STABILITY OF CODEINE: CHARACTERIZATION OF OXIDATION PRODUCTS OF CODEINE FORMED IN AQUEOUS SOLUTION

El-ahajBabiker Mohamed¹, ShehabNaglaa Ahmed² and Ali Heyam Saad^{3*}

¹Department of Pharmaceutical Chemistry, Ajman University of Science and Technology, Fujairah, UAE

²Department of Pharmacognosy and Phytochemistry, Dubai Pharmacy College, Dubai, UAE

³Department of Pharmaceutics, Dubai Pharmacy College, Dubai, UAE

*Corresponding Author Email: heyam57@hotmail.com

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ABSTRACT

The aim of the present study was to characterize some compounds resulting from storing codeine aqueous solutions under different conditions in search of an impurity observed in some codeine preparations and in the urine of their users. Acidic, neutral and basic aqueous solutions of codeine were stored in the dark or exposed to sunlight for one month. Aliquots of the solutions were withdrawn at 3-day intervals, extracted with organic solvent at basic pH and analyzed by GC-MS before and after trimethylsilyl derivatization; where applicable separated compounds were identified by database mass spectral libraries. Other compounds were tentatively characterized from GC-MS data and reaction mechanistic interpretations. At neutral pH, codeine was partially oxidized to norcodeine and codeinone while at basic pH only the former compound was formed. In both cases, light had no effect. In the acidic solutions exposed to light, codeine was autoxidized to two products which were tentatively characterized as epimeric forms of 10-hydroxycodeine. None of the three products was detected in pharmaceutical preparations of codeine or in opium. The nature of the oxidation products of codeine in aqueous solution is determined by the state of protonation of the molecule as well as by the presence or absence of photo effects. The results indicated that codeine aqueous pharmaceutical preparations should always be protected from light. On the other hand the stability of codeine in opium is most probably due to the co-presence of compounds with antioxidant properties.

Keywords: Codeine; Oxidation; Norcodeine; Codeinone; Autoxidation; GC-MS

INTRODUCTION

Codeine (Figure 1) is a narcotic analgesic drug, which is used in the alleviation of minor to moderate pain and as an antitussive. Its pharmaceutical preparations (analgesic tablets, capsules and antitussive syrups) are subject to abuse¹ and are sometimes used, in large doses, by heroin addicts to obtain a rewarding effect or to justify the presence of morphine, resulting from heroin, in their urine². Codeine is prepared either by the methylation of morphine or extraction from the natural product opium³. During routine confirmation of immunoassay-opiate-positive urine by GC-MS, we had detected the opium alkaloid thebainone-A (Figure 2) in the urine of codeine pharmaceutical preparation users. In investigative experiments designed to trace the source of thebainone-A in those preparations, aqueous solutions of codeine at different pH values were exposed to sunlight or kept in the dark and then analyzed by GC-MS. Thebainone-A was not detected in any of the test solutions, however, other compounds were, differing in nature according to the pH of the solutions and the presence or absence of photo effects. The main aim of this study was to characterize those compounds and investigate their possible formation in aqueous pharmaceutical preparations as they might be a source of toxicity. Furthermore, as opium forms an important source of codeine, it will be investigated for codeine-related products that might have been formed during processing and storage.

MATERIALS AND METHODS

Materials

Reference standard of codeine phosphate was a kind gift from Pharmicare Pharmaceuticals, Dubai, UAE. Opium samples were obtained as seizures submitted to our laboratory by the Drug Enforcement Department of Sharjah Police (UAE). Syrups containing codeine, pseudoephedrine and triprolidine

were obtained from local pharmacies. Bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was purchased from Sigma Aldrich, GmbH, Deisenhofen, Germany. Dichloromethane, isopropanol, ethyl acetate, methanol, concentrated ammonia, concentrated hydrochloric acid, sodium hydroxide and sodium sulphate anhydrous, all of analytical grades, were purchased from BDH Chemicals Ltd, Poole, England.

Methods

Codeine aqueous solutions: preparation, storage and GC-MS analysis

Two sets of three 100 µg/ml aqueous solutions of codeine phosphate were prepared in water, 0.1M HCl and 0.01M NaOH, in screw-capped Pyrex glass test tubes. The solutions of one set were exposed to the morning sunlight (at ~25-30°C) for 100 hours in 30 consecutive days, while those of the other set were kept in the dark at room temperature (25°C). At 3-day intervals, the pH of 1 ml portions of all the solutions was adjusted to ~10 with either 2 M HCl or 2 M NaOH, as appropriate and each of the solutions was extracted, first with a 9/1 mixture of dichloromethane/isopropanol (3 ml) and then with ethyl acetate (3 ml). After evaporation of the combined solvent extracts under nitrogen at 45°C, each of the residues was dissolved in methanol (1 ml) and a 2-µl aliquot was injected into the GC-MS. The methanol was then evaporated off each of the remaining solutions and the residues were separately heated with BSTFA/1% TMCS (100 µl) at 60°C for 10 min. Each reaction mixture was then diluted to 1 ml with ethyl acetate and a 2 µl aliquot was injected into the GC-MS.

