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Voltammetric Oxidation of Drugs of Abuse
II. Codeine and Metabolites

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Abstract
The oxidation of codeine on glassy carbon electrodes has been studied in detail using differential pulse voltammetry. The results obtained using a glassy carbon electrode clearly show a much more complex oxidation mechanism than that previously reported when platinum and gold electrodes were used. To clarify the codeine oxidative profile, several metabolites and analogues of this alkaloid, codeine *N*-oxide, norcodeine, dihydrocodeine, acetylcodeine and 6-chlorodesoxycodeine, were synthesized and studied. It was deduced that the anodic waves observed in codeine oxidation are related to the presence of methoxy, hydroxy and tertiary amine groups. Due to the similarity of potentials at which these oxidative processes take place, at some pHs an overlap of peaks occurs and only one anodic wave is observed.

Keywords: Codeine, Dihydrocodeine, Codeine-*N*-oxide, Norcodeine, Acetylcodeine, 6-Chlorodesoxycodeine, Oxidation, Voltammetry, Drugs of abuse

1. Introduction

Codeine is an alkaloid from the opiate drug class that has important effects on the central nervous system including analgesic and mood-altering effects [1]. For medical purposes, it is usually used either alone or in combination to treat mild to moderate pain and as cough suppressant, because it has good antitussive properties. Narcotic effects of codeine, although less potent, resemble those of morphine and hence addiction can occur. This ability constitutes one of the main limitations of its clinical use. In fact, about 40 percent of people who use it meet the criteria for codeine dependence [2] and the use of codeine-containing products is strongly associated with endogenous depression, again a dual-diagnosis problem [3].

The increment of drug misuse over the past decades led to an increase of codeine and dihydrocodeine, the 7,8-dihydro analogue of codeine (Fig. 1), in drug prescriptions as substitutes and alternative drugs for the management of heroin in some European countries [4, 5]. However, the presence of these drugs as a contributory factor in deaths from narcotic overdoses has also been reported [5, 6].

The determination of codeine and dihydrocodeine in biological samples is a common practice in many laboratories involved in forensic and clinical toxicology. In addition, it is also important for pharmacological studies to determine codeine and its metabolites in biological samples. Therefore the development of sensitive, rapid and simple methodologies for its analysis are welcome.

Although some electrochemical methods are described in the literature for determination of codeine in pharmaceutical and biological samples [7–9] little is known about its oxidative profile. Moreover, until the middle 80's codeine was stated to be devoid of electroactivity [10]. The voltammetric study of codeine and dihydrocodeine at platinum and gold electrodes revealed a single anodic wave related to the oxidation of the 6-hydroxy group (Fig. 1) from which resulted the formation of a quinonoid type structure [11]. Nevertheless, other studies involving the use of glassy carbon and of modified electrodes showed the appearance of more than one anodic wave that were not identified [12, 13].

In order to clarify the oxidation process occurring for codeine and to identify all anodic waves observed, the synthesis of several codeine metabolites and derivatives (Fig. 1) was performed as well as the study of their electrochemical behavior.

