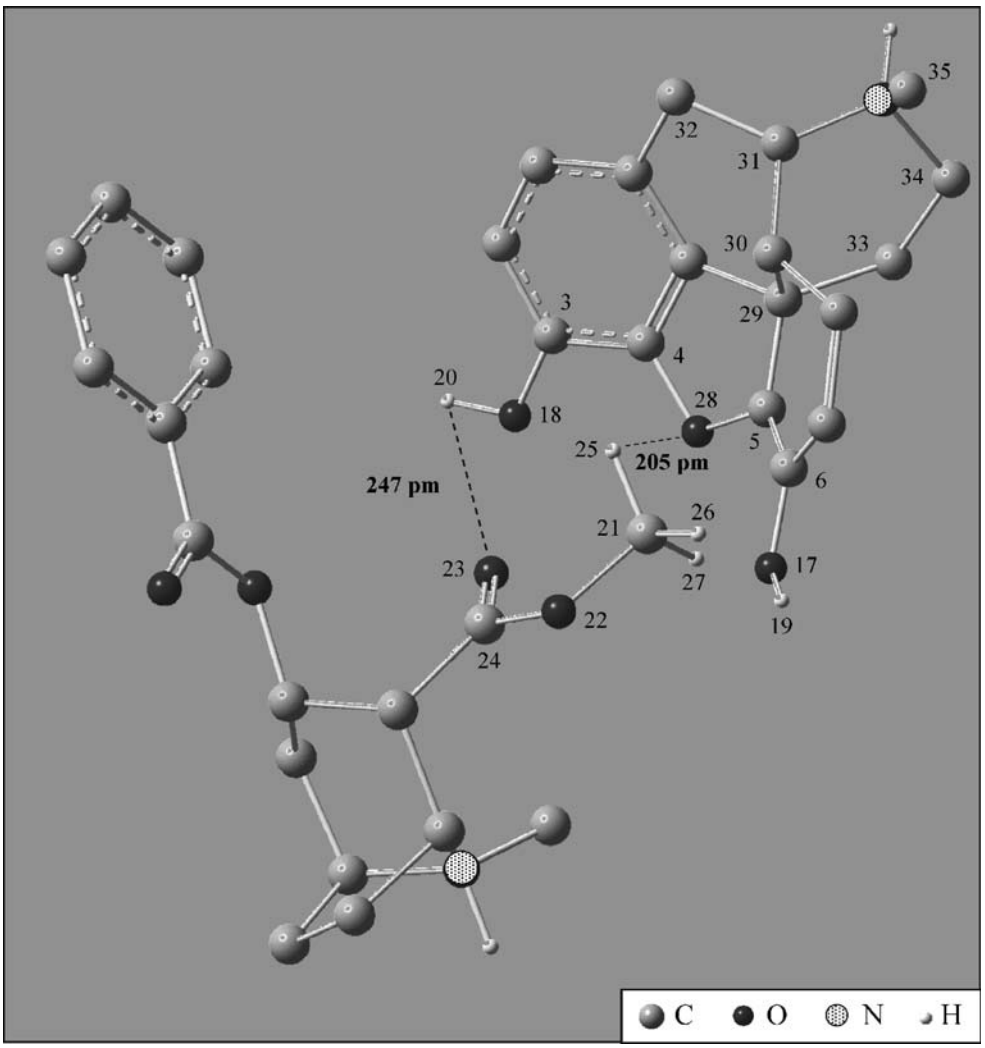


Fig. 4 Schematic representation of the model proposed for the cocaine–morphine salt (C–MH) interaction (structures calculated at the B3LYP/6-31G** level)

occurs between these two drugs in solution, with formation of a complex or adduct. The chemical integrity of each drug seems to be intact in the C–MH mixture, because, apart from the intensity change, no significant changes in the peak potential of the oxidation waves was observed. To clarify the cocaine–morphine interaction further a detailed study was conducted, both in the solid state and in solution, using theoretical methods coupled with spectroscopic techniques (Raman and NMR).