Codeine-containing syrups: extraction of codeine and GC-MS analysis

The pH of 5 ml of syrup containing codeine phosphate, pseudoephedrine and triprolidine (10 mg/5 ml) was adjusted to ~10 with conc. ammonia. The solution was extracted with a 9/1 mixture of dichloromethane/isopropanol (10 ml). A 100 μ l aliquot of the organic layer was diluted to 1 ml with the dichloromethane/isopropanol mixture and a 2 μ l aliquot was injected into the GC-MS. The solvent was then evaporated off the remaining solution under nitrogen at 45°C and the residue was heated with BSTFA/1% TMCS (100 μ l) at 60°C for 10 min. The reaction mixture was diluted to 1 ml with ethyl acetate and a 2 μ l aliquot was injected into the GC-MS.

Opium sample solutions: preparation and GC-MS analysis

The solutions were prepared assuming that the opium seizures contained ~1% w/w of codeine. A suspension of powdered dry opium or of resinous opium cut into small pieces (1 g) in 10% ammonia solution (10 ml) was shaken with a 9/1 mixture of dichloromethane/isopropanol (10 ml) for 10 min. After centrifugation at 3000 rpm for 5 min, the organic layer was separated and extracted with 1M hydrochloric acid (10 ml). The aqueous layer was separated and basified to pH 10 with conc. ammonia and then extracted with dichloromethane/isopropanol (10 ml). The organic layer was then separated and filtered through a phase separator filter paper containing anhydrous sodium sulphate. After evaporation of the solvent from a 100 μ L aliquot under nitrogen at 50°C, BSTFA/1% TMCS (100 μ l) was added and the reaction mixture was heated at 60°C for 10 min; a 2 μ l aliquot was injected into the GC-MS.

GC-MS instrumentation and analytical conditions

GC-MS analyses were conducted on an Agilent (HP) series 6980 GC interfaced with a 5973 HP quadrupole mass selective detector (MSD) (Palo Alto, CA, USA) using a HP 5MS capillary column (Cross-linked 5% methyl phenyl silicone, 30 m x 0.25 mm i.d., 0.25 μ m film thickness; Hewlett-Packard, Palo Alto, CA, USA). Analytical conditions: The injector port and transfer line were maintained at 250°C and 280°C, respectively. Helium was used as the carrier gas at a flow-rate of 1 ml/min. A 2- μ l splitless injection was performed with the following oven program: an initial temperature of 100°C held for 2 min and then ramped at 20°C/min to 280°C, with a final hold time of 7 min. The total run time was 18 min. Electron impact (EI) analysis was performed with the ionization energy set at 70eV. Data were acquired in the scan mode over a range of m/z 50-550 at a rate of 2 scans/second. The ion source temperature was 230°C.

Database mass spectral libraries

Three database mass spectral libraries were available for characterization of unknown compounds: Wiley7N.1, Nist98.1 and PMW_Tox3.1.

Figure 1: codeine. Figure 2: Thebainone-A

Figure 3: A: Total ion chromatogram obtained from a neutral-pH solution of codeine exposed to sunlight or in dark.

B: Mass spectrum of codeinone

**Figure 4: A: Ion chromatogram at m/z 315, obtained from aqueous acidic-pH solution of codeine exposed to sunlight
B and C: Mass spectra of compounds I and II as per labels**

B

Figure 5: A: Extracted ion chromatogram at m/z 459, obtained from aqueous acidic-pH solution of codeine exposed to sunlight B and C: Mass spectra of compounds III and IV as per labels. The GC-MS analysis was carried out after TMS derivatization

Figure 6: A: Extracted ion chromatogram at m/z 459, obtained from TMS-derivatized basic-pH extract of opium. B: mass spectrum of the compound V