2. Experimental

2.1. Apparatus

Voltammetric measurements were performed using an Autolab PGSTAT 10 potentiostat/galvanostat (EcoChimie, Netherlands) and a one-compartment glass 663 VA Metrohm cell with a three-electrode configuration (Metrohm). The electrodes used were a glassy carbon working electrode with a diameter of 2 mm (Metrohm), a glassy carbon rod
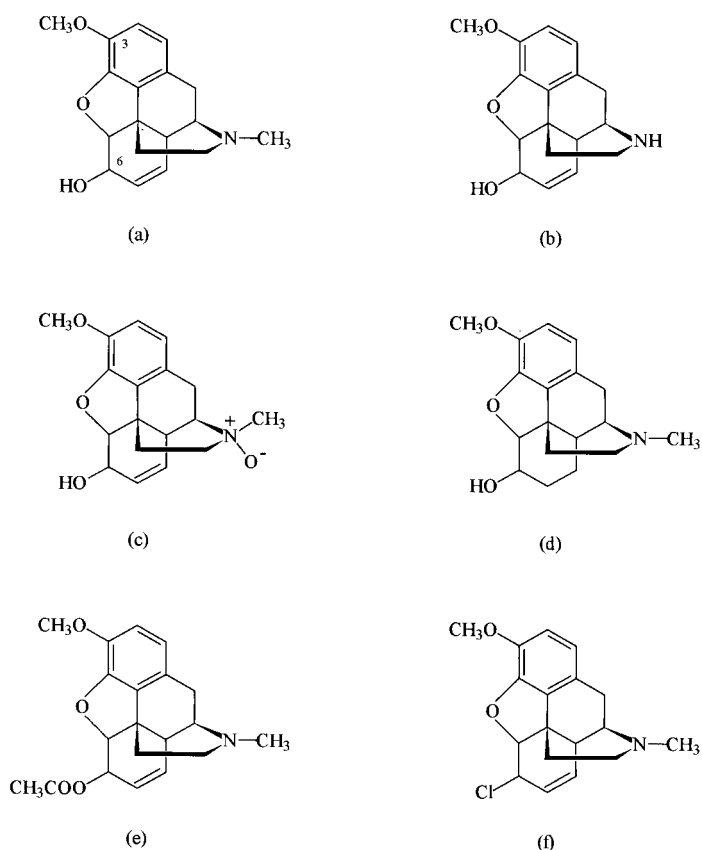
Fig. 1. Structural formulae of a) codeine, b) norcodeine, c) codeine *N*-oxide, d) dihydrocodeine e) acetylcodeine, and f) 6-chlorodesoxycodeine.

counter electrode (Metrohm) and an Ag/AgCl (3 M KCl) reference electrode (Metrohm). The working electrode was polished with alumina (BDH) on a microcloth pad and rinsed with water before use.

A Metrohm E-520 pH-meter and a Metrohm glass electrode were used for pH measurements. Melting points were obtained on a Köfler microscope (Reichert Thermovar). Infrared spectra were recorded on a ATI Mattson Genesis Series FTIR spectrophotometer using potassium bromide disks (Uvasol, Merck). ^{1}H and ^{13}C NMR (^{1}H decoupled) data were acquired, at room temperature, on a Brüker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Chemical shifts are expressed as δ (ppm) values relative to tetramethylsilane (TMS) as internal reference; CDCl$_3$ was used as sample solvent. Assignments were also made from DEPT (distortionless enhancement by polarization transfer) (underline values). Electron impact mass spectra (EI-MS) were obtained out on a VG AutoSpec instrument and data are reported as *m/z* (% of relative intensity of the most important fragments).

2.2. Other Conditions

Thin-layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 plates (E. Merck). The layer

thickness was 0.2 and 0.1 mm, respectively. The following chromatographic systems were used: chloroform/ methanol/ diethylamine (8:1.5:0.5), carbon tetrachloride/butanol/ methanol/ammonia (4:3:3:0.3), chloroform/acetic acid/ diethylamine (8:1.5:0.5). The spots were visualized under UV detection (254 and 366 nm) and iodine vapor. Purification of the compounds was performed by column chromatography using Merck silica gel 60 (0.2 – 0.5 mm, E. Merck) or aluminium oxide, activated basic, Brockmann I (ca. 150 mesh, 58 Å, Aldrich). Solvents were evaporated in a Büchi Rotavapor.

2.3. Reagents and Solutions

Codeine hydrochloride was obtained from Uquipa (Lisbon, Portugal) and was used without further purification. Norcodeine hydrochloride was kindly donated by the United Nations Drug Control Programme (Vienna, Austria). Dihydrocodeine bitartrate was a gift from Asta Medica (Lisbon, Portugal). Reagents used in the synthetic procedures were obtained from Sigma-Aldrich Quimica (Sintra, Portugal). All other reagents and solvents were *pro analysis* grade and purchased from Merck (Lisbon, Portugal). *m*-Chloroperbenzoic acid (free of *m*-chlorobenzoic acid) was purified according the procedure of Bortolini et al. [14]. Deionized water with conductivity less than 0.1 μS cm^{-1} was used throughout. Buffer solutions employed were 0.2 M in the pH range 1.2 – 12.2 [15].

2.3.1. Synthesis of Codeine N-Oxide (Codeine Oxide)

The preparation of codeine *N*-oxide was adapted from the procedure proposed by Craig and Purushothaman [16].

Codeine hydrochloride (1.0 g) was dissolved in 25 mL of water containing a trace of sodium metabisulfite at 10 °C. One equivalent of concentrated aqueous ammonia was added dropwise, with stirring. The cooling was continued for an additional 15 min. and during this time a white solid appeared in the solution. The crystals of codeine free base were filtered and washed with ice-cold water.