DFT Calculations

Quantum mechanical calculations at the density functional theory (DFT) level were performed for the molecules under study—morphine (both basic (M) and *N*-protonated (MH) species), heroin, and cocaine (protonated species)—and for the 1:1 (*w*/*w*) mixtures C–H, C–M, and C–MH. These calculations enabled determination of the conformational preferences of the drugs (i.e. the sites at which intermolecular interactions may occur), thus leading to a better understanding of the cocaine–opioid close contacts detected experimentally.

The lowest-energy conformations calculated for cocaine, heroin, and morphine (in their *N*-protonated forms) are depicted in Fig. 3a. The conformational differences between the basic and hydrochloride forms of morphine were also evaluated. It was found that *N*-protonation of this molecule leads to a slightly more open molecular conformation (Fig. 3b), which will probably favor interaction of this opioid with cocaine. The three-dimensional structures obtained also enabled identification of the steric and electrostatic constraints within each molecule and the geometrical requirements for intermolecular interactions.

Different possible modes of interaction of cocaine with either morphine or heroin were investigated. From these results it was verified that the presence of the two terminal $-O(C=O)CH_3$ groups in heroin probably hamper docking of the cocaine molecule whereas the approach to the morphine cavity defined by the two aromatic rings was found to be more favorable, because it does not involve any significant steric hindrance (Fig. 4). Theoretical calculations performed for the C–H and C–M systems yield shorter distances between the two components of the mixture for the latter, especially for protonated species (C–MH) (Table 1).

Table 1 Most significant conformational changes detected for morphine hydrochloride (MH) on formation of the cocaine–morphine binary complex (C–MH) (Fig. 4) (atoms are numbered in accordance with Fig. 4; atoms from cocaine are represented in bold)

Bond lengths (pm)	C–MH	MH
(C=)O_{23}–H_{20}	247.0	–
(O)$C_{21}H_{25}$–O_{28}	205.0	–
Bond angles (degrees)		
C_{34}–N–C_{35}	114.7	115.5
C_{31}–N–C_{35}	117.7	119.9
N–C_{34}–C_{33}	117.5	115.9
C_3–C_4–O_{28}	127.0	125.6
C_6–C_5–O_{28}	126.7	125.4
C_5–C_6–O_{17}	115.0	116.9
C_4–C_3–O_{18}	120.0	119.0
Dihedral angles (degrees)		
C_{35}–N–C_{34}–C_{33}	133.3	138.4
C_{35}–N–C_{31}–C_{32}	84.8	85.8
C_{35}–N–C_{31}–C_{30}	–67.2	–70.6
N–C_{34}–C_{33}–C_{29}	–31.6	–32.2
C_{30}–C_{29}–C_5–O_{28}	132.0	128.1
O_{17}–C_6–C_5–O_{28}	23.0	24.9
O_{18}–C_3–C_4–O_{28}	–16.2	–13.4

