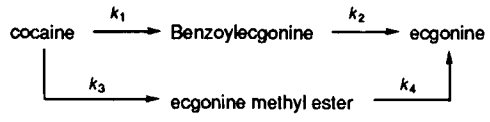
Prediction of Stability in Pharmaceutical Preparations. XXI: The Analysis and Kinetics of Hydrolysis of a Cocaine Degradation Product, Ecgonine Methyl Ester, Plus the Pharmacokinetics of Cocaine in the Dog

To the Editor:

The kinetics of hydrolyses of cocaine and benzoylecgonine, studied previously,[1] delineated the rate constants k_1, k_2, and k_3



at various temperatures and pH values on the premise that the rate-determining steps for the loss of cocaine were k_1 going to benzoylecgonine and k_3 as loss to nonchromophoric products. Since no specific assay for the nonchromophoric ecgonine methyl ester (EME) was available, the exact characterizations of k_3 and k_4 were not possible at that time. The procedures given here permitted the delineation of k_4 (see Table 1) and showed that there should be no great build-up of ecgonine methyl ester from the solvolysis of cocaine due to pH factors alone since the rate of hydrolysis of EME is greater than that of its formation.[1] The rate of hydrolysis of EME is also greater than the rate of hydrolysis of cocaine to benzoylecgonine.[1] Apparently, the presence of a benzoyl function on the 8-methyl moiety, as in cocaine, inhibits the hydrolysis of the methyl ester group. A possible alternative explanation is that the hydroxyl group facilitates the hydrolysis of the methyl ester group, possibly due to hydrogen bonding with the oxygens of the ester group. The log k–pH profiles of Figure 1 were constructed from the data given in the table based on the principles previously espoused.[1]

The ecgonine methyl ester excreted in urine has been claimed to have been formed from cocaine by human plasma cholinesterase.[2]

Rate Constants and Arrhenius Parameters for the Hydrolyses of Ecgonine Methyl Ester—The Arrhenius relations and parameters[3] for first-order rate constants at pH values of 6, 10.5, and 13 and for the second-order rate constant k_{OH^-} obtained in 0.2 N NaOH are given in Table 1. The estimated second-order rate constants k_{H^+} and k_{OH^-}, respectively determined from $k = k_{H^+}[HCl]$ and $k = k_{OH^-}[NaOH]$, as well as the second-order rate constants $k_{a_{H^+}}$ and $k_{a_{OH^-}}$, respectively determined from $\log k = \log k_{a_{H^+}} - pH$ and $\log k = \log k_{a_{OH^-}} - pK_w = pH$, are given in the table for various temperatures and pH values. The pH 6 studies were presumed to result solely from hydroxyl ion attack on the amine-protonated ecgonine methyl ester. The apparent first-order rate constant k at higher pH values must be multiplied by the fraction of EME protonated, $a_{H^+}/(a_{H^+} + K'_a)$, where the hydrogen ion activity is $a_{H^+} = 10^{-pH}$ and $K'_a = 10^{-pK'_a}$. Estimated apparent pK'_a values are 9.3 at 30 °C, 9.1 at 40 °C, and 8.5 at 60 °C. The pK_w (13.83 at 30 °C) value determination was discussed previously.[1] As the protonated amine is neutralized, the rate constant as a function of pH approaches an asymptote and this plateau is maintained until the slower rate of attack of hydroxyl ion on nonprotonated ecgonine methyl ester becomes significant and the plot deviates upward from this log k – pH plateau. A fuller discussion of this type of relation has been given previously.[1]

Detection and Analytical Methods—Ecgonine and EME lack chromophores and thus were not susceptible to direct UV detection with high-performance liquid chromatography, as were its precursors.[1] EME free base was prepared by dissolving 221.4 mg of EME·HCl (NIH, Bethesda, MD, Lots 3001-1022-151 and 3003-1022-97) in 2.5 mL of 2.5 M K_2CO_3 with a resultant pH of 11.79. The solution was immediately extracted twice with 40 mL of benzene (Fisher Scientific, Orlando, FL, HPLC grade) with shaking (5 min). Since benzene is a suspected human carcinogen, use should be confined to a fume hood or a glove box. The combined benzene extracts were passed through a 20-g anhydrous Na_2SO_4 column (with 10 mL of benzene as a chaser) to remove any residual water. EME free base was obtained by taking the benzene solution to dryness at 30 °C under nitrogen. Stability studies were effected on this material. Ecgonine hydrochloride (Lot 2460-1022-80) was also obtained from NIH.