A solution of *m*-chloroperbenzoic acid (0.15 g) in 5 mL of chloroform was added gradually, at 0 – 5 °C, to an ice-cooled solution of codeine free base (0.23 g) in 5 mL of chloroform. Stirring was continued for 4 hours, during which the mixture was allowed to come to room temperature. After solvent evaporation the residue was purified by column chromatography (alkaline alumina; chloroform → chloroform/methanol 9:1). The fractions containing the major product were collected, the solvent evaporated, and the residue recrystallized from ethanol. The crystals were washed with diethyl ether and dried in a oven.

The analytical data of the compound were similar to those found in the literature [17 – 23].

2.3.2. Synthesis of Acetylcodeine (6-O-Acetylcodeine; 3-O-Methyl-6-O-acetylmorphine)

Codeine hydrochloride (0.5 g) was dissolved in a mixture of 25 mL of dry pyridine and 30 mL of acetic anhydride and maintained at room temperature for 24 h. After complete reaction the solution was poured into a beaker containing ice. The crude product was extracted with chloroform (3 × 20 mL). The combined organic phases were washed with 5% hydrochloric acid and water and dried over anhydrous sodium sulfate. After evaporation of the solvent, a residue was obtained and recrystallized with diethyl ether. Acetylcodeine was obtained as white crystals. The analytical data of the compound was similar to that found in the literature [24].

2.3.3. Synthesis of 6-Chlorodesoxycodeine (6-Chloro-4,5α-epoxy-3-methoxy-N-methyl-7,8-didehydromorphin-an)

This codeine congener was prepared using a modified version of a reported procedure [25]. A solution of *p*-toluenesulfonyl chloride (0.34 g) in pyridine (2 mL) was added dropwise to an ice-cooled solution of codeine (0.5 g) in 3 mL of pyridine. The reaction mixture was allowed to stand at 5 °C for 4 h and then poured into ice-water with rapid stirring. The medium was alkalinized with concentrated aqueous ammonia and the mixture was extracted with chloroform (3 × 50 mL). The combined organic extracts were washed with water and dried over anhydrous sodium sulfate. The melting point of the tosylate was similar to that referred in the literature [25].

The tosylate was dissolved in a mixture of ether and hexamethylphosphoramide and dry lithium chloride (0.2 g) was added to the solution. The reaction mixture was stirred overnight at room temperature. After the usual workup a white solid was obtained (80% yield) [26–28].

M.p. 156–158 °C. ^{13}C NMR: 119.2 (CH, C1), 112.8 (CH, C2), 141.8 (C3), 145.6 (C4), 98.8 (CH, C5), 53.8 (CH, C6), 135.2 (CH, C7), 128.6 (CH, C8), 58.6 (CH, C9), 20.2 (CH$_2$, C10), 127.4 (C11), 130.0 (C12), 44.4 (C13), 39.9 (CH, C14), 36.2 (CH$_2$, C15), 46.6 (CH$_2$, C16), 56.2 (OCH$_3$), 43.1 (NCH$_3$). EI-MS: 319 ([M + 2]$^{+}$·, 44), 317 ([M]$^{+}$·, 63), 282 (100).

3. Results and Discussion

Codeine is fairly stable in the solid state, but in aqueous solutions degrades relatively rapidly in the presence of strong acids and bases [29]. In this degradation process, isomerization reactions play an important role [20]. Moreover, some factors, such as pH, temperature and counter-ion or buffering species could also contribute to the degradation of codeine [29].