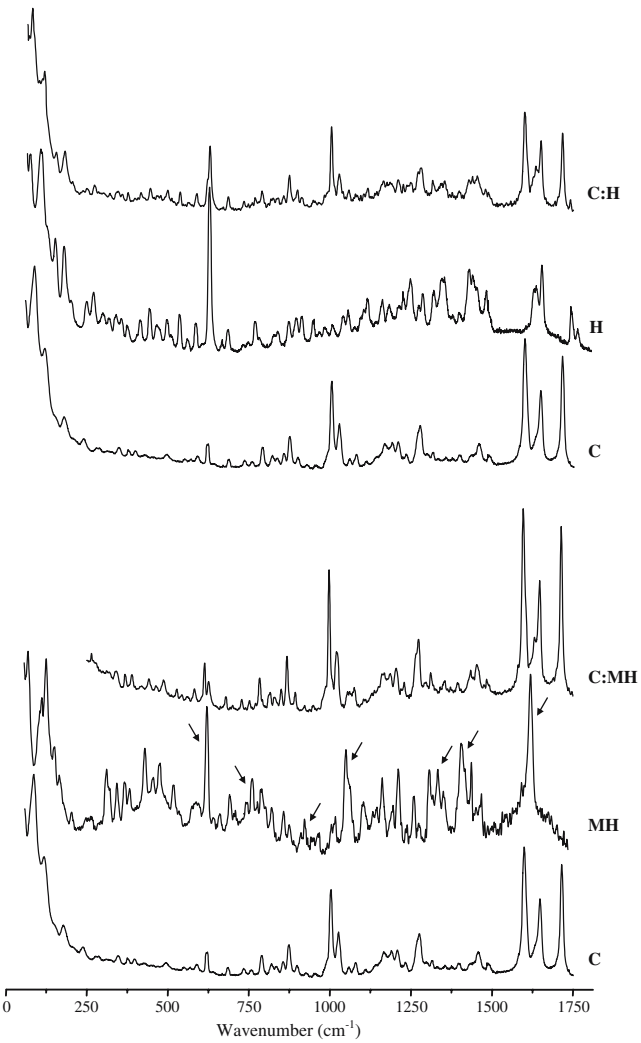


Fig. 5 Experimental Raman spectra (50–1750 cm^{-1}, solid state, at 20 °C) obtained for cocaine (C), morphine (MH), and heroin (H) salts, and for the 1:1 mixtures C–MH and C–H (*arrows* mark the most significant changes in the spectra)

Spectroscopic studies

Raman spectroscopy

Solid-state Raman spectra were recorded for morphine (both the basic (M) and *N*-protonated (MH) forms), heroin, and cocaine (protonated species), and for 1:1 (*w*/*w*) cocaine–heroin (C–H) and cocaine–morphine (C–M and C–MH) mixtures (Figs. 5 and 6), to check for the occurrence of the cocaine–opioid close contacts detected by electrochemical methods. These experiments were coupled with theoretical calculations which yielded the conformational preferences of the molecules under study, including possible sites of intermolecular interaction(s).