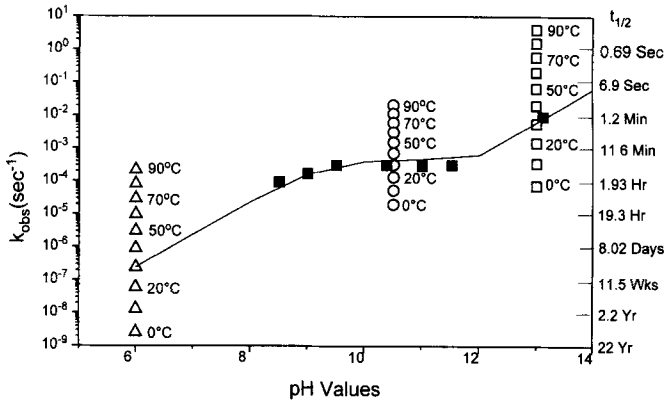


Figure 1—log k_{obs} vs pH for the hydrolysis of ecgonine methyl ester at 30 °C. Values for log k_{obs} at other temperatures for several pH values were predicted from Arrhenius parameters determined from three or four studies at different temperatures at the stated pH values (see Table I).

Calibration curves were prepared for 1–12 μg/μL EME·HCl in reaction vials (5–50 μL of a 0.12 μg/μL stock solution in pH 8 buffer admixed with pH 10.5 carbonate buffer to give 0.5 mL of solution of a final pH of 9.9). EME free base was extracted by 3.2 mL of benzene with shaking (2 min) and centrifugation (2 min) to separate the phases, and 3 mL of benzene extract was reacted overnight in the dark with 25 μL of freshly redistilled phenyl isocyanate (Fluka Co., Ronkonkoma, NY) and 50 μL of acetonitrile (Fisher, HPLC grade). Then, 50 μL of 2-propanol (Fisher, HPLC grade) was added with vortexing and reacted for 60 min. The solution was evaporated under nitrogen with low heat (40 °C) and the residue redissolved in 300 μL of 0.01 N HCl containing cocaine (NIH Lot 2563-1022-20C) standard (10 μL of 0.5 μg/mL pH 3.6 acetate buffer). Background cleanup was effected by subsequent extraction by 4 mL of benzene with 2 min of shaking, and the aspirated benzene phase was discarded. After pH readjustment with 250 μL of pH 9 buffer, the phenyl carbonate derivative of (EME-PC) and cocaine standard were extracted with 3.2 mL of benzene (15 min of shaking on the shaker) followed by 2 min of centrifugation to separate the phases. THe 3-mL benzene phase was transferred to a reaction vial and taken to dryness under nitrogen with low heat (40 °C), and the resulting residue was redissolved in 250 μL of mobile phase prior to injection into the HPLC of 15 μL onto a C^{18} 10-μm Bondapak 30-cm column (Waters Lot P52041C46; with a mobile phase of 27:73 acetonitrile:pH 6.5, 0.05 M phosphate buffer at a flow rate of 1.5 mL/min with ultraviolet spectral detection at 229 nm).

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Table 1—Dependencies of Rate Constant (k in s^{-1}) for Hydrolysis of Ecgonine Methyl Ester on pH and Temperature

A. Dependencies as Functions of pH, Acidity, and Alkalinity

Conditions	Based on HCl normality	Based on pH in HCl	Based on NaOH normality	Based on pH in Phosphate Buffers	Based on pH in Borate Buffers
Mechanism	$H^+ + EMEH^+$	$H^+ + EMEH^+$	$OH^- + EME$	$OH^- + EMEH^+$	$OH^- + (EMEH^+ \rightleftharpoons EME)$
Range	0.02–0.10 N	pH 1.12–1.77	0.01–0.2 N	pH 5–6	pH 9.5–11.5
N	3 at 90 °C	3 at 90 °C	3 at 30 °C	3 at 80 °C	4 at 30 °C
Dependency	$k = k_{H^+}[HCl] + intcpt$	$\log k = \log k_{a_{H^+}} - m$pH	$k = k_{OH^-}[NaOH] + intcpt$	$-m$pOH + $\log ka_{OH}$ = m(pH − pK_w) + $\log k_{a_{OH}}$ $\log k_{a_{OH}}$ = Intcpt + mpK_w	$\log k = -m$pH + Intcpt m(pH − pK_w) + $\log k_{a_{OH}}$ $\log k_{a_{OH}}$ = Intcpt + mpK_w
corr r^2	0.9915598	0.97313	0.9999992	0.9947273	−0.352
Values	$k_{H^+} = 3.01 \times 10^{-5}$ ($\pm 1.96 \times 10^{-6}$) Intcpt = 6.65×10^{-8} ($\pm 1.24 \times 10^{-7}$)	m = −0.9693(±0.1131) $\log k_{a_{H^+}} = -4.442(\pm 0.168)$ $k_{a_{H^+}} = 3.62 \times 10^{-5}$ L/mol/s	$k_{OH} = 0.00851$ ($\pm 5.33 \times 10^{-6}$) Intcpt = 8.19×10^{-5} ($\pm 6.89 \times 10^{-7}$)	m = 0.658 (±0.34) Intcpt = −8.03(±0.187) pK_w = 13.83 @ 30 °C $k_{a_{OH}} = e^{1.07}$ = 11.67 L/mol/s	$m = -2.56 \times 10^{-3}$ ($\pm 5.47 \times 10^{-3}$) Intcpt = −3.49(±0.058) pK_w = 13.83 @ 30 °C $k_{a_{OH}} = e^{-3.53}$ = 3×10^{-4} L/mol/s