Some specific congeners possessing important functionalities that could help to understand the oxidative profile of codeine were synthesized: codeine *N*-oxide, acetylcodeine
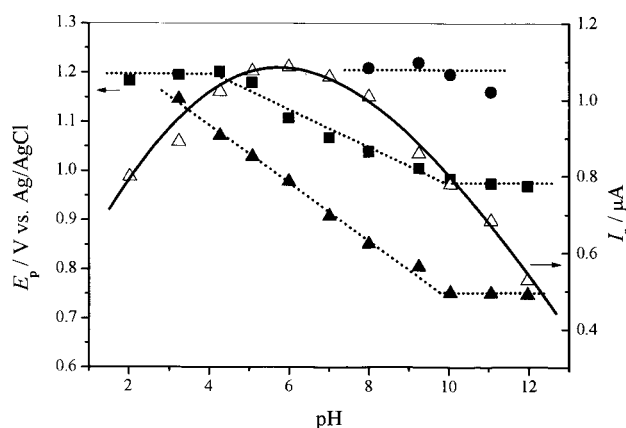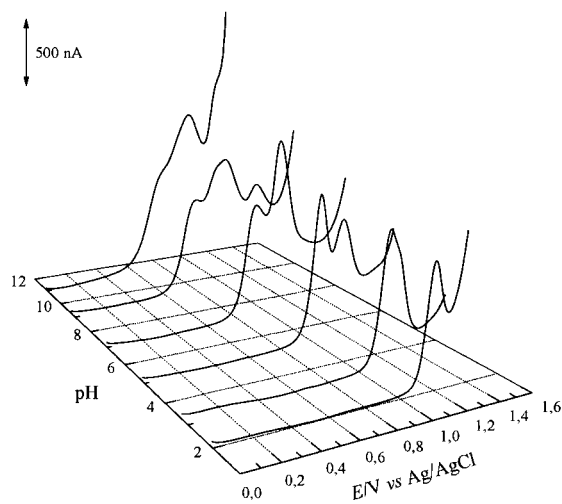
(a)

(b)

Fig. 2.   a) 3D plot and b) plots of E_p (filled symbols) and I_p (open symbols) vs. pH from differential pulse voltammograms of 100 μM codeine in different buffer electrolytes as function of pH. Scan rate 5 mV s^{-1}.

and 6-chlorodesoxycodeine, as described in the experimental section.

3.1. Electrochemical Oxidation

In order to understand codeine's oxidative pathways and to assess the influence of pH on its degradation, a detailed voltammetric study was carried out.

The electrochemical behavior of codeine was studied over the pH interval 1.2 to 12.2, at a glassy carbon working electrode using differential pulse voltammetry (Figs. 2 and 3a). It was seen that the anodic oxidation of codeine follow a very complex mechanism that is pH dependent.

The anodic wave observed starting at pH 2, E_p = + 1.19 V, appears to be the result of the sum of two close peaks. These peaks might be related with the oxidation of the tertiary amine and the 6-hydroxy groups (Scheme 1), based on two observations. First, at pH 3 the peak at E_p = + 1.15 V,
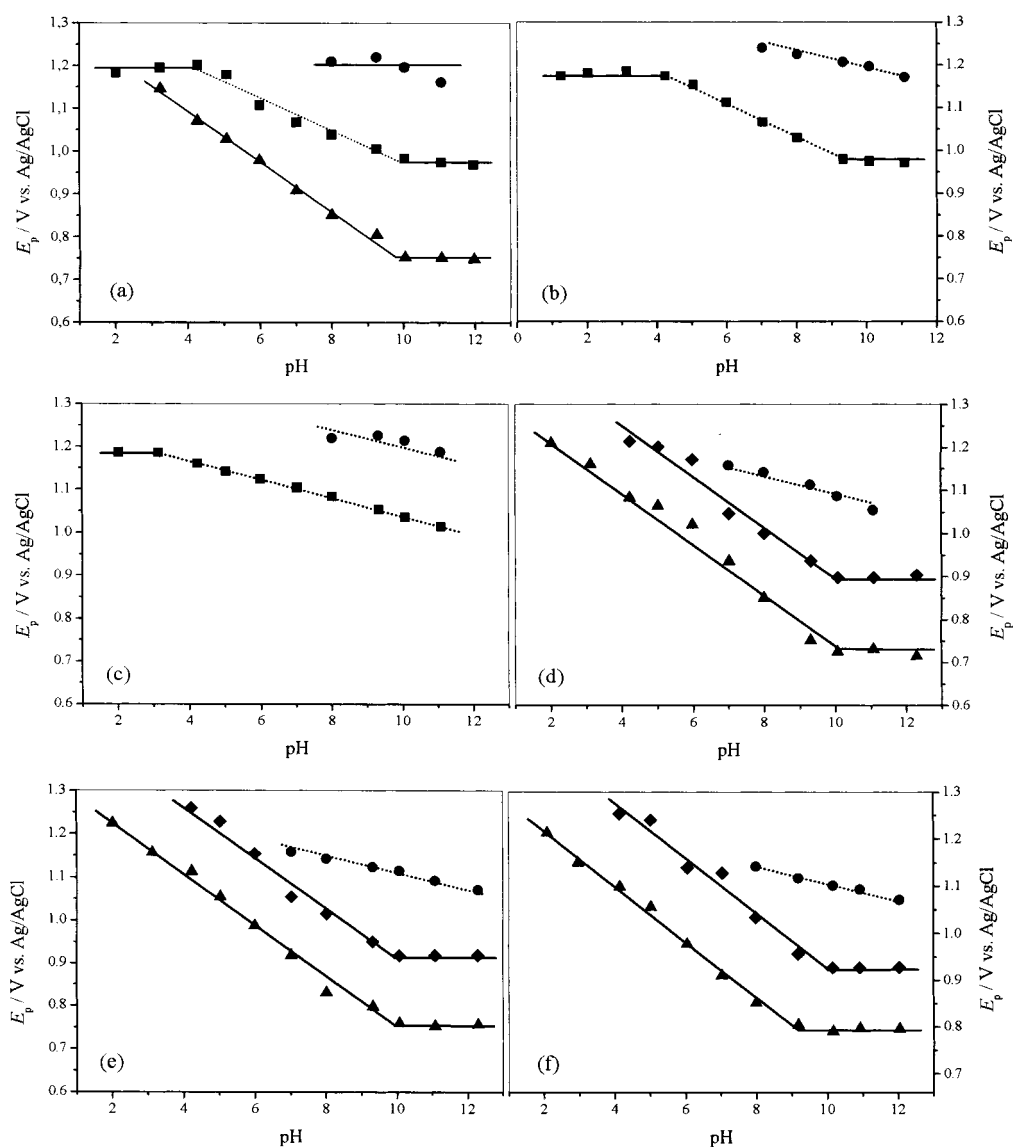
Fig. 3.   Plots of E_p vs. pH from differential pulse voltammograms of 100 μM solutions of a) codeine, b) norcodeine, c) codeine *N*-oxide, d) dihydrocodeine, e) acetylcodeine and f) 6-chlorodesoxycodeine in different buffer electrolytes. Scan rate 5 mV s^{-1}. (—) Slope 59.2 mV/pH unit.