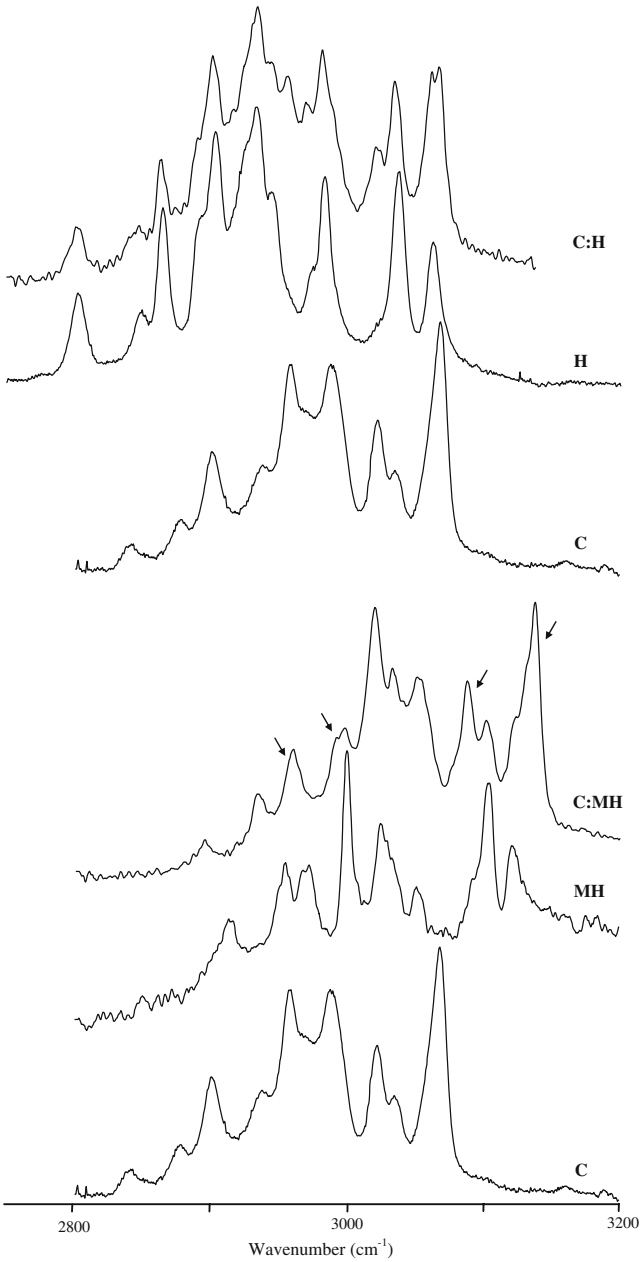


Fig. 6 Experimental Raman spectra (2800–3200 cm^{-1}, solid state, at 20 °C) obtained for cocaine (C), morphine (MH) and heroin (H) salts, and for the 1:1 mixtures C–MH and C:H (*arrows* mark the most significant changes in the spectra)

The vibrational pattern obtained for the C–M, C–MH, and C–H mixtures reflect a clear interaction occurring between cocaine and morphine, but not between cocaine and heroin. A noticeable interaction was detected for the former, particularly for the morphine salt (Figs. 5 and 6). For the C–H sample no variations in the Raman bands were observed (in either the low or high-frequency regions) compared with the individual molecules (Figs. 5 and 6), even one week after preparation of the mixture.

For the C–MH sample, however, clear changes, compared with the Raman bands of the free components, were detected immediately after mixing the two drugs. In the 50 to 1750 cm^{-1} range (which includes the CH_2, C=C and C–O deformation modes, Fig. 5), the Raman pattern of morphine, especially the bands at 632, 775, 1066, 1347, 1419, and 1635 cm^{-1}, was found to be strongly affected by the presence of cocaine. In the high-wavenumber region (which contains the CH-stretching modes, Fig. 6) new features were observed for the mixture, at 2959, 3019, 3052, and 3138 cm^{-1} whereas the bands at 2841 and 2877 cm^{-1} from cocaine and at 2998 and 3000 cm^{-1} from morphine either disappeared or decreased markedly in intensity. The most affected bands were, therefore, those ascribed to the aromatic (oxygen-containing) moiety of the morphine molecule, i.e. the $\nu(C{=}C)_{ring}$ (1635 cm^{-1}) and $\nu(CH/CH_2)_{ring}$ (ca 2900 to 3100 cm^{-1}) modes. This Raman pattern did not change on aging of the sample.

These experimental findings were corroborated by the theoretical calculations performed for the morphine base and hydrochloride species. Indeed, both the Raman and electrochemical data obtained for the C–MH mixture are readily explained if the interplay between the two molecules is proposed to occur predominantly through the inner cavity of the morphine, possibly via $C{=}O_{23}$ ... $H_{20}(O)$ and $(C)H_{25}$ ... O_{28} interactions (Fig. 4), which are favored by the *N*-protonation process because of the larger dimensions of the nitrogen-containing ring in this acidic form (Fig. 3b).

Relevant experimental and calculated vibrational wavenumbers for cocaine (C), morphine hydrochloride (MH) and the C–MH (1:1) mixture, and tentative assignment of these characteristic bands, are listed in Table 2.

Nuclear magnetic resonance spectroscopy

In the NMR study of the interactions between cocaine and the opioids morphine and heroin complete and unambiguous characterization of the drugs, alone or in 1:1 mixtures, was achieved by both 1D and 2D NMR experiments in CD_3OD solution. ^1H unequivocal assignments were

Table 2 Experimental and calculated Raman wavenumbers for the most stable conformers of cocaine (C), morphine hydrochloride (MH), and the 1:1 (*w*/*w*) cocaine–morphine mixture (C–MH)