B. Dependencies as Functions of Temperature: $\ln k_{obs}$ = slope × $1/T$ = ln P

pH	13 (0.2 N NaOH)	6	10.5
Temp Range	16.5–40 °C	70–90 °C	40–60 °C
N	4	3	3
Variable	$\ln k_{OH^-}$ – (L/mol/s)	ln k (s^{-1})	ln k (s^{-1})
Slope	−5.46 ± 0.47	−12.50 ± 1.05	−7.68 ± 1.73
r^2	0.97786	0.9859	0.90313
ΔH^a (kg cal/mol)	10.84(±0.94)	24.83(±2.09)	15.26(±3.44)
ln P	11.75 ± 1.57	26.04 ± 2.99	17.30 ± 5.37
ΔS^a (EU/mol)	23.4 ± 3.1	51.7 ± 5.9	34.4 ± 10.7

[a] From $\ln k = \Delta(S/R) + \Delta H/R(1/T)$, where R = 1.987 kcal/deg/mol.

The assayable UV peak at 232 nm, similar to that of cocaine,[1] was the basis for the aforementioned kinetic studies. EME-PC and cocaine had respective retention times of 14.3 and 17.5 min. HPLC assays of injected 37–187 ng/10 μL derivatized pure EME base gave an excellent concentration vs peak area and peak height ratio calibration curve with a standard error of regression of ±2.4 ng/10 μL.

Silylated glassware appeared to minimize variability (sodium hexafluorosilicate, PCR Inc., Gainesville, FL).

Kinetic studies were effected on pH-adjusted 12 μg/mL solutions of EME·HCl at various temperatures and times using similar analytical schemes.

Other Methods Essayed To Derivatize Ecgonine Methyl Ester—Attempts to obtain derivatives of EME for UV detection with HPLC by reacting 5 mg of dansyl chloride in 1.5 mL of acetone with 1 mg of EME in 0.5 mL of saturated Na_2CO_3 overnight at room temperature in the dark were unsuccessful. Excess reagent was removed from one-half of the solution by reacting it with 5 mg of proline in 20 μL of water for 30 min at room temperature and by adding 0.6 mL of 1 N NaOH to the other half and allowing that portion to react for 1 min at room temperature. Subsequently, the solutions were each extracted with 4 mL of benzene and no derivative that differed from blanks carried through similar procedures could be detected by TLC or HPLC with spectrophotometry.

Dry EME free base (0.5–2 μg of ecgonine hydrochloride, NIH, Lot 2460-1022-80) was derivatized for 1 h at 55 °C to an analog of cocaine [(3,5-dinitrobenzoyl)ecgonine methyl ester]. Ecgonine hydrochloride (National Institute on Drug Abuse, NIH Lot 2460-1022-30) yielded 3,5-dinitrobenzoyl chloride derivatized ecgonine. A 5 molar excess of 3,5-dinitrobenzoyl chloride (11.5 mg, 50 μmol, Lot P 80-26-2, Regis Chemical Co., Morton Grove, IL) in 2 mL of dichloromethane (Fisher, Orlando, FL) with 2 μL of pyridine (Aldrich Chem. Co., Milwaukee, WI) was used in the reaction. After evaporation under N_2 and redissolution in 500 μL of mobile phase, such derivatives were observable by HPLC (Waters) with UV detection at 254 nm (t_R = 16.7–17.6 min, with 10 μL injected on a Waters C^{18} Bondapak P27324 column with a mobile phase of 25% acetonitrile: 75% 0.05 M acetate buffer of pH 3.67) and by thin-layer chromatography. Unfortunately, yields were variable on repetitive assays and the resultant derivatives had many assay interferences even when conditions of the procedure were varied.