presents a well-defined shoulder after the main peak (Fig. 2), meaning that the two peaks are almost super-imposed. The second observation is the behavior of codeine derivatives at this pH.

By studying the electrochemical behavior of norcodeine, codeine *N*-oxide and 6-chlorodesoxycodeine (Figs. 1, 3b, 3c and 3f) it is possible to draw some conclusions. Norcodeine presents an anodic wave at pH 2 at $E_p = +1.17$ V, that can be attributed to the 6-hydroxy group, given the absence of the tertiary amine group (Fig. 4). 6-Chlorodesoxycodeine also presents an anodic wave, $E_p = +1.21$ V, at this pH. Since in the 6-position there is a chloride instead of a hydroxy group the peak should correspond to the oxidation of the tertiary amine group (Fig. 4). Since codeine molecules have both these two groups, it is not surprising that a single wave appears at $E_p = +1.19$ V corresponding to their oxidation.

At pH 4 it is possible to observe two well-defined anodic peaks for codeine, $E_p = +1.07$ V and $+1.20$ V (Figs. 2 and 3). These waves should result from oxidation of the tertiary amine and 6-hydroxy groups (Scheme 1) which are now separated. In fact, the decrease of peak potential with increasing pH observed for the oxidation of the tertiary amine group together with the pH independent potential corresponding to oxidation of the hydroxy group until pH 5 means there is a separation between the two peaks (Fig. 3).