C		MH		C–MH	Approximate description[a]
Exp.	Calc.[b]	Exp.	Calc.[b]	Exp.	
		632	630	613[c]	ν(CO) + ν(CC) + Γ(CC) (aromatic rings)
		775	774	786	γ(CH) (aromatic rings)
		1066	1109	1060[c]	ν(CO) + ν(CC) (aromatic ring)
		1347	1399	1312[c]	δ(OH) + δ(CH) + ν(C=C)
		1419	1401	1452[c]	δ(OH) + ν(C=C) (aromatic rings)
		1635	1621	1629[c]	ν(C=C) (aromatic rings)
2841	2917			—	$\nu_s(NCH_3)$
2877	2948			2886[c]	$\nu(CH/CH_2)$ (cycloheptane)
		2972	2970	2959	$\nu(CH_2)$ (non-aromatic rings)
		2998	2986	2992[c]	$\nu(CH_2)$ (non-aromatic rings)
		3000	2995	2997[c]	$\nu(CH_2)$ (non-aromatic rings)
		3025	3030	3019	ν(CH) (aromatic rings)
3068	3065			3052	$\nu_{as}(OCH_3)$
		3120	3085	3138	ν(CH) (aromatic rings)

[a] δ, in-plane deformation; γ, Γ, out-of-plane deformations; ν, stretching (s, symmetric; as, anti-symmetric)
[b] B3LYP/6-31G* level of calculation; frequencies scaled by 0.9614 [39]
[c] Displays a marked intensity decrease relative to the free C or MH bands

achieved by use of 2D-*g*COSY and NOESY and ^{13}C assignments were achieved by use of ^{13}C NMR DEPT and 2D-*g*HSQC and *g*HMBC experiments. Because the most significant shift variations were observed for the carbon atoms, the results from the ^{13}C NMR spectra were tabulated for correlation of the data (Table 3).

Analysis of the ^{13}C NMR spectra of cocaine (C), morphine (M), heroin (H), and the 1:1 (*w*/*w*) C–M and C–H mixtures revealed coherent chemical shift variations for the opioid and cocaine carbon resonances (Table 3). These variations suggest interaction of cocaine with the opioid molecules essentially through the nitrogen lone-pair electrons of the latter and the methyl ester carbonyl carbon of cocaine. This can be concluded from the deshielding effect detected for the C_9, C_{10}, and C_{16} atoms ($\Delta\delta$=−0.3 to −0.8 ppm) of the opioid molecule on complexation, and from the shielding of the carbon atoms of the methyl ester of cocaine ($\Delta\delta$=+0.5 to 0.8 ppm). This drug–drug interaction implies:

1. steric hindrance involving the *N*-methyl group of cocaine, which is the responsible for the shielding effect on its C_5 atom; and
2. formation of van der Waals interactions between cocaine and each of the opioid molecules, possible affecting their conformational geometries.

This conformational rearrangement is probably responsible for some of the shielding effects observed for the C_8, C_{11}, C_{12}, C_{13}, and C_{14} atoms of the opioid molecules ($\Delta\delta$=+0.5 to 1.5 ppm), and for the deshielding detected for several carbon atoms of cocaine ($\Delta\delta$=−0.4 to 1.5 ppm).

Table 3 Most significant NMR chemical shift variations ($\Delta\delta$, ppm) observed for the opioid (heroin and morphine) and cocaine carbon atoms (atoms are numbered in accordance with Fig. 1)

$\Delta\delta$ C–M	$\Delta\delta$ C–MH	$\Delta\delta$ C–H	Carbon atoms
			Opioid
1.33	−2.12	0.61	C_8
−0.72	1.22	−0.10	C_7
−0.79	1.38	−0.56	C_9
−0.76	0.01	−0.53	C_{10}
1.47	−2.30	0.56	C_{11}
0.73	−1.15	0.58	C_{12}
0.46	−0.43	0.46	C_{13}
1.07	−0.31	0.87	C_{14}
1.15	−0.83	0.89	C_{15}
−0.26	−4.49	−0.21	C_{16}
0.63	−0.70	0.47	NCH_3
			Cocaine
−0.67	−0.71	−0.49	NCH_3
−0.49	−0.49	−0.42	C_1
0.70	0.73	0.51	C_5
−0.93	−0.99	−0.61	C_3
−1.47	−1.54	−1.05	C_2
−0.93	−0.98	−0.69	C_4
−0.51	−0.54	−0.38	CH_{26}
−0.82	−0.86	−0.60	CH_{27}
0.72	0.75	0.53	$COOCH_3$
−0.34	−0.36	−0.24	ArCO
−0.35	−0.37	−0.26	$C_{1'}$