The Assayable Phenyl Carbamate Derivative of EME and Its Properties—Recrystallized, purified EME-PC was formed by overnight reaction of EME free base with redistilled phenyl isocyanate melted at 162–167 °C and had an NMR consistent with the assigned structure.

Attempts to apply this assay methodology to EME in biological fluids were unsuccessful because of high variability and low yield even through mixtures of EME-PC and mobile phase and evaporation of these mixtures from acetonitrile and benzene solutions gave excellent calibration curves.

EME-PC was reasonably stable under the conditions of the aforementioned clean-up procedures; concentrations were unchanged at pH 1.5 and 7 for at least 6 h at 23 °C with apparent half-lives of 6 h at pH 8.2 and 2.1 h at pH 8.8. Since EME-PC was extracted completely by benzene at pH 7 as well as at pH 9, the former pH was used so as to minimize any possible degradation during its clean-up extraction.

When the assay was performed on chloroform:ethanol 3:1 solutions of EME base that were evaporated to dryness and redissolved in acetonitrile for derivatization, highly variable and lessened yields of EME-PC were encountered.

The interference of a urine background in EME-PC analysis which had given the abovementioned variability was avoided when a mobile phase of 27:73 acetonitrile:0.05 M phosphate buffer with a flow rate of 1.5 mL/min was used with a μBondapak C^{18}

column. The standard error of the regression of concentration against the peak height ratio was ±7.5 ng/mL. Procaine was used as an internal standard. Calibration curves for EME in plasma had a standard error of regression of concentration vs peak height ratio of ±2.8 ng/mL. This was obtained with a mobile phase of 30:70 CH_3CN:0.5 M phosphate buffer, pH 6, at an 1.5 mL/min flow rate.

Optimum Extraction Procedures—When benzene, rather than chloroform, was used as the extracting solvent for cocaine,[1] impurity peaks that interfered with the retention time peak of cocaine did not occur. Sensitivities below 5 ng/mL plasma for cocaine and norcocaine were established with a standard error of regression of concentration vs peak height of ±1.4 ng/mL for plasma and ±14 ng/mL for urine.

Benzoylecgonine is not extracted into benzene. A clean background for its assay can be obtained by prewashing the benzene-extracted plasma with 10 mL of ethyl acetate, a clean-up procedure that does not extract any benzoylecgonine. This cleanup is important since the alcohols necessary for benzoylecgonine extraction possess miscibility with water and can extract potentially interfering polar compounds from the urine as well. There is no need to adjust the pH since benzoylecgonine is extracted well by 50:50 benzene:butanol (2 mL of benzene: butanol to 1 mL of benzene pre-extracted plasma) around a pH of 7. Due to the instability of cocaine in plasma, blood samples must be assayed immediately upon sampling and urine should be frozen immediately. To insure that cocaine remains stable before being assayed, the pH of all stored biological samples should be adjusted to 4, at which the half-life is *ca.* 30 years, before freezing.

Additional Studies Performed To Perfect the Assay—Some additional studies were conducted to perfect the assay and results were as follows.

(1) When EME-PC plus 200 μL of acetonitrile was evaporated under nitrogen, there was no loss of material.

(2) There did not appear to be any significant difference between silyated and nonsilyated reaction vials nor among 15, 30, and 60 min reaction times in the preparation of EME-PC.

(3) EME-PC concentration decreased significantly at 30 and 60 min in 9.36 pH buffer but showed no significant instability at pH values of 1.5 or 7.0 over these intervals. The average half-lives (four studies each) of EME-PC were 5.6 h at pH 8.2 and 2.2 h at pH 8.8.

(4) Solutions of EME free base showed degradation when the extracting 3:1 or 4:1 $CHCl_3$:ethanol was evaporated to dryness with or without heat (under nitrogen). Interfering spectrophotometrically observable peaks resulted. Increasing the $CHCl_3$ content did not prevent an apparent EME free base loss. Derivatization to EME-PC in solution without prior evaporation did not prevent loss or remove spectral interferences.