The identification of these two anodic waves, involving the tertiary amine and 6-hydroxy group, was confirmed by considering and comparing the voltammetric behavior of codeine and norcodeine at pH 4 the first peak observed for codeine is absent for norcodeine (Fig. 5). The only structural difference between codeine and norcodeine is in the amine
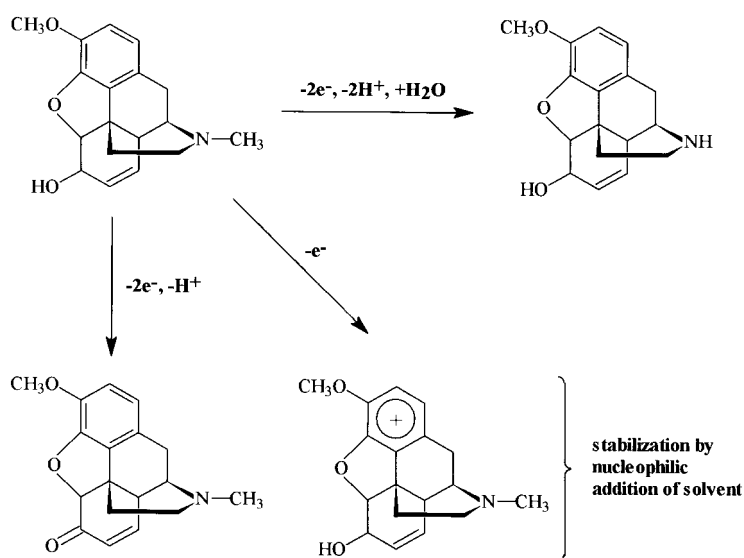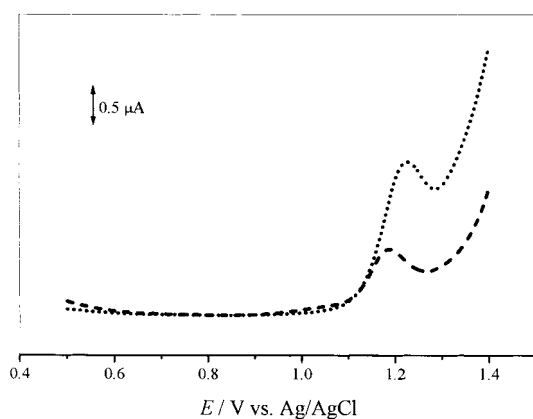
Scheme 1.

Fig. 4. Differential pulse voltammograms in pH 2 KCl/HCl supporting electrolyte of 100 μM solutions: (- - - -) norcodeine and (·····) 6-chlorodesoxycodeine. Scan rate 5 mV s^{-1}.
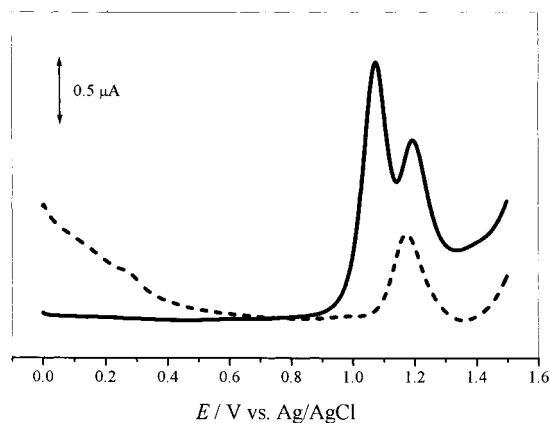

Fig. 5. Differential pulse voltammograms in pH 4 acetate buffer of 100 μM solutions: (—) codeine and (- - - -) norcodeine. Scan rate 5 mV s^{-1}.

group, codeine has a tertiary and norcodeine a secondary amine group (Fig. 1). Thus this peak could only be related with the oxidation of the tertiary amine group. The oxidation of the tertiary amine group leads to the formation of a secondary amine group (i.e., norcodeine) and an aldehyde [30]. The assignment of the second peak to oxidation of the 6-hydroxy group could be made by examining the data obtained for the other compounds studied and, particularly, considering the behavior of norcodeine (Fig. 3b). As seen, the peak position is pH independent until pH 5 and above pH 9. This result is undoubtedly important for peak identification made since it is in agreement with the pK_a values of 5.7 and 9.1 given in the literature for norcodeine [24, 31]. The results obtained for dihydrocodeine (Fig. 3d) are particularly significant in supporting this assignment. In fact, the nonexistence of the double bond in the neighborhood of the 6-hydroxy group of dihydrocodeine (Fig. 1) makes its oxidation practically impossible at these potentials.

The experimental evidence presented above and the data available in the literature [11] lead to assignment of the second peak to the oxidation of the 6-hydroxy group. In order to try and confirm this, further studies involving model compounds, dextromethorphan and 3-methoxymorphinan, were carried out by differential pulse voltammetry. These compounds are structurally related with codeine although they do not possess the 6-hydroxy function. In the literature, the occurrence of a new oxidative metabolite arising from oxidation of the benzylic carbon (C-10) present in the codeine and morphine structures is referred to [32–34]. Nevertheless, the results obtained were not sufficiently elucidative in order to prove or disprove the hypothesis of the oxidation of the benzylic carbon in codeine (data not shown).