Figure 7: Chemical structures of oxidation and autoxidation products of code

RESULTS AND DISCUSSION

Codeine

In aqueous solution, codeine exhibited different behaviors according to the state of protonation of the molecule, as governed by the pH of the solution, and the presence or absence of photo effects. The results are depicted in Figure 3, 4 and 5. In neutral/basic-pH solutions, exposed to sunlight or kept in the dark, codeine was N-demethylated to norcodeine (Figure 3 and 7), which is a known product of microbial oxidation^{4,5}. In the neutral solution, exposed to light or kept in the dark, codeine was oxidized to codeinone (Figure 3 and 7), which is a known product of chemical and microbial⁶ oxidation of codeine. Both norcodeine and codeinone have been characterized by database mass spectral libraries. At the end of the reaction time, i.e. 100 hours, the two degradation products were estimated to have been formed in approximately 17% concentrations of that of codeine as measured from peak heights in the corresponding chromatogram (Figure 3). In acidic solutions kept in the dark, codeine did not undergo any change; however, in the acidic solutions exposed to sunlight, it behaved in a different way to the neutral/basic solutions. At the end of the reaction time, i.e. 100 hours, almost all of the codeine was oxidized to a mixture of two hydroxycodeines of identical mass spectra (compounds I and II, Figure 4). The formation of the hydroxycodeines has been judged from: (a) increase of the molecular weight of codeine by 16 (i.e. the atomic weight of oxygen) giving rise to both compounds I and II (Figure 4) and (b) formation of di-trimethylsilyl derivatives of compounds I and II (compounds III and IV respectively, Figure 5). The identical mass spectra of compounds I and II (Figure 4) and of their corresponding TMS derivatives, compounds III and IV (Figure 5) indicate that the two compounds are diastereomeric. Hydroxylation of candidate compounds exposed to light for a long time is an autoxidation reaction⁷. The role of light is to generate free radicals to which molecular oxygen adds to form hydroperoxides (ROOH), which will react further to give alcohols⁸. Mechanistically, the two most favoured auto hydroxylation sites in the codeine molecule are the secondary-benzylic C10 and the tertiary allylic C14 (Figure 1) which is due to their high reactivity towards free radical formation. The latter carbon was excluded as the site of hydroxylation in

compounds I and II based on observed differences in the mass spectra: the m/z of the base-peak ions of the TMS derivatives of compounds I and II and that reported for 14-hydroxycodeine⁸ were 196, 196 and 229, respectively. Therefore, compounds I and II could be designated as 10-hydroxycodeine formed in two diastereomeric (α and β epimeric) forms (Figure 7). Epimerization is a process that takes place in compounds in aqueous solutions whereby a functional group at a chiral carbon undergoes partial inversion of configuration⁹. Usually, epimeric forms of a substance in aqueous solution exist in equilibrium¹⁰. It may be of interest to note that chemical hydroxylation of codeine by hydrogen peroxide had been reported¹¹ to take place at the aromatic ring; however, the authors did not give evidence of this nor did they specify the position of the hydroxy group. Analytical artifactual formation of the codeine degradation and autoxidation products under the experimental conditions used in this study was ruled out by analyzing fresh solutions of codeine phosphate; none of the degradation products was detected. Furthermore, contrary to previous reports¹², none of the degradation products found in this study was an isomer of codeine. Neither norcodeine nor codeinone was detected in syrups of codeine that had been stored in the dark for more than two years suggesting that other ingredients present in the syrups had played an antioxidant role. Although it is unlikely that codeine pharmaceutical preparations will be formulated at the acidic conditions that favor autoxidation in the presence of light, catalysis by excipients may not be completely ruled out and should be investigated.

Opium

As early as 1911, opium was reported to contain a hydroxycodeine¹³. The data shown in Figure 6 confirm this and almost certainly indicate the presence of 14-hydroxycodeine upon comparing the mass spectrum with that reported in the literature⁸. Since position 14 in opium is allylic and represents an active site for autoxidation, 14-hydroxycodeine in opium may be an autoxidation product the formation of which is influenced by the natural matrix of opium. Generally, the processing of opium involves, in its early stages, sun drying of the latex, which is an aqueous suspension.

Morphine

As a matter of interest, the same experiments performed with codeine were repeated with morphine. No oxidation products of morphine analogous to those of codeine found in this work were detected for morphine.

CONCLUSION

Light and pH of aqueous solutions are two important parameters to consider in stability investigations of medicinal substances in their pharmaceutical preparations and processed natural sources. Generally, the presence of compounds with antioxidant properties will help avert oxidation and autooxidation of pharmacologically active ingredients in pharmaceutical dosage forms and natural products. It is recommended that stability studies should involve investigations of antioxidants with the aim of their inclusion in pharmaceutical formulations of the subject substance. The dependence of the chemical nature of degradation of a compound on the state of protonation of the molecule in aqueous solution is an interesting subject for exploration.

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