(5) EME-PC did not completely partition into 3 mL of hexane from 0.3 mL of solutions (5 μL of 1 μg/mL EME-PC with 300 μL of buffer) at pH values of 7.0, 7.5, 8.0, 8.5, and 9.0. Only after two extractions at pH values of 8.5 and 9.0 was 100% extracted. Benzene (3 mL) did completely extract EME-PC from such solutions at pH values of 7.00–9.00.

(6) Interfering HPLC peaks were removed and there was no loss of EME-PC with use of the following procedures. After evaporation of the residual solvent from EME-PC prepared from phenyl isocyanate and EME free base, 300 μL of 0.01 N HCl was added to the residue and extracted with 3 mL of benzene. This benzene was discarded and from the remaining solution, adjusted to a pH 7, was extracted EME.PC into 3 mL of benzene which was evaporated to dryness. The EME-PC was redissolved in mobile phase for assay. It was no longer necessary to filter the white precipitate previously seen on drying derivatized solutions.

(7) EME free base (1.3–6.5 μg) could not be derivatized by 5 μL of phenyl isocyanate in a 200-μL solution (150–190 μL of dichloromethane with 50–10 μL of acetonitrile). It can be derivatized completely in acetonitrile (200 μL for 30 min) or benzene (1 mL for 30 min, 2 mL for 60 min, and 3 mL for 90 min) with 25 μL of phenyl isocyanate.

(8) EME free base (1.3–5.2 μg) could not be extracted from 0.5 mL into 3.2 mL of benzene at pH 7 (where it should be completely protonated) whereas at room temperature, 56% could be extracted at pH 9 with one extraction from 0.5 mL into 3.2 mL of benzene (partition coefficient = 0.197), 80% with two extractions. The extraction efficiency of 5.5 μg of EME free base from 0.5 mL of pH 10 borate buffer by benzene is 83.2% (partition coefficient = 0.774), from pH 10.54 is 87%, and from pH 11 and 11.6 is 94%. These extraction efficiencies as a function of pH are consistent with an EME of pK'_a *ca.* 9. Rough estimates of $t_{1/2}$ based on decreases in the peak area ratio at 30 °C were 67 min at pH 9.0, 56 min at pH 9.5, 42 min at pH 10.0, 40 min at pH 10.5, 26 min at pH 11.0, and 23 min at pH 11.55, (94% extracted) and indicate only minor changes in degrees of EME protonation in the pH range 9.5–11.5. These extractions were performed immediately after pH adjustment. Any delay would impair valid extraction of unhydrolyzed EME. These rough estimates were confirmed by the 30 °C log k–pH profile of Figure 1.

(9) Kinetic studies on EME·HCl in buffered solutions gave the same results as free base in the same buffers and were so performed when feasible.

Pharmacokinetic studies in a mongrel dog at 3 mg/kg of intravenously administered cocaine used the analytical methods developed for cocaine.[1] Admittedly, it does not provide any indication of variability among animals but is presented since there is no present opportunity to perform other studies. A three compartment body model for plasma was observed down to 15 ng/mL and the respective phases had 1.4, 12.9, and 55.4 min biological half-lives. Total body clearance was 1441 mL/min, and since hepatic blood flow in the dog is only 415 (266–612) mL/min,[4] the extrahepatic transformation of cocaine is indicated. Renal clearance showed no urinary pH or flow rate dependence. Only 9.3% of the unchanged cocaine was excreted into the urine. The apparent volumes of distribution were V_c = 21 L and V_d = 115 L (4.6 L/kg in a 25-kg dog). There were no apparent nonlinear kinetics.

As we observed in the dog, only small amounts of unchanged cocaine (<1% of the iv dose) appeared in human urine, 18–22% of the dose as ecgonine methyl ester and only very small amounts of norcocaine.[5,6] The probable reason for significant ecgonine methyl ester accumulation *in vivo* is that its kinetics of formation are enhanced by cholinesterase action on cocaine.[2,5] Benzoylecgonine was the most prevalent cocaine metabolite found in human urine (35–54% of the dose[6]). The apparent volume of distribution of cocaine was a relatively small 2.7 L/kg (216 L in an 80-kg human),[5] which is of similar magnitude to that observed in our dog. The renal clearance of cocaine in human was 13 ± 2 mL/min, <1% of the total clearance of 1.8 L/min or 1.2–1.4 L/h, which, as in the dog, is largely due to metabolic processes.[2,5,6] The terminal plasma half-life in humans[5] is a short 87 min, which is of the same magnitude as the 55 min $t_{1/2}$ in our dog and suggests that the dog may be a valid model for cocaine pharmacokinetics in man. It is realized that dissimilar enzymes may have identical reaction rates.

References and Notes

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