At pH 8 it is possible to observe three anodic peaks for codeine (Figs. 2 and 3a). The appearance of a new wave at $E_p = +1.21$ V can be attributed to the oxidation of the 3-methoxy group. In support of this assignment, the peak
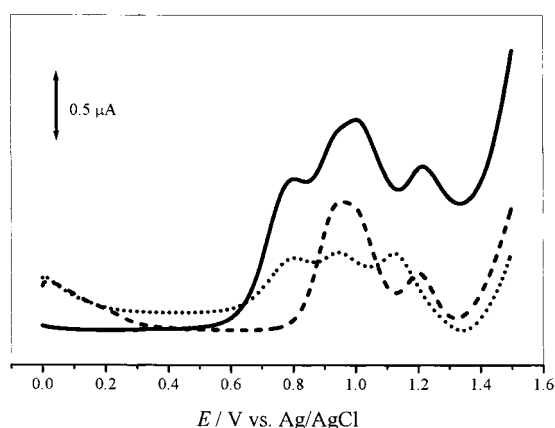
Fig. 6.　Differential pulse voltammograms in pH 9 borate buffer of 100 μM solutions: (—) codeine, (- - - -) norcodeine and (·····) acetylcodeine. Scan rate 5 mV s^{-1}.

potential does not depend on the solution pH, the peak potential is the same as that for more simple compounds with this group, such as anisole and *p*-methylanisole (data not shown), and the peak appears in all compounds studied (all have this substituent).

At pH 9 and 10 four anodic waves are observed (Figs. 2 and 6). A small shoulder is seen near the second peak, which means that probably the second peak is, for lower pHs, also the result of the sum of two anodic waves. This new peak, is probably related with a subsequent oxidative process involving the secondary amine group from the oxidation of the tertiary amine group present in codeine. The similar behavior of codeine and norcodeine at these pHs (Fig. 6) and the results obtained for the closely related compound norheroin where a secondary amine group is the only oxidizable group present (data not shown) reinforces this assignment. Moreover, this explains clearly the similarity in oxidative behavior of codeine and 6-chlorodesoxycodeine or acetylcodeine, compounds in which the 6-hydroxy group is not present (Fig. 6). If the second peak appearing for codeine at $E_p = +1.20$ V (pH 4) were merely a result of oxidation of the 6-hydroxy group, see Figure 5, it should not be present for acetylcodeine or 6-chlorodesoxycodeine. More experimental evidence is obtained from the results for codeine and acetylcodeine at pH 9 (Fig. 6). As seen, contrarily to that observed for codeine, acetylcodeine presents only one anodic peak at this pH at a potential similar to that of the shoulder.

4. Conclusions

The oxidation behavior of codeine and dihydrocodeine was studied over a large pH interval. It was verified that in aqueous solution codeine and dihydrocodeine oxidation mechanisms are coincident, follow a very complex pH-dependent mechanism. To understand and clarify its oxidative mechanism, a series of metabolites and analogues of codeine were synthesized and their voltammetric behavior

studied. From analysis of the data obtained for all the compounds it was concluded that the oxidative profile of codeine is a consequence of the oxidation of methoxy, hydroxy and tertiary amine groups present in the molecule. Moreover, it was found that further oxidation of the product deriving from the tertiary amine group occurs at the electrode surface. These results will contribute to a better understanding of the oxidative behavior of codeine and to the development of new analytical methodologies, based on electrochemical detection, for its monitoring.

5. Acknowledgements

6. Supporting Information Available

NMR, IR and EI-MS data of the synthesized compounds is available upon request from authors.