A completely different situation was encountered for morphine hydrochloride (MH). The ^{13}C NMR peaks recorded for the C_{10}, C_{13}, C_{14}, C_{15}, C_{16}, and N-CH_3 atoms are broad and of low intensity, suggesting significant mobility and flexibility of this part of the molecule. After addition of cocaine, this mobility is found to be restrained, because the signals of these carbon atoms become narrow and more intense. Because morphine is *N*-protonated, the C–MH interaction cannot occur through morphine's nitrogen atom. This conclusion is also supported by the most important shift variations obtained for the C–MH mixture compared with those for the C–M system (Table 3). The $\Delta\delta$ values obtained for the cocaine–morphine hydrochloride mixture suggest the formation of a bidentate complex, via interactions between the carbonyl oxygen atom of the cocaine methyl ester and the 3-hydroxyl group of morphine, and between the methyl protons of the cocaine methyl ester and the heterocyclic oxygen of morphine (Fig. 4). This interplay leads to a strong deshielding effect on the C_{11} and C_{12} carbon atoms ($\Delta\delta$=−1.2 to −2.3 ppm) and to a constrained conformation of the "flexible arm" of cocaine (as discussed above). This may be responsible for a significant steric hindrance between C_{16} and C_8 which is responsible for the marked deshielding effect observed for these atoms ($\Delta\delta$=−2.1 to −4.5 ppm) and for the shielding of C_7 and C_9 ($\Delta\delta$=+1.3 to 1.4 ppm). These effects are found to be propagated to the neighboring carbons atoms (Table 3).

Conclusion

Spectroscopic (Raman and NMR) and electrochemical techniques, coupled with DFT theoretical methods (yielding a complete conformational analysis), have been shown to be powerful tools for understanding the intermolecular interactions associated with co-abuse of drugs.

Overall, the results presented provide clear evidence of the occurrence of a cocaine–morphine interaction, both in the solid state and in solution, particularly for the hydrochloride form of morphine. A slight interaction, in solution, between cocaine and heroin was also detected by NMR.

It is important to mention that a cocaine–morphine combination such as that described in this study can also occur with heroin in vivo (e.g. after "speedball" intake), because heroin has ester bonds that are described as quite unstable, both in vitro and in vivo. Because heroin has a very short half-life (2 to 5 min), it is widely believed it acts mainly through its more stable agonistic metabolites [40–43]. It is well established that this semi-synthetic drug suffers rapid enzymatic hydrolysis to 6-monoacetylmorphine (6-MAM) and morphine [40, 42, 43].

The experimental information obtained in this work enables better understanding of the controversial biological data reported for this type of cocaine–opioid system [2, 18, 21–23]. Pharmacological data clearly show that combination of cocaine with morphine produces an enhanced effect relative that of each drug per se [2, 44] and/or may change the toxicity profile of the isolated drugs [2, 45, 46]. There is, in addition, clinical evidence (although arguable) of a particular activity of the cocaine–morphine combination (Brompton mixture) against chronic pain [47]. For the cocaine–heroin mixture it was found that each drug act largely per se. In fact, the combination of cocaine and heroin is often described as synergistic [48], or responsible for reinforcing and discriminative stimulus effects that are similar to those of the drug alone [2, 18].

This work provides a new insight into cocaine–opioid interactions at the molecular level. The development of experimental methods combined with theoretical calculations has proved of utmost relevance to understanding the molecular basis of drug–drug interactions. This type of study, which clarifies the mechanisms of action of drugs of abuse, may, hopefully, also lead to the development of effective therapy, because no specific and reliable method for the treatment co-drug abuse is yet available.

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