7. References

[1] E. J. Simon, J. M. Hiller, *Ann. Rev. Pharmacol. Toxicol.* **1978**, *18*, 371.
[2] B. A. Sproule, U. E. Busto, G. Somer, M. K. Romach, E. M. Sellers, *J. Clin. Psychopharmacol.* **1999**, *19*, 367.
[3] M. K. Romach, B. A. Sproule, E. M. Sellers, G. Somer, U. E. Busto, *J. Clin. Psychopharmacol.* **1999**, *19*, 373.
[4] M. Backmund, K. Meyer, D. Eichenlaub, C. G. Schutz, *Drug Alcohol Depend.* **2001**, *64*, 173.
[5] A. Seymour, M. Black, J. Jay, J. S. Oliver, *Scot. Med. J.* **2001**, *46*, 143.
[6] K. Klinder, G. Skopp, R. Mattern, R. Aderjan, *Int. J. Legal Med.* **1999**, *112*, 155.
[7] J.-M. Zen, M.-R. Chang, H.-H. Chung, Y. Shih, *Electroanalysis* **1998**, *10*, 536.
[8] T. Zhou, H. Yu, Q. Hu, Y. Fang, *J. Pharm. Biomed. Anal.* **2002**, *30*, 13.
[9] J. M. P. J. Garrido, C. D. Matos, F. Borges, T. R. A. Macedo, A. M. Oliveira-Brett, *Anal. Lett.* **2002**, *35*, 2487.
[10] C. W. McLeod, T. S. West, *Analyst* **1982**, *107*, 1.
[11] E. Bishop, W. Hussein, *Analyst* **1984**, *109*, 143.
[12] P. Surmann, *Arch. Pharm.* **1979**, *312*, 734.
[13] M. P. Prete, J.-M. Kauffmann, J.-C. Vire, G. J. Patriarche, B. Debye, G. Geuskens, *Anal. Lett.* **1984**, *17*, 1391.
[14] O. Bortolini, S. Campestrini, F. Di Furia, G. Modena, *J. Org. Chem.* **1987**, *52*, 5093.
[15] A. M. Oliveira-Brett, M. M. M. Grazina, T. R. A. Macedo, C. Oliveira, D. Raimundo, *J. Pharm. Biomed. Anal.* **1993**, *11*, 203.
[16] J. C. Craig, K. K. Purushothaman, *J. Org. Chem.* **1970**, *35*, 1721.
[17] I. J. Holcomb, R. B. Luers, S. A. Fusari, *J. Pharm. Sci.* **1973**, *62*, 1504.

[18] J. D. Phillipson, S. S. Handa, S. W. El-Dabbas, *Phytochemistry* **1976**, *15*, 1297.

[19] A.-F. Hsu, R. H. Liu, E. G. Piotrowski, *Phytochemistry* **1985**, *24*, 473.

[20] P. Gundermann, R. Pohloudek-Fabini, *Pharmazie* **1980**, *35*, 296.

[21] H. G. Theuns, R. H. A. M. Janssen, H. W. A. Blessels, C. A. Salemink, *Org. Magn. Reson.* **1984**, *22*, 793.

[22] J. T. M. Linders, M. A. Prazeres, T. S. Lie, L. Maat, *Magn. Reson. Chem.* **1989**, *27*, 980.

[23] G. W. Caldwell, A. D. Gauthier, J. E. Mills, *Magn. Reson. Chem.* **1996**, *34*, 505.

[24] Clarke's Isolation and Identification of Drugs (Eds: A. C. Moffat, J. V. Jackson, M. S. Moss, B. Widdop), The Pharmaceutic Press, London **1986**.

[25] H. Rapoport, R. M. Bonner, *J. Am. Chem. Soc.* **1951**, *73*, 2872.

[26] G. Stork, P. A. Grieco, M. Gregson, *Tetrahedron Lett.* **1969**, 1393.

[27] S. Makleit, G. Somogyi, R. Bognár, *Acta Chim. Acad. Sci. Hung.* **1976**, *89*, 173.

[28] F. F. Knapp Jr., P. C. Srivastana, A. P. Callahan, E. B. Cunningham, G. W. Kabalka, K. A. R. Sastry, *J. Med. Chem.* **1984**, *27*, 57.

[29] M. F. Powell, *J. Pharm. Sci.* **1986**, *75*, 901.

[30] J. R. L. Smith, D. Masheder, *J. Chem. Soc. Perkin Trans. 2* **1977**, 1732.

[31] H. Rapoport, S. Masamune, *J. Am. Chem. Soc.* **1955**, *77*, 4330.

[32] H. Rapoport, G. W. Stevenson, *J. Am. Chem. Soc.* **1954**, *76*, 1796.

[33] B. Proksa, *Pharmazie* **1984**, *39*, 687.

[34] G. Krowech, P. S. Caldera-Munoz, K. Straub, N. Castagnoli, M. A. Correia, *Chem. Biol. Interact.* **1986**, *58*, 29.